Sangeeta Saxena · A. K. Tiwari *Editors*

Begomoviruses: Occurrence and Management in Asia and Africa

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Editors
Sangeeta Saxena
Department of Biotechnology
Babasaheb Bhimrao Ambedkar University
Lucknow, UP, India

A. K. Tiwari
Division of Seeds
U.P. Council of Sugarcane Research
Gola, Khiri, UP, India

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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

V.G. Malathi (✉)
Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore 641003, India
e-mail: vgmalathi@rediffmail.com

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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.

P. Roshan • A. Kulshreshtha • V. Hallan (✉)
Academy of Scientific & Innovative Research (AcSIR), CSIR-Institute of Himalayan Bioresource Technology, Palampur, HP 176061, India

Plant Virology Lab, CSIR-IHBT, Palampur, HP 176061, India
e-mail: hallan@ihbt.res.in; rnaivi@gmail.com

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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

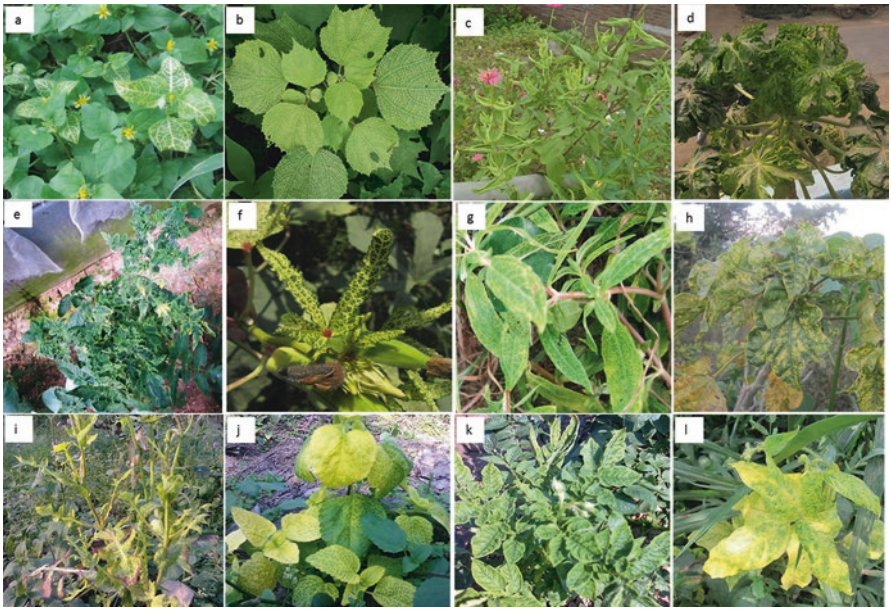

Fig. 2.1 Symptoms of *Begomovirus* on (a-l) *Synedrella* sp., *Urena* sp., *Zinnia elegans*, papaya, tomato, okra, *Eclipta* sp., *Jatropha* sp., *Sonchus asper*, *Ageratum conyzoides*, potato, and *Vigna* sp

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).

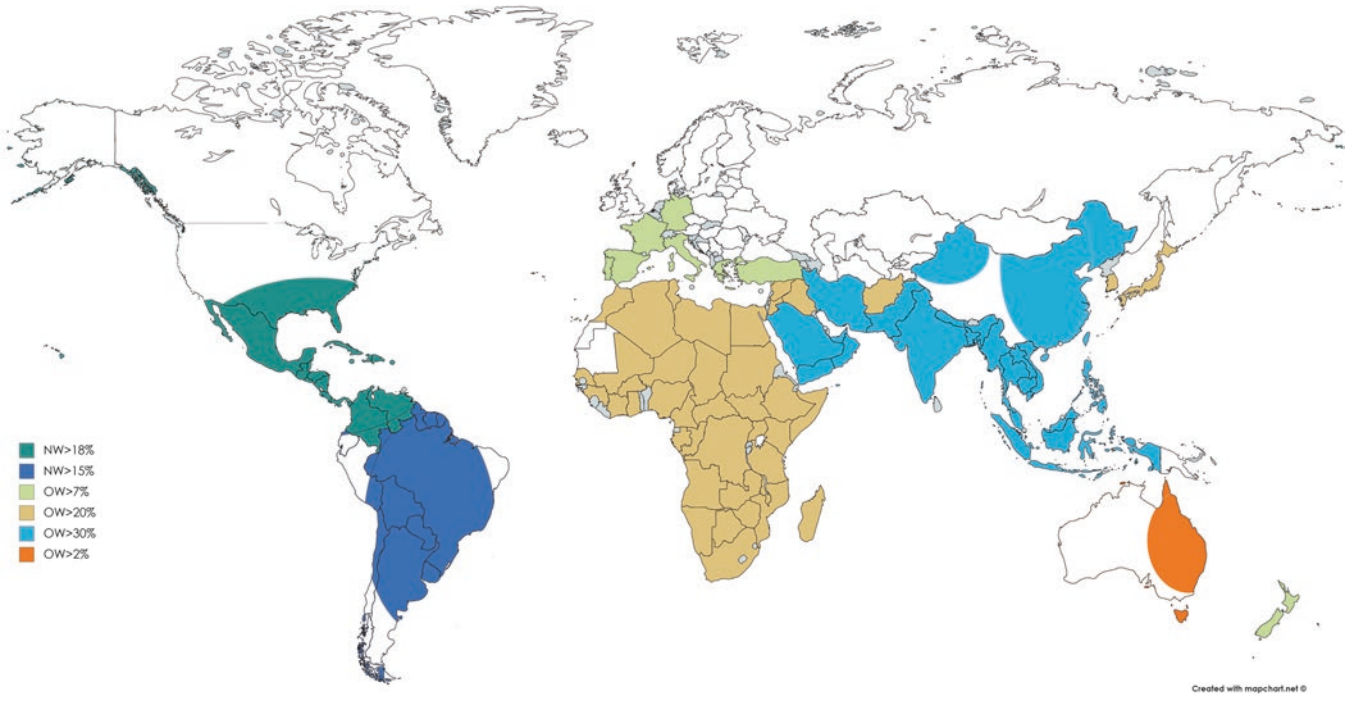

Fig. 2.2 Geographical distribution of begomoviruses indicating the highest percentage of OW begomoviruses in Southeast Asia, followed by Africa and Mediterranean region; whereas NW begomoviruses are predominantly present in America

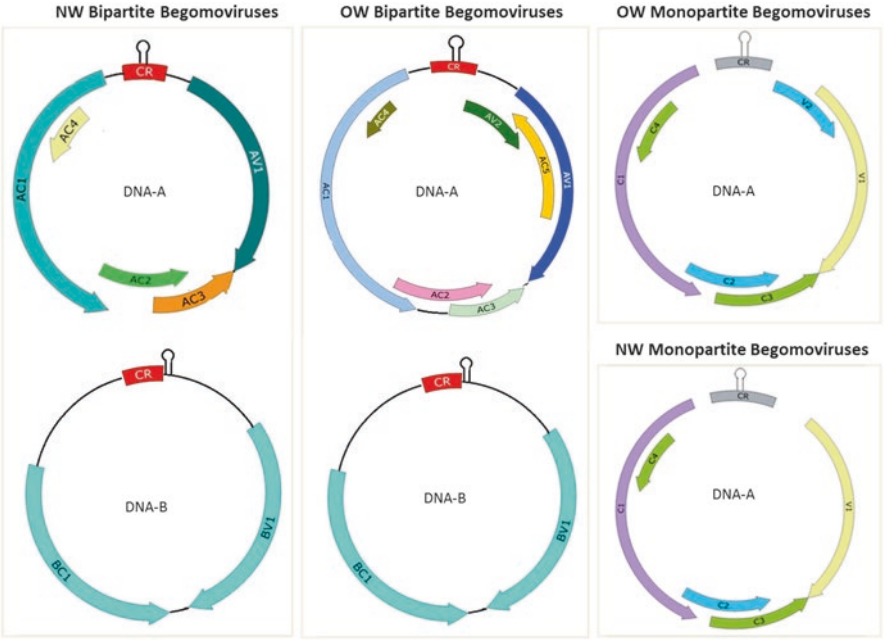

Fig. 2.3 Schematic representation of *Begomovirus* genome organization: New World bipartite, Old World bipartite, Old World monopartite *Begomovirus*, and New World monopartite *Begomovirus*

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

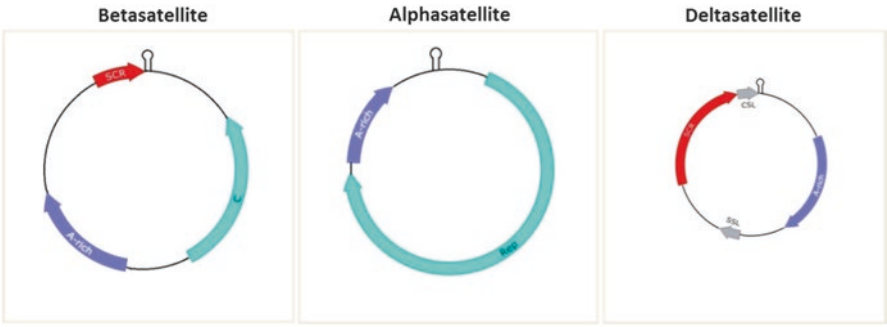

Fig. 2.4 Schematic representation of ssDNA satellites associated with begomoviruses: betasatellites, alphasatellites, and deltasatellites

(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

are few reports which have shown their association with the NW bipartite begomoviruses also (Paprotka et al. 2010, Romay et al. 2010). They encode a single replication-associated protein (Rep) of 315 amino acids, similar to nanovirus replication protein. Consequently, they have the capability to replicate autonomously but depend on the helper virus for the encapsidation and whitefly transmission. Alphasatellites have an A-rich region, immediately downstream of Rep gene of 180–200 nt (adenine content 46–52%; overall adenine content 29–33%), and a predicted hairpin loop structure with loop sequence, TAGTATTAC, similar to the nanoviruses (Fig. 2.4). It has been suggested that A-rich region might function as a "stuffer sequence" to increase the size of the molecule to half that of a begomovirus component (Briddon et al. 2004; Zhou 2013). Ageratum yellow vein Singapore alphasatellite associated with *Tomato leaf curl Oman virus* was shown to attenuate viral symptoms by significantly reducing the accumulation of betasatellite (Idris et al. 2011). This study claimed that alphasatellite plays no role in the disease development. The Rep protein of *Gossypium darwinii* symptomless alphasatellite and Gossypium mustelinium symptomless alphasatellite was reported to suppress the PTGS (Nawaz-ul-Rehman et al. 2010).

2.4.3 Deltasatellites

A novel class of DNA satellites, associated with the NW bipartite begomoviruses, were identified in two malvaceous plants "*Malvastrum coromandelianum*" and "*Sidastrum micranthum*" (Fiallo-Olive et al. 2012). These satellites were structurally similar to the first reported DNA satellite ToLCV-sat (Dry et al. 1997). These are small noncoding (630–750 nt) DNA satellites, approximately one quarter of the size of begomovirus genome, which share sequence similarity to the SCR of betasatellites and have an A-rich region, a primary stem-loop structure (containing the conserved nonanucleotide, TAATATTAC); and a secondary stem-loop structure (located between the A-rich region and SCR-like sequences) (Fig. 2.4). Like betasatellites, they are also dependent on helper begomovirus for their replication (Fiallo-Olive et al. 2012). Later on, similar satellite molecules were also found associated with begomoviruses, infecting *Ipomea* sp. However, these satellites are distinct from the betasatellite as these do not encode the βC1 protein, and therefore the name deltasatellite was proposed (Lozano et al. 2016). Recently, it has been reported that deltasatellites are transmitted by *B. tabaci* and reduce the accumulation of a helper virus (Fiallo-Olive et al. 2016).

2.5 Factors Responsible for the Emergence of New Begomoviruses and Satellites

During the DNA replication, the incorporation of noncomplementary nucleotide causes point mutations, and ~10^{-4} substitutions/site/year mutation rate has been reported in TYLCV and EACMV infection. This high mutation rate can be attributed due to the fact that DNA viruses do not utilize the host proofreading and repair

mechanisms (Roossinck et al. 1997). The mutation rate in the begomoviruses depends upon the virus type, host, host age, and homogeneity of inoculum (Isnard et al.1998; Padidam et al. 1999). Genetic exchange of the segments between the two DNA strands leads to the recombination. It has been believed that the recombination is the main cause of diversity, establishment, and evolution of the new begomoviruses (Rojas et al. 2005). The CR including "ori" is considered as hot spot for the recombination events among the begomoviruses (Padidam et al. 1999; Tao and Zhou 2008). In nature, the recombination occurred between the CR of *Potato yellow mosaic virus* (PYMV) and *Potato yellow mosaic Panama virus* (PYMPV); (Urbino et al. 2004). It has been found that the exchange of DNA not only occurs between the DNA-A molecules but also between the DNA-B and/or satellite molecules, leading to the emergence of new begomoviruses (Nawaz-ul-Rehman and Fauquet 2009). Recombinant betasatellite comprising the portions of DNA-A has been characterized in TYLCCNV infection (Tao and Zhou 2008). In several cases, the SCR is missing and replaced by the CR of begomoviruses, but a functional βC1 gene is present (Saunders et al. 2001). These examples of recombination explained the evolutionary relationship between the begomoviruses and satellite molecules. The pseudo-recombination or reassortment of genomic components is described as the genetic exchange of DNA segments between DNA-A and DNA-B components of different viruses and that involves a process known as "regulon grafting" in which CR is donated by the DNA-A to the DNA-B component (Saunders et al. 2002). CR and *Rep* of *Tomato severe rugose virus* (ToSRV) were found in *Tomato rugose mosaic virus* (ToRMV), and both isolates displayed pseudo-recombination in their host tomato (Silva et al. 2012). During the 1990s a recombinant between the EACMV and ACMV showed enhanced virulence and was responsible for the devastating epidemic of cassava mosaic disease (Zhou et al. 1997).

2.6 Conclusion

Begomoviruses are widespread, circular single-stranded DNA viruses that are a serious threat for the crop production. In case of bipartite begomoviruses, the presence of an additional genomic component created a size-based constraint in the encapsidation, and therefore, it was proposed that DNA-B encodes only two proteins to avoid this constraint. This suggests that DNA-B might have been introduced as a satellite molecule initially, that has become a genomic component during the evolution. However, the presence of betasatellite molecule along with begomovirus genomic components renders the selective advantage in terms of infectivity, host range, and pathogenicity. The *trans*-replication of betasatellites by different begomoviruses can lead to the emergence of new disease complex, and these associations may be responsible for severe crop losses. Alphasatellites are considered to be evolved from a nanovirus Rep-encoding component, but the functions of alphasatellites are still unclear. The small noncoding deltasatellites were found to reduce the level of a helper virus suggesting their role in the viral fitness. Further investigation of begomoviruses and satellites relationship with the host will be helpful for the development of crop protection methods and strategies against the virus infection.

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Recent Advancement in Diagnosis of Begomoviruses

3

Saurabh Verma and Sangeeta Saxena

Abstract

The diagnostics techniques are required for the detection of disease-causing organisms in plants. It is necessary for these techniques to be easy, specific, and efficient in virus detection at early or late stages of the disease. The timely and efficient detection of these viruses is essential in the control of diseases and in farm management practices. The diagnostics has evolved from simple symptomatic detection to the level where the disease-causing organisms are detected in non-symptomatic plants also that act as a reservoir for *Begomovirus* during unfavorable season. This chapter will give an update about the status of the established techniques for virus detection and recent advances in virus diagnostics. The techniques discussed here are available for effective farm management of the viral diseases caused by *Begomovirus* and other associated genera of geminivirus.

Keywords

Begomovirus • ELISA • qRT-PCR • RCA • LAMP • Genomics

3.1 Introduction

Although there were many techniques available for *Begomovirus* detection and identification, only those techniques reached the apex of diagnostic application which favored simplicity, sensitivity, and accuracy with the probability of scaling them up to high-throughput screening (HTS). Therefore, the diagnostic techniques

S. Verma (✉) • S. Saxena (✉)
Department of Biotechnology, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raebareli road, Lucknow 226025, UP, India
e-mail: saurabhv1982@gmail.com; dr_sangeeta_saxena@yahoo.com

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associated with polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) gained popularity. Both research and development laboratories and the on-field diagnostic laboratories applied the above mentioned tools especially ELISA being cost-effective, simple, sensitive, and scalable to testing of large number of samples even when the viral particles need not be purified, i.e., crushed leaf extract can be tested directly. This aspect provided an edge to ELISA-based diagnostics, and therefore it became a part of routine laboratory and on-field screening of plant viruses. The only limitation being the availability of a high-quality antiserum whose production requires expertise in virology and protein biochemistry to isolate and purify the specific, sensitive, and stable antiserum in bulk amount. The ELISA also faced a tough competition from PCR-based diagnostic techniques like RT-PCR and qRT-PCR (see Sect. 2.3) as these techniques were highly customizable and sensitive for differentiation of various virus strains, e.g., *Potyvirus*, and in detection of families where virus coat protein is highly conserved, e.g., geminivirus. However, despite having few limitations, the ELISA-based techniques like DAS-ELISA, TAS-ELISA, and PAS-ELISA (see Sect. 2.2) are highly recommended methodologies for surveillance, eradication, certification, sanitation, and quarantine of parent seedlings prior to storage and planting. On the other hand, the PCR-based techniques are more generic and fulfill the need for a stand-alone diagnostics for detection, identification, and quantitative estimation of *Begomovirus* and, thus, a suitable technique to address a wide range of single-stranded DNA plant viruses for on-field diagnostics.

3.2 Principles and Tools for *Begomovirus* Diagnostics

3.2.1 Plant Immune Response

The plant immune response (PIR) is basically a pattern-triggered immune (PTI) response or effector-triggered immune (ETI) response upon recognition of virus-associated molecular patterns by plant pattern recognition receptors and virus effector molecule recognizing receptor-like proteins (R proteins) triggering the hypersensitive responses (HRs). The HR is symptomatically identified as necrotic lesions, ringspots, and chlorotic lesions over leaves, stem, and fruits. Therefore, we need to target these viral effector molecules or capsid regions in order to detect and identify plant viruses in specific and effective manner (Mandandi and Scholthof 2013; Zvevreva and Pooggin 2012).

The biological diagnostics is based upon the visual symptoms along with detection of virus-specific proteins and nucleic acids responsible for transmission and amplification in host, e.g., whole or a region of coat protein or capsid, and viral nucleic acid such as DNA or RNA. The capsid or capsomeres forming coat proteins are responsible for immunity-like response in plants. These viral particles inside the host interact with host defense components thus producing immunogenic responses. These responses are sensed, decoded, identified, and trapped by host immune

machinery to direct them toward either quarantine mechanism or destruction through various defense pathways (Cann 2011).

3.2.2 Serum-Based Diagnostics

The diagnostics research in immunology has developed sensitive, quick, and quantitative techniques based upon PIR factors present either in viruses or hosts, e.g., precipitation, agglutination, immunofluorescence, coprecipitation, radioimmunoassays, Western blotting, and several types of enzyme-linked immunosorbent assays (ELISAs). These techniques involve identification and detection of epitopes, i.e., specific region of viral antigen using antibodies labeled with radioisotopes or fluorescent dyes that can be detected or imaged using suitable detectors present in phosphoimagers, spectrophotometers (fluorescent), and light microscopes (Hull 2014; Van Regenmortel and Dubs 1993; Verma et al. 2013).

Immunodiagnostics in plant virology focus upon the principle to detect the viral particles responsible for immunogenic response in host with the virus-specific antibody. These viral-specific antigens (Ag) or immunogens, i.e., coat protein, are produced in vertebrates which act as factories to produce viral coat protein-specific antibody (Ab). These viruses might be symptomatically similar but serologically distinct on the basis of interacting epitopes. The assays can be performed by immobilizing the antigens present in plant sap over suitable matrix such as nylon membrane (Dot-ELISA) (Banttari and Goodwin 1985) or agar/agarose gel (radioimmunoassay) and then detecting them by adding specific antisera (Hampton et al. 1990). Early application of such techniques, i.e., Dot-ELISA or dot immunobinding, has been utilized to detect various strains of *Potato virus X*, *S*, *M*, and *Y*, *Potato leafroll virus* (Van Regenmortel and Dubs 1993, Heide and Lange 1988), and several cereal and legume viruses (Makkouk and Kumari 2002).

ELISA ELISA technique involves immobilization of analyte or a reagent on a microtiter plate surface generally made up of adsorbing-type synthetic material. Several types of ELISA variants are illustrated in Fig. 3.1a–e (Crowther 2000). The ELISA provides high specificity, cost-efficiency, an opportunity for scaling up of the screening process, and flexibility in modifications for customization of the technique. ELISA was first applied for plant viral diagnostics by Clark and Adams in 1977, and since then many variants of ELISA have been introduced such as indirect, sandwich, double-antibody sandwich (DAS-ELISA), direct antigen coating (DAC-ELISA), antigen-coated plate (ACP-ELISA), and trapped antibody sandwich (TAS-ELISA). These techniques vary in mainly capturing and detecting antibodies from same or different sources.

Among above techniques, DAS-ELISA is virus strain specific and necessitates the conjugation of every detecting antibody to be conjugated to an enzyme. The TAS-ELISA is more efficient in detection of Ag than DAS-ELISA; a mAb from a different animal source such as goat, sheep, horse, mouse, or rabbit (as compared to

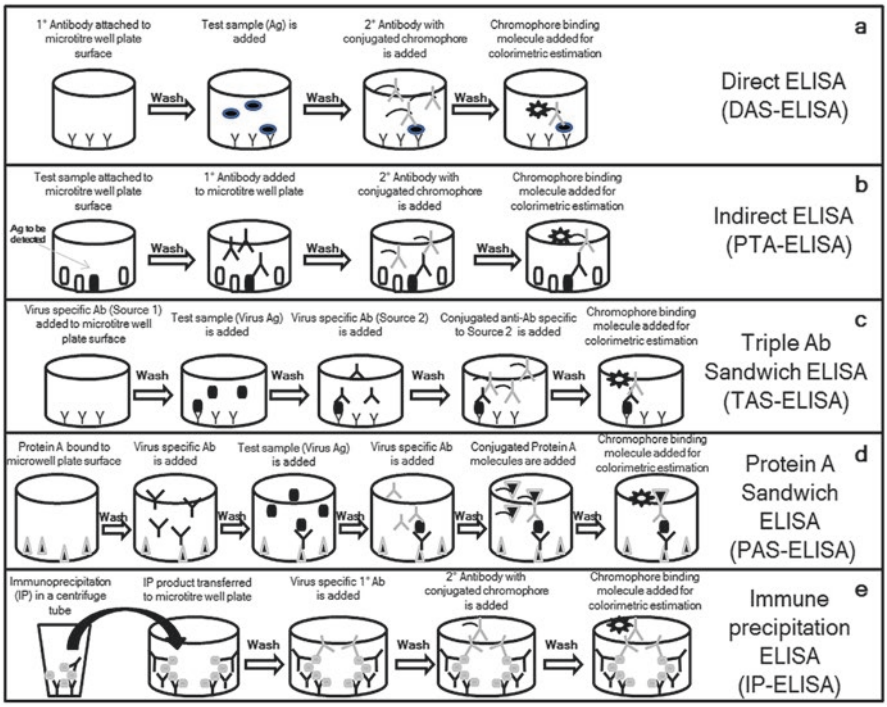

Fig. 3.1. ELISA variants used in plant virus diagnostics. (**a**) DAS-ELISA, (**b**) PTA-ELISA, (**c**) TAS-ELISA, (**d**) PAS-ELISA, (**e**) IP-ELISA (Cooper and Patterson 2008; EPPO 2005; Ferreira et al. 2008: 125)

surface-trapped virus-specific antibody) is used before adding anti-antibody or a secondary antibody raised in the similar host animal. Presently, PAS-ELISA utilizes protein A-coated surface to specifically bind to the Fc region of trapping antibody such that Fab' region is only available for subsequent Ag-Ab interactions. This increases the efficiency and specificity of viral coat protein detection. The ELISA has proved to be simple, versatile, fast, and efficient in the immunodiagnostics of plant viruses. It is only limited by the cross reactivity of heterologous Ag-Ab complexes whose exact nature is not simple to be studied at diagnostic level. Another limitation of ELISA is that the identification of an unknown virus is almost impossible without further confirmations (Abou-Jawdah et al. 2004; Baker et al. 1991; Cooper and Patterson 2008; Desbiez et al. 2007; Hornbeck et al. 2001).

The serology-based detection became an integral part of large-scale agricultural surveys and disease eradication programs in the past, and it is still a popular approach for the detection of many horticultural important crop viruses, i.e., *Plum pox virus*, *Papaya ringspot virus*, *Papaya mosaic virus*, *Citrus tristeza virus*, *Tomato ringspot nepovirus*, and *Banana streak virus*. These surveys had been detrimental in establishing the geographical distribution of viral strains, their vectors, and their host range. In general, the indirect ELISA technique was more sensitive and

efficient than DAS-ELISA in the detection of serologically similar viruses, e.g., *Tymovirus*, *Tombusvirus*, and *Tobamovirus* (EPPO 2004a, b, 2005).

Western blot technique, also referred to as protein immunoblotting, is a widely employed analytical technique used to detect the PVDF or nitrocellulose membrane transferred viral proteins electrophoretically resolved over native or denaturing PAGE gels. The principle of detection is similar to that in ELISA using the same type of antibodies and chromogenic reagents for the purpose of detection and colorimetric measurements (Burnette 1981). This technique is useful in the detection of new virus strains and also in the characterization of novel viral proteins only limited by the availability of suitable and costly Abs, the sophisticated laboratory instruments, and technically trained hands. These techniques are very sensitive but not simple, cheap, and easy to be included into the farm virus control strategy.

3.2.3 Nucleic Acid-Based Diagnostics

The principle of detection of viruses involves "hybridization." In hybridization, the "probe" nucleic acid can bind against its complementary "test" sequence bound or trapped on a solid material such as blotting membranes or microsphere beads. This probe can be later either detected by radioisotopes or amplified using polymerase chain reaction-based techniques and labeled using fluorescent nucleic acid binding dyes, e.g., ethidium bromide (EtBr), SYBR Green, propidium iodide (PI), etc. The techniques developed based on the abovementioned principle are Southern blotting, allele-specific PCR, Nested PCR, multiplex PCR, DNA sequencing, reverse transcriptase PCR, real-time PCR, and rolling circle replication (RCR) (López et al. 2003).

3.2.3.1 Polymerase Chain Reaction

The polymerase chain reaction (PCR) technique developed by Karry Mullis (1983) is used to generate thousands to millions of amplified copies of a genomic fragment starting with a single or few copies of that particular fragment. The method relies on the isolation of total plant genomic DNA, thermal cycling (in the presence of a DNA polymerase enzymes) of genomic fragments with the help of primers (18–30 nucleotide long) containing sequences that are complimentary to the target region, thus generating million copies of the target regions known as amplicons (Fig. 3.2a). This technique has revolutionized the field of diagnostics with ever-evolving and improvised versions of PCR techniques enhancing the specificity and efficiency and simplifying the early detection, quantification, and cloning of viral particles in the infected plants (Dasgupta et al. 1996; Patel et al. 1993; Saxena et al. 1998a, b; Rojas et al. 1993). The PCR along with its variants has been instrumental in establishing plant species variations (Saxena et al. 2005; Mishra et al. 2007).

Degenerate PCR Most simple and widely employed technique in viral diagnostics is the use of degenerate primers for early detection of DNA viruses, i.e., geminiviruses (Briddon and Markham 1994; Wyatt and Brown 1996; Rojas et al. 1993; Sinha

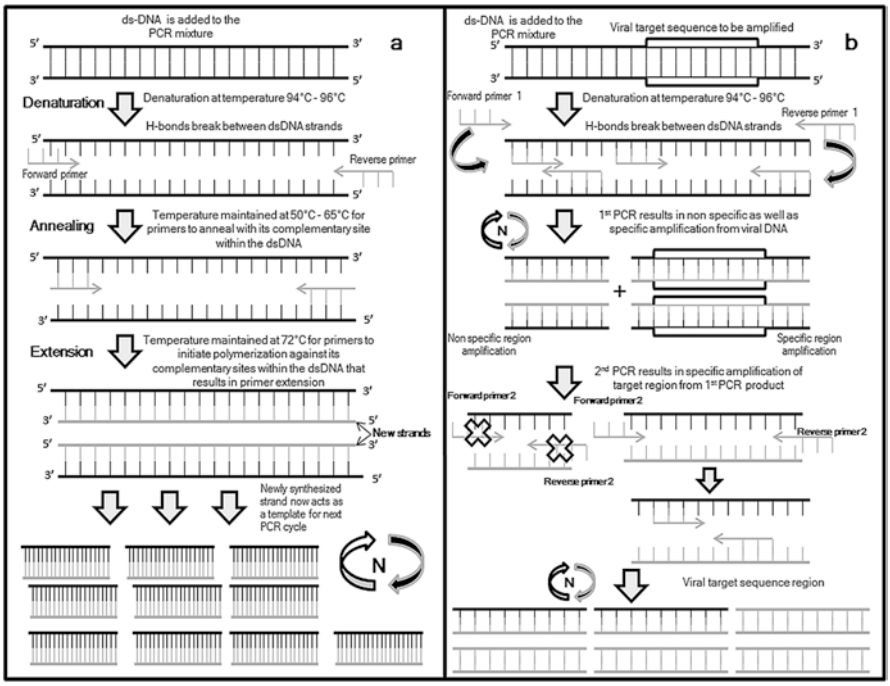

Fig. 3.2. (**a**) PCR showing various steps of template denaturation, primer annealing, primer extension, and the product (amplicon) after N cycles. (**b**) Nested PCR showing various steps involved in the diagnostic process using nested primers. The primer set 1 is designed external to the viral target region, the first PCR cycle results in a mixture of a specific and nonspecific amplicon population. The second PCR is performed with primer set 2, designed very near to the specific viral target region. This step results in the omission of nonspecific amplification, and thus, the amplicon population corresponds to the specific viral target only

et al. 2011; Srivastava et al. 2010). The degenerate primers is a cocktail of oligonucleotides possessing degeneracy due to inclusion of possible bases at various positions selected on the basis of nucleotide sequences of virus obtained by DNA sequencing. Thus, the degenerate PCR is efficient in detecting genera of plant virus sharing sequence similarity, e.g., *Begomovirus*, *Mastrevirus*, and *Curtovirus* belonging to family *Geminiviridae*. The limitation of this technique is that it lacks specificity; hence, it must only be used at the early stage of diagnosis.

Nested PCR The Nested PCR is often required to achieve highly specific amplification of target regions which is useful in the confirmation of a specific virus. This technique requires two sets of primers where the first could be a degenerate primer set used to amplify a generic region. The second set of primer is designed to amplify an internal region, which is more specific and contains a marker region of a particular virus. The products can be used for sequencing and further analysis (Fig. 3.3b). Nested PCR in combination with RT-PCR in a single tube was employed in the

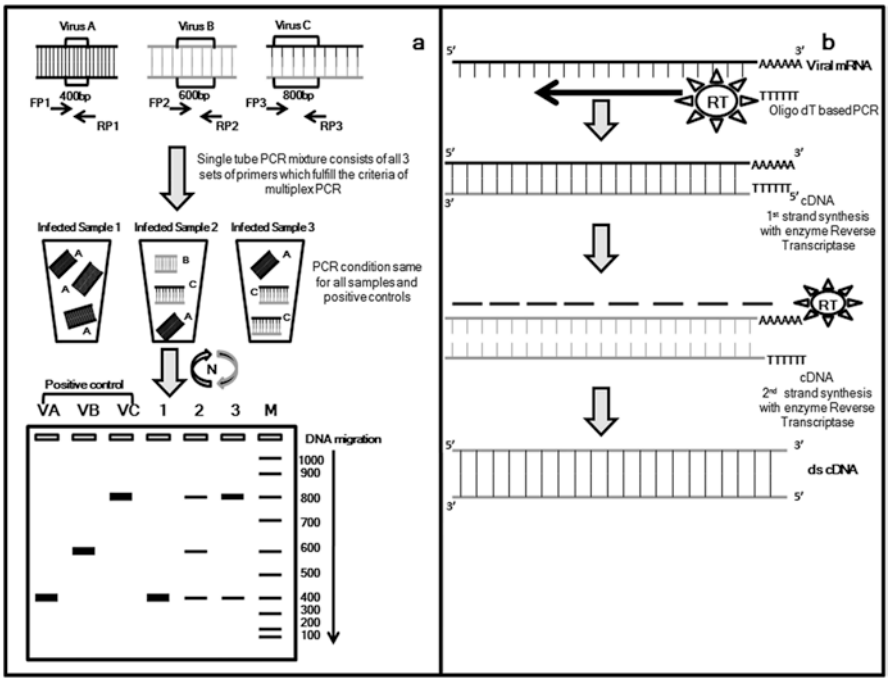

Fig. 3.3 (**a**) A multiplex PCR, illustrating the principle of multiplexing the different diagnostic viral target regions in a single reaction tube. Three different primer sets are designed against the regions such that their melting temperature (T_m) is close to each other, so that a single reaction condition could be set up to amplify all the target regions in a sample, if present. The figure illustrates how three different viral target regions could be amplified to detect and identify types of viruses infecting a particular sample. The positive controls, i.e., VA, VB, and VC, have been used as comparative markers for the correctly amplified bands in infected samples. (**b**) Reverse transcriptase-PCR (*RT-PCR*), the figure illustrates the simple principle of RT-PCR when used for viral diagnostics. A viral mRNA is isolated; oligo-dT primers are employed to transcribe the complementary strand with the help of enzyme *reverse transcriptase*. The first strand synthesis is followed by synthesis of complementary strand to the newly synthesized first cDNA strand followed by degradation of the original viral mRNA strand. Thus, the newly synthesized molecule is a viral ds-cDNA that can be detected using any virus-specific or degenerate primer set

detection of *Citrus tristeza virus* (CTV) and *Plum pox virus* (PPV) (Yourno 1992; Olmos et al. 2003; Adkins et al. 2008; Lee et al. 2013). The limitation imposed by this technique is that the ratio between the external and internal primers needs to be accurately established for the success of this reaction in a single tube to bring down cost and time of the diagnosis.

Multiplex PCR The Multiplex PCR is another technique that permits simultaneous detection of several viral targets in a single reaction (Fig.3.3a). It involves the use of two or more primer pairs that anneal at different viral target sequences (Bariana et al. 1994). The Multiplex PCR has been applied to detect viruses in various horticulture and vegetative crops (Hyun et al. 2009), banana (Sharman et al.

2000), cucurbits (Kwon et al. 2014), etc. It has been successfully used to simultaneously detect six major viruses infecting olive tree (Bertolini et al. 2001) and nine grapevine-infecting viruses (Gambino and Gribaudo 2006). The versatility of Multiplex PCR lies in the usage of carefully designed primer pairs which have very close annealing temperature. Any mistake in the designing may lead to greater chances of primer-dimer formation; more mispriming, hence more probability of amplifying artifacts on the agarose gel. Therefore, the experimental setup needs to be planned and executed by a technically skilled person having specialized laboratory facilities. However, this technique once standardized is quite simple, time saving, sensitive, and efficient for early diagnosis of plant viruses and also determination of sex in case of tropical fruits such as papaya (Saxena et al. 2016). It can also be employed to find out the molecular complexity of the interaction between virus and vector host complex (Saxena and Verma 2016). It is mainly employed by molecular diagnostic laboratories involved in field diagnostics that are limited by cost and test sample volume.

Reverse Transcriptase (RT) and Quantitative Real-Time (qRT) PCR The quantitative analysis of virus in the plant samples provides the information about the etiology, stage of virus infection, and the relative expression of host genes at the time of initiation till the death of the plant tissue. This necessitates the need for a quantitative PCR where the growth and gene expression patterns could be studied along with the detection of virus infection. The quantitation could be absolute or relative depending upon the information required for analysis. The absolute quantitation states the exact number of viral nucleic acid present in the sample, whereas the relative quantitation describes the change in amount of target sequence compared with the level in a relative matrix. The relative quantitation provides more information and is relatively simpler to analyze as compared to absolute quantitation. However, the absolute quantitation is required when we study the viral load kinetics in a plant specimen. The success of qRT-PCR lies in the fact that it can be optimized for both types of studies. In conventional real-time PCR, an 18–20 nucleotide long oligoprobe labeled with a fluorescent tagged at 5′ end is employed in both forward and reverse orientation to amplify a 150–250 bp region which is highly specific. As the reaction progresses, the Taq polymerase employs its 5–3′ exonuclease activity to remove the probe, thus resulting in rise in fluorescence intensity in the reaction tube which is detected by fluorescent detectors with suitable filters attached in front of them (Fig.3.4). The test sample is studied along with a "housekeeping gene" which also amplifies along with the viral nucleic acid and acts as a reference for comparative or relative estimation of viral gene expression. The reference gene expression remains constant throughout; hence, it never changes even though the viral gene expression might alter dramatically during the course of reaction. There are several discrepancies though which can be removed by employing appropriate standard measures as described in qRT-PCR protocols. There are various reporter chemistries available such as TaqMan®, Molecular Beacons®, SYBR® Green, and Scorpions. The chemistry of these probes is based upon the principle of Forster resonance energy transfer (FRET) to generate fluorescence signals through coupling

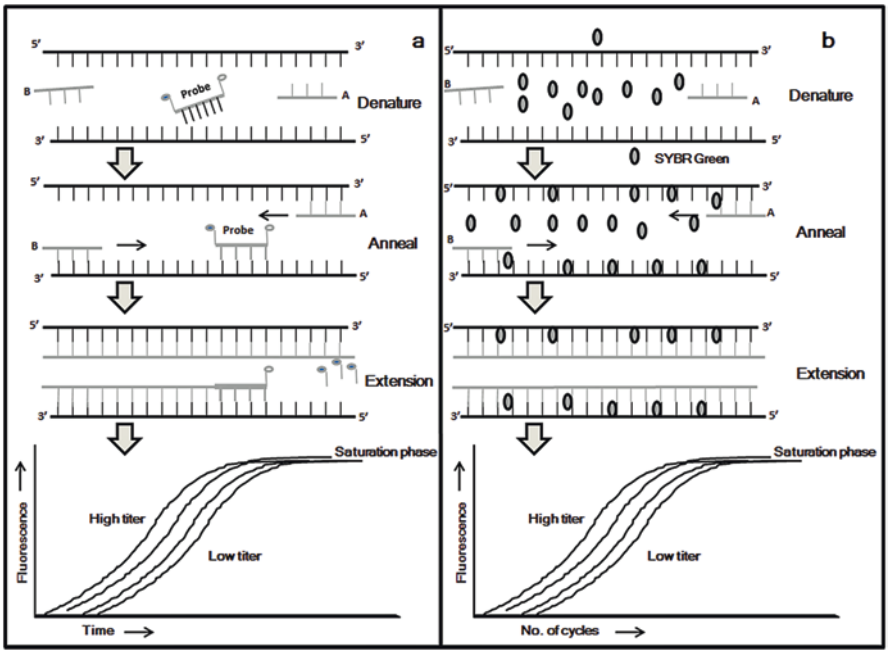

Fig. 3.4 Quantitative real-time-PCR (*qRT-PCR*). (**a**) TaqMan® probe chemistry. TaqMan® probes are 19–24 nt long primers labeled with fluorescent or chemiluminescent fluorophores and chromophores, respectively. The principle of this probe chemistry is that, with incorporation of each nonlabeled dNTPs during extension step, the fluorophore or chromophore will be released into the reaction tube, thus enhancing the fluorophore or chromophore excitation level. Each reaction sample finally reaches equilibrium, i.e., saturation phase according to the basal viral cDNA amount, means the sample harboring more viral cDNA population initially will attain saturation phase before those having lesser initial viral cDNA population. (**b**) SYBR® Green chemistry. SYBR® Green is a commercially available DNA-binding dye that nonspecifically intercalates between any two nucleotide bases during each primer annealing and extension step. Thus, after N cycles in a PCR reaction, the dye will incorporate invariably at any position toward 5′ end of extending primers in both forward and reverse direction. Thus, the emission intensity of free dye will decrease with every cycle, and the intensity of bound SYBR® Green dye will increase with amplification of each new viral target double-strand amplicon. Thus, the viral titer can be determined in a similar way, through analysis of melting curves

of a fluorescence dye and a quencher molecule to the same or separate oligoprobe substrates (O'Connell 2002).

The RT- and qRT-PCR techniques are different from conventional PCR techniques as they are used to study gene expression. The reverse transcriptase PCR (RT-PCR) is a technique relying on the ability of a viral enzyme called *reverse transcriptase* to generate double-stranded complementary DNAs (cDNAs) from single-stranded RNA molecules. The cDNA template thus generated can be further amplified by traditional virus-specific PCR. The RT-PCR end product can be visualized on agarose gel (Fig.3.3b). RT-PCR is thus a qualitative technique and popularly used to detect the ss-RNA virus, e.g., *Bunyaviridae* and *Rhabdoviridae*. It is capable

of specifically detecting RNA virus from a mixed virus population even when the viral population is too low to be detected by any other technique, e.g., distinct *Potyvirus*, *Tospovirus*, and several potato tuber necrosis-causing viruses (Raj et al. 1998; James 1999; Nie and Singh 2001; Okuda and Hanada 2001; Cating et al. 2015).

The earlier version of RT-PCR was limited to detection of a single virus in a single-tube reaction (Raj et al. 1998; O'Connell 2002). The development of random primers and oligo-dT for RT- PCR has led to the development of duplex and multiplex RT-PCR-based techniques to detect various RNA viruses (Nemchinov et al. 1995; Nassuth et al. 2000; Nie and Singh 2000, 2001; Singh et al. 2000; Halgren et al. 2007). Multiplexing in RT-PCR allows detection and quantification of two or more viral DNAs with the help of oligoprobes tagged with fluorophores, e.g., simultaneous RT-PCR detection of five tobacco viruses [*Tobacco mosaic virus*, *Cucumber mosaic virus subgroup I*, *Tobacco etch virus*, *Potato virus* Y^O, and *Tobacco vein banding mosaic virus* amplifying five distinct fragments 237, 273, 347, 456, and 547 bp, respectively] (Dai et al. 2012). Thus, multiple amplicons could be discriminated on the basis of the emission spectrum of the fluorogenic probes, i.e., TaqMan® probe chemistry (Fig. 3.4a). Multiplexing is somewhat limited in use due to limited numbers of fluorophores available and the restriction to use a monochromatic light source for excitation (Wei et al. 2012). The Nested RT-PCR has also been used to detect viruses in a single-tube reaction when the stringency has to be increased (Olmos et al. 2002, 2003). A rapid single-tube immunocapture RT-PCR has been used to detect two yam *Potyvirus* (Mumford and Seal 1997).

The real-time detection and quantification methods have proved to be a rapid, reliable, yet simple technique for the detection and simultaneous quantitative analysis of plant viruses through melting curve analysis using SYBR® Green chemistry (Fig.3.4b) (Varga and James 2005). The qRT-PCR popularly used in the diagnostics of ss-RNA and a ds-RNA virus for detection, amplification, and quantitation of these difficult to detect virus due to unavailability of ss- or ds-DNA template for normal PCR techniques. Therefore, qRT-PCR is employed in their diagnostics as it serves all of the abovementioned purposes providing quantitative values of viral presence and gene expression levels.

The RT- and qRT-PCR has enhanced the sensitivity, speed, efficiency, and scope of measuring viral strains and titer differences in various host plants showing different symptoms due to the presence of the same virus. It has also improved the epidemiological studies by rapid identification of target DNAs in a single reaction using multiplex qRT-PCR. New chemistries have allowed efficient discrimination of various multiple virus genotypes in a single reaction tube. With advancements in hybridization and labeling techniques along with the development of precision detectors and high-performance softwares, the researchers are now able to study and perform in-depth analysis of large number of samples using multiplexing techniques and also dissect the whole transcriptome or genome of viruses via next-generation sequencing platforms like Illumina RNASeq, Roche 454, and Ion torrent sequencing (Sambrook et al. 1989; Boonham et al. 2014).

3.2.3.2 Isothermal Amplification of Viral Genome/Fragments

The viral diagnostics has taken a huge leap since PCR and multiplexing techniques became a norm in diagnostic laboratories. The rise of isothermal NA-based diagnostics like rolling circle amplification (RCA), nucleic acid sequence-based amplification (NASBA) (Compton 1991; Leone et al. 1997), helicase-dependent amplification (HDA) (Vincent et al. 2004), and recombinase polymerase amplification (RPA) (Pipenburgh et al. 2006) is slowly displacing the technically complex and resource-demanding diagnostic techniques as they can be performed at low temperature ranging from 30 to 42 °C.

Rolling Circle Assay (RCA) RCA is a very simple, sequence-independent, and very efficient technique to amplify a closed circular viral DNA from total plant DNA for amplification, cloning, sequencing, and identification of novel plant viruses and to study the variability (Haible et al. 2006; Inoue-Nagata et al. 2004). RCA involves Phi29 DNA polymerase, an enzyme with multiple-strand displacement activity, which is preferable for amplification of single-strand DNA templates into double-strand products. The enzyme due to its displacement activity produces concatemers of variable lengths with debranching at different intervals. Random hexamers are used to prime the multiple-strand displacement reaction due to which the RCA is independent of the sequence information. Thus, enhancing its application in isolating, cloning, and sequencing the unknown virus from symptomatic samples. The enzyme provides many advantages for diagnostic purpose, such as high processivity and high proofreading activity, and generates large fragments (more than10 kb) in high amounts as compared to PCR (few μg when starting material used is few pg). The time taken for initial investigation that took few weeks to months has reduced by many folds, and a new isolate can be identified and cloned within a week following simple RCA protocols (Ferreira et al. 2008). It has been successfully used to detect several viral satellite nucleic acid components, which were earlier not known in Old World begomoviruses (Briddon et al. 2003, 2004; Alberter et al. 2005; Lozano et al. 2016). The low cost and simplicity of this technique makes it a preferable workhorse of any diagnostic lab in the world.

Loop-Mediated Isothermal Amplification (LAMP) An isothermal technique is a potential substitute for on-field diagnostics as it is a simple technique used to amplify a single-stranded loop containing amplification product with the help of internal, external, and loop primers at 65 °C without the need for template denaturation. It relies on the auto-cycling strand displacement synthesis of DNA strands by DNA polymerases with high displacement activity and two or more specially designed specific primers set. The primers are designed to amplify initial dumbbell-shaped template. Later, only inner primers (forward and backward) are required to amplify concatemeric products with several loops in them (Notomi et al. 2000). The simplicity and specificity of the LAMP and the time required for amplification of detectable amplicons are a huge improvement over the time taken by RCA, i.e., 18 h for RCA and 1 h for LAMP. It has been used in diagnosis of PVY (Nie 2005), *Potato leafroll virus* (Ju 2011), Squash leaf curl virus (Kuan et al. 2010), and many more

with slight modifications. Therefore, the viral diagnostics seem to be getting closer to a simple water bath-based diagnostics, thus surpassing the need for expensive and sophisticated instruments and laboratory requirements. The isothermal amplification-based kits are easy to handle and require less technical expertise hence amenable for on-field diagnostics development in plant virology.

3.2.4 Genomics in Diagnostics

The genetic relatedness is one of the most fundamental principles of molecular evolution, and extensive sequence similarity implies sequence homology among group of sequences and often conservation of function. When we refer to genomic information, we describe a sequence of information related to gene number, sequence length, and location along with other coding and noncoding features, which provide us an insight about the phylogeny and conservation of function among a group of sequences. Such type of study is called comparative genomics and requires dedicated algorithms, programmable pipelines, and huge computational engines for the analysis of large datasets such as group of sequences that might range from 10 kb to 100 mb.

Sequence Analysis and Phylogenetic Analysis The sequence analysis involves data mining, local and global alignment of genomic sequences, and then analysis of conservation; based upon all of these, the researchers draw a cladogram which is obtained after computing percent identity matrix and distances, providing useful phylogenetic information about molecular evolution and conservation of function. The advent of powerful computers and efficient algorithms of sequence analysis, e.g., hidden Markov model (HMM), neural network models (NNM), models based upon dynamic programming, and many other similar algorithms, has made the comparative study of viral genes and protein sequences easier for virologists. A comparative sequence study of highly divergent virus isolates resulted in the establishment of three new genera in *Geminiviridae*, i.e., *Becurtovirus*, *Eragovirus*, and *Turncurtovirus* (Varsani et al. 2014). The species demarcation and classification of highly divergent alpha, beta, gamma, and delta satellites have been possible because of the intensive and in-depth analysis of their sequence, their recombination frequency analysis for the determination of their parental origin for accurate and specific nomenclature of these particles (Briddon et al. 2008; Rosario et al. 2016; Lozano et al. 2016). The principle behind comparative sequence analysis of viral genomic sequences lies in the fact that the viral genome is an ever-evolving entity and undergoes high rates of recombination during "horizontal gene transfer" and thus each region can have a viral sequence which is unique and specific to the population or different variants might be possibly infecting the same region. This probability of recombination has resulted into the evolution of host-virus relationship and the viruses as well, e.g., geminivirus and *Bemisia tabaci*. Apart from diagnostics and diverse studies (Saxena et al. 2005; Mishra et al. 2007; Sinha et al. 2012; Abu-Samah and Randles 1981; Guindon and Gascuel 2003; Halgren et al. 2007;

Koonin and Dolja 1993), the sequence information can also be used for designing an efficient antiviral strategy, e.g., the information gathered from comparative analysis can be utilized to design siRNA strategy against both the viruses and vectors (Saxena et al. 2011, 2013).

Therefore, it becomes a prerequisite for molecular virologists to identify these variations and suitably design strategies to combat these plant viruses and prevent huge crop losses incurred due to viral infections across the globe. Hence, bioinformatics provided a handy tool to study the evolutionary origin and phylogenetics related to viruses.

3.3 Conclusion

The enormous prospects of customization and technical flexibility are always a huge advantage for PCR-based diagnostics as many multiplexing techniques like duplex RT-PCR (d-RT-PCR) (Halgren et al. 2007), quadruplex RT-PCR, and multiplex real-time PCR (Agindotan et al. 2007; Kogovšek et al. 2008) have revolutionized the field of plant virus diagnostics and enabled simultaneous detection of six to nine types of plant viruses infecting the same crop. The PCR technique provides a single-handed advantage over serum-based techniques in rapid detection of *Begomovirus* reservoir in weed plants such as *Triumfetta*, *Ageratum*, *Croton*, *Malvastrum*, and *Solanum* (Hallan et al. 1998a, b). The plant virus diagnostics is necessary for regular surveillance, quarantine, and eradication programs to keep our crops safe from virus infections, thus preventing huge losses incurred every year in crop productivity and total food production chain. The diagnostics is an important component of farm management practice and required regular update in techniques and methodologies to effectively detect ever-evolving viral entities to keep a check on their global spread; hence, the pandemic rise in viral population could be restricted if we try to detect them at the right time and at early stages of infection. Therefore, a simple, easy, robust, efficient, and high-throughput viral diagnostic methodology would be able to help human beings to tackle the problem of global food security and help in developing a sustainable future.

Present-day virus diagnostic strategies employ techniques adapted from various research areas ranging from immunology to advanced genomics like ELISA, Western blotting, multiplex PCR, allele-specific polymerase chain reaction (PCR), real-time PCR, gene sequencing, and microarray and RNA sequencing. These techniques might be a stand-alone confirmatory test, e.g., qRT-PCR, or could be a supplementary test for other techniques like next-generation sequencing which employs large-scale viral transcriptome analysis and later confirmation through real-time PCR for whole-genome sequencing of viral pathogens (Wang et al. 2003; López et al. 2003). Recently, the need for high-throughput screening paved the way for the development of microfluidic and multiplexing-based techniques in the area of PCR-based diagnostics in the plant sector. The protein-based techniques included in the viral immunodiagnostics are ELISA, Western blotting, and dot blot for plant virus detection and identification with the help of specific antibodies. The nucleic

acid-based techniques include Southern blotting (Saxena et al. 1998c), allele-specific PCR, real-time PCR, multiplex PCR, rolling circle assay, and RNA sequencing as a means to detect, identify, and dissect the plant viral diseases and their infection mechanism. In this chapter, the emphasis was built to provide the information about the principles and recent developments in the field of *Begomovirus* diagnostics and the latest techniques that could potentially replace early diagnostic tools in future.

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Transmission of Begomoviruses

4

Priyanka Varun and Sangeeta Saxena

Abstract

Transmission is the mechanism of pathogen transfer from an infected plant to another host. Begomoviruses are emerging and economically very important phloem-bound plant pathogens that choose the single species of whitefly, i.e. *B. tabaci*, as vector for their spread in many crops. Mouthparts of whiteflies are designed to detain begomoviruses while feeding on phloem sap of plants. An interaction between mouthparts and coat protein of virus confers *Begomovirus*-whitefly specificity. High-degree conservation of capsid protein of begomoviruses is the main reason for the choice of their vector. Once virus particle enters, it further moves along in the body of vector in a persistent circulative manner and is introduced back into the plant with salivary secretion during next feeding. There are many proteins present inside the vector that facilitate the efficient transmission of begomoviruses. Variations in the begomoviral coat protein can change their vector preferences. Viruses have the ability to manipulate the behaviour of their vector to enhance their transmission; as a result, begomoviruses negatively affect the longevity and fertility of their whitefly vector, whereas behaviour of whiteflies and their feeding habits can also affect the population genetics, behaviour and evolution of viruses. Whitefly-*Begomovirus* relationship is an example of co-evolution, and the studies on transmission mechanism, virus-vector interactions and proteins involved in virus translocation inside the vector can help in developing new virus management strategies.

P. Varun • S. Saxena (✉)
Department of Biotechnology, School of Bioscience and Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow, UP, India
e-mail: priyanka.varun@yahoo.com; dr_sangeeta_saxena@yahoo.com

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4.1 Introduction

Plant viruses affect many commercially important crops causing diseases that are responsible for great economic loss to growers. Viruses are obligate intracellular parasites that do not possess the molecular machinery to replicate without a host; thus, they need living cell for their survival and stability. As viruses cannot penetrate the cell wall of plant cell which is mainly composed of cellulose and pectin barrier, they are unable to transmit themselves from host to host for their survival, multiplication and spread. Transmission is considered as one of the most important assets of viruses to overcome this problem and to spread from one susceptible host to another. Most of the time, virus transmission is done either mechanically or by some vectors. More than 400 species of insect vectors have been reported to transmit plant viruses belonging to the Homoptera order in the Orthopoda phylum. Mainly the insects that play an important role as vectors for viral transmission are plant hoppers, leafhoppers, beetles, whiteflies, thrips, mealy bugs, aphids and mites. Among these insects, whiteflies are the only vectors that transmit begomoviruses naturally causing huge crop losses through direct feeding damage, honeydew secretion and plant virus transmission. More than 200 species of begomoviruses are known to be transmitted by whiteflies causing severe losses to a range of crops (Castillo et al. 2011), and the number is increasing day by day. These viruses are phloem-bound icosahedral-shaped single-stranded DNA viruses which are exclusively transmitted by whitefly in a persistent, circulative manner (Czosnek et al. 2002). Whiteflies are reported to feed on the sap flowing in the phloem sieve elements present in the vascular bundle, and as we know, begomoviruses are phloem-bound viruses; whiteflies naturally become a very good vector to transmit begomoviruses (Hogenhout et al. 2008). The location, movement and sites of viral transport inside the whitefly were visualized by indirect fluorescent microscopy using polyclonal and monoclonal primary antibodies, and virus transport from the gut lumen of whitefly into the haemocoel was found to be located in the filter chamber and anterior part of the midgut. Further, from haemocoel, the virus moves into the salivary glands and finally is introduced back into the plant host during insect feeding (Hunter 1998; Czosnek et al. 2002). Although begomoviruses are not known to replicate in their insect vectors and apparently do not negatively affect their insect host, there are some reports suggesting the possible interactions between begomoviruses and their exclusive vectors, the whitefly *Bemisia tabaci*, which may lead to a possibility of begomoviruses as an insect pathogen (Czosnek and Ghanim 2012). This chapter describes the methods of transmission, vectors, mechanism and factors responsible for transmission of begomoviruses.

There are two types of transmission: vertical transmission and horizontal transmission. These two terms describe the movement of any pathogen that moves from one host to another by different means. Horizontal transmission is more common as compared to vertical transmission. Vertical transmission involves the infection of a progeny from its parents; hence, transmission is limited to host and its other relative crops. During vertical transmission, viruses can be passed on either through vegetative propagation, sexual reproduction or via infected seeds in plants. On the

contrary in horizontal transmission, the spread of virus is into much wider host range by means of penetration of viruses in the plant cells through direct wound damage by some mechanical means like pruning shearing, direct external contamination of wound or indirectly through feeding damage by vectors (Ghanim et al. 2007). In such kind of transmission of begomoviruses, whiteflies play a major role with respect to virulence, mobility and host range of virus. Studies on whiteflies-mediated transmission of begomoviruses indicate that these viruses are transmitted in a persistent circulative manner and are also transferred from generation to generation of insects. In some earlier reports according to initial transmission studies begomoviruses were not considered to be passed on to offspring of insect vector mainly whitefly *B. tabaci.* However, later on, extensive studies on *tomato yellow leaf curl virus* (TYLCV) revealed an exception of this concept by detecting virus between male and female whiteflies during sexual intercourse (Ghanim and Czosnek 2000) and in their progenies (Ghanim et al. 1998; Bosco et al. 2004, Sinisterra et al. 2005; Accotto and Sardo 2010). As a consequence, whiteflies make their progenies viruliferous to transmit viruses form one plant to another and cause extensive damage to the crops.

4.2 Modes of Transmission Through Insect Vectors

Many insect vectors involved in viral transmission use different mechanisms to transmit viruses; currently three transmission mechanisms are known for virus transmission by insect vectors – non-persistent, semi-persistent and persistent (Fig. 4.1). Watson and Roberts (1939) classified plant viruses into two groups, non-persistent and persistent considering transmission. Afterwards, an intermediate category of viruses, i.e. semi-persistent, has been introduced (Murant and Goold 1968; Johnson et al. 2002).

In non-persistent transmission, viruses can be inoculated by vectors to the plant host only after a few minutes of acquisition and loses viruses within few minutes; no latent period is required for viruses in non-persistent transmission. Viruses involved in this type of mechanism are retained in the stylet of the insects, hence called stylet-borne viruses (Martin et al. 1997; Collar et al. 1997); on the other hand, semi-persistent transmission is an intermediate category in which vectors take few hours to few days after acquisition to inoculate the virus into plant host and lost the virus after moulting. Viruses retained in the foregut of insect and do not require latent period for their transmission. Potyviruses, cucumoviruses and caulimoviruses are transmitted through non-persistent and semi-persistent manner (Ng and Falk 2006).

Persistent transmission involves the inoculation of viruses by insect vectors after long time retention following acquisition through the salivary secretion of insects to the plant host. Persistent plant viruses move through the insect gut and reach to the haemolymph after crossing barriers of gut lumen or other tissues. Further, they entered in the salivary glands from where these viruses spit out into the plant with salivary secretion of insects during feeding. Viruses involved in persistent

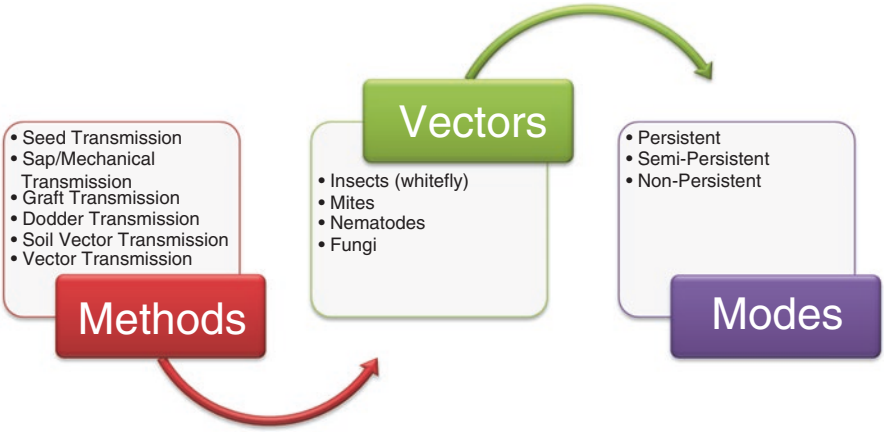

Fig. 4.1 Transmission of viruses is the way of disease transfer. There are many methods by which infection can be transferred from one infected host to another. Vector transmission is the most common natural transmission method of begomoviruses. There are many vectors that can carry and transmit viral diseases, but begomoviruses are transmitted only through the insect vector whitefly. Viruses transmitted through insect vectors follow different routes for their transfer, and begomoviruses move in a persistent manner inside their vectors to reach the plant host

transmission can be further categorized into circulative, non-propagative and circulative, propagative persistent transmission (Nault 1997). Circulative, non-propagative viruses pass through the insect gut, haemolymph and barrier of salivary glands to reach the salivary glands from where the viruses are inoculated into the plants via insects feeding. Non- propagative viruses do not replicate in their insect vectors and restricted to the phloem tissues in the host plants (Hogenhout et al. 2008). Luteoviruses, nanoviruses and geminiviruses are vectored by phloem-feeding insects in a persistent, circulative and non-propagative manner, whereas viruses transmitted in a persistent propagative manner can replicate in different organs of their insect vectors as well as in several plant tissues. In contrast to the non-propagative viruses, propagative viruses enter to the salivary glands from haemolymph and other connecting tissues like the trachea or nervous system. There is also an evidence of propagative virus transmission in vector's progeny through virus-infected embryos/germ cells in the female insects (Silvester 1980). In case of persistent viruses, either propagative or non-propagative, it can be transmitted to plants after their introduction into the vector haemocoel (Silvester 1980; Dietzgen et al. 2016).

4.3 Methods of Transmission

Different ways involved in virus transmission are given below:

1. Mechanical or sap transmission
2. Seed transmission
3. Dodder transmission

4. Vegetative transmission
5. Graft transmission
6. Transmission by soil vectors: fungal and nematode
7. Insect transmission: aphids, leafhoppers, whiteflies, mealybugs, mites, thrips and beetles

Although all begomoviruses are naturally transmitted by the insect vector whitefly *Bemisia tabaci* (Gennadius) in a persistent manner (Chang et al. 2010), rapid spreading of begomoviral diseases needs to be monitored, and transmission studies of viruses are very imperative to investigate plant virus interactions and virus resistance. Some viruses have been reported to be mechanically sap transmitted, seed transmitted and also through graft inoculation artificially to their original hosts. Mechanical or sap transmission is the method in which rubbing or injecting the infectious sap of diseased plant leaves causes the infection in the healthy plants (Wege and Pohl (2007). Construction and introduction of infectious clones of viral genomic components are a very popular method to know about the causal viruses by recreating the disease (Chen et al. 2016; Zhang et al. 2010). This method has helped scientists and researcher in every area to study the transmission of virus by observing similar viral symptoms and onset of disease in plant. Chang et al. (2010) first time reported a new *tomato leaf curl New Delhi virus* (ToLCNDV) isolate on oriental melon causing severe disease in Taiwan and observed similar diseased symptoms on oriental melon after mechanical inoculation of virus and naturally diseased melon in the field. Saha et al. (2014) studied the apical leaf curl disease of potato in sub-Himalayan West Bengal to detect causal pathogen and first time recorded the *Begomovirus* infection on cultivated potato through molecular characterization of the virus and transmission assays performed for the disease and confirmed that viruses causing apical curl disease of potato can be transmitted through mechanical sap inoculation and whitefly vector. Further, a method for mechanical inoculation was developed by Lopez et al. (2015) to identify the sources of tolerance against ToLCNDV in melon. They have screened melon germ plasms and confirmed mechanical transmission of *tomato leaf curl New Delhi virus* (ToLCNDV) in 4 genera and 13 species including cucumber, watermelon, melon, pumpkins and some exotic germ plasm used for cucurbits breeding. Plants showed diverse response against virus, and two of them, namely,wild agrestis and *Cucumis melo*, were tolerant to ToLCNDV. In few cases, injection as an effective method for artificial infection of geminiviruses chilli peppers has also been observed (Jamsari et al. 2015).

Transfer of viruses in further progeny of plants through seeds is known as seed transmission. Many researchers study the seed transmissible property of begomoviruses and confirmed that begomoviruses can be transmitted through infected seed (Wang and Maule 1994; Kil et al. 2016; Kothandaraman et al. 2016). Graft inoculation is used for transmission and maintenance of pathogens invading the vascular system of susceptible host. Stem cuttings of infected plants are used to implant on meristematic tissues, older stem parts or distal vines of healthy plant for graft transmission. In order to conduct screen house and field trial experiments, Akhtar et al. (2003) standardized a graft inoculation method for *mungbean yellow*

mosaic virus (MYMV) transmission which was simple, faster, inexpensive and effective. Two approaches, i.e. graft inoculation method and biolistic inoculation, to screen resistant cassava genotypes against cassava mosaic virus were used by Ariyo et al. (2003) who found that graft inoculation is more effective for specific viruses, while biolistic inoculation method can be used to screen many begomoviruses efficiently. In the same way, Boissot et al. (2008) have used vectors and graft inoculation method to ensure the level of resistance against potato yellow mosaic virus (PYMV) (bipartite begomovirus) in wild accessions of *Solanum lycopersicum* and confirmed that *Solanum pimpinellifolium* reveals recessive resistance against PYMV. All the transmission methods implied by humans like grafting, physical damages and mechanical inoculations are contributing in transmission processes. These methods are commonly used by viruses to move from one plant to another and can also be used in the rapid screening of viruses to study their transmission mechanisms. Since begomoviruses are transmitted by whiteflies, their transmission by vector *B. tabaci* will be discussed further.

4.4 Whitefly: The Insect Vector

Whitefly is the only vector that transmits begomoviruses naturally. Along with begomoviruses, carlaviruses, criniviruses and potyviruses can also be transmitted through whitefly vectors. Whiteflies (*Bemisia tabaci* Gennadius) are the widely distributed small insects which belong to the Aleyrodidae family in the order Hemiptera. *B. tabaci* are found throughout the subtropics, tropics and mild or Mediterranean climates. These are sap-sucking insects and reside on the undersides of plant leaves (Fig. 4.2). Both nymphs and adults of whitefly become viruliferous by ingesting the phloem sap from the leaves of diseased plants that cause damage by making plants weak and susceptible to disease which results in leaf yellowing, stunted growth and reduced yields of crops. Whiteflies secrete a sticky substance, honeydew, which gives the suitable conditions for growth of a black sooty mould. This affects the proper functioning of leaves by covering them and further acts as a substrate to many fungi to cause infection. The whitefly *B. tabaci* is named as cryptic species complex because they have more than 31 species in the group that are morphological indistinguishable but greatly diverse in their genetic and biological characteristics (Bedford et al. 1994; De Barro et al. 2005; Zang et al. 2006; De barro et al. 2011; Lee et al. 2013). Visual identification of all the species in the complex is not

Fig. 4.2 Adult whitefly photographs. They are found and feed on the undersurface of plant leaf and transmit begomoviruses in plants

possible, but molecular methods provide the platform to differentiate the species in the *B. tabaci* complex (Costa and Brown 1991; Bedford et al. 1994). Various molecular methods such as amplified fragment length polymorphisms (AFLP) (Cervera et al. 2000), random amplified polymorphic DNA (RAPD) (Gawell and Bartlett 1993), microsatellites (De Barro et al. 2003; Wang et al. 2014), mitochondrial cytochrome oxidase 1 (mtCO1) (Frohlich et al. 1999; Liu et al. 2012; Lee et al. 2013) and nuclear ribosomal internal transcribed spacer 1 (ITS1) (De Barro et al. 2000) have been used to differentiate several groups in this complex. The most accepted method for the identification of several *B. tabaci* complex species is the characterization of mitochondrial cytochrome oxidase subunit I (COI) gene because Dinsdale et al. 2010 analysed the sequence difference and phylogenetic relationship of different species of *B. tabaci* complex and observed a divergence threshold of 3.5%. They have proposed a speciation system by taking this threshold value as a discrimination criterion; further Lee et al. (2013) again worked on similar aspect and raised the divergence threshold from 3.5% to 4% to study the species of *B. tabaci* complex. Ashfaq et al. (2014) studied the barcode region of mtCOI- 5′ to analyse genetic diversity among cryptic species of *B. tabaci* complex of cotton and found DNA barcoding approach effective for successful discrimination among them.

Biotype nomenclature of *B. tabaci* complex is widely accepted in whitefly community. Among the 31 species complex of *B. tabaci*, B and Q biotypes are worldwide now termed as the Middle East-Asia Minor 1 (MEAM) 1 and the Mediterranean MED species, respectively (Brown et al. 1995; Dinsdale et al. 2010; De Barro et al. 2011). The B biotype whiteflies are found in arid, irrigated cropping system and can transmit both New World and Old World begomoviruses efficiently (Gottlieb et al. 2010; Gotz et al. 2012), whereas Q biotypes can adapt greenhouse environments easily and are able to develop resistance against certain insecticides (Horowitz et al. 2005, 2014; Dennehey et al. 2006, 2010). Comparative studies of whitefly biotypes/cryptic species and begomoviruses transmitted by them revealed that begomoviruses and their whitefly vectors are clustered according to their geographic origin and showed a clear parallel grouping (Brown and Czosnek 2002; Brown 2007). B biotype of *B. tabaci* was the first biotype recognized as a cosmopolitan pest and vector during 1990–1994 from the Mediterranean region of Europe, China, Japan, Pakistan, Australia, Egypt, Israel and Turkey. The Q biotype, described first from Spain and Europe/North Africa/Middle East region, is native to the Mediterranean region and has an extensive host range that comprises cultivated and noncultivated crops (Brown 2007). Owing to intermediate names of biotypes (MEAM1 and MED species), Dinsdale et al. (2010) worked on cryptic complex of *B. tabaci* to define their clear and consistent genetic separation and suggested a threshold of 3.5% mtCOI for species differentiation. Further, Boykin et al. (2012) made it more complex by using numerous species delimitation measures to define the species in the *B. tabaci* species complex. As per these studies, there are overall 37 species of *B. tabaci* (Dinsdale et al. 2010; Boykin et al. 2012). However still work is being carried out, and changes in nomenclature of *B. tabaci* species complex are documented suggesting further revision of *B. tabaci* nomenclature (Boykin et al. 2014).

4.5 Whitefly Transmission of Begomoviruses

Begomoviruses are known to be transmitted by insect vector, whitefly (*B. tabaci*); therefore, they are known as whitefly-transmitted geminiviruses (WTGs). There is a tripartite relationship among plants, whitefly and begomoviruses. The interplay between them is revealed in Fig. 4.3. Whiteflies transmit begomoviruses in a persistent, circulative and non-propagative manner by means of both acquisition and inoculation of viruses while feeding on the plants (Czosnek et al. 2002). Some proteins encoded by insect vector and bacterial symbionts present inside the vector play an important role in transmission mechanism during viral transmission.

4.5.1 Circulative Pathway of Begomoviruses in Whitefly

Whiteflies play an important role in begomovirus transmission through feeding on the host plant that causes disease. Virus translocates in the vector in a persistent circulative manner (Fig. 4.4). An infection cycle (the internal route) of a *Begomovirus* begins by acquiring the virus particle from the plant phloem through the insect stylet and move from oesophagus to midgut and filter chamber (the structure where membrane of midgut, hindgut and caeca integrates and acts as a filter for food substances); this process is characterized by an acquisition access period (AAP). After acquisition, begomoviruses are retained in whitefly vector for a long period (latency period) and circulate in the body of the vector. During latency period, viruses

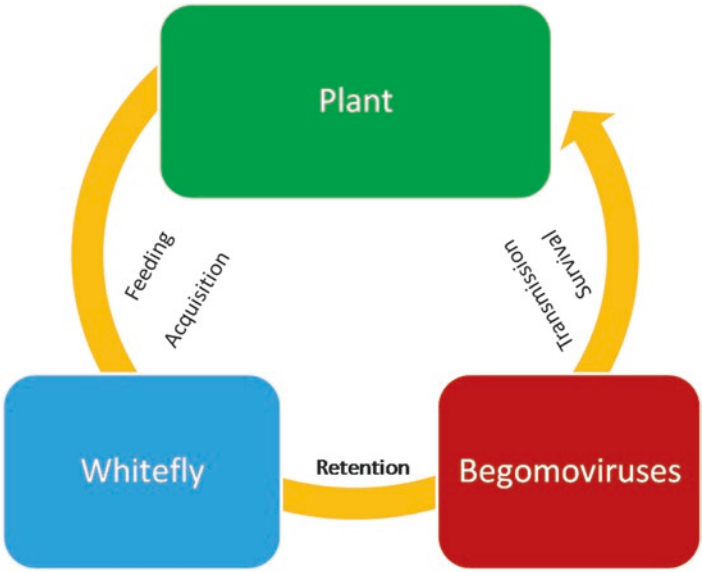

Fig. 4.3 Tripartite interactions between whitefly, begomoviruses and plants. Begomovirus transmission is mediated by whitefly insect vector. Vectors carry the viruses and become mediator for their spread from plant to plant

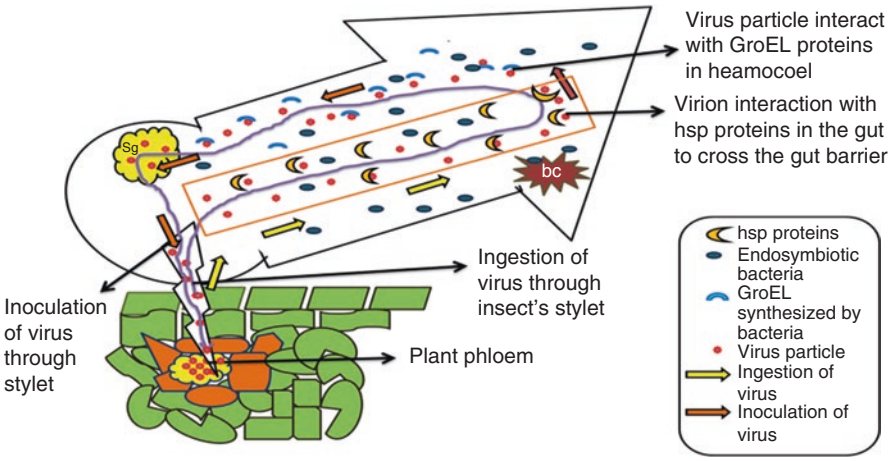

Fig. 4.4 Persistent circulative transmission pathway for begomoviruses (*red colour* particles) acquired and transmitted by whitefly (*B. tabaci*) vector. After acquisition, viruses reach to the gut via food canal where viruses interact with hsp proteins, which help the virions in crossing gut epithelia to reach the haemolymph, where virus particles interact with GroEL protein (*light blue colour*) secreted by primary symbiotic bacteria in the bacteriocytes (*bc*). After that, virions enter into the salivary glands (*Sg*) and transmit to the host plants through salivary secretion during feeding. Secondary symbiotic bacteria (*dark blue*) present in most of the body cavity of insects and influence virus-vector interactions

interact with molecular chaperone proteins, HSP70, and cross the midgut epithelial to reach the haemolymph (insect blood) where virions interact with GroEL proteins produced by endosymbiotic bacteria. Further viruses are translocated to the primary salivary glands through endocytosis from where they are inoculated into a host plant with salivary secretions of whitefly during feeding (Ghanim and Czosnek 2015; Rosen et al. 2015). Minimum time required by whiteflies for virus acquisition and virus inoculation from plant phloem ranges from 15 to 60 min and 15 to 30 min, respectively. In between acquisition and inoculation, a minimum latent period of approximately 8 h is needed for successful transmission of begomoviruses by whiteflies (Ghanim et al. 2001; Czosnek 2008).

4.6 Factors Affecting Whitefly Transmission

As discussed earlier in the chapter, *B. tabaci* are the complex of different species. Different *B. tabaci* species have diverse transmission abilities because of different life history parameters, feeding habits, host preferences and bacterial symbionts present in whiteflies (Pan et al. 2013; Jiang et al. 2000; Polston et al. 2014). Transmission ability of any insect vector is chiefly dependent on AAP, and increased AAP enhanced their transmission frequency (Czosnek et al. 2002). Age and gender of *B. tabaci* have also influenced their virus *transmission* efficiency. Studies on

transmission efficiency of male and female whiteflies revealed that female whiteflies transmit virus with higher efficiency than males (Cohen and Nitzany 1966; Muniyappa et al. 2000). Effect of age of whitefly on its transmission efficiency was studied by Rubinstein and Czosnek (1997) with respect to *tomato yellow leaf curl virus* (TYLCV), and they concluded that transmission efficiency decreases as the age of whitefly increases. They have also correlated the inoculation capabilities of whiteflies with their age and found that the decreased inoculation capability of ageing female whiteflies is due to fewer amounts of viruses acquired during AAP. It is reported that 24-day-old adults ingested only about 10% as compared with 1–2-week-old adult (Czosnek 2008).

4.7 Role of Viral Coat Protein in Transmission

All begomoviruses are transmitted through whitefly *B. tabaci* because of having very high specificity to their vectors. Whitefly-mediated transmission of begomoviruses confers virus-vector specificity with extremely specific intramolecular interactions between insect receptors and the viral-encoded determinants. Mouthparts of whitefly vector are designed to capture the specific virus through their stylet; once virion is acquired by the vector, it circulates in its body. *B. tabaci* stylet follows a convoluted path to reach the phloem from where they acquire begomoviruses (Pollard 1955). Coat protein encoded by begomoviruses is the only protein that determines insect specificity and transmission. Coat protein is the most conserved protein that plays a role in specific binding of begomoviruses with their insect vector, its receptors in the gut and salivary glands. Many whitefly-encoded factors including heat shock proteins govern the efficient circular transmission of begomoviruses (Brown and Czosnek 2002). Change in coat protein amino acids of viruses can make a virus transmissible/non-transmissible and also alter the vector choice of a virus. To ensure the role of CP amino acids of viruses in their vector selection, Briddon et al. (1990) in a very interesting experiment demonstrated the effect of exchange of coat protein gene of African cassava mosaic virus (ACMV) with beet curly top virus (BCTV) and found altered insect specificity of ACMV from whiteflies to leafhoppers. Similarly, the exchange of non-transmissible viral coat protein (CP) amino acids with the transmissible viruses was found to be helpful in the restoration of transmissibility of viruses (Hofer et al. 1997; Noris et al. 1998). Coat protein mutant analysis has resolved the necessity of CP amino acid for efficient transmission and proved that mutant viruses unable to synthesize viral capsid cannot be acquired by whiteflies (Azzam et al. 1994; Liu et al. 1997; Hohnle 2001).

4.8 Endosymbionts in Whitefly

Symbiotic association of bacteria and insects is a good example of co-evolution. These bacteria strengthen their host by providing essential nutrients to enrich their diets (Baumann 2005) and play a very important role in the biology of the insects.

Symbiotic bacteria (symbionts) are predominantly found in arthropods including whiteflies, localized in the mycetocytes (bacteriome) that protect begomovirus in the haemolymph (insect blood). Whiteflies like other insects established different types of interactions with a diverse bacterial community found inside them (Chiel et al. 2007; Zchori-Fein et al. 2014). These interactions are found to be important for insect survival, nutrition and fitness (Moran et al. 2003). Endosymbionts also play an important role in biological diversification of their insect vectors by enhancing their physiological capabilities.

A population dynamics study of endosymbionts revealed that influence of extrinsic factors and location of symbionts inside their host reflect the symbiotic homeostasis of endosymbiont and their insect host (Su et al. 2014). Bacterial flora of whiteflies and other insects includes primary and secondary endosymbionts. Primary (obligate) endosymbionts are ancient and highly specialized bacteria that reside in bacteriome which is an aggregation of some cells known as bacteriocytes (Baumann 2005; Moran 2007). Primary endosymbiont "*Candidatus Portiera aleyrodidarum*" (Oceanospirillales) offers essential amino acid as well as carotenoids to their host (Sloan and Moran 2012), whereas secondary endosymbionts are not essential for host survival but play a crucial role in defence against pathogens (Oliver et al. 2003), genetic differentiation and adaptation to different plants for food (Tsuchida et al. 2002, 2004) and reproduction (Engelstadter and Hurst 2009). Till now, seven genera of secondary (facultative) endosymbionts found in *B. tabaci* are *Arsenophonus* (Enterobacteriales), *Hamiltonella* defence (Zchori-Fein E and Brown JK. 2002), *Candidatus Cardinium hertigii* (Bacteroidales) (Weeks et al. 2003), *Wolbachia* species (Rickettsiales) (Nirgianaki et al. 2003), "*Candidatus Fritschea bemisiae*" (Chlamydiales) (Everett et al. 2005), *Rickettsia* spp. (Rickettsiales) (Gottleib et al. 2006) and *Candidatus Hemipteriphilus asiaticus* (*Orientia*-like organism) (Bing et al. 2013). Occurrence of secondary endosymbionts is not restricted to bacteriocytes; it may vary within their host, for example, secondary symbionts have been distinguished in Malpighian tubules (Bution et al. 2008), salivary glands (Macaluso et al. 2008), primary and secondary bacteriocytes (Gottlieb et al. 2008), haemolymph (Fukatsu et al. 2001; Braquart et al. 2008) and reproductive organs (Frydman et al. 2006).

Bacterial symbionts produce GroEL proteins that are possibly found to be specific for begomoviruses and protect virions from the proteolytic action (degradation) of the insect's immune system in the haemolymph (Morin et al. 1999, 2000; Gottlieb et al. 2010). Morin et al. (1999) gave an evidence for the importance of secondary endosymbionts in safe and efficient viral transmission. They have described the interaction of viral coat protein with the GroEL proteins produced by secondary endosymbiont *Hamiltonella* in *B. tabaci* B biotype that facilitates the translocation of tomato yellow leaf curl virus, whereas GroEL proteins produced by other secondary endosymbionts in both B and Q biotypes do not reveal any interaction with the coat protein of TYLCV (Gottlieb et al. 2010). Another facultative endosymbiont *Rickettsia* found in the digestive tract of *B. tabaci* (B biotype) helps in more virus accumulation in filter chamber and hence becomes responsible for increased transmission efficiency of TYLCV (Kliot et al. 2014).

Apart from proteins produced by bacterial symbionts, *B. tabaci* itself synthesizes a protein, i.e. 70S heat shock protein (HSP70), during virus retention that limits virus transmission and protects insects from lethal effects of viruses (Gotz et al. 2012). HSP70 protein helps in the refolding of virion particles that facilitate their translocation across membrane barriers particularly from the midgut to haemolymph and the haemolymph to salivary glands. Further, Xiao et al. (2016) studied the effect of temperature to check the tolerance capacity of two invasive whiteflies (Middle East-Asia Minor 1 (MEAM1) and the Mediterranean (MED)), found MED whiteflies more tolerant to high temperatures as compared to MEAM1, mainly in adult stage by studying the effect of three heat shock protein-related genes, and concluded that heat shock-related genes hsp90 and hsp70 may be responsible for thermal tolerance ability of whiteflies. On the other hand, peptidyl prolyl isomerase proteins (PPIases/cyps) are also contributing in viral transmission regulation; recently Kanakala and Ghanim (2016) confirmed the role of cyp B protein present in *B. tabaci* in translocations of TYLCV during circulative transmission as well as transovarial transmission of virus between generations.

4.9 Effect of Viruses on Their Whitefly Vector

Begomoviruses are retained in whitefly vector for many weeks (Jiang et al. 2000) and sometimes for whole life of insect vectors (Ghanim et al. 2001; Rubinstein and Czosnek 1997). The structure of capsid protein (CP) of virus determines the vector specificity by interacting with the receptors and insect chaperones present inside whitefly (Hofer et al. 1997). Long-time association of viruses may affect the fitness of their whitefly host and produce the effect on longevity and fertility of *B. tabaci*. Rubinston and Czosnek (1997) studied the long-term association of tomato yellow leaf curl virus (TYLCV) with *B. tabaci* and correlate with the decrease in longevity and fertility of *B. tabaci* in comparison with non-host *B. tabaci*. Similarly, Jiu et al. (2006) have seen the association effect of *tomato yellow leaf curl china virus* (TYLCCNV) and *tobacco curly shoot virus* (TobCSV) on two different B and China-ZHJ-1 biotypes of *B. tabaci* on cotton and concluded the same results for TYLCCNV (Liu et al. 2009) but total opposite results for monopatite TobCSV. *B. tabaci* B biotype infected with TobCSV showed higher fertility and longevity than non-viruliferous B biotype, while TobCSV showed minor effect on China-ZHJ-1 biotypes of *B. tabaci.* Further, they studied the performance of two different *B. tabaci* biotypes on plants infected with TYLCV and TYLCCNV and found differential effects of begomoviruses on performance and preference of whitefly vector biotypes. Similarly, Sindhu et al. (2009) studied the deleterious effects of cotton leaf curl virus on *B. tabaci* and found reduced longevity and fertility of viruliferous whitefly. In contrast, Moreno-Delafuente et al. (2013) found positive effects of begomoviruses on viruliferous whiteflies in comparison with non-viruliferous whiteflies that improve virus transmission efficiency and spread. Matsuura and Hoshino (2009) did not found any differences in survival or fecundity while studying the effect of a Japanese isolate of TYLCV on healthy and infected tomatoes.

Further, some researchers worked on whitefly and their plant host interactions and interestingly concluded that changes in phenotypic and physiological behaviour in the plant hosts of whiteflies determine their preferences for infection. Non-viruliferous and viruliferous whiteflies prefer virus-infected and noninfected plants for infection, respectively (Lapidot et al. 2001). Further, McKenzie (2002) found that viruliferous B biotype whiteflies infected with ToMoV laid more eggs on healthy tomato as compared to non-viruliferous whiteflies. An interesting study on whitefly mobility was conducted by Moreno-Delafuente et al. (2013), and it was suggested that viruliferous whiteflies move slower than non-viruliferous whiteflies and also gave the evidence of increased inoculation efficiency of virus by comparing the duration of salivation phase of viruliferous and non-viruliferous whiteflies.

These observations suggested that different begomoviruses produce diverse effects (positive, negative or neutral) on their whitefly vectors and these contradictory results may be due to their origin, genetic background and adaptation to local whiteflies. Although plant viruses influence the vector physiology and behaviour to increase their transmission rate, on the contrary, insect activities can also modulate the population genetics, behaviour and evolution of virus (Gutierrez et al. 2013).

4.10 Summary

Begomoviruses (whitefly-transmitted viruses), vectored through *Bemisia tabaci*, are very emergent and diverse pathogen having a great importance on global agriculture. Life cycle of begomoviruses is mainly dependent on transmission by vectors that ensure spread and maintenance of virus on host plants. It is interesting to note that viruses influence the vector physiology and behaviour for their transmission, whereas lifestyle and feeding habits of insect vector influence the behaviour, evolution and population genetics of viruses inside the infected plants ultimately influencing the efficiency of virus transmission (Gutierrez et al. 2013). The use of resistant cultivars also produces an impact on virus acquisition and transmission by whiteflies by inhibiting virus multiplication inside the host. Lapidot et al. (2001) evaluated the effect of resistance on viral transmission while studying the TYLCV acquisition and rate of transmission by whiteflies after feeding them on different tomato cultivars and observed that plants with high resistance produce lower transmission rate of viruses as compared with the susceptible plants. Consequently, following acquisition from a highly resistant plant, TYLCV transmission by whiteflies was found to be less efficient. Additionally, the timing of infection and host resistance is responsible for vector preference and development. Less viral accumulation on resistant genotypes than susceptible ones suggested that modifications in phenotypic and physiological traits of the plant hosts could change the fitness of insect vectors, their host preference and ultimately virus spread (Legarrea et al. 2015).

The existence of different cassava and sweet potato whiteflies in India (Lisha et al. 2003) and a different whitefly (*Trialeurodes ricini*) (Idriss et al. 1997) has been reported as the vector for begomoviruses. So, it is important to study and differentiate among the whiteflies and their biotypes with respect to their host range and

insecticide resistance for developing a management strategy against begomoviruses. Although acquisition, retention and transmission property of *B. tabaci* are well known, there is lack of understanding about molecular basis of transmission pathway. Molecular mechanisms, proteins synthesized by insects and their symbiotic bacteria involved in the circulation of virus inside insects that facilitate virus transmission are poorly understood. So, studies on proteins encoded by whiteflies and symbiotic bacteria housed within them may reveal their accurate function in virus-vector interactions and indirectly in transmission of begomoviruses. Technologies like next-generation sequencing and proteomics can be used to identify proteins involved in circulation of begomoviruses. Transgenics, gene silencing and mutagenesis in whiteflies are the very powerful tools to verify the role of proteins involved in transmission. Efficient knowledge about the diversity of viruses and their vectors will extensively help in developing such tools to improve our understanding about molecular basis to choose *B. tabaci* as a very efficient and exclusive vector for their transmission.

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Begomovirus Disease Management Measures, Now and Then

5

Susheel Kumar, Ashish Srivastava, Aarti Kumari, Rashmi Raj, Meraj Jaidi, and S.K. Raj

Abstract

The diseases caused by genus *Begomovirus* (family *Geminiviridae*) are limiting factors in the sustainable crop production throughout the world and majorly in the tropical and subtropical regions of the world. Furthermore, liberalization of agriculture led to change in the agricultural practices and developed significant opportunities for whitefly-borne begomoviruses to disseminate subsequently to newer climatic zones of the favorable environment. As a result, they negate the subsistence agriculture as well as the socioeconomic of the geographic area where they intrude. Consequently, the crop production failure led to considerable financial losses and food insecurity not only for resource-poor directly dependent farmers of developing nations but also indirectly for the developed nations, and therefore management of begomoviruses is essential. As there is no "one-size-fits-all" approach for management of begomovirus diseases, thus together with the prophylactic measures, in the modern era, the integrated pest management strategies (IPMS) have come into existence to provide developing sustainable and environment-friendly novel approaches to limit the crop losses. The present compilation of leading researches in this realm would provide the vast view and understanding of begomovirus diseases and a glimpse of approaches employed for management of begomovirus disease in Asia and Africa, where they greatly affect agriculture.

S. Kumar • A. Kumari • R. Raj • M. Jaidi • S.K. Raj (✉)
Plant Molecular Virology Laboratory, Council of Scientific and Industrial Research-National Botanical Research Institute, Rana Pratap Marg, Lucknow 226 001, UP, India
e-mail: skraj2@rediffmail.com

A. Srivastava
Plant Molecular Virology Laboratory, Council of Scientific and Industrial Research-National Botanical Research Institute, Rana Pratap Marg, Lucknow 226 001, UP, India

Amity Institute of Virology and Immunology, Amity University Uttar Pradesh, Noida 201 313, UP, India

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Keywords
Begomovirus • Whitefly • Disease management • Biotechnological approaches • Conventional approaches • Integrated pest management

5.1 Introduction

Virus diseases are responsible for the severe crop losses throughout the world, and adopted insect (whitefly) transmission makes their spread favorable in warmer regions of the globe. The geminiviruses are responsible for many of these diseases and infect monocot and dicot plants (Stanley et al. 2005). From the agriculturally important crops like cereals, legumes, vegetables, ornamentals, medicinal, and commercially important fiber crops, the geminiviruses infect a large range of weed plants and cause substantial losses (Legg and Fauquet 2004; Shepherd et al. 2010; Navas-Castillo et al. 2011; Scholthof et al. 2011; Sattar et al. 2013; Khan et al. 2014). More of it, the disease incidences as well as the propensity to infect larger group of economically important crop plant are increasing day by day. Studies have provided evidence that many begomovirus species as a disease complex exist very often (Nawaz-Ul-Rehman and Fauquet 2009). This association of complex, therefore, enhances the possibilities of mutation, recombination, and reassortment in the viral genomes (Martin et al. 2011) to further increase viral diversity (Duffy and Holmes 2008) for better adaptability of virus to the environment as well as to increase their host range (Harkins et al. 2009; Lima et al. 2012). Geminiviruses have one or two components of single-stranded circular DNA genome of ~2.8 kilo bases (kb) DNA packaged into geminate virions (Zhang et al. 2001), with limited coding capacities both by the virion and complementary sense DNA strands (Rojas et al. 2005). To pursue the life cycle, to duplicate the genome, and consequently to cause the disease, they completely depend on the host cellular machineries and interact with several plant proteins and processes during this infection.

Recently, family *Geminiviridae* has divided into nine genera: *Begomovirus*, *Mastrevirus*, *Curtovirus*, *Becurtovirus*, *Eragrovirus*, *Topocuvirus*, *Turncurtovirus*, *Capulavirus*, and *Grablovirus* by the International Committee on Taxonomy of Viruses (Varsani et al. 2017) on the basis of their genome organization and transmitting vectors. Among these, the genus *Begomovirus* is of particular importance which has 288 of the 325 total species in geminiviruses (recognized by the ICTV, Fauquet et al. 2008; Brown et al. 2012, 2015) and also infect a large number of hosts mainly of dicotyledonous plants, affecting the agriculture system.

Begomoviruses either possess bipartite genome containing DNA-A and DNA-B components or monopartite genome having a single DNA-A-like genome (Fig. 5.1). The DNA genome of monopartite begomoviruses encodes for six to seven open reading frames (ORFs), the V1 and V2 transcribed from the virion sense strand and four ORFs, C1–C4, transcribed from the complementary sense strand. Many monopartite begomoviruses are commonly associated with satellite DNA molecules

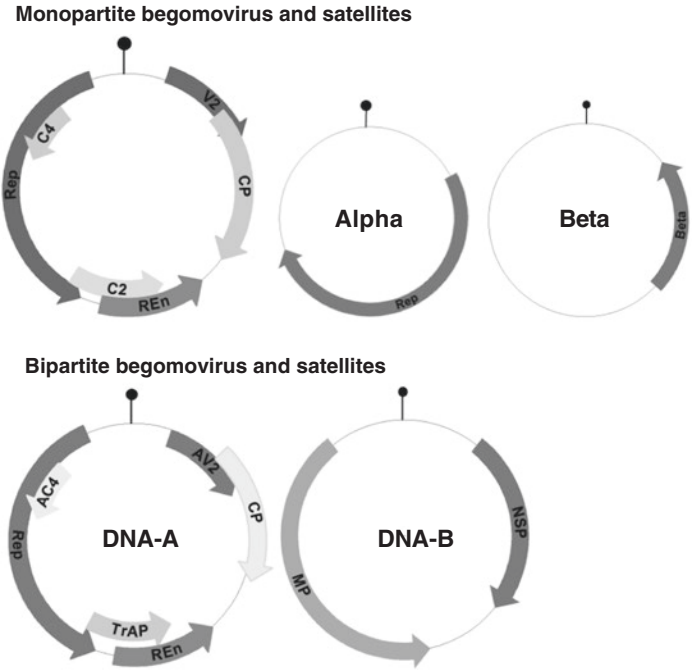

Fig. 5.1 Cartoon showing the arrangement of begomovirus genome (*monopartite* and *bipartite*) and associated alphasatellite and betasatellite

such as betasatellites and alphasatellites (Srivastava et al. 2013, 2014) which depend on genomic DNA for replication, encapsidation, and movement. The betasatellite is known as symptom-modulating component and reported to exacerbate the symptom expression by suppressing host gene silencing (Kumar et al. 2014). However, the role of alphasatellite is not very much identified. In addition to these satellites, recently a small noncoding subviral molecule in association with several distinct sweepoviruses is discovered and named as "deltasatellites." They share structural similarities in the conserved region of betasatellites and have A-rich sequence and a stem-loop structure containing the nonanucleotide TAATATTAC as reported in ToLCV-sat (Lozano et al. 2016). A few of the monopartite begomoviruses also have an additional C5 ORF that encodes a protein which has recently shown to be involve in perturbation of the regulation of miR156, miR159, miR160, and miR164-170 (Melgarejo et al. 2013). Figure 5.1 clearly depicts the arrangement and function of begomovirus genomes.

Some begomoviruses have bipartite genomic organization where association of two molecules of 2.6 kb size, DNA-A and DNA-B, is reported. The DNA-A encodes for five to six ORFs, AV1 in the virion sense strand and AC1–AC4 in the complementary sense strand, whereas DNA-B encodes for BV1 and BC1 ORFs in virion and complementary sense strands, respectively. The AV1/V1 of begomoviruses

encodes for coat protein (CP) and AV2/V2 for pre-coat protein in virion sense. AC1/C1 encodes the replication-associated protein (Rep), AC2/C2 the transcriptional activator protein (TrAP), AC3/C3 the replication enhancer (Ren), and AC4/C4 for RNA silencing suppressor protein in complementary sense. The BV1 and BC1 codes for nuclear shuttle protein (NSP) and movement protein (MP), respectively, are required for nucleus-to-cytoplasm and cell-to-cell movement of the begomoviral DNA. The region does not encode for any ORFs and is considered as an intergenic region (IR) containing origin of replication (a place where Rep protein binds for initiating rolling circle replication) and bidirectional promoter for begomovirus DNA transcription (Brown et al. 2012). Both monopartite and bipartite begomoviruses occur in the Old World (OW) countries (Africa, Asia, Australasia, and Europe), whereas the begomoviruses native to the New World (NW, the Americas) are almost exclusively bipartite with few exceptionally monopartite (Sanchez-Campos et al. 2013; Melgarejo et al. 2013). The polyphagous whitefly (especially the biotype-B)-mediated transmission of begomovirus makes the condition severe (Navas-Castillo et al. 2011) and poses major threats to food security (De Bruyn et al. 2012).

5.2 Disease Management, Need of the Hour

Begomoviruses are emerging as serious problems in crops like tomato, chili, cassava, cotton, legumes, and vegetables as well as in non-crop plants known as alternative host (Varma and Malathi 2003). They mostly induce yellowing, vein enation, mottling, curl, crumpling, leaf distortion, and growth stunting (Fig. 5.2) and transmitted one host to another through whitefly vectors. The plants once infected by begomoviruses cannot be controlled by any chemical means, and no treatment methods are reported till date (Valkonen 1998), thus limiting for the search of facile management except preventive measures. The recommended preventive measures are modification of cultural practices like rouging, intercropping, avoidance, use of barriers, and crop residue disposal. For better results, the preventive measures may also be combined with the use of insecticides and/or resistant varieties (Polston and Anderson 1997; Faria and Zerbini 2000; Hilje et al. 2001). The present chapter deals with the information on both prophylactic and modern methods used for control of viral diseases. Prophylactic measures include pest control, development of virus-free plants, breeding, culture control, and quarantine regulation, while modern methods include pathogen-derived resistance, RNAi-mediated resistance, and ribozyme-mediated resistance.

5.2.1 Traditional Measures

The prophylactic measures are mainly based on the trained agriculture practices to control and/or limit the transmission of the disease. The first line of management measures relies on regular surveillance to observe the natural begomoviral disease symptoms and their vectors in fields. Sanitation to provide clean cultivation of crops

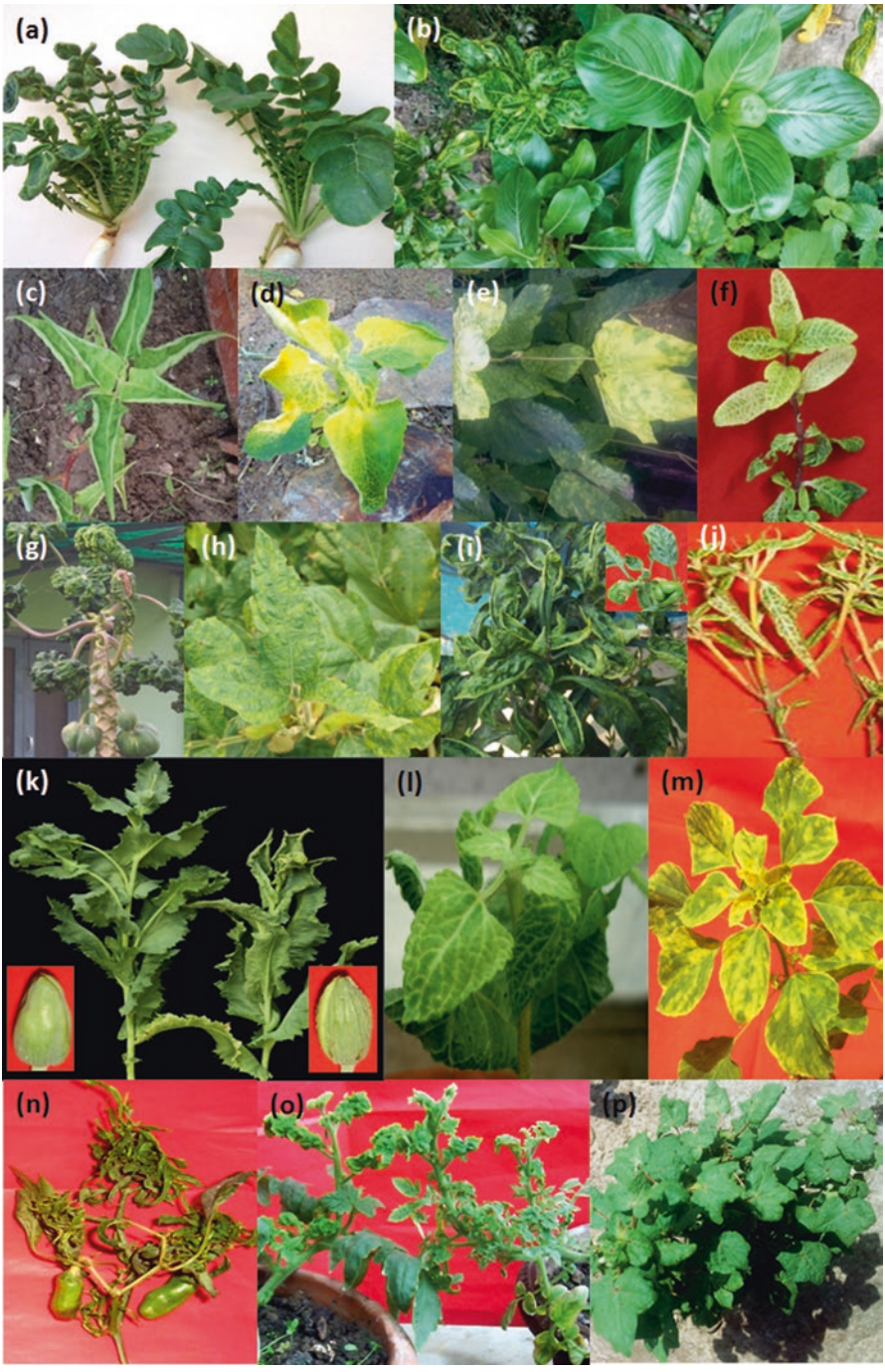

Fig. 5.2 Bird's eye view of begomovirus-induced symptoms on various crop and non-crop plants. (**a**) *Raphanus sativus*, (**b**) *Catharanthus roseus*, (**c**) *Mirabilis jalapa*, (**d**) *Helianthus* sp., (**e**) *Dolichos lablab*, (**f**) *Eclipta alba*, (**g**) *Papaya carica*, (**h**) *Glycine max*, (**i**) *Capsicum annuum* (sweet pepper), (**j**) *Croton bonplandianum*, (**k**) *Papaver somniferum*, (**l**) *Ageratum conyzoides*, (**m**) *Malvastrum coromandelianum*, (**n**) *Capsicum annuum* (hot pepper), (**o**) *Solanum lycopersicon*, and (**p**) *Gossypium hirsutum*

may improve the scenario. Elimination of the insect vectors and source inoculum (symptomatic plants) along with the use of insecticides like Malathion, Rogor, Confidor, etc. on non-symptomatic hosts may also add strength to control the disease. The use of sensitive diagnostic methods for virus detection, the use of virus-free propagating materials for mass production through tissue culture or breeding, efficient cultural practices, and quarantine regulation during import or export of any plant material are also some of the recommended conventional methods which are described in details for control of begomovirus diseases in the following paragraphs.

5.2.1.1 Culture Control

The non-crop plants growing in and around the cultivated field including weeds are one of the major sources of begomoviruses during non-crop seasons and become key sources of begomoviral inoculum during the next cropping season as well as for a long-time inoculation. Cleaning and eradication of infected plant materials together with the potential reservoir of begomovirus from the fields were found to be very effective methods. The weeds like *Croton*, *Acalypha*, *Malvastrum*, *Eclipta*, *Ageratum*, *Jatropha*, *Parthenium*, *Sida*, and *Sonchus* have been found to be a potent begomovirus inoculum (Khan et al. 2006; Somvanshi et al. 2009). A study revealed more than 18 different weed species for reservoir of tobacco leaf curl virus in southern India (Valand and Muniyappa 1992).

During the detection of alternative reservoir of tomato leaf curl virus in India, 13 weed species were identified as an alternative host (Singh et al. 1994). *Sida* sp. and *Abutilon indicum* were identified as alternate reservoir of cotton leaf curl virus in Punjab and *Croton bonplandianum* of tomato leaf curl New Delhi virus (ToLCNDV), and *Parthenium hysterophorus* was found to host tomato leaf curl Karnataka virus (ToLCKV) (Reddy et al. 2005) and *Ageratum enation* virus. *Nicotiana plumbaginifolia*, *Physalis minima*, *Coccinia grandis*, *Solanum nigrum*, *Momordica charantia* (wild), and *Luffa* sp. have been found to be the hosts of geminivirus (Khan et al. 2003). In literature eradication of perennial weeds from around greenhouses, gardens, and fields to eliminate possible sources of virus may prove helpful. Therefore, such weed plants may be eliminated from and nearby cultivated fields for possible management of begomoviruses. Along with this, the practices such as early plantation and plant spacing use of silver- or white-colored mulches were also found effective in reducing disease incidence and obtaining maximum crop yield.

5.2.1.2 Quarantine Control

Plant and seed health testing, known as quarantine, is an essential measure for the control of pathogens. The European and Mediterranean Plant Protection Organization (EPPO) has defined certification scheme as a system to produce, propagate, and sale the disease-free vegetative propagated plants, obtained from nuclear stock after several propagation stages under conditions ensuring that stated health standards are met. Since then, researchers were carried out in pathology, and now there is greater

international cooperation in certification activities (Waterworth and Kahn 1978). These certification safeguards both the nursery man who sells vegetatively propagated plant material and the grower who buys the nursery man's products. Hence, certification schemes have been established to comply with official standards/regulations set by the national and international authorities to guarantee the quality of propagative material to trueness-to-type (genetic purity). The process basically involves assessing the risks (pathogens and pests), selection of planting material that is claimed as clean, pre-detection of the virus, micro-propagation, and tests for genetic fidelity. Ultimately, a certificate is only provided to the plants that are produced as per the directive of the scheme.

5.2.1.3 Pest Control and Monitoring of Host-Vector Populations

Begomoviruses are transmitted by insect vectors from one plant to other plants. Hence, diseases can be efficiently controlled by limiting the populations of whitefly vectors with the applications of appropriate pesticides. The use of non-host "trap plants" may also be considered to attract vectors to reduce the number of individuals feeding on the crop of interest and thus the transmission of the disease (Bragard et al. 2013).

Pest control comprises of controlling the insect (vectors which act as carriers for transmitting the viruses from infected plant to the healthy ones). The use of insecticides is as early since 1930s as a means of direct attack on vectors (Du 1948; Bradbent et al. 1956). A study by Mehrotra (1991) suggested the early seed sowing, crop rotation, avoidance by growing plants in isolated areas, and putting physical barriers like screens or cages toward off insect vectors. Sastry and Singh (1973) also did extensive studies and field evaluation of different insecticides for control of whitefly in relation to the incidence of yellow vein mosaic of okra (*Abelmoschus esculentus*). Khan et al. (2006) and Somanshi et al. (2009) suggested that sprayings of Malathion (50% E.C.) insecticide three times at 21-day intervals on chili cultivars in field conditions can minimize the whitefly population.

Verma and Awasthi (1980) and Kunkalikar et al. (2006) suggested the spry of viricides, pesticides, oils, and botanicals can reduce the yield losses.

In addition to this, spraying of oils, viricides, pesticides, and botanicals has been found to be significant in reducing the yield loss by many workers for various viral diseases (Verma and Awasthi 1980; Kunkalikar et al. 2006). Management of begomovirus diseases by oil sprays (Sastry 1984) with an example of control of tomato leaf curl virus by carbofuran plus oil combination (Sastry 1989; Sastry et al. 1974) has also been reported. However, overdose as well as repetitive use of insecticides may also prove ecologically harmful in the long run besides providing resistance to the insect vectors. Insects feeding on perennial hosts may cause early infection, and the use of insecticides on those hosts and weeds may be more helpful (Hull and Davies 1992).

5.2.1.4 Breeding for Resistance

Breeding resistance technique against virus or vector is the best approach for controlling begomoviral diseases. According to Valkonen (1998), resistant variety

against virus insect has superiority, and they will require no extra inputs for virus-free planting materials or virus/vector control and rise profitability of production as it increases yields in both qualitative and quantitative manner.

Genes introgressed from *Solanum peruvianum*, *S. chilense*, *S. pimpinellifolium*, and *S. habrochaites* are one of the best examples of breeding-mediated resistance against begomovirus infection in tomato (Ji et al. 2007a).

A partially dominant major resistance gene, Ty-1, was introgressed from *S. chilense* and mapped to the short arm of chromosome 6 (Zamir et al. 1994). To develop begomovirus resistance in *S. pimpinellifolium*, a dominant resistance QTL derived from its material hirsute-INRA was mapped to a different position on chromosome 6 (TG153-CT83) (Chague et al. 1997). Hanson et al. (2000) also identified and mapped a dominant resistance gene, Ty-2, in *S. habrochaites*-derived line H24, to the short arm of chromosome 11. Similarly, a partially dominant major gene of *S. chilense* materials LA2779 and LA1932, Ty-3, was mapped to chromosome 6 (Ji et al. 2007a, 2009). Recently, a recessive resistance gene (Ty-5) was identified on chromosome 4 in the lines derived from cultivar Tyking (Hutton et al. 2012), which is suspected to be similar to the Ty-5 locus that accounts for more than 40% of the variation (Anbinder et al. 2009). Most of these resistance sources are known to support virus replication. However, the level of virus accumulation is lower than the levels in susceptible cultivars. It is well established that the virus level in tomato lines carrying Ty-1/Ty-3 is <10% of the level found in susceptible cultivars (Verlaan et al. 2013). Similarly, a low level of virus accumulation and a positive correlation between virus level and disease severity were found in Ty-2-carrying lines (Barbieri et al. 2010).

There is limitation in conventional method for controlling the virus diseases because single dominant genes usually conferred by any breeders for the producing resistance cultivars (Fraser 1990), and this failed in the field (Pelham et al. 1970). Easy identification of resistance genes is quite difficult because the mechanism involved for resistance in many crop species has largely remained unknown in most of the cases (Grumet 1994; Valkonen 1998).

5.2.2 Novel Measures

The traditional measures for managing begomovirus diseases have been found to possess their own drawbacks like natural resistance to several important viruses which is not known. The breeding programs have limitations to produce cultivars with effective resistance in a reasonable period of time. Therefore, development of begomovirus-resistant transgenic plants through recombinant DNA technologies emerged as one of the reliable strategies. The use of genetically modified resistant plants is one of the most efficient, sustainable, and frequently employed strategies to control virus infections in fields. The stable expression of gene of interest from organism of different species or kingdom into desired plants represents one of the most significant developments in a series of advances in bio-agriculture and biotechnology that includes modern plant breeding, hybrid seed production, farm

mechanization, and the use of agrichemicals to provide nutrients and control pests (Gasser and Fraley 1989). The establishment of *Agrobacterium tumefaciens* as foreign gene carrier to develop transgenic plants made major breakthrough in the management of viral diseases. Powell-Abel et al. (1986) were the first to produce the coat protein (CP)-mediated genetically engineered virus resistance in tobacco plants against the tobacco mosaic virus (TMV). This proved to be a major breakthrough in several ways. Along with engineered herbicide resistance and insect resistance using the *Bacillus thuringiensis* toxin gene, virus resistance was one of the first successes in the genetic engineering of a useful trait into plants (Horsch et al. 1985). Resistance to begomoviruses has been achieved in various plants either by the use of begomoviral genes (known as the pathogen-derived resistance or PDR) or through the expression of non-begomoviral genes from different organisms, as under.

5.2.2.1 Pathogen-Derived Resistance

The research established by Powell-Abel et al. (1986) opened up the new possibilities for developing the protection against viral diseases in plant, and genetic engineering catalyzes this process to a greater extent (Gonsalves and Slightom 1993). Following their work, at present, as many as 30 different virus groups have been utilized for which engineered resistance has been achieved utilizing different virus genes (Lomonossoff 1995; Pappu et al. 1995; Varma 1997; Prins and Goldbach 1998; Reimann-Phillipp 1998; Bendahmane and Beachy 1999; Jain and Varma 2000; Callaway et al. 2001; Varma et al. 2002). The pathogen-derived resistance (PDR) is inducing the resistance to pathogens by transformation with genes derived from the genome of the pathogen. The term was first postulated by Hamilton, and later Sanford and Johnston expanded it into a generalized concept. The PDR had correlation with cross-protection wherein inoculation host with a milder/symptomless strain of virus can cross-protect the plant from infection by more severe strains of the same virus or very closely related viruses. Accordingly, it was postulated that the expression of a pathogen's own genes in a host in a dysfunctional form, in excess or at an inappropriate stage, could disrupt the normal pathogenic cycle of the invading pathogen. To achieve resistance against begomoviruses, the native or altered genes derived from begomovirus might be used to interfere with various stages in the viral life cycle such as uncoating of genome, replication, cell-to-cell or long-distance movement, or vector-mediated transmission by Rep, NSP, and MP, respectively. Such interference with host-pathogen interactions renders the host resistant (Grumet 1994; Lomonossoff 1995). Among which, the CP gene is the most widely and commonly used transgene for which virus-resistant transgenic plants have been developed followed by replicase and the movement protein genes (Varma et al. 2002). The mechanism of PDR includes protein-mediated resistance and RNA interference (RNAi). Both mechanisms have been shown to confer geminivirus resistance in transgenic tomato, common bean, cassava, and others (Vanderschuren et al. 2009; Abhary et al. 2006; Zhang and Gruissem 2003; Vanderschuren et al. 2007). Some of the examples of PDR are

discussed here showing their potential to control the begomovirus infection to a considerable level with their pros and cons.

Coat Protein (CP)-Mediated Resistance

The CP of begomovirus has to perform several essential functions during the life cycle of the virus. CP binds to and protects viral ssDNA and forms the geminate particles. CP also performs some cellular functions such as self-binding, nuclear targeting, and nuclear export and, with monopartite genome begomoviruses, mediates systemic movement in plants. CP is needed for transmission by the whitefly vector and protects virus particles from degradation inside the whitefly. The use of CP-mediated protection has been successful and applied against several RNA viruses (Beachy et al. 1990; Mayo 1992; Kumar et al. 2012; Pratap et al. 2011, 2012). Kunik and coworkers (1994) were the first to demonstrate that tomato cultivars transformed with coat protein gene of TYLCV were resistant to the virus, and transgenic plants remained asymptomatic or showed delayed disease symptoms. Later, CP gene of tomato leaf curl virus (TLCV) was cloned into a plant expression vector, and transgenic tomato plants of Pusa Ruby were developed by cotyledon leaf explants (Raj et al. 2005). They have cloned TLCV-CP gene into pROK vector, and transgenic tomato cv. Pusa Ruby plants expressing coat protein were generated.

The putative transgenic plants obtained by Raj et al. (2005) at T0 generation were screened by PCR and rescreened by Southern and Northern hybridization tests and Western blot assay, which confirmed the incorporation and expression of the CP gene. Seven transformed plants of three CP lines obtained were found to contain detectable levels of CP mRNA and 30 kDa CP. T0 generation transgenic plants of two lines were as fertile as untransformed control plants and produced flowers, fruits, and seeds. This observation is like the past work of Zhuk and Rassokha (1992), who regenerated TMV resistance in tomato plants Pusa Ruby and found that most regenerated plants were fertile and suitable for breeding.

Further, plants regenerated up to T1 generation from these lines, and screened by challenge inoculations of TLCV using viruliferous whiteflies, showed variable degrees of resistance/tolerance. Many of the challenged inoculated CP1 and CP3 plants were symptomless after 10 weeks. Highest (30%) resistant plants were found in CP1 line, while 50% of tolerant plants were observed in line CP2 at 7 weeks post inoculation. Further, PCR with Rep-specific primers supported that transgenics did not allow replication and accumulation of challenged begomovirus, suggesting CP gene (TLCV-CP)-mediated resistance in these plants. However, during assessment of T1 generation plants, we could not observe the absolute correlation between the degree of resistance and the expression of CP in them.

It was also demonstrated by Kunik et al. (1994) that the transgenic tomato plants expressing the TYLCV coat protein, when challenged with TYLCV, responded either as tolerant or showed delayed disease symptoms. Earlier, Mayo (1992) has established in the case of TMV that CP-mediated resistance operated by inhibiting an early event of infection prevents the uncoating of the virion, therefore blocking the systemic spread. During our experiments, Rep-specific primers failed to detect

TLCV by PCR in T1 generation plants of our CP lines, indicating the lack of accumulation and inability of begomovirus spread after infection. These findings would strengthen the understanding on the phenomenon of virus encapsidation by the capsid protein and its cell to cell transport in infected plants. Additionally, efforts made in this direction were preliminary processes to achieve resistance against TLCV in transgenic tomato plants. The protocols standardized for efficient tomato transformation have a wide scope in generation of transgenic plants using other genes of interest.

Replication-Associated Protein (Rep)-Mediated Resistance

Another gene often used to obtain PDR-derived resistance to begomoviruses is the replication-associated protein (Rep) gene, usually expressed by C1. The first evidence of the high levels of resistance in *N. benthamiana* plants was conferred by the expression of a truncated tomato yellow leaf curl Sardinia virus (TYLCSV) Rep (encoding the first 210 amino acids of the Rep protein and potentially co-expressing the C4 protein) given by Noris et al. (1996). Interestingly, the resistance was high in the early growth stage; however, it overcame with time. Transgenic tomato plants expressing high level of the truncated Rep were found resistant to TYLCSV infection during challenge inoculation by whiteflies. Tomato transgenic lines not expressing Rep (lines containing the antisense *Rep* or both sense and antisense *Rep* gene) were susceptible to TYLCSV infection. However, the TYLCV-resistant tomato exhibited an undesired and altered phenotype and did not seem to be effective against a distinct species of begomovirus, tomato leaf curl Australia virus (Brunetti et al. 1997). Similar studies were performed in *N. benthamiana* plants; however, it showed that transgenic tomato was resistant to the homologous virus but susceptible to the related strain TYLCV Murcia (TYLCV-ES). According to the authors, the truncated *Rep* acts as a trans-dominant-negative mutant that inhibits the transcription and replication of TYLCSV, but not of TYLCV-ES (Brunetti et al. 2001; Freitas-Astúa et al. 2002).

Distinct functional domains of bipartite begomovirus encoded by AC1 perform several important biological processes. Two truncated forms of Rep (tAC1) gene, capable of expressing only the N-terminal 669 bp (50AC1) and C-terminal 783 bp (30AC1) nucleotides cloned under transcriptional control of the CaMV35S, were introduced into cotton (*Gossypium hirsutum* L.), making use of an interference strategy for impairing cotton leaf curl virus (CLCuV) infection in transgenic cotton. Compared with non-transformed control, we observed that transgenic cotton plants overexpressing either N-terminal (50AC1) or C-terminal (30AC1) sequences confer resistance to CLCuV by inhibiting replication of viral genomic and b satellite DNA components. Molecular analysis by Northern blot hybridization revealed high transgene expression in early and late growth stages associated with inhibition of CLCuV replication.

Most of the T1 transgenic lines tested were showing delayed and mild symptoms as compared to non-transgenic control lines. These lines developed severe disease symptoms after 2–3 weeks of whitefly-mediated viral delivery. Virus biological assay and growth of T2 plants proved that transgenic cotton plants overexpressing

50- and 30AC1 displayed high resistance level up to 72% and 81%, respectively, as compared to non-transformed control plants, giving significantly high cotton seed yield. Assessment of progeny of these plants by PCR, blotting, and immunological assays showed stable transgene, integration, inheritance, and cotton leaf curl disease (CLCuD) resistance, some of the transgenic lines having single or two transgene insertions. Partial CLCuV AC1 expressing transgenic cotton can be used as cotton leaf curl disease resistance source in cotton breeding programs aiming to improve begomovirus resistance in cotton (Hashmi et al. 2011).

Recently, a dominant resistance gene, *Ty-1* resistance gene from tomato against tomato yellow leaf curl virus (TYLCV) encoding an RNA-dependent RNA polymerase (RdRp), is proposed to confer resistance to TYLCV by amplifying the RNAi signal.

siRNA Mediated

Small RNA (siRNA) regulates expression of several genes in all plants and constitutes a natural immunity against viruses (Blevins et al. 2011). siRNA-based genetic engineering (SRGE) technology had been explored for crop protection against viruses for nearly 30 years. Viral resistance has been developed in diverse crops with SRGE technology, and a few viral resistant crops have been approved for commercial release. Here, we summarized the efforts generating viral resistance with SRGE in different crops and analyzed the evolution of the technology, its efficacy in different crops for different viruses, and its application status in different crops.

Jatropha curcas, a biodiesel plant, is grown in tropical and subtropical regions and is found susceptible to a number of begomoviruses such as jatropha mosaic India virus, Indian cassava mosaic virus (ICMV), etc., and very often viral disease outbreaks severely limit productivity. Since jatropha grow as shrub and tree and they are perineal, therefore the development of durable begomovirus resistance remains crucial and poses a major biotechnological challenge. In a study by Ye and coworkers (2014), the transgenic *J. curcas* plants expressing hairpin, double-stranded (ds) RNA with sequences homologous to five key genes of ICMV-Dha strain DNA-A were generated which silenced the viral genes expression, thereby conferring ICMV resistance. The durability and heritability of resistance conferred by the dsRNA were further tested by two rounds of virus challenge inoculation via vacuum infiltration of ICMV-Dha to ascertain that T1 progeny transgenic plants were resistant to the ICMV-SG strain, which were 94.5% identical to ICMV-Dha strain. Begomovirus could not be detected in transgenic lines by quantitative PCR analysis (Ye et al. 2014).

miRNA-Mediated Resistance

microRNAs (miRNAs) are small noncoding RNA molecule (22–24 nt) present in and regulate expression of genes in plants and animals. Virologists have employed them to control begomovirus diseases. In a study, cotton leaf curl Multan begomovirus (CLCuMV) causes devastating cotton leaf curl disease (CLCuD) in cotton plants. Baig and Khan (2013) using in silico approaches identified the cotton miRNA targets in the genomes of CLCuMV and betasatellite. Total 18 nt sequences

representing full-length DNA-A of CLCuMV and 58 nt sequences of full-length betasatellite were screened against a set of 69 mature miRNAs of *G. hirsutum*. The antiviral activity of cotton miRNAs against putative viral mRNA targets was analyzed on the basis of complementarily of miRNA-mRNA target pairings. Their study revealed 34 putative miRNA targets in DNA-A-encoded proteins loci and two putative miRNA targets in βC1 gene of betasatellite above threshold values. The most potential miRNAs against identified DNA-A were miR168, miR169, miR390, miR395, miR399, miR414, miR779, miR2948, miR2950, and miR3476which could target viral genome with perfect complementarities at multiple gene loci. For betasatellite, two miRNAs targets were identified, viz., miR398 and miR2950. Out of the total miRNA identified, seven miRNAs (miR168, miR169, miR398, miR399, miR779, miR2948, and miR2950) strongly cleaved the mRNA target sites, while three miRNAs (miR390, miR414, and miR3476) were probably targeting the translational. The hotspots for miRNA targets in DNA-A genome AC1 gene, while in betasatellite, the satellite conserved region (SCR) is the host spot for miRNA target. In their study, the AC1 gene was significantly targeted by 14 miRNAs while the βC1 by two miRNAs. Interestingly, a common miR2950 was identified that could target miRNAs of both DNA-A and betasatellite. Artificially designed miR168, miR169, miR390, miR395, miR398, miR399, miR414, miR779, miR2948, miR2950, and miR3476 targeting DNA-A of CLCuV and βC1 gene of DNA-β may have the potential to confer effective resistance against CLCuD infection in transformed cotton (Baig and Khan 2013).

In another study, the in silico analysis of mung bean yellow mosaic India virus (MYMIV) and mung bean yellow mosaic virus (MYMV) reveals the micro-RNA (miRNA) target (Ramesh et al. 2016). The recent study showed that the MYMV genome is targeted by 70 miRNAs. The miRNAs derived from soybeans (*Glycine max*), wild soybean (*Glycine soja*), and chickpea (*Cicer arietinum*) display 63, 18, and 8 potential target sites on the begomovirus genomes, respectively. Begomoviruses also exhibit seven and six potential target sites for non-host crops like *Oryza sativa*- and *Populus trichocarpa*-derived miRNAs, respectively. Begomoviral movement proteins (MP) reveal greater vulnerability for *G. max*-derived miRNA binding and repression. In silico analysis with ssDNA animal virus genome as negative control sequences further emphasizes that plant miRNAs preferentially target begomovirus genomes. The nine potential soybean-derived miRNAs targeting begomovirus genes have been shown to play a role in host-microbe interactions and abiotic stress responsiveness. The study thus provides in silico evidence for the plant-derived miRNAs in antiviral immunity (Ramesh et al. 2016).

amiRNA Mediated

miRNAs play an important role in plant development, signal transduction, and response to biotic and a biotic stress. The miRNAs can be engineered to alter their target specificity and such artificial miRNAs (amiRNAs) that have been shown to provide resistance against many begomovirus infection. Begomovirus-linked cotton leaf curl disease is a major constraint to cotton cultivation across Pakistan and northwestern India. Based on the studies on amiRNA, the two amiRNA constructs,

related to cotton miRNA169a sequences, were produced that contained 21 nt of the V2 gene sequence of cotton leaf curl Burewala virus (CLCuBuV), and *Nicotiana benthamiana* were transformed. The first amiRNA construct (P1C) maintained the miR169a sequence except for the replaced 21 nt, whereas in the second (P1D), the sequence of the miRNA169a backbone was altered to restore some of the hydrogen bonding of the mature miRNA duplex. A high-level resistance was observed in P1C transgenic plants challenged with CLCuBuV, plants being asymptomatic with low viral DNA levels. The heterologous virus resistance was lower and correlated with the numbers of sequence mismatches between the amiRNA and the V2 gene sequence. The P1D plants showed overall poorer resistance when challenged with all viruses tested. The results show that the amiRNA approach can deliver efficient resistance in plants against a monopartite begomoviruses, and multiple target site incorporation can make it the potential broad-spectrum, virus resistance method. However, the drawback of the findings is that the levels of resistance depend upon the levels of complementarities between the amiRNA and the target sequence and the sequence of the miRNA backbone, consistent with earlier studies (Ali et al. 2013).

Ribozyme Mediated

Cleavage of target RNA was effected by antisense RNA ribozymes which have intrinsic endonucleolytic activity. However, double-standard RNA mechanism or antisense dominance controlled the endonucleolytic activity in vitro. According to Mishra et al. (2014), hammerhead ribozyme was designed to target rep-mRNA of MYMIV and was developed as an antiviral agent, and during in his study, it was also found that RNA silencing is induced on introduction of catalytically active as well as inactive ribozymes. It was also demonstrated that endonucleolytic activity of ribozymes is a true phenomenon, while muted version may have similar downregulation of target RNA by using RNA silencing suppressors.

Artificial tasiRNA

AC2, AC4, and AV2 proteins have been identified to act as RNAi suppressors in case of geminiviruses. Trans-acting siRNA (tasiRNA) are siRNA that repress gene expression through PTGS in plants. A gene silencing vector designed by Singh et al. (2015) using the features of tasiRNA can be further utilize to target multiple genes and it is simple also, and this was also used to target two RNAi suppressor proteins of TOLCNDV because this vector has fragments of TOLCNDV, AC2, and AC4 genes, and during filtration, it produced large quantities of proteins.

TOLCNDV-infiltrated plants showed no symptoms and low accumulation of viral DNA. Amounts of siRNA produced against AC2 and AC4 genes of viruses are proportional to resistance, and the use of tasiRNA-generated resistance against virus was first reported (Singh et al. 2015).

5.2.2.2 Non-pathogen-Derived Resistance

Other nonconventional approaches rely on the use of genes that are derived from the host plant instead of the pathogen. Here we discuss the examples that utilized the plant genes and show significant resistance against the plant viruses.

Tma12-Mediated Resistance

Different control strategies based on physical barriers, pesticides, biotic agents, and host plant resistance have been used to combat hemipteran pests in general and whiteflies in particular (Horowitz et al. 2011). Integrated pest management, involving one or more of these strategies, is also practiced in some countries, but none of these strategies can effectively control, and therefore the concept to engineer the dual begomovirus-*Bemisia tabaci* resistance in transgenic plants was conceived (Shukla et al. 2016). As the technology could be supplied as seeds, the transgenic crops' resistant to whiteflies is vulnerable. However, no transgene has been reported to date that provides reliable and stable resistance to whitefly. Sap-sucking whitefly also damages field crops by transmitting viral diseases. Complete eradication of whitefly by the use of insecticidal proteins expressed in genetically modified (GM) crops is effective against whitefly to date. With the idea that ferns and mosses are rarely infested by phytophagous insects in the wild-insect infestation on ferns is 30-fold lower than in flowering plants. The work of Shukla and coworkers reported the identification and use of a fern, *Tectaria macrodonta* (Fee) C. Chr., protein (Tma12) which shows its potential as insecticidal to whitefly with a median lethal concentration, 1.49 mg/ml, during in vitro feeding assays, and interferes with its life cycle at sublethal doses. They express Tma12 in cotton lines under 35S promoter of pNBRI719 cassette.

Whitefly-resistant transgenic cotton lines expressing Tma12 were resistant to whitefly, with no detectable yield penalty. The transgenic lines were also protected from whitefly-borne cotton leaf curl viral disease. Sensitivity of this protein was also tested in rats that showed no detectable histological or biochemical changes, and absence of allergenic domains in Tma12. Therefore, Tma12 may be used for deployment in GM crops to control whitefly and the viruses it carries (Shukla et al. 2016). Proteins that can control whiteflies effectively in transgenic crops would be invaluable, provided that they do not affect host plant biology and are safe to humans and other nontarget organisms. In search of such proteins, 38 ferns for protein-based insecticidal activity have been identified, and *T. macrodonta* is the most promising source. TSP from rhizomes as well as fronds had insecticidal activity. The target protein was purified from fern fronds. Activity-guided purification yielded the insecticidal protein Tma12. We note that Tma12 was isolated from an edible plant, which could make it a more promising candidate for the development of whitefly-resistant GM crops than lectins. Although the use of RNA interference to control whitefly has been suggested, the multigenic nature of target genes and nonspecific recognition make it a poor choice for GM crop development.

CRISPR-Cas9-Mediated Resistance

Conventional strategies can fail to control rapidly evolving and emerging plant viruses. Genome engineering strategies have recently emerged as promising tools to introduce desirable traits in many eukaryotic species, including plants. Among these genome engineering technologies, the CRISPR (clustered regularly interspaced palindromic repeats)/CRISPR-associated 9 (CRISPR/Cas9) systems have evolved recently, because of its simplicity, efficiency, and reproducibility. CRISPR/Cas9 technique is being used to engineer virus resistance in plants, either by directly targeting and cleaving the infecting viral genome or by modifying the host plant genome to introduce viral immunity. Till date, CRISPR/Cas9 technique has been used to develop resistance against many begomoviruses such as tomato yellow leaf curl virus (TYLCV), bean yellow dwarf virus (BeYDV), and cotton leaf curl Kokhran virus (CLKCoV). These resistant plants were developed by targeting the virus genome using this technique. We conclude by pinpointing the gaps in our knowledge and the outstanding questions regarding CRISPR-/Cas9-mediated viral immunity (Zaidi et al. 2016).

5.3 Conclusion

Valkonen (1998) suggested that viruses are obligate parasites, and chemical control is not possible in field conditions. However, there is a possibility to check the further spread of the disease by controlling vectors of begomoviruses. Management of begomovirus is based on the strategies that deal with either by arrest of their replication/expression or by prevention of plant-to-plant movement through whiteflies. The conventional methods mainly deal with the extermination of transmitting vector whitefly.

Using of insecticides, plant extracts, biological methods, and nonconventional methods may be suggested to control the infection caused by begomoviruses on different crop species. As begomoviruses have small genome size and are capable to find new hosts, they have received much attention in the last decades by the researchers across the world especially in the tropical and subtropical region as climates favor the multiplication and ability of vectors.

Emergence of new begomoviruses (by recombination/pseudo-recombination) and increasing incidence of the disease are causing increased severity of the disease in many crop species worldwide (Varma and Malathi 2003). The association of strong host silencing suppressor, that is betasatellite molecules with both monopartite and bipartite begomoviruses, is the other major factor having a role in disease severity. Begomoviruses are mainly transmitted by whitefly vectors; however, recently few reports proved seed transmission in some begomoviruses. Due to the ability of whiteflies and begomovirus to adapt to new host growing nearby the crop fields, their control is particularly difficult in the open field. Therefore, the disease management may also include the elimination of alternate host/virus reservoir and vector reservoir plants. Perennial weeds growing near the fields may be the possible

sources of begomovirus infection, and these weeds have already been identified as alternative hosts of natural reservoirs of begomovirus (Agrios 1978; Khan et al., 2012, 2013), and removing such kind of weeds from the field may be helpful in preventing further spread of the disease in nature. The infected material may be immediately destroyed and should not be left to compost near adjacent to developing fields. The development of integrated disease management strategies by spray of oils, efficient pesticides, and tested plant extracts may be significant in controlling begomoviral disease. Many viruses, fungi, or abiotic stresses cause similar symptoms; therefore, development of sensitive diagnostic protocol is very essential to identify the actual begomovirus infection in cultivated crops. Several PCR-based, probe-based, and antibody-based detection tools are available commercially; however, minimizing the cost of testing is highly required. As viruses are obligatory parasites, therefore, development of virus-resistant crop is an easy way to get rid of this disease.

Genetic engineering in crop has been proven to be highly effective for the management of begomovirus infection in crops. However, in case of RNA viruses, transgenic crop expressing CP gene was reported to be successful (Kunik et al. 1994). Hamilton and Baulcombe (1999) attempted transgenic expression (antisense AC1) against tomato golden mosaic virus. Further, CRISPR-/Cas9-based begomovirus resistance plays an important role in broad-spectrum resistance in crops.

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Begomoviruses Associated with Horticultural Crops

6

Swati Kumari and Maneesh Mishra

Abstract

The horticultural crops are severely threatened by many insects, pests and diseases. In this backdrop, viral diseases assume much greater significance as most of these viral diseases are transmitted through insects. The source of resistance for these viral diseases is scanty. Begomoviruses affect a large number of vegetables and few fruit crops. Begomoviruses cause significant crop losses in horticultural crops like tomato, okra, chilli, papaya, brinjal, cassava, squash, sweet potato, potato, etc. Despite the amount of efforts that has gone into the control of begomoviruses, sustained resistance has not been acquired in many crops. Obtaining crops resistant to begomoviruses is very difficult because the insect vector is whitefly (*Bemisia tabaci*) which develops resistance against insecticides, and it is increasingly spreading over large parts of the world. Molecular markers and other genomic information are allowing more precision breeding for greater tolerance to viral diseases in general and begomoviruses in particular. This chapter highlights many fruit and vegetable crops which are affected by begomoviruses.

Keywords

Begomovirus • Whitefly • Molecular markers • Genomic

S. Kumari • M. Mishra (✉)
ICAR-Central Institute for Subtropical Horticulture,
Rehmankhera, P.O. Kakori, Lucknow 226106, India
e-mail: maneeshmishra.cish@gmail.com

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6.1 Introduction

The horticultural industries are one of the most important sectors in the world. Recently, fruit and vegetable production has grown beyond leaps and bounds owing to lifestyle changes, health consciousness and stress and also due to the growing economy (USAID 2005). Global fruit production stands at 548 million tonnes. After China, India is the second largest producer of fruits in the world. China is the largest producer of fruits (83.24 mt) as fruit production in China has shot up by 246% in the last 15 years, whereas India contributes 49 million tonnes and Brazil contributes 36 million tonnes of fruits. Global production of vegetables was 990 million tonnes in 2011. Preceding China, India is the second largest producer of vegetables with 11% production of vegetables across the world, while Brazil stands third in the category (Chadha and Chaudhary 2007). India ranks first in production of okra; second in production of brinjal, cabbage, cauliflower, pea, onion and tomato; and third in production of potato across the globe. India is the second leader player in terms of flower production accounting 1,15,921 under floriculture, producing 6,54,837 mt of loose flowers annually. Similarly the medicinal plant-based industries are also growing at the rate of 7–15% annually. The world trade of medicinal plants is about 62 billion USD which was expected to increase by 5 trillion USD (Chadha and Chaudhary 2007). The present international state in production and trade of essential oils and aroma chemicals is very composite and vital in which Indian industries have taken the lead and are the largest producer, consumer and exporter of all spices and spice products globally. The spice production in India is of 3.72 mt from 0.226 million hectare area.

6.2 Begomoviruses in Horticultural Crop Species

There has to be a balance between growing fruit demands of the world and global agricultural output (Smil 2000). Plant production in general and protection of crops against various fungal, bacterial and viral plant diseases in particular have an apparent role to perform in scaling up with the tune of meeting the growing demand for food quality and quantity (Strange and Scott 2005). As per an estimate, direct yield losses by pathogens, weeds and diseases range between 20% and 40% of global agricultural productivity (Teng and Krupa 1980; Savary et al. 2012). Geminiviruses are one of the most potent plant pathogens whose vector carrier *B. tabaci* belongs to the genus *Begomovirus*, family *Geminiviridae* (van Regenmortel et al. 2000). The virus morphology shows its non-enveloped and small size (22 × 38 nm geminate particle) comprising two joined incomplete icosahedra encapsidating a single-stranded genome of approximately 2800 nucleotides (Goodman 1977). Begomoviruses cause significant crop losses in horticultural crops like tomato, okra, chilli, papaya, brinjal, cassava, squash, sweet potato, potato, etc. In spite of all the efforts employed in the control of begomoviruses, sustained resistance has still not been acquired in many crops. Generally all begomoviruses cause symptoms that include bright yellow to chlorotic mosaic on leaves, usually with some leaf

distortion or leaf curling. Plants infected with TYLCV or ToLCV at an early growth stage get severely stunted. Leaflets that develop at a later stage of infection are upright with yellowing between veins, and their leaf margins are rolled upwards. Young emerging leaves are cupped downwards. Plants infected at younger age lose vigour and are unable to produce marketable fruits. Fruits already present in the plant ripen normally, but no further fruits are formed when infection is sought in older plants. Begomoviruses affect a large section of horticultural crops (Thompson 2011). Mahesha and Manjunath (2015) have compiled different strains of begomoviruses which infect vegetable crops in India (Table 6.1 and Fig. 6.1).

6.3 Papaya Leaf Curl Virus Disease

Papaya (*Carica papaya* L.) is a giant herbaceous plant native to tropical America. It is a rapidly growing, hollow-stemmed and short-lived perennial. Papaya has a long history of cultivation and is used in tropical and subtropical regions between 32° latitude, north and south of the equator. The main papaya-producing countries are Brazil, Nigeria, Congo, Indonesia, Malaysia and India. Papaya has become a high-ranking fruit crop for its great nutritive and commercial value (Van Droogenbroeck et al. 2004).

Papaya leaf curl virus (PaLCuV) disease has been outlined from different parts of the world such as India (Singh et al. 2006), Pakistan (Nadeem et al. 1997), Taiwan (Chang et al. 2003) and China (Wang et al. 2004; Zhang et al. 2005; Huang and Zhou 2006). PaLCuV disease has been reported from different parts of India, viz. Tamil Nadu (Thomas and Krishnaswamy 1939) Bihar, Karnataka (Govindu 1964) and Uttar Pradesh (Saxena et al. 1998a, b). The disease is known to be transmitted by the vector carrier whitefly (*B. tabaci*) in a persistent manner, and a begomovirus was detected from papaya by nucleic acid hybridization tests (Haber et al. 1981; Hamilton et al. 1983; Stanley 1983; Saxena et al. 1998a, b). It is interesting to note that the whitefly is unable to feed continuously on papaya to complete the acquisition, latency and effective inoculation periods. The first report on association of ToLCNDV with leaf curl disease of papaya in India predicts that the disease is characterized by severe curling, crinkling and distortion of leaves accompanied by vein thickening and reduction in leaf size. The leaf margins are rolled downwards and inwards to form inverted cup followed by thickening of veins. The affected leaves become cup shaped, leathery and brittle; and petioles get twisted in a zigzag manner. The interveinal areas are raised on the upper surface due to hypertrophy which gives rugosity to leaves. The affected plants fail to flower or bear fruits. In advanced stage, defoliation occurs and plant growth is arrested (Saxena et al. 1998a). The association of *Tomato leaf curl New Delhi virus* with leaf curl disease of papaya was detected by using begomovirus-specific primers for polymerase chain reaction (PCR) and confirmed by highest sequence similarities and close phylogenetic relationships (Raj et al. 2008). Natural defence mechanism in plants is done by RNAi activity which plays an important role in maintenance in genome. In geminiviruses RNA silencing is done by genes like AV2, AC2 and AC4.

Table 6.1 The major begomoviruses infecting vegetables in India

Crop	Disease	Group	Transmission	Diagnostic techniques
Tomato	*Tomato leaf curl New Delhi virus*	Begomovirus	Whitefly	PCR
	Tomato leaf curl Gujarat virus	Begomovirus	Whitefly	PCR
	Tomato leaf curl Palampur virus	Begomovirus	Whitefly	PCR
	Tomato leaf curl Joydebpur virus	Begomovirus	Whitefly	PCR
	Tomato leaf curl Ranchi virus			
	Tomato leaf curl Kerala virus	Begomovirus	Whitefly	PCR
	Tomato leaf curl Bangalore virus	Begomovirus	Whitefly	PCR
	Tomato leaf curl Karnataka virus	Begomovirus	Whitefly	PCR
	Tobacco curly shoot virus	Begomovirus	Whitefly	PCR
Chilli and pepper	*Chilli leaf curl virus*	Begomovirus	Whitefly	PCR
	Tomato leaf curl New Delhi virus	Begomovirus	Whitefly	PCR
	Chilli leaf curl Palampur virus	Begomovirus	Whitefly	PCR
	Chilli leaf curl Bhavanisagar virus	Begomovirus	Whitefly	PCR
Brinjal	*Tomato leaf curl New Delhi virus*	Begomovirus	Whitefly	PCR
	Tomato leaf curl Joydebpur virus	Begomovirus	Whitefly	PCR
Cucurbits				
Bitter gourd	*Tomato leaf curl New Delhi virus*	Begomovirus	Whitefly	PCR
	Indian cassava mosaic virus	Begomovirus	Whitefly	PCR
	Pepper leaf curl Bangladesh virus	Begomovirus	Whitefly	PCR
Cucumber	*Tomato leaf curl New Delhi virus*	Begomovirus	Whitefly	PCR
Musk melon	*Tomato leaf curl New Delhi virus*	Begomovirus	Whitefly	PCR

(continued)

Table 6.1 (continued)

Crop	Disease	Group	Transmission	Diagnostic techniques
Pumpkin	*Tomato leaf curl New Delhi virus*	Begomovirus	Whitefly	PCR
	Tomato leaf curl Palampur virus	Begomovirus	Whitefly	PCR
	Squash leaf curl China virus	Begomovirus	Whitefly	PCR
Ridge gourd	*Tomato leaf curl New Delhi virus*	Begomovirus	Whitefly	PCR
Bottle gourd	*Tomato leaf curl New Delhi virus*	Begomovirus	Whitefly	PCR
Sponge gourd	*Tomato leaf curl New Delhi virus*	Begomovirus	Whitefly	PCR
Winter and summer squash	*Squash leaf curl China virus*	Begomovirus	Whitefly	PCR
	Tomato leaf curl Palampur virus	Begomovirus	Whitefly	PCR

Source: Mahesha and Manjunath (2015)

Post-transcriptional gene silencing by specific gene targets is involved in suppression of plant RNA silencing machinery, thereby, bringing resistance against PaLCuV. To some extent PaLCuV could be managed by controlling whitefly vector through insecticide, growing barrier crops, etc. (Fig. 6.2).

6.4 Potato Yellow Mosaic *Begomovirus* Disease

Potato (*Solanum tuberosum* L.) is the world's most important staple food crop and is very critical to global food security. It is the world's fourth largest food crop. Potatoes are mostly cross-pollinated by insects such as bumblebees. The Food and Agriculture Organization reports that the global production of potatoes in 2013 was about 368 million tonnes. Two thirds of the world production is consumed by humans, while the rest is used as animal feed or used to produce starch. Venezuela experienced *Potato yellow mosaic virus* in 1986 on potatoes (Roberts et al. 1986). It was observed that these may be strains or isolates of PYMV infecting tomato. It is clear now that begomoviruses affecting tomato and potato are the same. This disease has been reported in different parts of the world including India, Martinique, Puerto Rico, Trinidad, Tobago and Venezuela. Usharani et al. 2003 reported leaf curl disease in potato for North India which causes leaf distortion, bright yellow mosaic and eventually stunting of plants. There is limited information about the control of the disease; however, controlling *B. tabaci* would limit its spread is still

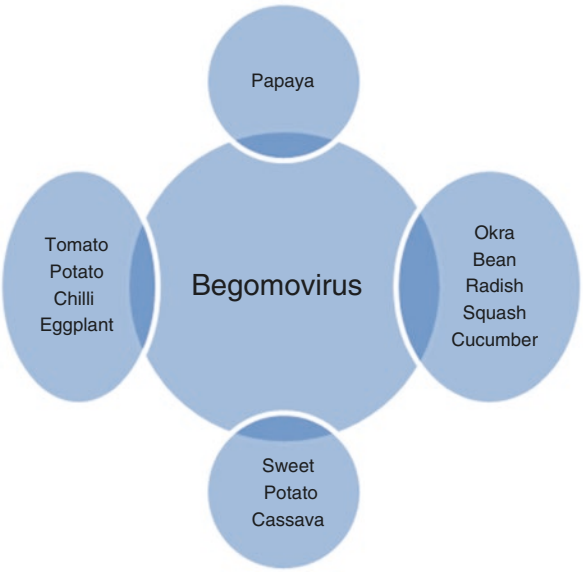

Fig. 6.1 Horticultural crops associated with begomoviruses

questionable as it is not clear whether the disease is tuber transmitted or not. In India, infection is more common in crops planted during October due to large white-fly population (Chandel et al. 2010).

6.5 Tomato Leaf Curl Virus Disease

Tomato (*Solanum lycopersicum*) belongs to family Solanaceae. Global production of tomatoes was recorded to be 170.8 million tonnes in 2014 with China accounting for 31% of the total, preceded by India, the United States and Turkey. It is estimated that around 7 million hectares could experience *Tomato yellow leaf curl virus* (TYLCV) infection annually, and around 30 tomato-growing countries in the world are under its influence. The virus was first reported in Israel in 1930, and now it has spread in Asia, Africa, North and Central America and Australia. TYLCV is a monopartite virus which causes the most destructive disease of tomato in tropical and subtropical areas creating severe economic losses. The primary host of TYLCV is tomato, whereas other hosts include brinjal, potato, tobacco, beans and pepper. The female plant usually gets infected from the diseased male plant and vice versa. It is considered that the virus had evolved from the Middle East region around eight decades ago (Lefeuvre et al. 2010). The symptoms include stunting of plantlets, reduction in leaf size, puckering, upward curling, distortion and yellow margins. The infection interferes with fruit setting and development. The virus is transmitted in a persistent and circulative manner by whiteflies (*Bemisia tabaci*) that are attracted to young leaves and growing tips (Pandey et al. 2010a). Analysis revealed that ssDNA genome encodes six ORFs and four complementary orientations.

Fig. 6.2 Papaya plantlets infected with papaya leaf curl disease

Usually *coat protein* encapsulates ssDNA, and it protects the viral RNA from the virus particle, whereas movement of the virus is associated with precoat protein (Glick et al. 2009). It has been found that GroEL homologue is prerequisite for protein-folding high binding affinity and TYLCV has been found to have high binding affinity to GroEL. Studies conducted revealed that feeding whitefly with antiserum against GroEL eventually reduced TYLCV transmission.

It is considered now that ToLCVV and ToLCGV recombination gave rise to a novel begomovirus in China which infects tomato (Yang et al. 2011). Geminiviruses transmitted by whitefly have bipartite genome having DNA-A and DNA-B. In Peru, a geminivirus which lacks DNA-B was found to be associated with tomato leaf curl disease. Later, it was renamed as tomato leaf deformation virus. ToLDeV is a monopartite begomovirus which is also found in Ecuador and Peru. This was the first report of an indigenous NW (New World) monopartite begomovirus, and evidence presented that it emerged from the DNA-A component of a NW bipartite progenitor via convergent evolution and recombination. Melgarejo et al. (2013) reported a virus previously only identified in the Indian subcontinent as chilli leaf curl virus (ChLCV) was found associated with tomato and pepper diseases in all vegetable-grown areas of Oman (Khan et al. 2014). A few of the infected plant samples were also found to have a beta satellite. A total of 19 potentially full-length

begomoviruses and eight beta satellite clones were sequenced. ChLCV-OM is the fourth begomovirus identified in tomato in Oman and the first in capsicum.

Tomato yellow spot virus (ToYSV) is isolated from tomato. However, it is genetically similar to viruses from *Sida* sp., having the capability to form viable pseudo-recombinants with tomato viruses. According to Andrade et al. (2006), severe symptoms in *Nicotiana benthamiana* were observed due to pseudo-recombinant formed between DNA-A and tomato crinkle yellow virus DNA-B. The result suggested that formation of pseudo-recombinant cannot be explained solely on the basis of phylogeny and conserved iteron sequences. Morin et al. (1999) suggested TGMV Rep protein may be more versatile in terms of heterologous DNA components than that of TpYSV.

Pandey et al. (2010a) studied the genetic diversity of two isolates of monopartite ToLCV infecting tomato in North and South India and observed that K3/K5 and CTM are novel isolates of ToLCV and possibly evolved by recombination between viruses related to two or more viral ancestors. High genome diversity in India was observed in ToLCV isolates, and this poses a serious threat to tomato production in the Asian countries.

Efforts are on to develop virus-resistant tomato plants. Advances in breeding lines for resistance to TYLCV and ToMoV were reported by Yuanfu et al. (2007). Agrobacterium-mediated genetic transformation was done to produce transgenic tomato against tomato leaf curl disease by using *rep* gene. *Rep* gene along with *nptII* gene was used for transformation through binary vector. Mendelian pattern of inheritance in two of the six transgenic lines having single transgene insertion was showed by the progeny of the developed plants (Praveen et al. 2005).

6.6 Eggplant Yellow Mosaic Disease

Brinjal (*Solanum melongena* L.) is one of the most commercial vegetable crops grown in India (Anonymous 2007). The brinjal production gets affected in India due to pest and disease. Brinjal is one of the preferred hosts for rearing whiteflies and was known to be immune to begomoviruses till 2010 (Pratap et al. 2011). Brinjal has been reported to be a host of cucumber mosaic virus in India, Israel, Lebanon, Jordan and Italy. Brinjal which is commonly known as eggplant is severely affected by eggplant yellow mosaic disease (EYMD) showing symptoms such as severe yellow mosaic and mottling of leaves at later stage of infection. EYMD symptoms were also observed in Thailand (Green et al. 2003). The tomato- and eggplant-infecting viruses in Thailand constitute a distinctly novel bipartite begomovirus species (Green et al. 2003). Pratap et al. (2011) reported that begomovirus which affects brinjal is a variant of ubiquitous *Tomato leaf curl New Delhi virus* – India (ToLCNDV-IN). The pathogenicity of ToLCNDV-IN isolate was confirmed by agroinfiltration and dimeric clones of DNA-A- and DNA-B-induced characteristic yellow mosaic symptoms in eggplants and leaf curling in tomato plants. The complete DNA-A and DNA-B genomic components of the causative virus were cloned and sequenced.

6.7 Chilli Leaf Curl Virus Disease

Chilli leaf curl is a serious problem in chilli (*Solanum annum* L.) growing areas of India. Chilli leaf curl was first time reported in India by Senanayake et al. (2006) and recently by Khan et al. (2014). Chilli leaf curl complex is caused by geminivirus which may be transmitted by thrips, whitefly or chilli mite. In case of infection, the plant showed typical leaf curling, bunching of leaves and possible puckering. Reduction in size of leaves and fruits is also a main symptom of geminiviral infection (Venkatesh et al. 1998). Khan et al. (2006) reported the association of *Tomato leaf curl New Delhi virus* with chilli leaf curl disease in Lucknow of Uttar Pradesh, India. From Pakistan, cotton leaf curl Multan virus (Hussain et al. 2004) and, from Indonesia, pepper leaf curl Indonesia virus (Tsai et al. 2006) were also recorded on chilli leaf curl disease.

Very recently *chilli leaf curl virus* was found to be associated with *Mentha* (*M. Spicata* var. Neera) in India (Saeed et al. 2016) which shared highest sequence identity with the chilli leaf curl virus of Pakistan. Chattopadhyay et al. (2008) for the first time demonstrated Koch's postulates by using cloned DNA. Upgradation of several defence-related genes was also observed through comparative study between resistance and susceptible chilli plants by Kushwaha et al. (2015).

The management should start from the seedling stage itself by using seeds from virus-free plants. The nursery should be covered with nylon net or straw to protect the seedling from viral infection. Raising barrier crops such as sorghum, maize and pearl millet around chillies in two to three rows will reduce the disease incidence level. The management of virus by plant products showed that neem seed kernel extract 5% was found the most effective for controlling the whiteflies. Management by insecticide imidacloprid 17.8 SL (0.003%) was also found to be effective (Pandey et al. 2010b).

6.8 Okra Yellow Vein Mosaic Virus Disease

Okra yellow vein mosaic virus was reported in 1924 in India (Kulkarni 1924). It is a major limitation in production of okra (*Abelmoschus esculentus*) in India (Jose and Usha 2003). Plants infected with begomovirus showed vein clearing, chlorosis and yellow patches on leaves. In case of severity of the disease, chlorosis may extend up to the interveinal area which resulted in complete yellowing of the leaves and up to 90% yield losses (Pun and Doraisamy 1999). Affected plants have dwarfed and malformed fruits with yellow-green colour. Insect vectors of the disease are whiteflies, and the causal agent is ssDNA (bhendi yellow vein mosaic virus), which is associated with beta satellite.

The management strategy includes burning of infected plants, vector control through insecticide and use of moderately resistant cultivars (Balamurugan 2003). Decrease in minimum temperature is conducive to disease development (Ali et al. 2012). Okra samples containing leaf curl and yellow vein symptoms were sequenced which contain 36 beta satellites. Four groups sequences were segregated, in which

two groups correspond to beta satellites okra leaf curl satellite and bhendi yellow vein beta satellite that have been previously identified in okra from subcontinent.

One sequence was distinct from all other, previously isolated beta satellites and represented a new species for which the name bhendi yellow vein India beta satellite (BYVIB) was proposed. This new beta satellite was nevertheless closely related to BYVB and OLCuB. Most surprising was the identification of croton yellow vein mosaic beta satellite (CroYVMB) in okra: a beta satellite not previously identified in a malvaceous plant species. The okra beta satellites were shown to have distinct geographic host ranges with BYVB occurring across India, whereas OLCuB was only identified in North-Western India (Venkataravanappa et al. 2011). *Croton yellow vein mosaic virus* has also been reported from India in tomato plant with the symptoms of leaf curl, accompanied with puckering, vein swelling and stunting of the whole plant (Khan et al. 2015).

6.9 Sweet Potato Leaf Curl Virus Disease

Sweet potato (*Ipomoea batatas*) is an important root and tuber crop; and worldwide it is next to potato and cassava, although the major production and cultivation is confined to sub-Saharan Africa and China, but it is cultivated in many tropical and subtropical parts of the world as well. The monopartite sweet potato leaf curl is sometimes also termed as sweepovirus (Fauquet et al. 2008). Leaf curl symptoms were first reported in sweet potato in Taiwan (Liao et al. 1979; Chung et al. 1985). Sweet potato leaf curl virus (SPLCV) symptoms cause severe yield reduction without showing any visible foliar symptoms such as leaf curl, yellowing, growth reduction and vein yellowing (Clark and Hoy 2006). As sweet potato is vegetatively propagated, viruses can accumulate in seed stock of stored roots (Clark and Hoy 2006). Sweet potato-related *Ipomoea* species are frequently infected by monopartite begomoviruses (genus *Begomovirus*, family *Geminiviridae*), known as sweepoviruses. *Bemisia tabaci* transmits SPLCV in a persistent and circulative manner. SPLCV has been managed through meristem tip culture with non-persistent results. Control of whitefly through insecticide has also not been very successful (Gilbertson et al. 2011). Sweet potato leaf curl Lanzarote virus, sweet potato leaf curl Spain virus and sweet potato leaf curl Canary virus were identified from Spain as novel species of sweepovirus. Trenado et al. (2011) confirmed Koch's postulates for the first time in case of sweepovirus.

6.10 Cassava Mosaic Disease

Cassava (*Manihot esculenta* Crantz) is a staple food of Africa. It is a drought-tolerant crop and it even yields in poor soils. Cassava is propagated asexually and vulnerable to viruses. Around 800 million people are using cassava as food and source of income in Africa, Asia and Latin America. Africa accounts for more than 50% global production of 233.8 million metric tonnes. Cassava is vulnerable to

broad range of diseases, out of which cassava mosaic disease (CMD) is the most severe and widespread among all other diseases. CMD-affected plants produce few or no tubers depending upon the severity of the disease and the age of plant at the time of infection. CMD was first reported from Tanzania in 1894. It produces a variety of foliar symptoms which includes mosaic, mottling, misshapen, twisted leaflets and overall reduction in size of leaf and plant. The disease could spread through whitefly. Recently, Kim et al. (2015) proved that SPLCV could be transmitted through seeds as well. Two bipartite begomoviruses, *Indian cassava mosaic virus* (ICMV) and *Sri Lankan cassava mosaic virus* (SLCMV), have been isolated from mosaic-diseased cassava originating from Central India and Sri Lanka, respectively. SLCMV is more closely related to ICMV (DNA-A, 84%; DNA-B, 94% nucleotide identity) than African cassava mosaic virus (ACMV) (DNA-A, 74%; DNA-B, 47% nucleotide identity). Sequence comparisons suggest that SLCMV DNA-B originated from ICMV DNA-B by a recombination event involving the SLCMV DNA, intergenic region. Thus, SLCMV DNA-A has biological characteristics of a monopartite *Begomovirus*, and the virus probably evolved by acquisition of a DNA-B component from ICMV. Khan et al. (2011) from India reported the association of ICMV with chilli crop with leaf curling and yellow mosaic symptoms. CMD symptoms, collected in Burkina Faso, revealed four DNA-A begomovirus components when cloned and sequenced, showing 99.9% nucleotide identity among them. These isolates were most closely related to African cassava mosaic virus (ACMV) but share less than 89% nucleotide identity (taxonomic threshold) with any previously described begomovirus. A DNA-B genomic component, sharing 93% nucleotide identity with DNA-B of ACMV, was also characterized. Since all genomic components have a typical genome organization of Old World (OW) bipartite begomoviruses, this new species was provisionally named African cassava mosaic Burkina Faso virus (ACMBFV). Recombination analysis of the new virus demonstrated an interspecies recombinant origin, with major parents related to West African isolates of ACMV and minor parents related to tomato leaf curl Cameroon virus and *Cotton leaf curl Gezira virus*. CMD is endemic in Africa, and seven distinct species are found associated with the disease: African cassava mosaic virus, East African cassava mosaic virus, East African cassava mosaic Cameroon virus, East African cassava mosaic Kenya virus, East African cassava mosaic Malawi virus, East African cassava mosaic Zanzibar virus and South African cassava mosaic virus (Tiendrébéogo et al. 2012). The molecular variability of CMGs was also evaluated using partial B component nucleotide sequences of 13 EACMV isolates from Tanzania (Ndunguru et al. 2005). Complete nucleotide sequence of begomovirus infecting sweet potato in Argentina has been reported by Pradina et al. (2012).

6.11 Radish Leaf Curl Virus Disease

Radish (*Raphanus sativus*) is an edibleroot vegetable of the Brassicaceae family. Radishes are grown and consumed throughout the world, being mostly eaten raw as a crunchy salad vegetable. Radishes owe their sharp flavour to the various chemical compounds produced by the plants, including glucosinolate, myrosinase and isothiocyanate. They are sometimes grown as companion plants and suffer from few pests and diseases. Singh et al. (2006) for the first time observed the radish leaf curl disease at Varanasi of UP, India, in kitchen gardens with symptoms such as leaf distortion, typical leaf curling (downward and upward) enations and leaf size reduction with an incidence of 10–40%. Singh et al. (2012) used an electron microscope and found typical germinate particle in infected samples. Singh et al. (2012) investigated the interaction of radish-infecting begomoviruses and their associated satellites, with tomato leaf curl Gujrat virus and *tomato leaf curl New Delhi virus*, which showed a contrasting and differential interaction with DNA satellites, not only in the capacity to interact with these molecules but also in the modulation of symptom phenotype by the satellites.

6.12 Cucumber Yellow Vein Mosaic Virus Disease

Cucumber yellow vein mosaic virus disease (CYVMVD) is another devastating disease. Yellow vein mosaic virus disease symptoms include chlorotic mottling, yellowing, crumpling and leaf distortion (Suresh et al. 2013). Sequence analysis of PCR amplicons showed that the disease was found to be associated with five closely related begomovirus species. Three viruses identified in majority of the tested samples were *Tomato leaf curl New Delhi virus* (ToLCNDV), *Croton yellow vein mosaic virus* (CYVMV) and *Tomato leaf curl Karnataka virus* (ToLCKaV). Two identified begomoviruses showed highest identity (96–97%) in coat protein region with *Tomato severe leaf curl virus* (ToSLCV) and *Pepper golden mosaic virus* (PepGMV) (Suresh et al. 2013). Natural occurrence of yellow mosaic disease was observed on Armenian cucumber (*Cucumis melo* var. flexuosus) with a disease incidence of ~36. *Tomato leaf curl Palampur virus* was reported on *C. melo*, and *C. callous* was recently studied by Raj et al. (2015) from Lucknow. Earlier, *Tomato leaf curl Palampur virus* was reported by Tiwari et al. (2012) on *Cucurbita pepo* from Eastern Uttar Pradesh, India, with the symptoms of leaf curling and leaf yellowing.

6.13 Squash Leaf Curl Virus Disease

Squash leaf curl virus (SLCV) shows severely curled leaves with mottled areas, leaves with shorter petioles, sterility and, in some cultivars, even the distortion of fruit. SLCV mostly affects squash plants (*Cucurbita pepo* L). *Watermelon chlorotic stunt virus* and SLCV are both bipartite begomoviruses infecting several cucurbitaceous crops across the world. These were mainly found in Eastern Mediterranean

basin. Sufrin-Ringwald and Lapidot (2011) introduced the melon plants with whiteflies in greenhouse with SLCV and WmCSV or both.

Plants infected with SLCV showed mild symptoms which disappeared after a shorter period of time and did not affect the plant height. However, at the same time, plants infected with the WmCSV showed severe symptoms, and infected plants were dwarfed in comparison to control plant. In case of infection of both, plants showed 20–25% reduction in size of the plants, and yield was reduced up to 54% in the summer.

Virus isolate Y23V, obtained from squash showing leaf curl symptoms in China, was readily differentiated from four studied Chinese begomovirus isolates. The complete nucleotide sequence (2,714 nucleotides) of the DNA-A-like molecule of Y23V was determined. The DNA-A of Y23V is most closely related to that of *Tomato yellow leaf curl Thailand virus*-[1] (TYLCTHV-[1]) (84% sequence identity). However, the *AC1* and *AC4* genes of Y23V DNA-A resembled to *Pepper leaf curl virus* from Bangladesh (PepLCBDV). The DNA-A of Y23V had three distinct regions: the region from 74 to 2,071 nucleotides was 95% identical to TYLCTHV-[1] excluding a 27-nucleotide deletion; the following 386 nucleotides were 91% identical to PepLCBDV, and the rest of the DNA-A was not closely related to any reported begomovirus. Y23V, therefore, was considered to have arisen by recombination. The 84% sequence identity of Y23V with TYLCTHV-[1] allowed Y23V to be considered as a distinct begomovirus species, for which the name *Squash leaf curl Yunnan virus* (SLCYNV) was proposed (Xie and Zhou 2003). Cucurbit leaf curl virus was first identified from the United States and Mexico and is a distinct bipartite begomovirus species, and it has been reported to be transmitted in nature by whitefly (*Bemisia tabaci*). CuLCv is genetically very similar to *Cucurbit leaf crumple virus* identified from California. The virus has been found to be associated with several cucurbitaceous crops as well as bean and tobacco.

6.14 Bean Calico Mosaic Virus Disease

Bean calico mosaic virus, which was first reported from Sonora, Mexico, is a whitefly-transmitted geminivirus which follows Koch's postulates. The virus having bipartite genome and DNA-A of BCMoV is very close to *Squash leaf curl virus* E strain and *Cabbage leaf curl virus*. Plants belonging to Fabaceae, Malvaceae and Solanaceae are the hosts of BCMoV. Brown et al. (1999) reported that BCMoV isolated from bean in Americas are distinct begomovirus species on the basis of biological and molecular characteristics.

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Leaf Curl Disease of *Carica papaya*

7

Priyanka Varun and Sangeeta Saxena

Abstract

Papaya leaf curl disease is caused by *Papaya leaf curl virus* (PaLCuV), a begomovirus naturally transmitted through whitefly (*Bemisia tabaci*). Main symptoms of papaya leaf curl disease are inward/outward curling of plant leaves, vein thickening, and stunted plant growth with small distorted fruits or no fruits. *Papaya leaf curl virus* is a major threat for the crop production, and the virus has the capability to adapt new plant hosts very rapidly which helps in their host range extension that also has emerged as an evolving risk in papaya production. Whitefly management is the main method to control the spread of this virus so far. Several diagnostic techniques especially molecular techniques have been developed to detect the begomoviruses at early stages of infection to control the further spread of the begomovirus, but so far not much reports are available to control the begomoviral infection at later stage. This chapter provides the information about many aspects like causal pathogen, vector responsible for disease spread/transmission, host range and phylogenetic analysis of virus associated with the papaya leaf curl disease, and different resistance approaches for possible management of the disease.

7.1 Introduction

Papaya (*Carica papaya*) is a widely distributed agricultural crop in the tropical and subtropical regions and has been grown on a wide commercial scale throughout the globe. Papaya is very popular among kitchen gardeners, as packed delicious fruit

P. Varun • S. Saxena (✉)
Department of Biotechnology, School of Bioscience and Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow, UP, India
e-mail: priyanka.varun@yahoo.com; dr_sangeeta_saxena@yahoo.com

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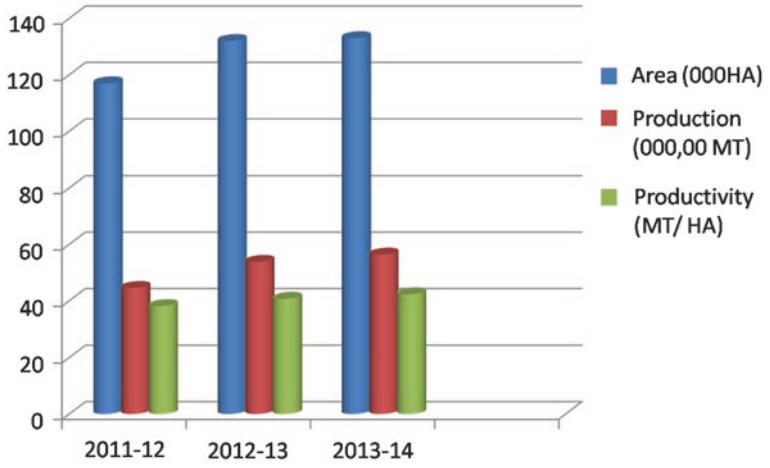

Fig. 7.1 Bar diagram showing area, production, and productivity during 2011–2014

contains numerous nutritive values. Consumption of papaya fruit has many health benefits related to diabetes, heart diseases, and obesity. It can be used as therapeutics in gastrointestinal disorders, intestinal infections, and neuralgia (nerve pain). Raw papaya fruits contain chemicals like papain and carpin that are useful as meat-tenderizing agent and to remove parasites, respectively. Papaya leaves are known for their medicinal benefits and are extensively used by industries involved in manufacturing of medicines. Papaya is originated from tropics of Americas and it was first cultivated in Mexico. Papaya has grown majorly in Asia, North Central America, South America, and Africa. India is the largest producer of papaya covering the highest cultivated area (133,360 ha) with 42.3 mt/ha productivity and shares 43.7% of the total papaya production globally (Fig. 7.1) (Indian horticulture database 2015). Brazil is the second largest producer of papaya followed by Indonesia, Dominican Republic, Nigeria, Mexico, and China-Taiwan province contributed 11.8%, 7%, 6.3%, 6%, 5.5%, and 1.7% of total papaya production, respectively. In India, significant increase has been observed in papaya production in the last few years. Ideal climatic conditions are the main reason for the growth of papaya crop. Andhra Pradesh is the highly papaya-producing state in India, followed by Gujarat, Maharashtra, Karnataka, Madhya Pradesh, West Bengal, etc. (Fig. 7.2) (Indian horticulture database 2015). In many tropical and subtropical countries, papaya cultivation largely suffers due to extreme climatic conditions like frost and heat along with several fungal, bacterial, and viral diseases (Prasad and Verma 1980; Paula et al. 2007; Rawell 2010; Lopez and Pantoja 2012). One of the severe diseases observed in papaya fields is papaya leaf curl causing major loss to its production. The disease being fatal to the plant is of concern as the papaya fields are completely wiped out and farmers are at economic loss while cultivating papaya. Though this disease affects papaya crop in many parts of the world like China, Nepal, Pakistan, Taiwan, Africa, and Korea, its occurrence in India is of great concern. In India papaya belt

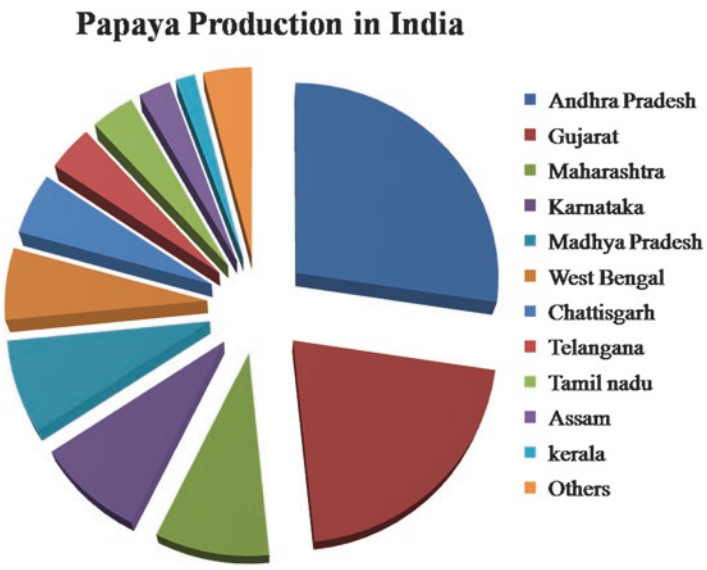

Fig. 7.2 Leading papaya-producing states in India

in the northern region is seriously affected by papaya leaf curl disease (Reddy et al. 2010; Dubey et al. 2015b) to the extent that farmers have stopped growing it on large commercial scale and the crop is limited to the kitchen garden only. Many countries including India are trying to do intensive management of the disease and improvement program by producing a number of improved hybrids. However, none of the breeding program has resulted in developing leaf curl-resistant variety in papaya (Mishra et al. 2007). Analysis of genetic diversity among papaya cultivars resulted in polymorphism among Indian papaya cultivars; still extensive study is required to select efficiently the progeny with viral resistant characters (Saxena et al. 2005, 2016). Papaya leaf curl disease is reported to be caused by *papaya leaf curl virus*, a DNA virus causing a major threat to papaya production (Thomas and Krishnaswamy 1939; Saxena et al. 1998a).Virus infections are the basis for many physiological and biochemical changes during multiplication inside the plants and are responsible for yield losses and reduced fruit quality and quantity of papaya crop. Therefore, cellular constituent's determination can provide the better understanding of damage level and host cell activities after viral infection (Tajul et al. 2011). Khalil et al. (2014) worked on *Tomato yellow leaf curl virus* (TYLCV)-infected plants to study the changes in cell ultrastructure and physiological and biochemical changes. In their study they have observed reduced carbohydrate contents, growth, photosynthetic pigments, and Mg^{++} ions while increase in sodium ion level as genus begomovirus a defensive response of plants in stress condition leading to various symptoms like curling which were obvious in nature. Curling of leaves may be due to many reasons like wind damage, herbicide damage, broad mites, and viruses (Masabni et al. 2011). *Papaya leaf curl virus* (PaLCuV) causing

leaf curl disease in papaya belongs to begomoviruses of Geminiviridae family, and currently authors are working on the genetic variability of PaLCuV isolates. Recombination and mutations in the genome are the reasons for the emergence of new variants of viruses; these new variants along with the use of susceptible varieties of plants and climatic changes are the main problems in begomovirus aggravation from the last two decades and need to be monitored urgently. This chapter summarizes the current status of PaLCuV causing leaf curl disease in papaya, transmission, variable host range, understanding of phylogenetic relationships of virus, and their management strategies.

7.2 Leaf Curl Disease of Papaya

Papaya leaf curl disease is caused by PaLCuV belonging to the family Geminiviridae. Leaf curl-like symptoms on host papaya plant are the basis for its name, i.e., papaya leaf curl in the same manner as we refer other diseases like yellowing, ring spot, crumpling, etc. Papaya plants infected with leaf curl disease show severe downward/upward curling, thickening of veins, rugosity of leaves, and overall stunted plant growth (Fig. 7.3). Diseased plants produce distorted fruits of small size with latex oozing out resulting in very poor quality of fruits with less sugar content. Some *papaya leaf curl viruses* are reported to induce symptoms like crumpling, crinkling, mottling, and vein thickening also in the literature (Dubey et al. 2015a). Severity of the disease is observed more in young plants in field as compared to grown-up plants or plants grown initially in nurseries under controlled environment conditions. This could be due to less exposure to vector as well as low virus

Fig. 7.3 Papaya plants showing typical leaf curl symptoms

inoculums in controlled condition during initial phase of growth. In severe cases petiole is twisted with complete defoliation of the leaves. This virus infects not only papaya but also many other crops and produce same symptoms. Papaya leaf curl disease was first time reported in India in 1939 (Thomas and Krishnaswamy 1939), and further Saxena et al. (1998a, b; Srivastava et al. 2010) confirmed the presence of virus in infected plants by using nucleic acid-based diagnostics and its sequence homology with other begomoviruses. Occurrence of papaya leaf curl disease has been reported in many countries. Nadeem et al. (1997) have reported the disease in Pakistan, and further during a questionnaire survey on *Carica papaya*, Taylor found the presence of papaya leaf curl disease in Africa in 2001. Reports of PaLCuV infecting papaya plants in Taiwan and China were given by Chang et al. (2003) and Wang et al. (2004), respectively. Recently, PaLCuV infecting papaya plants has been reported in Korea by Byun et al. (2016). Extensive survey of papaya fields in north India undertaken by the authors revealed the severity of virus attack with prominent leaf curl symptoms. The incidence of the disease was recorded to be as high as 100% in the fields surveyed (unpublished data). Similar high incidence reports of this virus in north Karnataka, Gujarat, and eastern Uttar Pradesh from India have been published (Sagar et al. 2012; Hemambara and Yogesh 2014; Dubey et al. 2015b). Such impact of leaf curl disease in papaya has led to overall reduced fruit production and marketing constraints of papaya growers all over the world.

7.3 Transmission of *Papaya leaf curl virus*

Transmission simply means dispersal or scattering. Viruses need host cell for their replication/multiplication, but viruses themselves cannot penetrate the cell wall and need a carrier to enter inside the plant cell. Method by which viruses spread from one plant to another is called transmission of virus. Whiteflies are reported as the most appropriate and the only vector to spread begomoviruses horizontally from one infected host plant to a new healthy plant. Whiteflies are known as potential pests, and eight species of whiteflies have been recorded as pest worldwide (Culik and Martins 2004). *Bemisia tabaci* (*Gennadius*) biotype B is a chief insect vector that acquires begomoviruses while feeding on phloem sap of plants and transmits on another healthy plant during next feeding (Vieira and Correa 2001). *Trialeurodes variabilis* whiteflies are similar to other whiteflies that feed on and cause damage to papaya plants. The presence of *T. variabilis* as a pest has been found in the Caribbean (Pantoja et al. 2002), Florida (Culik et al. 2003), and Brazil (Culik and Martins 2004). Lee et al. (2013) worked on the cryptic complex of *Bemisia tabaci* species to determine their taxonomic status based on cytochrome oxidase subunit I (COI) gene and concluded that *B. tabaci* is a complex of 31 putative species of different genera or subfamilies.

Whiteflies are the sap-sucking insects that feed on the dorsal side of plant leaves by inserting their stylet into phloem tissue of veins. The transmission process begins as soon as whiteflies insert their stylet into the infected plant leaves for feeding; as

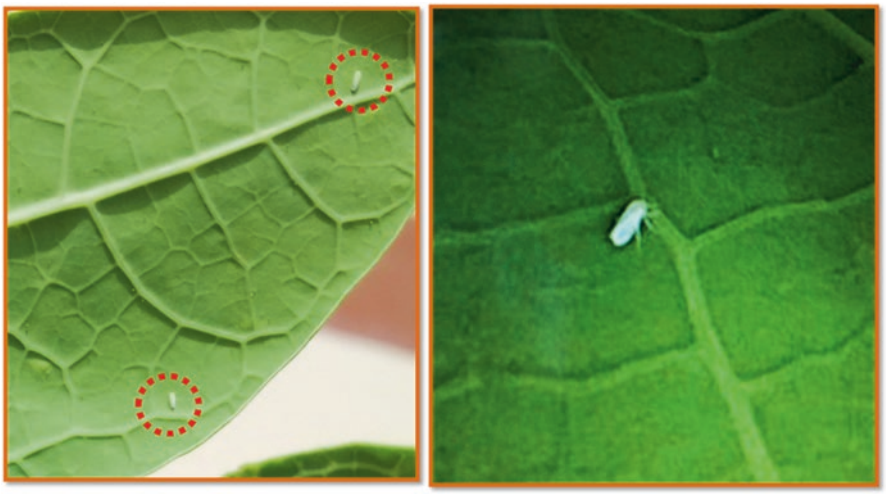

Fig. 7.4 Whitefly vector—an insect that transmits begomoviruses from infected plant to healthy

a consequence begomoviruses enter in the whitefly through their stylet and circulate in its body. This process of acquiring the virus through whitefly vector is called as acquisition. Further, begomoviruses travel in the whitefly system via the esophagus to gut lumen and are collected into filter chamber. After crossing the gut epithelia, they reach to the hemolymph and from there to the primary salivary gland. Finally, the whiteflies spit out the virus from salivary gland into plant phloem through salivary secretion during next feeding. This process of introducing virus particle into the plant is called as inoculation (Czosnek 2008).

Whitefly (*B. tabaci*) is the only transmitting vector that spreads *papaya leaf curl virus* in nature. It was found that *B. tabaci* could transmit the virus from infected plants showing typical leaf curl symptoms in both tobacco and papaya plants. An acquisition period of 24 h was required for transmission studies on a group of about 50 viruliferous whiteflies which were allowed a minimum 48 h of inoculation access period on healthy seedlings (Fig. 7.4) (Saxena et al. 1998b). During this experiment, it was observed that whitefly had a disliking to papaya sap and didn't feed continuously on papaya plants only. The survival rate of whitefly was always found to be more when alternate food like healthy clitoria was provided during the study. We assume that papaya is not a preferred choice of plant for the whitefly to feed upon though occasional visit of viruliferous whiteflies on papaya may result in inoculation of infective virus particle to phloem cells leading to transmission of the disease. Guo et al. (2015) worked on four cryptic species of whiteflies (Mediterranean (MED), Middle East-Asia Minor 1 (MEAM1), Asia 1, and Asia II 7) to compare their efficiency to acquire, retain, and transmit the *Papaya leaf curl china virus* and found MEAM1 as the most efficient vector for virus among all four species.

Since, in India, papaya is grown during summer season when the climate is warm, population of whiteflies is also persistent during these months, resulting in

infection of papaya plants with begomoviruses transmitted by whiteflies. Also, during these months and in rainy season, plenty of whiteflies are found on vegetable crops like tomato, brinjal, chilli, etc. which are generally grown in the adjacent fields during the same period. Thus, such persistent and broad occurrence of whiteflies throughout the year on various crops leads to massive damage of crops susceptible to begomoviruses in general and papaya in particular.

7.4 Host Range of *Papaya leaf curl virus*

An organism/parasite needs a living cell for its survival, and begomoviruses can utilize different plants for this purpose. Host range is the collection of different hosts on which viruses can maintain their existence. Host and viral proteins play an important role in specific inter- and intracellular trafficking of geminiviruses in crop plants (Gafni and Epel 2002). The rapid extension of host range is a big challenge for disease management of viral diseases. Dawson and Hilf (1992) have reviewed various hosts of plant viruses and their interactions with each other emphasizing the significance of host range determinants of plant viruses. Further, Gillette et al. (1998) studied the genetic determinants responsible for host specificity of bipartite begomoviruses and identified the role of a region of the DNA-A component in host adaptation. Increase in host range among begomoviruses in India was shown by Sahu et al. (2015) analyzing molecular diversity of monopartite begomoviruses and their associated satellites isolated from different crops. Their results showed that CP and betasatellite gene exhibit very close sequence similarity within begomoviruses infecting a range of various crops. *B. tabaci* was termed as super vectors which can transmit geminiviruses (begomoviruses), ipomoviruses, and torradoviruses by different mechanisms by Gilbertson et al. (2015). It was concluded that super vectors play a crucial role in evolution, mixed infections, host switch, and spread of viruses. Both whiteflies and the viruses transmitted by them are expanding their natural habitats and are emerging as new plant-virus complex due to highly adaptive capacity of *B. tabaci* (Brown 2001).

A literature survey on PaLCuV revealed that PaLCuV is also reported in other hosts: *Gossypium* sp. (Mansoor et al. 2003), *R. capitata* (Ilyas et al. 2010), *A. alpinus* (Srivastava et al. 2013), *A. cruentus* (Srivastava et al. 2015), tomato (Raj et al. 2008), aster (Srivastava et al. 2013), and tobacco (Kumar et al. 2009). Besides this, *papaya leaf curl virus* from India (accessions JN807765 and HM143914) isolated from alternate host *G. max* and *N. glutinosa* is available in GenBank database. One of the weeds *Ageratum conyzoides* was found to grow frequently in and around papaya plantation in India. Sequence homology revealed that the virus infecting ageratum was remarkably close to PaLCuV in coat protein sequences (80.5%) as well as in complete DNA-A (75.8%) to PaLCuV (Saxena et al. 1998c). The presence of *papaya leaf curl Guangdong virus* on passion fruit was reported in Taiwan (Cheng et al. 2014). In other studies *papaya leaf curl china virus* was reported on three hosts, namely, *Corchoropsis tomentosa* (Haung and Zhou 2006), *Sigesbeckia orientalis* (Yang et al. 2011), and tomato (Zhang et al. 2010) in China. As we have

mentioned earlier in the chapter that during transmission experiments, it was observed that whiteflies dislike the papaya sap and don't feed continuously on papaya alone when caged under control condition and need some alternate plants to survive. This may be the reason that *papaya leaf curl virus* is transmitted to other crops and weeds resulting in broad host range. Pramesh et al. (2013) provided molecular evidence for weeds as an alternative host for monopartite begomoviruses that cause leaf curl disease in tomato. Many crops including weeds are a good choice for viral vectors and viruses themselves as secondary host. This may be due to diverse climatic changes making it difficult for the host crop to survive or increase in vector populations throughout the year. This results in modified host range due to the crops and weeds which can grow in extreme climates along with availability of whiteflies. Weeds serve as an alternative host and play an important role to maintain the viral inoculums during the non-cropping season and spread of the virus in the next cropping season when climatic conditions are suitable and primary hosts are available (Khan et al. 2012, 2014). Weeds like *Triumfetta*, ageratum, croton, and *Malvastrum* are known to harbor geminiviruses during off-season (Hallan et al. 1998a, b). The above reports regarding such broad host range of begomoviruses especially in tropical and subtropical region suggest that new begomoviruses may evolve and chimeric begomoviruses along with satellite molecules may pose serious threat to crops susceptible to begomoviruses.

7.5 *Papaya leaf curl virus*

Papaya leaf curl virus is a whitefly-transmitted begomovirus and belongs to the family Geminiviridae. Though the disease was identified to be caused by a begomovirus long back (Saxena et al. 1998a), still a lot of information regarding its genomic composition and genetic variability among PaLCuV is required. As we know that begomoviruses are single-stranded DNA viruses with a bipartite or monopartite genome, bipartite begomoviruses contain two genomic components (DNA-A and DNA-B) of ~2.7 Kb each, and monopartite begomoviruses have a single genomic component similar to DNA-A component of bipartite (Briddon et al. 2008). Genomic component of these viruses encodes for coat protein, replicase, and five to seven multifunctional proteins that are involved in viral replication, movement, transmission, and pathogenesis. Some viral proteins have evolved to serve different functions not only different viruses but also between closely related species. Viral proteins, such as replication initiator protein (*Rep*), are highly conserved across the family Geminiviridae, whereas others, such as coat protein, confer unique properties to a given genus and are evolved in response to insect vectors determining vector specificity. Apart from the above DNA-A components, satellite molecules like alpha- and betasatellites are often reported to be associated with the disease, and their accessions are available in database.

In case of *Papaya leaf curl virus*, few studies have been done using infectious clones to know about its genome organization. Zhang et al. (2010) reproduced the disease using only DNA-A and concluded that DNA-B or any satellite molecule is

not associated with papaya leaf curl disease found in China. Similarly, from the Guangdong Province, China, the disease was introduced in tobacco and other host plants by inoculation of infectious clones of PaLCuGdV clone of DNA-A alone, confirming it to be a monopartite virus (Chen et al. 2016a). Interestingly in India and China many isolates of PaLCuV have been reported to have DNA-A along with associated satellites (GenBank accession on the basis of sequencing AY244706.1, EU126826.1, EU126825, KJ642219) that have been completely sequenced which throws some light on its genomic composition. Earlier, the PaLCuV from India was reported as bipartite on the basis of southern hybridization (Saxena et al. 1998a). On the basis of all these studies reporting different genomic components of PaLCuV, it is assumed that papaya leaf curl disease is diverse in nature, and studies on genetic diversity of PaLCuV isolates along with their genomic components and host range are required.

Papaya leaf curl viruses are reported from most of the part of the world, and the phylogenetic analysis revealed their association on the basis of their geographic distribution. Isolates from the same region are very closely related to each other but differ from isolates that belong to different geography.

7.6 Phylogenetic Analysis of DNA-A Component of PaLCuV

Evaluation of some PaLCuV isolates available in database to know about their relationship and distribution has already been done. Studies on PaLCuV complete genome sequences using MEGA 6.0 revealed their association on the basis of their geographic distribution. *Papaya leaf curl China virus* (PaLCuCNV) is reported as a different species in the genus begomovirus and forms a separate cluster in the tree of all the PaLCuCNV isolates. Neighbor-joining tree of *papaya leaf curl virus* isolates using MEGA 6.0 (Tamura et al. 2015) depicted clustering based on geographical locations of the isolates (Fig. 7.5). Isolates of Korea and Taiwan clustered together, whereas *papaya leaf curl China virus* isolates and *papaya leaf crumple virus* isolates make different clusters. Isolates of PaLCuV from India and Pakistan grouped together in a separate cluster which further divided into subgroups of different locations as isolates of Gujarat, Lucknow, and Pratapgarh. Interestingly isolate of Haryana shows the association with the Lucknow isolates, whereas isolates of Pakistan show close relationship with the papaya leaf curl isolates from Pratapgarh.

7.7 Phylogenetic Analysis of CP

Similarly, evolutionary studies of coat protein amino acids of Indian papaya leaf curl isolates available in GenBank database provide the same results as of whole genome sequences and produce two main clusters on the basis of their geography. Tree obtained through MEGA 6.0 (Tamura et al. 2013) gives an idea about their phylogenetic relationship (Fig. 7.6). Three different clusters of geographical

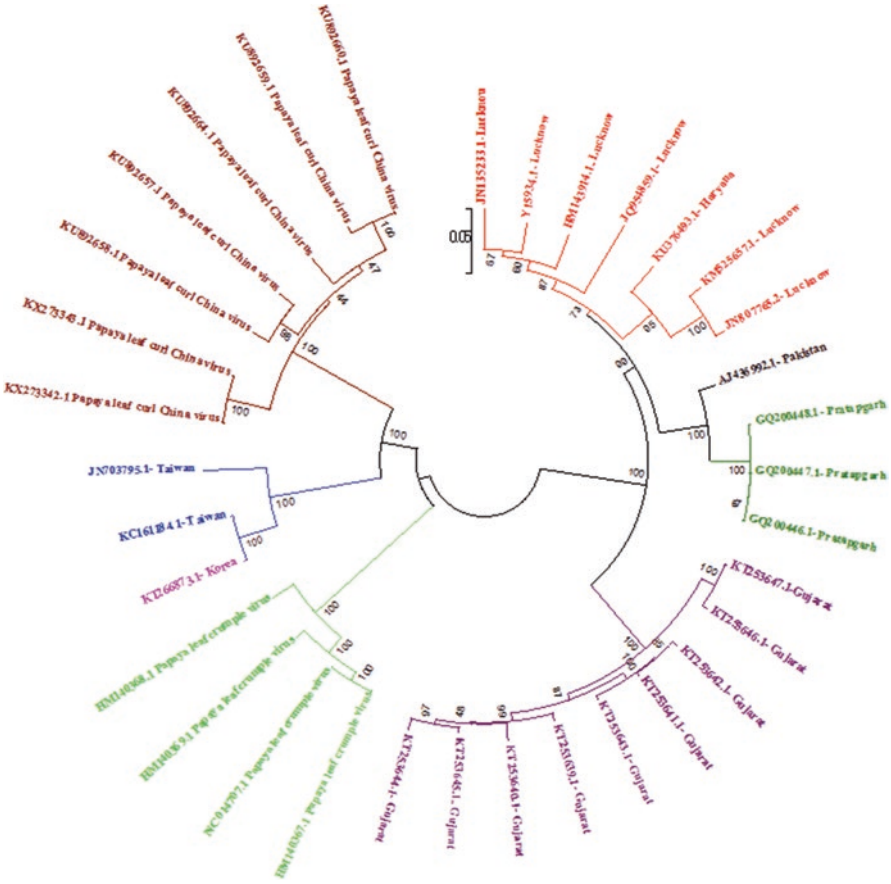

Fig. 7.5 Phylogenetic tree of complete DNA-A of *papaya leaf curl virus* isolates available in database

locations, i.e., Lucknow, Gujarat, and Pratapgarh, were observed. Isolates of Lucknow clustered together, and interestingly one isolate of Haryana also shows close relationship with the Lucknow isolates. Isolates of Pratapgarh region were subclustered together, while PaLCuV isolates of Gujarat were distantly apart and form the separate cluster.

7.8 Management of Disease

Papaya leaf curl disease mostly affects the young papaya plants at seedling stage and its apical meristematic region. Though there are many management practices followed all over the world to control viruses and vectors to save the host plants, however, if these practices fail, removal, burning, and destruction of the infected plant are the only control measure followed by farmers to reduce the initial

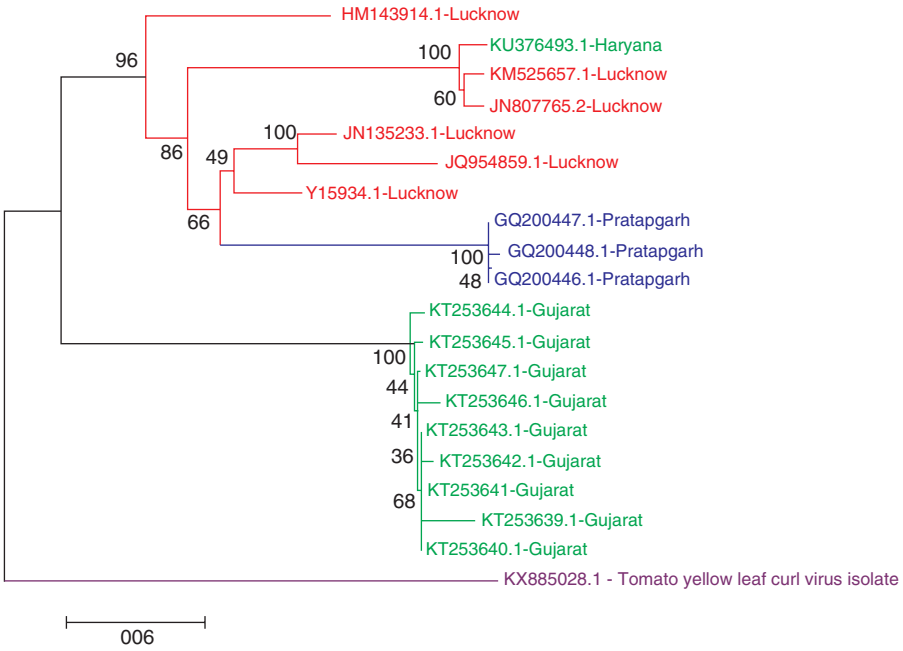

Fig. 7.6 Phylogenetic tree of coat protein amino acids of different *papaya leaf curl virus* isolates from India

inoculums and spread of disease. Considering this advising farmer to buy seedlings from nurseries or tissue culture raise healthy plants which are certified virus-free and have attained a certain height is a good idea to prevent the disease at the first place. Also to prevent papaya plantation from heavy infestation by whiteflies, the use of certain innovative idea like yellow traps (Fig. 7.7) can reduce the whitefly population. Here we emphasize that prevention of young plants/saplings/seedlings from viral infection and controlling the vector whiteflies are anytime better than cure as no certain measures to control the disease are available till date. Simultaneously freeing the papaya fields from secondary hosts and weeds along with a suitable barrier crop thus hindering the virus vector host nexus can lead to sustainable management of this disease which we have discussed in detail below.

Papaya crop is economically very important because of having many nutritional and medicinal values. Different climatic conditions offered the opportunities for many pathogens to cause infections in the crop plants. Many diseases including fungal diseases, leaf spot, mosaic, leaf blight, foot rot, ring spot, and leaf curl are affecting the sale and export of papaya fruits due to huge crop losses. Among all the papaya diseases, viral diseases are the main constraints in papaya cultivation and need to be managed effectively. Effective management of diseases requires better understanding of pathogens, host pant, vectors, and their interactions with the knowledge of environmental conditions. So, there is an urgent need of accurate

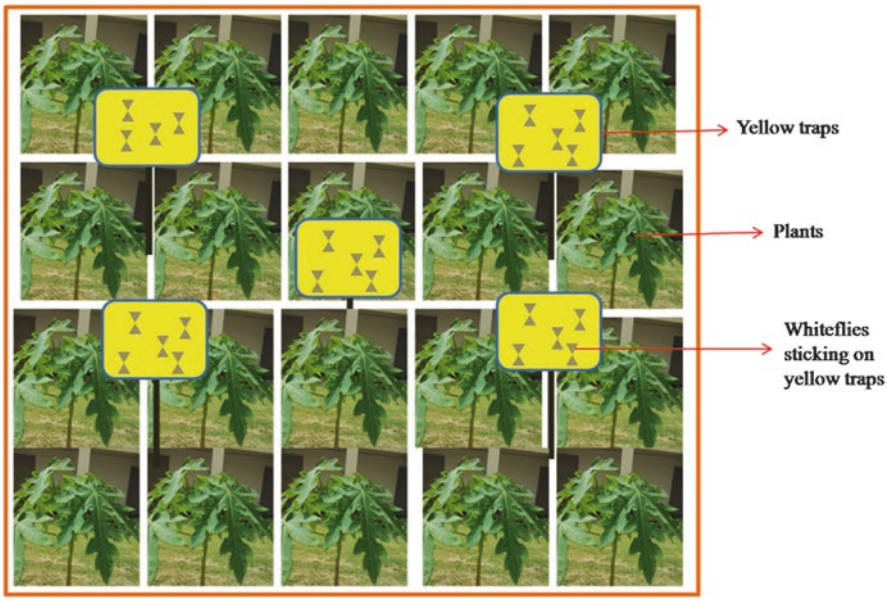

Fig. 7.7 A schematic representation of papaya field with sticky yellow traps to catch the vector whiteflies

diagnosis and understanding of disease cycle and vectors responsible for disease to develop an effective management strategy. Different curative measures and use of resistant cultivars are the commonly used practices for viral disease management. There may be two approaches to deal with virus diseases; the first approach is by minimizing the viral infection sources and another is decreasing the rate of virus spread. Many methods like integrated pest management, vector control, variation of cultural practices of crops, cross protection, use of virus-free propagules, phytosanitation, breeding for disease resistance, pathogen-derived resistance, transgenic management, and RNA interference have been used for management of begomoviral diseases in general.

7.9 Vector Control

Whitefly *B. tabaci* is the known vector for the leaf curl disease of papaya. Early detection of viral vectors, mode of transmission of virus, and viral host range including weed and non-weed plants provide opportunities to manage plant viruses and vector populations. Management of whiteflies and viruses they transmit is a major challenge for agriculture. Destruction and removal of crop residues and weeds, combination of cultural practices, and insecticidal applications can be effective practice to control whitefly populations. The use of insecticides cannot be sufficient for management of whiteflies and begomoviruses because application of insecticides and use of insect-resistant plants will become the cause for development of

resistant whiteflies (Gilbertson et al. 2011). Integrated pest management (IPM) approach including traditional methods like roughing of infected plants and weeds, effective postharvest sanitation, use of insecticides, and implementation of host-free period or regional crops are considered as an effective whitefly management strategy (Flint 2015). Due to low toxicity and better repellent property, oils are generally used to control whitefly populations (Butler and Henneberry 1991), but results are not very promising. When Sieburth et al. (1998) evaluated the combined effects of oil and oil surfactant on whitefly nymph on collards (*Brassica oleracea* L.), they found it 94–99% effective. Legaspi and Simmons (2012) studied the effect of selected commercial oils like garlic oil (11%), horticultural petroleum oil (1%), mustard oil (3%), and hot pepper wax oil (3%) to repel the whiteflies which is termed as "push" and then to trap them as "pull" strategy for whitefly management in vegetable and horticultural crops. Studies reveal that mustard oil is most effective when used as a repellent in the push-pull management plan. UV light is also reported to play a crucial role on the effectiveness of whiteflies as a vector of begomoviruses as it affects the normal behavior resulting in reduced dispersal activity and insect flight orientation. So, the use of UV-absorbing greenhouse sheets can be employed as an effective management strategy against the spread of begomoviruses through whiteflies (Antignus et al. 2001). Apart from abovementioned oil treatment, physical control of whiteflies, and use of UV-absorbing greenhouse, biocontrol of whiteflies can also be done. Ateyyat et al. (2009) found some culturable bacteria like *Erwinia persicinus*, *Bacillus pumilus*, and *Exiguobacterium acetylicum* associated with whiteflies and listed them as a potential biocontrol agent by reducing second nymphal instar populations of *Bemisia tabaci*.

As checking the population of whiteflies reduces the infection severity, frequent use of chemicals and insecticides has been done in recent years. Insecticides like carbofuran, dimethoate, metasystox, and nuvacron have been found to be very effective to control whitefly population (http://agropedia.iitk.ac.in). Such insecticides can be applied directly to the soil while sowing the seed followed by several foliar sprays at regular intervals on leaves and canopy till the plant attains good size, whitefly population is reduced, and there is little or no inoculums of virus to start with. Research is also going on the role of volatile compounds against many viruses including whitefly-transmitted begomoviruses. Volatile metabolites are a class of chemical compounds known to be produced primarily by plant leaves in response to insect feeding and damage and induce defense response. Further these chemicals also communicate with surrounding plants inducing the production of volatile metabolites resulting in activation of pathways involved in defense response against insect feeding. Interestingly these volatile metabolites are majorly produced only when plant is a host to certain insects which feed and damage the plant tissue and not by any other kinds of physical damage. Bleeker et al. (2009) worked on specific tomato volatiles and found that certain volatile mono- and sesquiterpenes emitted by tomatoes repel the whiteflies, thus making the crop less preferred to feed upon. Consequently it assumed that if the plant is not accessible to whiteflies due to its repellent nature, it will remain free from whitefly-borne viruses, i.e., begomoviruses.

7.10 Molecular Approaches for Whitefly Management

Though insecticide sprays are used worldwide as pest management practices of *B. tabaci*, still it has its own drawback. Pesticides are not only hazardous in nature but also a reason for increase in pesticide-resistant whitefly. Improved agricultural practices and breeding for virus resistance trait are the main virus control strategies. Several transgenic approaches that directly target begomoviruses either via RNA interference (RNAi) or with genome editing system such as clustered regularly interspaced short palindromic repeats (CRISPRs)/CRISPR-associated 9 (Cas9) have been widely used along with artificial zinc finger (AZF) and transcription activator-like effectors (TALEs). However, till now, no transgenic lines have been released for cultivation with respect to above strategies. Studies on begomovirus interactions with their host cellular machinery reveal the evolving nature of begomoviruses and pave the way for direct targeting of begomoviruses through RNAi and genome engineering. Mixed infections and nature of host expansion are the reasons for failure of field trial practices of resistant crops. Recently many researchers worked on RNAi strategy to control whitefly by silencing the actin ortholog (v-ATPases) (Thakur et al. 2014) and osmoregulators (Raza et al. 2016), whereas Javaid et al. (2016) worked on broad-spectrum resistance against multiple sap-sucking insects by expressing lectin (a plant defense protein) and a toxin (Hvt) from a spider's venom. Consequently, Shukla et al. (2016) from India provided the most promising strategy to develop transgenic cotton by expressing an insecticidal protein from fern that confer resistance to both *B. tabaci* vector and begomovirus causing leaf curl disease in cotton. Whitefly endosymbionts found inside them are able to interact with virus particles and protect them from degradation during viral movement inside the vector. This being an interesting phenomenon was used against the diseases vectored through whitefly-transmitted geminiviruses (WTGs) by disturbing the virus-GroEL interaction. This particular property was considered as an effective approach to develop transgenic tomatoes for multiple virus resistance by expressing whitefly GroEL in the plant phloem (Edelbaum et al. 2008). Degradation of virus particles inside the whitefly may be due to immune response induced after viral infection in the whitefly. There is not much information available regarding the mechanism of whitefly immune response against begomoviruses (Luan et al. 2011). Autophagy is a physiological (self-degradation) process that plays an important role in cell survival, development, differentiation, and homeostasis that protects plants/mammals against pathogen infection. The role of autophagy in insect vectors and plant-virus interaction is not well known. Wang et al. (2016) studied the role of autophagy in whitefly against circulative-transmitted plant virus (TYLCV) infection and found that viral infection could activate autophagy pathway in *B. tabaci* (MEAM1) that can be used as an intrinsic antiviral program for viral resistance.

Begomovirus being the largest genera of family Geminiviridae comprises of many viruses infecting commercially important crops. They are considered as most destructive pathogens of many crop plants mainly in tropics and subtropical regions and lately being reported to be found in temperate regions as well which may be due to global warming. Some begomoviral diseases have been considered as emerging

infectious diseases (EIDs) of plants affecting the economy by causing huge crop losses to growers. Climate changes, host pathogen evolutions, and pathogen pollution are the main factors for emerging infectious diseases (EIDs) in plants (Anderson et al. 2004). Evolutionary histories of the viruses, vector population, and associated satellite molecules are the major issues in the emergence of more virulent strains and adaption to the new host of viruses due to recombination among viral genomes. Human activities like the use of susceptible varieties of crops, transfer of infected planting material, and alteration in cropping systems are also the major reasons in the spread of viral diseases across the world. Genetic flexibility and wide host range of the vector and begomoviruses are the major threat to crop production (Inoue-Nagata et al. 2016). Weeds serve as alternate hosts for both virus and vector. Biological relationship between weed, virus, and vector provides a clear understanding of the mode of virus spread and helps to develop strategies against weed hosts and vectors to minimize the occurrence of virus diseases. Many management approaches have been used to minimize the losses to the crops (Raj et al. 2010; Khan et al. 2013; Tiwari and Rao 2014; Saeed and Samad 2016; Mishra et al. 2016). Some begomoviral management approaches that can be employed for PaLCuV resistance are given below.

7.11 Pathogen-Derived Resistance

Pathogen-derived resistance (PDR) is a term used when a part/gene of the viral genome is introduced into the host plant, thus making it a transgenic plant expressing viral proteins corresponding to the introduced segment. It has been seen that such expressed proteins may interfere with the normal pathway virus follows after entering the cell and thus inhibits its multiplication inside the cell. Such transgenic plants show tolerance/resistance against the respective virus.

Expression of coat protein (CP) of TMV in transgenic tobacco was the first successful PDR (Abel et al. 1986). Although the majority of PDRs are based on viral coat protein, other viral genes like movement proteins and replicases have also been used to engineer resistance against viruses (Morroni et al. 2008; Galvez et al. 2014). Interactions of Rep protein and plant factors with replication initiation (REn) protein can be targeted to develop resistance against geminiviruses (Settlage et al. 2005). CP-mediated resistance is the most popular strategy reported against many viruses (Dasgupta et al. 2003; Yeam 2016). Coat protein is responsible for systemic infection by monopartite begomoviruses (Rojas et al. 2001), but altered level of CP expression in plants may result in the delayed symptoms of disease (Kunik et al. 1994). CP-mediated resistance is not much effective in bipartite begomoviruses because systemic spread of bipartite geminiviruses is not only due to CP (Azzam et al. 1994), but several other movement proteins are responsible for the same.

7.12 Breeding Resistance

Breeding resistance is the best way to reduce begomovirus damage either by conventional breeding or genetic engineering though it is not successful in case of papaya crop as most of the crosses are sterile due to incompatibility between *C. papaya* and resistant species (Mishra et al. 2007). Morales (2001) has discussed some of the conventional intra- and interspecific hybridization strategies in three major crops: cassava, common bean, and tomato to develop genetic resistance against whitefly-transmitted begomoviruses. Further, Lapidot and Friedman (2002) have discussed the breeding approaches for four crops (tomato, cassava, bean, and cotton) against viral diseases. Molecular breeding strategies provide the better opportunities for engineered disease resistances due to availability of advanced molecular marker systems. Marker-assisted selection (MAS) combining multiple resistance genes using various DNA-based molecular markers has been reported to generate disease resistance (Foolad and Sharma 2005; Miedaner and Korzun 2012). NSP interacts with plasma membrane receptor-like kinases (NSP-activating kinases (NsAKs) and NSP-interacting kinases (NIKs). NIKs are serine/threonine kinases which act as a component of antiviral plant immune system (Fontes et al. 2004). NIK receptor has also been used to make transgenic plants resistant to begomoviruses by constitutive overexpression, and this antiviral signaling was found to be effective for broad-spectrum tolerance in cultivated plants (Brustolini et al. 2015). Recently, Hanson et al. 2016 have used conventional methods, molecular-assisted selection (MAS), and gene pyramiding to develop transgenic tomato lines against many diseases including tomato leaf curl disease under a program initiated by AVRDC—the World Vegetable Center.

7.13 Gene Silencing

The most prevalent defense strategy against begomoviruses in plants is antiviral RNA silencing. R gene-mediated resistance and host factor-related recessive resistance are the other forms of resistance (Maule et al. 2007). RNA silencing is also known as RNA interference (RNAi) or posttranscriptional gene silencing (PTGS) in which double-stranded (ds) RNA generated either by secondary intramolecular RNA folding (hairpin) structures or by replication intermediates (Ratcliff et al. 1997; Marathe et al. 2000) that triggered the response of infection (Grishok et al. 2001) and regulate the gene expression during defense against biotic and abiotic stresses in plants (Carrington and Ambros 2003). RNAi technology is a sequence-based gene silencing tool which can be exploited in plant functional genomics as well as have a role in expression of transgenes (Travella et al. 2006). This technology provides a great potential in crop improvement by control of sap-sucking pest (whitefly), seedless fruit development, abiotic/biotic stress tolerance, prolonged of shelf life, etc. (Jagtap et al. 2011). Cells that respond to virus infection can also generate small RNAs that target the mRNA and provide protection against viruses (Voinnet and Baulcombe 1997). Napoli et al. (1990) reported PTGS for the first

time by co-suppression of chalcone synthetase gene in transgenic petunia plants where they observe complete absence of pink color instead of dense color in flowers. After that Lindbo et al. (1993) proposed PTGS as an effective mechanism for virus resistance in plants. One of the landmarks was transgenic papaya resistant against papaya ring spot virus (PRSV) developed by Gonsalves et al. (2007) in Hawaii. "Rainbow" and "SunUP" were the first commercial PRSV-resistant varieties developed by genetic engineering technology. These transgenic papayas resistant to PRSV and having best horticultural characteristics of host variety were boon to the papaya growers in Puna, Hawaii. In fact papaya is one of the first deregulated transgenic fruits with respect to the above.

Antisense technology is a gene silencing method in which antisense RNA (complementary to target mRNA) hybridizes with mRNA and regulates the gene expression of the corresponding gene (Erckson and Izant 1992). As this technique is effective at transcriptional level and targets multiple gene copy number, it can be ideally used against viral infections (Day et al. 1991; Bendahmane et al. 1997). Broad-spectrum resistance against begomoviruses is becoming very difficult through RNAi strategy whether siRNA-expressing transgenic lines of common bean have been found effective against bean golden mosaic virus (BGMV) to develop resistance (Aragao and Faria 2009). Strategies based on siRNAs were reported to give generic resistance against geminiviruses infecting tomato and papaya crops (Saxena et al. 2011). Recently, Khatoon et al. (2016) designed an intron hairpin (ihp) construct expressing the dsRNA homologue of intergenic region of *Cotton leaf curl Rajasthan virus* (CLCuRV) and demonstrated the effective RNAi strategy to control CLCuRV infection in cotton. Saxena et al. (2013) also designed a putative siRNA against suppressors of RNAi to develop geminivirus-resistant papaya crops. As virus encoded suppressors do not directly target miRNAs so the use of amiRs is more useful and the specificity of amiRs for their targets present it as a more stable resistance against viruses. Artificial microRNAs (amiRs) can be exploited as a new technique to target viral RNA for its degradation and develop resistance against viruses in plants. Multiple virus infections in a particular crop plant are very common nowadays, and it can affect the antiviral efficacy of transgenic crop plants in fields. To overcome this problem, multimeric amiRNA strategy has been used as an effective approach to develop resistance against mixed viral infections. In this technology expression of other viral suppressors can be used by generating a dimeric amiRNA precursor that produces two amiRNAs, thus providing resistance to two different viruses (Niu et al. 2006). Similarly in Taiwan, Chen et al. (2016b) made bi-viral RNAi constructs to develop resistance against leaf curl disease in tomato caused by mixed infection of bipartite *tomato yellow leaf curl Thailand virus* (TYLCTHV) and the monopartite *tomato leaf curl Taiwan virus* (ToLCTWV) and found it effective in reducing symptoms and viral DNA accumulation of these viruses. Although RNAi is a very effective approach against begomoviral diseases, results of field trials with certain time limit are yet to come and are necessary to evaluate the potential of this technology in nature. Emergence of new species of begomoviruses through rapid recombination during evolution is the main cause for the failure of RNA silencing against begomoviruses.

7.14 Ribozyme

Ribozymes are naturally occurring RNA molecules capable of site-specific cleavage of target mRNA catalytically but require access to bind their target sites. A ribozyme folding into an appropriate (active) confirmation is required to precede the catalysis (Lilley 2003). Hammerhead and hairpin are the main subtypes of ribozymes (Schubert and Kurreck 2004) that differ in their catalytic responses. Ribozymes have been investigated for some diseases in humans like, cancer therapy (Zinnen et al. 2002; Weng et al. 2005), anti-HIV ribozymes (Macpherson et al. 2005), and hepatitis C virus HEPTAZYME (Usman and Blatt 2000). Mishra et al. (2014) revealed the activity of a hammerhead ribozyme as an antiviral agent in plants by targeting rep-mRNA of mungbean yellow mosaic India virus (MYMIV). This opens a new era of ribozyme technology against plant begomoviral diseases.

7.15 Aptamers

Aptamers are multimeric structures attached to the small molecular targets and inhibit their functions and confer resistance. Peptide aptamers confer strong resistance against four different tospovirus species by interacting with their nucleocapsid proteins while expressing in transgenic *N. benthamiana* (Rudolph et al. 2003). The Rep-binding peptide aptamer emerges as an efficient strategy to develop resistant plants against many begomoviruses. Sunitha et al. (2011) have screened the expression of single-stranded DNA binding protein (virE2) from agrobacterium against mungbean yellow mosaic virus (MYMV) and found it effective in reducing viral DNA accumulation but not for resistance. Further, Reyes et al. (2013) have developed transgenic tomato lines with enhanced tolerance to tomato yellow leaf curl virus/tomato mottle virus by expressing peptide aptamers that bind and inhibit the replication protein (Rep) of begomovirus. So, peptide aptamer approach can be used to develop transgenic plants with better resistance against viral diseases.

7.16 CRISPR/Cas9

After failure of many management strategies against begomoviruses, some better techniques have been explored to control begomoviral diseases. CRISPR/Cas9 technology has emerged as a versatile, multifunctional genome editing technology which is successfully exploited against tomato yellow leaf curl virus (TYLCV) (Ali et al. 2015), bean yellow dwarf virus (BeYDV) (Baltes et al. 2015), beet severe curly top virus (BSCTV) (Ji et al. 2015), and cotton leaf curl virus (CLCuV) (Iqbal et al. 2016). This system provides broad-spectrum resistance not only to begomoviruses but also to the associated satellite molecules. Whole plant genome has been sequenced in case of papaya, so it will be very helpful to develop leaf curl resistance in papaya plants using this technology.

7.17 Summary

Papaya leaf curl disease is a major limiting factor for papaya production all over the tropical and subtropical countries. Being whitefly-transmitted viruses, they are more prevalent where whitefly population find conducive weather and climatic conditions to habitat. Rapid mutations and recombination in viral genome make them more suitable to adapt in new place and host resulting in their wide host range. Whiteflies also play the major role in viral distribution from one geographical location to another and host range expansion. Papaya leaf curl viruses may be monopartite or bipartite begomoviruses, and satellite molecules like alphasatellites and betasatellites are found to be associated with the virus. Phylogenetic studies on whole genome (DNA-A component) and coat protein of papaya leaf curl virus isolates available in database revealed their close relationship based on the geography from where they have been isolated. This indicates that viruses from the same geographical conditions having more or less similar weather, climate, whitefly vector biotypes, and secondary hosts are found to be genetically close to each other, as these factors can be a reason to introduce genetic diversity considering the fact that several domains in coat protein and replicase genes are vector and host specific. Many management approaches have been described in the chapter that can be used for viral control, and one strategy based on RNA interference using siRNA has been proposed for PaLCuV infections. Emergences of new viral strains are the major problem in their management, and genetically engineered resistance approaches are very encouraging against begomoviruses. The new genome editing technology using CRISPR/Cas9 opens a new era of viral management to develop broad-spectrum resistance either by using the viral genome or plant genome, and as whole genome of papaya plant is sequenced, this technology can be used for effective PaLCuV management.

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Strategy for Generic Resistance Against Begomoviruses Through RNAi

8

Nikita Shukla, Saurabh Verma, G Sunil Babu, and Sangeeta Saxena

Abstract

RNA interference (RNAi) is a natural gene regulatory mechanism that limits gene expression either by suppressing transcription (transcriptional gene silencing) or by promoting the sequence-specific mRNA degradation (posttranscriptional gene silencing). RNAi utilizes dsRNA molecule along with a group of proteins consisting of Argonaute (AGO), Dicer, and few RISC-associated proteins for generation of small noncoding RNAs (ncRNAs), i.e., microRNA (miRNA) and small interfering RNA (siRNA) of 21–23 nt in length which actually bind with target mRNA and regulate their gene expression. However, there is a slight difference in their mechanism of action; for instance, miRNA partially binds to target mRNA and mainly results in translational suppression, while siRNA shows complete complementarity to putative mRNA and cleaves it resulting in gene silencing. With growing evidence every day, one of the important functions of RNAi in molecular biology seems to be protection of host genome against viruses. In case of plant viruses, begomoviruses impose a serious threat to mankind as they infect several crops like tomato, cotton, papaya, etc. leading to huge economic losses. Though several physical, chemical, and transgenic strategies are in practice to provide resistance against begomoviruses, none of them have proved out to be successful. Here we propose a strategy to develop generic resistance against begomoviruses by generating small siRNAs using various in silico strategies.

Nikita Shukla and Saurabh Verma contributed equally to this work.

N. Shukla • S. Verma • G.S. Babu • S. Saxena (✉)
Department of Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow, UP, India
e-mail: dr_sangeeta_saxena@yahoo.com

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8.1 Introduction

Genus *Begomovirus* belonging to family *Geminiviridae* subgroup III (bean golden mosaic virus group) is the largest and most devastating group of plant viruses. Begomoviruses commonly found in tropical, subtropical, and warm climates, infecting mainly dicot crops, are transmitted by *Bemisia tabaci* (*Gennadius*) also known as whiteflies. These whiteflies suck the plant sap and secrete a sugary, sticky liquid that attracts several fungi and other pests. These pests in turn infest fields of crops and cause huge losses to farmers. Once these viruliferous whiteflies, i.e., carrying begomovirus, settle on leaves, they ingest phloem sap of plants and transmit virus resulting in curling, distortion, and yellowing of leaves with reduced overall plant growth.

Non-enveloped virions are 38 nm long and 22 nm in diameter, while the enveloped forms have geminate twinned appearance with icosahedral symmetry. The capsid contains 22 pentameric capsomeres, which are made up of 110 capsid proteins (CP) with single circular ssDNA in each geminate particle. The begomovirus genome is present in either a monopartite or a bipartite form. Monopartite begomoviruses have only DNA-A as a genomic component, whereas bipartite begomoviruses contain DNA-A and DNA-B, and both the DNAs are equally required for complete systemic infection. Both the components A and B are approximately 2.6 Kb in size, whereas the satellites may have genomic component ranging from 700 bp to 1.5 kb. The coding regions or open reading frames (ORFs) are present over ssDNA genome in both the virion (+) and complementary (−) sense strands. DNA-A comprises six ORFs in sense and complementary orientation. DNA-A virion (+) orientation comprises two ORFs, i.e., AV1 and AV2, which encode coat protein (CP) and precoat protein, respectively (Padidam et al. 1996), while complementary (−) sense has four ORFs, namely, AC1, AC2, AC3, and AC4. AC1 encodes very important replication-associated protein required for viral DNA replication (Hanley-Bowdoin et al. 1999), AC2 and AC3 encode transcription activator protein (TrAP) and replication enhancer protein (REn), respectively (Sunter et al. 1990; Sunter and Bisaro 1992), and AC4 is mainly reported to counteract against post-transcriptional gene silencing (Vanitharani et al. 2003; Hanley-Bowdoin et al. 2013). DNA-B component contains two proteins, namely, BC1 in sense (+) strand which encodes movement protein (MP) and BV1 in sense (−) strand encoding nuclear shuttle protein (NSP). Both the DNA components contain a common region, acting as origin of replication, and help in bidirectional transcription. On the other end, few monopartite begomoviruses such as *Tomato yellow leaf curl virus* (TYLCV), *Tomato leaf curl virus* (ToLCV), *Tomato yellow leaf curl Sardinia virus* (TYLCSV), and *Ageratum yellow vein virus* (AYVV) comprise single genomic component equivalent to DNA-A that encodes on an average six proteins, Rep, TrAP, CP, REn, C4, and V2 (Begomovirus 2017).

Monopartite begomoviruses are often found to be present along with satellite (incomplete defective genomic components) components. In this case the DNA-A component acts as a helper DNA and provides machinery to the satellite molecules for replication and transcription of its ORF. Three types of satellite DNAs

associated with begomoviruses are alphasatellite (α), betasatellite (β), and deltasatellite (δ). α- and β-satellites have circular ssDNA with single ORF that encodes *alpha-Rep* and *βC1*, respectively. Both coding regions are highly conserved and contain adenine-rich sequence regions: a hairpin containing the conserved nonanucleotide region (TAATATTAC). These α- and β-satellites coinfect with helper DNA and cause disease symptoms and devastating diseases (Briddon et al. 2003; Zhou 2013). The α-satellites have no obvious function in symptom development but to help in β-satellite replication. However, βC1 is a symptom determinant, i.e., it can suppress both transcriptional and posttranscriptional gene silencing and thus antagonize plant defense mechanisms (Cui et al. 2004, 2005). The begomoviruses and their associated satellites cause economically significant diseases in a wide range of crop plants worldwide. The δ-satellite molecules share similarity to β-satellites except that they have one more hairpin loop-containing region and are mostly noncoding in nature (Lozano et al. 2016).

Once present inside their host tissue, these viruses impose an imminent threat and are very difficult to control even through various approaches such as physical, mechanical, chemical, or even biological. Management of virus infection in commercial crops greatly affects total agricultural yield, farmers, horticulturist, etc. The control measures are not so easy as virus-infected diseases are not amenable to direct methods of intervention. Symptoms of virus-infected crops can occur right from early to late stages of plant development depending upon virus inoculum load and vector population. The first step in control of begomovirus is to stop their transmission through whiteflies by using mechanical barriers and biological predators of *Bemisia* sp. The chemical approaches though sound very attractive, but they result in heavy metal pollution in environment due to slow degradation rate of most insecticides and ever-increasing resistance against them. Considering these above facts, various transgenic approaches using pathogen-derived resistance, antisense RNA and siRNA, and other genomic intervention strategies have emerged as a necessary tool to develop begomovirus-resistant crops for sustainable crop production.

8.2 RNA Interference (RNAi)

Discovery of small (20–30 nt) noncoding RNA molecule is one of the most important scientific breakthroughs in the past 20 years and has contributed to a significant advancement in research areas under molecular biology. Andrew Z. Fire and Craig C. Mello shared the Nobel Prize in Physiology or Medicine in 2006 for their work on RNA interference (RNAi) (Napoli et al. 1990; Cogoni et al. 1996; Fire et al. 1998). RNAi is a novel gene regulatory mechanism in which small RNAs (sRNAs) suppress transcription via degradation of target mRNA in a sequence-specific manner earlier known as co-suppression. The suppression which occurs at transcriptional level is known as posttranscriptional gene silencing (PTGS), whereas at chromatin level, it is known as transcriptional gene silencing (TGS). The recent information on translational repression of target mRNAs suggests that even partial complementarity of miRNAs leads to translational suppression of mRNA transcript

in mammals (Meister and Tuschl 2004). PTGS involves production of 19–30 nt long sRNAs, produced from their lengthy precursor dsRNAs. These dsRNA precursors interact with silencing machinery consisting of Argonaute (AGO), Dicer, and other associated proteins of RNA-induced silencing complex (RISC). This interaction with RISC results into formation of 21–23 nt long sRNAs, and one of the strand known as guide RNA gets loaded onto RISC complex. This guides RNA-RISC complex and then binds to their target mRNA site in a homology-dependent manner resulting in cleavage of target site into smaller fragments incapable of any function. Thus, a posttranscriptional gene silencing phenomenon occurs resulting in RNA silencing (Hamilton and Baulcombe 1999; Matranga et al. 2005; MacRae et al. 2006; Siomi and Siomi 2009).

Based on the origin and nature of precursor molecule and their biological function, the sRNAs can be categorized into microRNAs (miRNAs), small interfering RNA (siRNAs), and piwi RNAs (piRNAs). piRNAs are the largest (26–31 nt) class of small noncoding RNA (sncRNA) molecules (Molecular biology select 2006; Seto et al. 2007), whereas the miRNAs and siRNAs are smaller counterparts (19–24 nt). These small regulatory RNAs are present in eukaryotes only and are derived from dsRNA; however, piRNAs are derived from long single-stranded precursor molecules (Lu et al. 2005). The biogenesis of these piRNAs is distinct from that of other sRNAs and is hypothesized to be either based on piRNA phasing or a ping-pong mechanism of generation (Brennecke et al. 2007). Both the groups of regulatory RNAs need different subsets of effector proteins, i.e., siRNA and miRNA need AGO proteins, whereas piRNA interacts with piwi proteins to mediate epigenetic and posttranscriptional gene silencing (Carmell et al. 2002; Carthew and Sontheimer 2009; Siomi and Siomi 2009; Siomi et al. 2011; Burgess 2013). Being endogenous in origin, miRNA regulates expression of host genes, whereas the exogenous origin of siRNAs allows them to protect genome integrity of host from foreign or invasive nucleic acids derived from viruses, transposons, or transgenes. In *Drosophila*, the endogenous siRNAs have been shown to provide resistance against *Drosophila* transposons; hence, the siRNAs can have both types of biogenesis mechanisms inside hosts (Chung et al. 2008; Czech et al. 2008). Exogenously supplied synthetic siRNAs or endogenously produced siRNAs are reported to regulate the expression of endogenous genes, e.g., in mice and various cell lines (Watanabe et al. 2008; Stein et al. 2011).

Transgenic approaches to develop virus-resistant plants involve introduction of viral protein-coding sequences conferring pathogen-derived resistance (PDR). Usually the mRNAs targeted are those involved in the translation of capsomeres, viral replication, suppression of host immune response, and expression of RNA-dependent RNA polymerases (RdRPs) (Abel et al. 1986; Hong et al. 1995; Ahlquist 2002). The most popular transgenic plant developed using PDR strategy targeting viral coat protein was the Hawaiian papaya resistant against *Papaya ringspot virus* (Fitch et al. 1992; Ferreira et al. 2002; Gonsalves et al. 2004). The papaya transgenics thus produced saved a huge amount of papaya produced in Hawaii thus helping farmers to incur financial gains from its export. Now we have the technical know-how about the exact mechanism of RNAi conferring virus resistance in plants and

realize that antisense RNA technology was nothing but a step into the RNAi realms. In this chapter we intend to propose a strategy to develop a generic resistance based on siRNA technique against begomoviruses infecting various crops.

8.3 RNAi-Based Strategies

RNAi vectors consisting of inverted repeats of siRNAs in sense and antisense orientation under the control of a strong promoter have produced mixed results in conferring resistance against begomoviruses. The precursor regions of putative siRNAs are introduced into host plants that provide significantly effective resistance against begomovirus challenge in the first generation. Eventually, the efficacy of resistance is lost or diluted as further progenies are studied. This is due to recombination, higher than critical inoculum of virus in the beginning, or due to the uncontrolled plant growth conditions (Ghoshal and Sanfaçon 2015). Though various strategies are reported for construction of RNAi vectors (Hirai and Kodama 2008), the two most popular strategies for RNAi experiments are inverted repeats containing short hairpin construct (shRNA) and artificial microRNA (amiRNA) harboring target siRNA within an miRNA backbone, thus acting like a natural miRNA (Zhou and Luo 2013).

Several studies have reported siRNA approach as a key tool to develop virus-resistant crops, e.g., transgenic *Nicotiana benthamiana* using antisense RNA technique designed against C1 (recently known as AC1) encoding Rep protein mRNA of *Tomato yellow leaf curl virus* (TYLCV). This transgenic tobacco line expressing C1 antisense RNA shows effective resistance to TYLCV through at least two generations of progeny (Bendahmane and Gronenborn 1997). A study has reported a transgenic tobacco resistant to *Cotton leaf curl virus* (CLCuV). CLCuV is a begomovirus that causes severe leaf curl symptom leading to overall economic losses to farmers in Asian subcontinent. This transgenic tobacco carries various siRNA fragments derived from CLCuV. The resistance conferred by these fragments is sustained for up to 120 days upon exposure to viruliferous whiteflies from T1 to T3 generations (Asad et al. 2003) suggesting it to be an effective resistance in case the occurrence of whiteflies overlaps this time period and thus giving a sustainable resistance approach.

Further, as one step ahead, black gram (*Vigna mungo*) infected with *Vigna mungo yellow mosaic virus* (VMYMV) is reported to recover successfully after expressing dsRNA against promoter region of viral DNA-A and established the fact that DNA of a replicating virus can also be an effective target of transcriptional gene silencing (Pooggin et al. 2003). Another study reported the recovery of *Potato spindle tuber viroid* (PSTVd) infected tomato plants using RNAi via sequence-specific degradation of viroid (Sano and Matsuura 2004). Zhang et al. (2005) developed *African cassava mosaic virus* (ACMV) resistant cassava transgenics using antisense RNA technology by targeting viral mRNAs of Rep (AC1), TrAP (AC2), and REn (AC3).

Thus, various siRNA-based resistance strategies were effective in controlling whitefly-mediated infection of begomoviruses, yet the transfer of resistance across

generations of transgenic progenies is not effective probably due to dilution of siRNA expression. Therefore, there is a need to develop effective strategy for resistant transgenic crops that are able to withstand viral attacks through many generations irrespective of initial viral titer.

Several geminiviral proteins with silencing suppressor activities have been identified which makes them potential target for RNA interference-based resistance, e.g., *Citrus tristeza virus* (CTV) resistant transgenic Mexican lime was developed by posttranscriptional gene silencing of p23, a silencing suppressor of *Citrus tristeza virus* (Fagoaga et al. 2006). In another interesting study, designing siRNA construct was reported for the first time, targeting v1 gene encoding coat protein (CP) of *Tomato yellow leaf curl virus* (TYLCV) (Zrachya et al. 2007). The transgenic tomato plants expressing siRNA did not develop disease symptoms 7 weeks postinoculation with the virus, while non-transgenic control plants developed disease symptoms within 2 weeks postinoculation. Hence, this strategy confers resistance to the TYLCV in transgenic plants and enables a good yield of flowers and fruit.

In case of cassava crop, Patil and coworkers developed RNAi-mediated resistance against different isolates of *Cassava brown streak virus* (CBSV) and *Cassava brown streak Uganda virus* (CBSUV) causing severe cassava brown streak disease (Patil et al. 2011). In this strategy, CP gene regions of the two *Potyvirus* species were targeted; the region consists of N-terminal 397 nt and C-terminal 491 nt constituting the full-length coat protein (FL-CP) gene of CBSV and CBSUV. The transgenic tobacco homozygous line expressing FL-CP region showed significant resistance (approximately 85% plants were completely resistant) against CBSUV-[UG:Nam:04] challenge, while the N-terminal and C-terminal expressing transgenic tobacco lines showed resistance in decreasing order. Therefore, the RNAi strategy appeared to be effective in case of cassava-infecting *Potyvirus* species.

Using a novel approach, designing siRNA against the most conserved region among coat protein (AV1) and replicase (AC1) genes of different isolates of geminiviruses infecting *Papaya leaf curl virus* (PLCV) and *Tomato leaf curl virus* (TLCV) was done to address generic resistance by Saxena et al. (2011). Subsequently, Saxena et al. (2013) proposed an improvised strategy to target three important genes, i.e., AV2, AC2, and AC4, which suppress plants' RNA silencing machinery. In this study, an approach to control begomovirus-infecting papaya crop was given by designing siRNA against these three suppressors using bioinformatics tools. Studies have also reported that these viral silencing suppressors may regulate functioning of the host plant with reference to resistance against the viruses by interfering with regulatory activity of some miRNAs in host gene expression (Kasschau et al. 2003; Chen et al. 2004; Bisaro 2006).

8.3.1 Artificial microRNA (amiRNA)

Structurally, amiRNAs span 21 nt length, generally designed by manipulation of seed region of mature miRNA sequences within double-stranded pre-miRNA. These artificial sRNAs are designed to target one or more endogenous genes in an

organism or a cell line; amiRNA precursors are generated either by PCR overlapping methods or by substitution of a natural stem loop for artificial one with restriction sites between upstream and downstream regions in the backbone of natural miRNA (Liang et al. 2012). The amiRNA sequence selection requires that there should be no mismatches within seed region (9–11 bp) though one or two mismatches are allowed near 18–21 nt positions. The hybridization energy of target and amiRNA should also favor their strong interaction leading to efficient silencing (Alvarez et al. 2006). The above parameters are not the only factors required for amiRNA-mediated silencing. Various miRNAs have been employed to introduce amiRNAs in *Arabidopsis thaliana*, e.g., miR159a, miR167b, miR169d, miR171a, miR172a, and miR319, and rice, e.g., miR528 and miR395. The use of miRNA backbone is limited, and not one miRNA can be employed for all types of silencing. Therefore, careful selection of miRNA backbone is essential for amiRNA-based strategy to be effective in viral RNA suppression (Khraiwesh et al. 2008; Tiwari et al. 2014; Carbonell et al. 2014).

The efficacy of target RNA silencing through amiRNA technology depends not only on the nature of amiRNAs but also on the accessibility of the 3′ untranslated region (UTR) of target mRNAs. The accessibility in turn depends upon the local secondary structure of the target mRNA, e.g., the tRNA-like structure found within the 3′UTR region of cucumber mosaic virus (CMV) was found to restrict target site access thus inhibiting amiRNA-RISC-mediated silencing of putative target viral RNA (Duan et al. 2008). Therefore, the target site accessibility governed by local structure at 3′UTR is as important as sequence specificity, and the target site cleavage is greatly dependent upon the interaction and thermodynamics of the small RNA-programmed RNA-induced silencing complex (siRISC) (Tafer et al. 2008; Pratt and MacRae 2009).

The concept of amiRNA with an aim to develop virus-resistant transgenic *Arabidopsis* plants was validated by modifying miR159a precursor backbone. This amiRNA backbone targets two gene silencing suppressors of viral mRNA sequence; one is P69 of *Turnip yellow mosaic virus* (TYMV) and the other is HC-Pro of *Turnip mosaic virus* (TuMV). Transgenic *A. thaliana* plants expressing amiR-P69^{159} and amiR-HC-Pro^{159} were resistant to TYMV and TuMV, respectively. The transgenic plants carrying the amiR-P59^{169} and amiR-HC-Pro^{159} within the same construct were resistant against both the viruses (Niu et al. 2006). A comparative study was conducted to evaluate efficacy of amiRNA and short hairpin RNA (shRNA) construct targeting viral silencing suppressor protein 2b derived from cucumber mosaic virus (CMV). Here, 2b gene expression was inhibited in a much more effective manner by amiRNA strategy when compared to the shRNA construct in transient assays (Qu et al. 2007). Therefore, the amiRNA technique has a potential to provide an effective and specific resistance against viral suppressors of plant RNAi machinery.

amiRNAs were studied in *Arabidopsis*, rice, wheat, maize, *Chlamydomonas reinhardtii*, etc. where they actually function like natural miRNAs (Schwab et al. 2006; Alvarez et al. 2006; Qu et al. 2007; Warthmann et al. 2008; Molnar et al. 2009; Yan et al. 2012; Fahim et al. 2012, Fahim and Larkin 2013; Xuan et al. 2015) (refer Table 8.1). These small regulatory RNAs based on miRNA precursors to

Table 8.1 Different RNAi strategies against *Begomovirus*

S. no.	Virus	Host	Approach	Target gene/ region	Transgenic plant	References
1	*Turnip yellow mosaic virus* (TYMV) and *Turnip mosaic virus* (TuMV)	*Brassica rapa*	Gene pyramiding	Gene silencing suppressor P69 of TYMV and HC-Pro of TuMV	*Arabidopsis thaliana*	Niu et al. (2006)
2	Cucumber mosaic virus (CMV)	*Cucumis sativus*	amiRNA mediated	Gene silencing suppressor 2b of CMV	*Nicotiana benthamiana*	Qu et al. (2007)
3	*Wheat streak mosaic virus* (WSMV)	*Triticum aestivum*	amiRNA mediated	5′UTR, ORF pipo region of P3 cistron, P1 gene, P3 cistron upstream of pipo, Hc-Pro of WSMV genome	*Triticum aestivum*	Fahim et al. (2012)
4	*Cotton leaf curl Burewala virus* (CLCuBuV)	Cotton	amiRNA mediated	V2 gene sequence of CLCuBuV	*Nicotiana benthamiana*	Ali et al. (2013)
5	*Rice black-streaked dwarf virus* (RBSDV)	*Zea mays*	amiRNA mediated	RBSDV coding gene and gene silencing suppressor	*Zea mays*	Xuan et al. (2015)
6	*Wheat dwarf virus* (WDV)	*Triticum aestivum*	amiRNA mediated	Different conservative sequence elements of WDV strains	*Hordeum vulgare*	kis et al. (2016)
7	*Tomato yellow leaf curl virus* (TYLCV)	*Solanum licopersicum*	Antisense RNA	C1 encoded Rep protein	*Nicotiana benthamiana*	Bendahmane and Gronenborn (1997)
8	*Cotton leaf curl virus* (CLCuV)	Cotton	Antisense RNA	Rep (AC1), TrAP (AC2), and REn (AC3)	*Nicotiana benthamiana*	Asad et al. (2003)
9	*Vigna mungo yellow mosaic virus* (VMYMV)	*Vigna mungo*	hpRNA construct	Promoter sequence of VMYMV 209 bp long (2650–130 nt) position	*Vigna mungo*	Pooggin et al. (2003)

(continued)

Table 8.1 (continued)

S. no.	Virus	Host	Approach	Target gene/ region	Transgenic plant	References
10	*African cassava mosaic virus* (ACMV)	Cassava	Antisense RNA	Rep (AC1), TrAP(AC2), and REn (AC3)	*Cassava*	Zhang et al. (2005)
11	*Citrus tristeza virus* (CTV)	*Mexican lime*	Antisense RNA	P23	Mexican lime	Fagoaga et al. (2006)
12	*Tomato yellow leaf curl virus* (TYLCV)	*Solanum licopersicum*	siRNA construct	V1 gene encoding coat protein	*Solanum licopersicum*	Zrachya et al. (2007)
13	*Banana bunchy top virus* (BBTV)	*Banana* sp.	ihpRNA	Rep	*Banana* sp.	Shekhawat et al. (2012)
14	*Cassava brown streak virus* (CBSV) and *Cassava brown streak Uganda virus* (CBSUV)	Cassava	RNAi construct	Coat protein (CP)	*Nicotiana benthamiana*	Patil et al. (2011)
15	*Papaya leaf curl virus* (PLCuV)	*Carica papaya*	siRNA mediated	AV2, AC2, and AC4	In silico	Saxena et al. (2013)
16	*Papaya leaf curl virus* (PLCV) and *Tomato leaf curl virus* (TLCV)	*Carica papaya* *Solanum lycopersicum*	siRNA mediated	AV1 and AC1	In silico	Saxena et al. (2013)
17	*Indian cassava mosaic virus* (ICMV)	*Jatropha curcas*	Hairpin dsRNA	Fragment1 target gene encoding 250 bp (CP/ AV1and AC5), Fragment2 250 bp (TrAP/ AC2 and Ren/ AC3), Fragment3 609 bp (Rep/ AC1 and AC4)	*Jatropha curcas*	Ye et al. (2014)

express artificial amiRNAs are capable enough to induce silencing of putative target gene and confer virus resistance in plants.

Studies discussed above were successful experiments where the construction and introduction of artificial miRNA precursor has been one of the most effective strategy to develop broad-spectrum resistance against multiple regions of viral genes at a time, e.g., miRNA construct expressing multiple artificial miRNAs (amiRNAs) against various viral genomic regions (Bucher et al. 2006). The advantage with amiRNA strategy is that it is easier to optimize amiRNA sequences for broad-spectrum targeting and has minimal off-target effect, i.e., enhanced specificity with high efficacy.

8.3.2 *In Silico* Strategy for Designing Effective siRNA Target in Plants

In silico analysis of target sequence is a critical step in the development of an effective strategy against begomoviruses. The selection parameter evaluation of siRNA should be performed in such a way that ensures optimal conditions and effective targeting of the gene of interest. The previously suggested guidelines are subdivided into first-, second-, and third-generation rules that have laid the foundation for the modern and most advanced parameters followed nowadays (Liu et al. 2012).

8.3.2.1 General Guidelines for a Potent siRNA Selection

The siRNA selection criterion is derived from popular studies and algorithms, which tried to validate parameters for potent siRNA prediction (Freier et al. 1986; Zecherle et al. 1996; Tuschl et al. 1999; Elbashir et al. 2002; Yu et al. 2002; Harborth et al. 2003; Khvorova et al. 2003; Reynolds et al. 2004; Ui-Tei et al. 2004; Jackson et al. 2006; Klingelhoefer et al. 2009; Wang et al. 2010; Liu et al. 2012). The following is the summary of various parameters based upon the above studies that control efficacy and specificity of the siRNA thus designed:

1. The target site must lie deep inside coding sequence or open reading frame (ORF), generally advised to start from 100 bp from the initiation codon and avoid the last 100–200 bp region.
2. Regions of high homology, i.e., 15–20 bp, tend to be more specific in silencing the gene expression of target mRNA.
3. Targets with polymorphic loci must be avoided.
4. Isoforms tend to decrease specificity; thus, the regions that are amenable to alternative splicing should be avoided.
5. The presence of secondary structure, i.e., stem loops in the siRNA sequence, decreases the RISC accessibility and hinders the formation of RISC-siRNA complex.

6. A siRNA antisense strand with low 5′ end thermodynamic energy is favorable as it eases the loading of RISC complex. Therefore, the sense and antisense strands should have a difference in binding energy.
7. Target sites with low GC content, i.e., less than 50%, have higher potential to be processed as functional siRNA regions.
8. The siRNA designed against target regions should not be having off-targets, i.e., seed region homology, with other functional mRNA sequences of the host and associated organisms, e.g., plants and their pests (helpful as well as harmful). It is also to be considered that the off-targets if present should not be a functional component of the upstream pathway or an important regulator of plant development and functions which might lead to nonspecific and insufficient handling of miRNA-like off-target effects (Schultz et al. 2011).

Elsewhere, useful guidelines for efficient designing of amiRNA-based backbone have been proposed (Vu and Do 2016). The guidelines intend to improve amiRNA design, backbone, efficiency, and specificity. However, it is impossible to design an amiRNA that fully mimics the natural miRNA function, specificity, and efficacy.

Apart from abovementioned parameters, the siRNA datasets, efficacy prediction models, and algorithms provide a useful tool to inspect the siRNA prior to its introduction into a plant. This helps to save time, resources, and labor involved in the process and enhances the probability of success.

8.3.2.2 General Strategy for siRNA Designing

The *Begomovirus* replicates by forming double-stranded intermediate after entering the host nucleus. The replication starts by replication-associated protein, and here, the virus genome might undergo recombination with plant genome or any other virus present in the vicinity (in case of mixed infection). This gives rise to genetic variations that help begomoviruses to evade the siRNA machinery of host. In present context, we have seen that the begomoviruses impose an imminent threat to global crop production due to evolution of genome, thus giving rise to genetic diversity which cannot be handled by a specific siRNA approach. It is however possible to generate some resistance if we target the genetic diversity by choosing conserved sequences to design siRNAs. This strategy will help in providing a solution to more than one isolate of same species or even phylogenetically similar begomovirus species. A study proposed that if we can find out the siRNA-generating "hot spots" in the begomovirus genome, then it is quite possible to target genera of begomoviruses (Sharma et al. 2015). This seems quite possible, as an in silico analysis of various leaf curl-causing begomoviruses has yielded regions ranging from 88% to 100% conservation among phylogenetically similar isolates around the world (unpublished data).

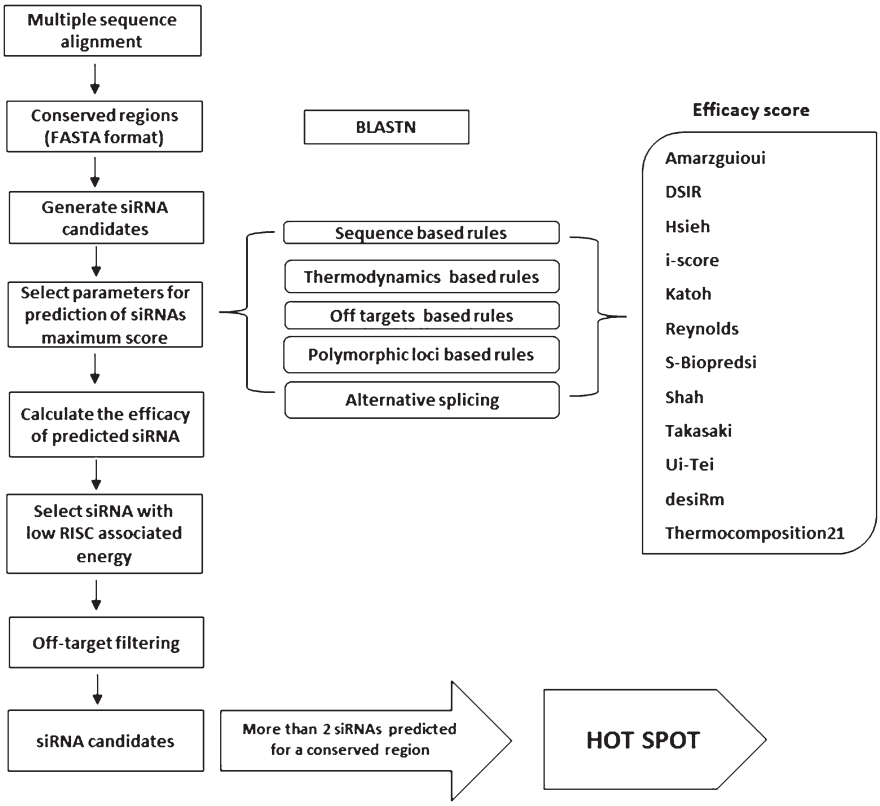


Fig. 8.1 Outline of the siRNA designing strategy

Here we present a strategy for efficient siRNA designing that considers and fulfills the latest guidelines and rules (Fig. 8.1).

STEP 1: Sequence retrieval from various databases such as NCBI (https://www.ncbi.nlm.nih.gov), EMBL (http://www.ebi.ac.uk/embl.html), or Swiss-Prot (http://web.expasy.org/docs/swiss-prot_guideline.html).

STEP 2: Multiple sequence alignment using popular tools such as Clustal X (http://www.clustal.org/clustal2), Clustal Omega (http://www.clustal.org/omega), T-COFFEE (http://www.tcoffee.org/), and MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle). In order to find out conserved region among different viral isolates, we prefer multiple sequence alignment using software package MEGA 6.0 (http://megasoftware.net/mega.php).

STEP 3: Selection of conserved regions (85–100%) (Fig. 8.2).

STEP 4: Use of siRNA prediction tools, i.e., pssRNAit (http://plantgrn.noble.org/pssRNAit/), for prediction of siRNA target regions. Here one has to choose species according to the model being used for conducting silenc-

Fig. 8.2 Multiple sequence alignment of the *Begomovirus* sequences using MEGA 6 package. Selection of conserved sequence regions

ing experiments. The software accordingly selects the cDNA/transcript library database for off-target effect-based analysis.

STEP 5: Analyze the parameters for the most effective siRNA-generating region. This process requires "hit and trial" approach. It is important to vary the parameters according to the sequence under study and refine them in order to achieve maximum specificity and efficacy (Figs. 8.3 and 8.4).

STEP 6: Choose siRNAs with minimal off-target score (Fig. 8.5).

STEP 7: Select hot spot regions, i.e., conserved regions producing more than two efficient siRNAs, fulfilling all rules and parameters.

Several studies have introduced large inverted hairpin siRNA construct in *N. benthamiana* without prediction of siRNAs and yet found some success in begomovirus intervention just on the basis of conservation criteria (Bucher et al. 2006; Medina-Hernandez et al. 2013). Few studies have proposed to incorporate a conserved nonanucleotide (hairpin loop) region along with large inverted repeat fragments for RNAi-based resistance due to its conserved nature (Wesley et al. 2001;

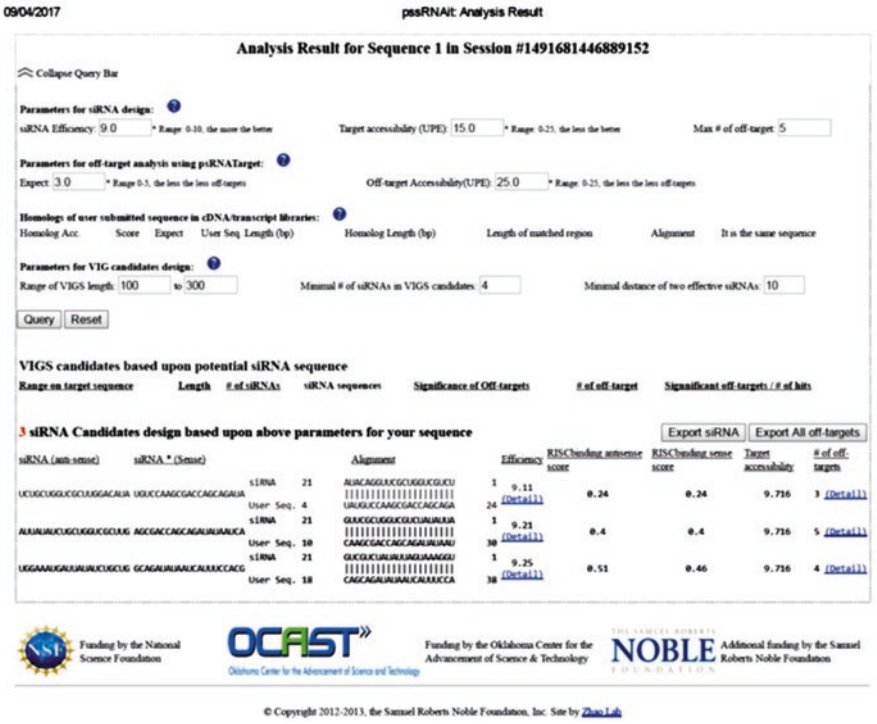


Fig. 8.3 Designing siRNAs using pssRNAit online tool available with various parameters

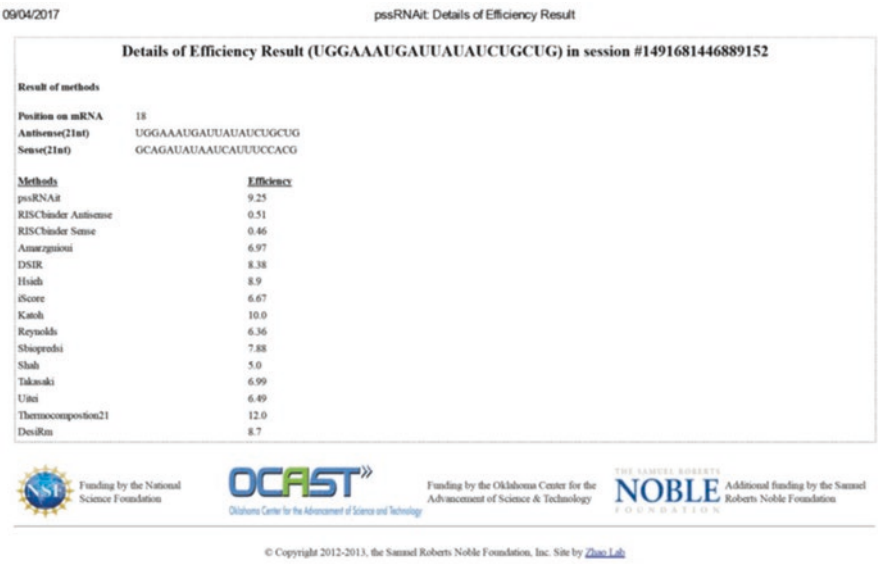
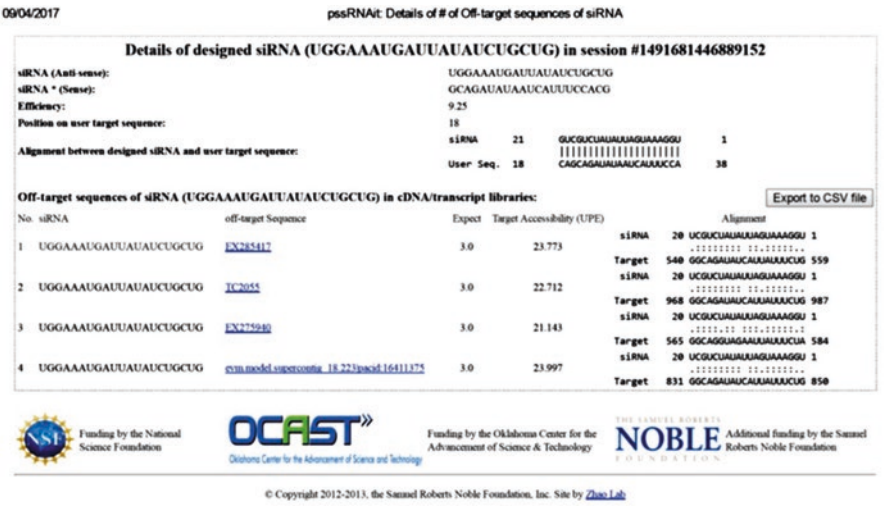


Fig. 8.4 Details of the pssRNAit result providing various efficiency scores for a predicted siRNA (http://plantgrn.noble.org/pssRNAit/)


09/04/2017 pssRNAit: Details of # of Off-target sequences of siRNA

Details of designed siRNA (UGGAAAUGAUUAUAUCUGCUG) in session #1491681446889152

siRNA (Anti-sense):	UGGAAAUGAUUAUAUCUGCUG
siRNA * (Sense):	GCAGAUAUAAUCAUUUCCACG
Efficiency:	9.25
Position on user target sequence:	18
Alignment between designed siRNA and user target sequence:	siRNA 21 GUCGUCUAUAUUAGUAAAGGU 1 User Seq. 18 CAGCAGAUAUAAUCAUUUCCA 38

Off-target sequences of siRNA (UGGAAAUGAUUAUAUCUGCUG) in cDNA/transcript libraries: Export to CSV file

No.	siRNA	off-target Sequence	Expect	Target Accessibility (UPE)	Alignment
1	UGGAAAUGAUUAUAUCUGCUG	EX285417	3.0	23.773	siRNA 20 UCGUCUAUAUUAGUAAAGGU 1 Target 540 GGCAGAUAUCAUAUAAUCUG 559
2	UGGAAAUGAUUAUAUCUGCUG	TC2055	3.0	22.712	siRNA 20 UCGUCUAUAUUAGUAAAGGU 1 Target 968 GGCAGAUAUCAUAUAAUCUG 987
3	UGGAAAUGAUUAUAUCUGCUG	EX275940	3.0	21.143	siRNA 20 UCGUCUAUAUUAGUAAAGGU 1 Target 565 GGCAGGUAGAAUUAUAUCUA 584
4	UGGAAAUGAUUAUAUCUGCUG	evm.model.supercontig_18.223|pacid:16411375	3.0	23.997	siRNA 20 UCGUCUAUAUUAGUAAAGGU 1 Target 831 GGCAGAUAUCAUAUAAUCUG 850

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Fig. 8.5 Details of the predicted off-targets for one of the siRNA

Khatoon et al. 2016). Therefore, the strategy of introducing hot spots rather than a single specific siRNA has an advantage over the later strategy.

8.4 Conclusion

RNAi being a popular tool to downregulate the gene expression at posttranscriptional level has helped in the development of various strategies that succeeded in conferring some amount of resistance against begomoviruses. Yet, the suppression of resistance is imminent in case of begomoviruses due to their recombination-dependent replication and RNAi suppression components that antagonize the plant immune response and bypass the siRNA-based silencing. Therefore, no strategy could be a complete solution for the global problem of begomovirus diseases; still, the generic solution for a particular crop in a specific region could be a possible solution until the virus isolate evolves itself into a new recombinant. Here, we have discussed few aspects of RNAi and the basis of siRNA-based strategies employed in the past in an attempt to develop begomovirus-free transgenic crops for sustainable food resources and global food security. The strategy presented here may not be the ideal solution but provides a perspective for researchers to pursue research in the very area in order to develop generic resistance against begomoviruses.

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9 Computational Analysis and Predicting Ligand Binding Site in the *Rose leaf curl virus* and Its Betasatellite Proteins: A Step Forward for Antiviral Agent Designing

Avinash Marwal, Megha Mishra, Charvee Sekhsaria, and R.K. Gaur

Abstract

Computational approach was done in protein molecules of the *Rose leaf curl virus* and its betasatellite component isolated from Rose plants. Moreover in-depth study was done using in silico approach such as restriction map, GC profile and prediction of binding sites for ligand molecule analysis. Hence, an approach has been taken into consideration to unearth a treatment against geminiviruses, resulting in huge yield loss across the globe. This study provides a great deal of novel knowledge and will be employed for the selection of inhibitors in opposition to geminivirus proteins focusing on begomovirus and paves a way for developing antiviral agents in the near future.

Keywords

Rose • Geminiviruses • Begomovirus • Satellites • Proteins • Binding sites

9.1 Introduction

Geminivirus is the leading group of plant viruses, widespread along the tropic and subtropic section of the earth and comprising a circular single-stranded DNA encapsulated within twin geminate icosahedral symmetry. Sometimes geminivirus has been found to be associated with their other genomic components, i.e. betasatellite and alphasatellite. Begomoviruses are a huge diverse virus family causing

A. Marwal • M. Mishra • C. Sekhsaria • R.K. Gaur (✉)
Department of Biosciences, College of Arts, Science and Humanities, Mody University, Lakshmangarh, Sikar 332311, Rajasthan, India
e-mail: gaurrajarshi@hotmail.com

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infections in a large number of plants like weeds, ornamentals, crop species, etc., resulting in a remarkable failure to horticulture and agriculture across the globe (Mansoor et al. 2003; Marwal et al. 2013b, c; Lima et al. 2013). Ornamental plants are expansively speckled throughout the world and show great ecological adaptability. Such ornamental plants are well-thought-out to be a host of undiscovered geminiviruses and a pool of new geminiviruses which are sometimes never taken into consideration while performing diversity analyses (Marwal et al. 2013d; Urbino et al. 2013).

Numerous study results suggested that ornamental plants harbour as a substitute host for the endurance of geminiviruses which multiply during the off season of the main crops (Raj et al. 2007; Ilyas et al. 2013). Hence, there is an urgent necessity for further knowledge regarding the distribution and diversity of geminiviruses among ornamental species. Proteins represent the vertebrae for the various functions attributed by the cell performing the job planned by the genes in a given type of cell. Moreover, it remains exigent to competently categorize the equipped responsibility of sole protein entities recognized in such events. Practical characteristics exhibited by protein domain, for example, enzymatic action or the capability to work together in the company of additional proteins, are possible due to the predictable spatial arrangement of the amino acid chain present in the folded manner (Bairoch 2000; Hannum et al. 2009).

Identifying the structure and functions of newly revealed proteins is quite important in understanding the job it performs in biological processes. Hence RaptorX and 3DLigandSite are convenient online available softwares for prediction of ligand-binding sites in protein molecules. For the 3DLigandSite web resources, the ligands are vault to the structures comparable to the query and are superimposed onto the model, employed to predict the binding site. These online portals help the client to deposit the query protein sequence or its PDB structure. All the prediction structures are generally illustrated with the help of user-friendly Jmol application. 3DLigandSite can be accessible for exploit at http://www.sbg.bio.ic.ac.uk/3dligandsite (Ivashchenko et al. 2016; Wass et al. 2010).

RaptorX sets apart from rest of the online softwares due to its superiority of the alignment bordered by the objective sequence and one or numerous imprecisely linked proteins template and with a novel nonlinear scoring principle and a probabilistic evenness algorithm. As a result, RaptorX conveys an elevated class structural model for several targets with only remote templates at http://raptorx.uchicago.edu/. We have previously identified *Rose leaf curl virus* and the satellite DNA associated with it and further deposited the geminivirus DNA at the National Center for Biotechnology Information, USA. The FASTA format of the proteins encoded by *Rose leaf curl virus* and its satellite molecule was deposited on the 3D-Jigsaw (https://bmm.crick.ac.uk/~populus/) acting as a protein comparative modelling server. This 3D-Jigsaw helps in the construction of 3D models of proteins laid on the fact of the already known homologue structure. After uploading the sequence on the 3D-Jigsaw online portal, it generates the PDB file format of the proteins and sends on the e-mail address registered at the modelling server (Heinrichs 2008; Marwal et al. 2013a; Källberg et al. 2012).

The online software presents the characteristic of all the amino acids liable for binding and moreover the file of ligand molecules acting as heterogens, which is make available by the Uniprot which is unlikely to be there in the protein structures as solvent. The protein and their predicted binding sites are illustrated in the form of spacefill model, as cartoon or wireframe formats. The protein displayed in coloured 3D format depicts the predicted binding site or residue conservation (Balakrishnan et al. 2010). Ornamental plants serve as a substitute host of geminiviruses and the satellite DNA linked to it in lack of main crop. Proteins frequently carry out their job via ligands (such as enzyme substrates). Hence the detection of ligand-binding sites is quite imperative (Marwal et al. 2013b). This paper presents highlights and supports the verification that geminivirus harbouring in Rose plants was characterized by means of computational tools using online available softwares such as RaptorX, GC profile, NEBcutter, etc.

9.2 Mapping of Restriction Sites of *Rose leaf curl virus* and Its Betasatellite

Before the advent of the polymerase chain reaction (PCR), restriction enzyme digestion was the chief method for transferring the gene of interest from one DNA source to the other. For an instance, it was suggested that the potential of polymerase chain reaction to empower precise amplification of individual fragment of genome might restrict the utility of restriction enzymes. Nevertheless, restriction enzymes still have new efficacy acting as a diagnostic reagent to reveal that the DNA constructs have been ligated or placed in correct fashion. Restriction enzymes still attribute the inexpensive and the best suitable method to characterize DNA constructs (Roberts et al. 2003; Pingoud and Jeltsch 2001).

Restriction enzyme sets along with the magnitude of open reading frame were employed. The software exhibited the exact position of all the cut sites of restriction enzyme, and it also identifies the number of ORFs present in the geminivirus DNA. It subsequently demonstrates a schematic illustration of the geminivirus DNA comprising the ORFs sets which is laid on the guidelines of one cut only by all the restriction enzymes. The initial display also reveals that the enzymes can also be taken into consideration with a complete digestion to expunge every ORF (Table 9.1). Restriction enzymes further establish its use for characterizing the DNA of higher organisms via restriction fragment length polymorphisms (RFLPs) working as a physical marker or by directly identifying the occurrence of single nucleotide polymorphisms (SNPs). For analysing and constructing the restriction map (created by restriction enzymes by cleaving the DNA sequence) of the geminivirus DNA sequence, the software NEBcutter, version 1.0, was employed which is accessible online at http://tools.neb.com/NEBcutter and resulted in a diversity of displays (Vincze et al. 2003).

The *Rose leaf curl virus* (RoLCV: KF584008) and *Rose leaf curl betasatellite* (RoLCB: KF584009) sequences were subsequently mined from the database NCBI in the form of GenBank file through its accession number. For displaying the open

Table 9.1 Locations and the coding capability of the predicted ORFs for geminivirus DNA and the satellite molecule associated with it as extracted from the Rose plants

Components	Description	ORFs	Strand	Frame	Predicted molecular weight (kDa)
DNA-A	Precoat protein	AV2	Sense	3rd frame (+)	13.87
	Coat protein	AV1	Sense	1st frame (+)	29.61
	Replication enhancer protein	AC3	Complement	3rd frame (−)	15.63
	Transcriptional activator protein	AC2	Complement	2nd frame (−)	15.29
	Replication associated protein	AC1	Complement	1st frame (−)	39.05
	C4 protein	AC4	Complement	2nd frame (−)	7.07
DNA-β	Symptoms inducing protein	C 1	Complement	2nd frame (−)	12.37

reading frame, its magnitude was determined along with the restriction enzymes used to cut it. The six main ORFs in *Rose leaf curl virus* (RoLCV: KF584008), i.e. AV2, AV1, AC3, AC2, AC1 and AC4 (Fig. 9.1a), are flanked by sites DraI and Bs1I, BfaI and ApoI, AlwI and EcoNI, PshAI and AcuI, and MwoI and BsrGI, respectively (Fig. 9.1b).

Determining the mapping of restriction sites in a DNA molecule is quite an important method while formatting the position of gene of interest in a cloning vector, by identifying the location of an off-centre restriction site in an insert. Restriction maps can be represented in a linear or circular fashion. In *Rose leaf curl betasatellite* (RoLCB: KF584009), the single ORF, C1 (Fig. 9.2a), is flanked by sites PsiI and NdeI (Fig. 9.2b). The software employed analysed the geminivirus DNA sequence to discover large nonoverlapping open reading frames taking into account the *E. coli* genetic code. Further it also identifies the restriction sites which make a single cut in the genome using all type II and commercially available type III restriction enzymes. Initially restriction enzymes provided by New England Biolabs were taken into consideration, while other sets of enzymes can be selected.

9.3 Estimating GC Profile of the Virus Sequences

For gaining perspective knowledge regarding the function, evolution and structure of genomes, it is imperative to understand the DNA sequence compositional features. A novel fragmented algorithm having quadratic divergence has come up which distinguishes a DNA sequence or a genome on the basis of its compositionally divergent domains. Taking the help of the skill of cumulative GC profile (which incorporates a windowless technique for the G + C content estimation), the allocation of fragmentation points can be demonstrated naturally. Hence GC profile approach endows with a qualitative and quantitative outlook of genomic

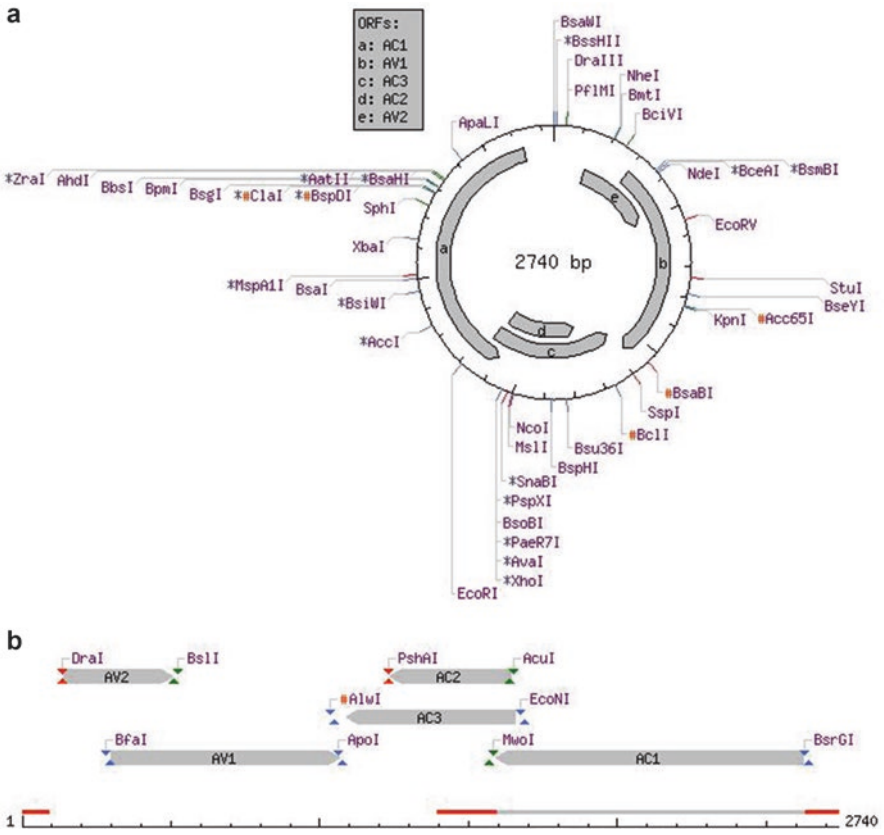

Fig. 9.1 (**a**) Circular representation of the restriction map of *Rose leaf curl virus* (RoLCV: KF584008) DNA-A component highlights the arrangement of restriction enzymes which cut the DNA only once. **(b)** Linear demonstration of restriction digestion for *Rose leaf curl virus* (RoLCV: KF584008)

arrangement in a graphical manner and facilitates us to ascertain the interactions among the G + C content and other features of genomic origin (Gao and Zhang 2006).

If the GC profile shows a negative value (cumulative) in case of genomic islands as discrete from the entire genome, it depicts that the genomic islands suggest moderately stumpy GC content as depicted by sudden plunge in the negative cumulative GC profile, thus suggesting clear boundaries amid the genomic islands and their neighbouring area. Such type of variability in nucleotides (purine and pyrimidines) influences the sequences responsible for coding and in turn imitates an elementary echelon of genome architecture. This architecture illustrates the difference in a variety of significant biological properties, such as gene extent, speed of recombination, model of codon usage, replication period and gene compactness.

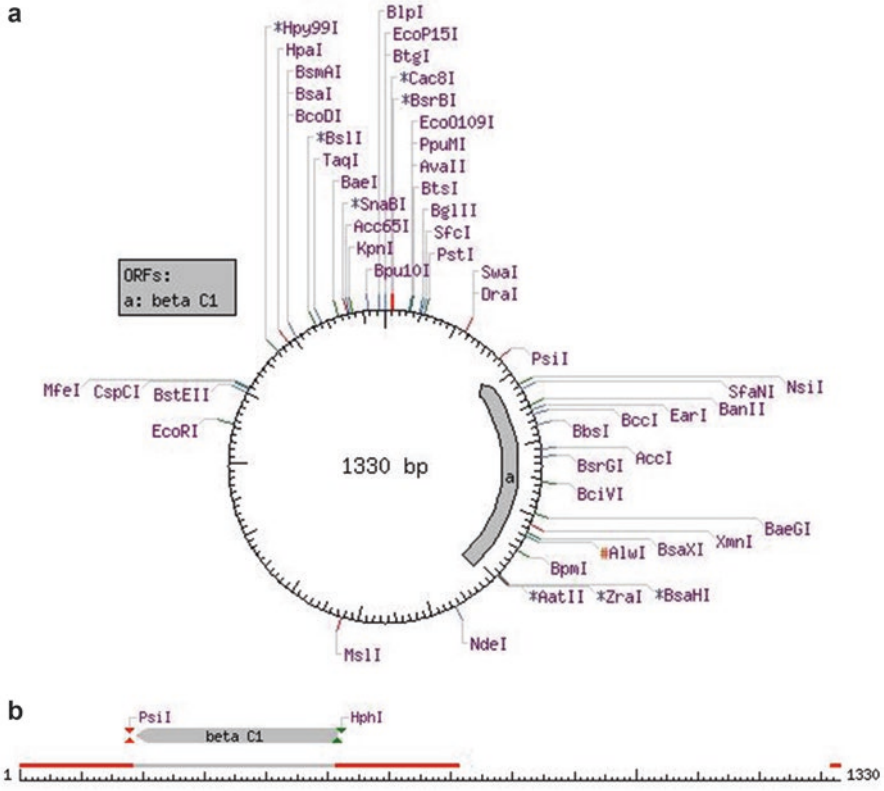

Fig. 9.2 (**a**) Circular representation of the restriction map of *Rose leaf curl betasatellite* (RoLCB: KF584009) infecting Rose highlights the arrangement of restriction enzymes which cut the DNA only once. (**b**) Linear demonstration of restriction digestion for *Rose leaf curl betasatellite* (RoLCB: KF584009)

The chief power governing the microbial evolution is the horizontal gene transfer, thus leading to evolution in quantum leaps. Horizontal gene transfer is also responsible for the activity of mobile genetic elements also known as genomic islands. These mobile genetic elements many a times comprise of DNA sequences which vary from the core genome in their G + C content and codon usage. Based on the purpose they exhibited (encoded), genomic islands are categorized as secretion islands, pathogenicity islands, symbiosis islands, resistance islands and metabolic islands (Hacker and Carniel 2001; Groisman and Ochman 1996; Hentschel and Hacker 2001; Koonin et al. 2001).

Here in the case of *Rose leaf curl virus* (RoLCV: KF584008), two boundaries were detected from the GC profile (Fig. 9.3a). Here a decrease in the GC profile was observed, marked as the first boundary from 200 to 350 bp. Second boundary has an intense decrease in the negative region (G + C) ranging from 1100 to 1600 bp. The

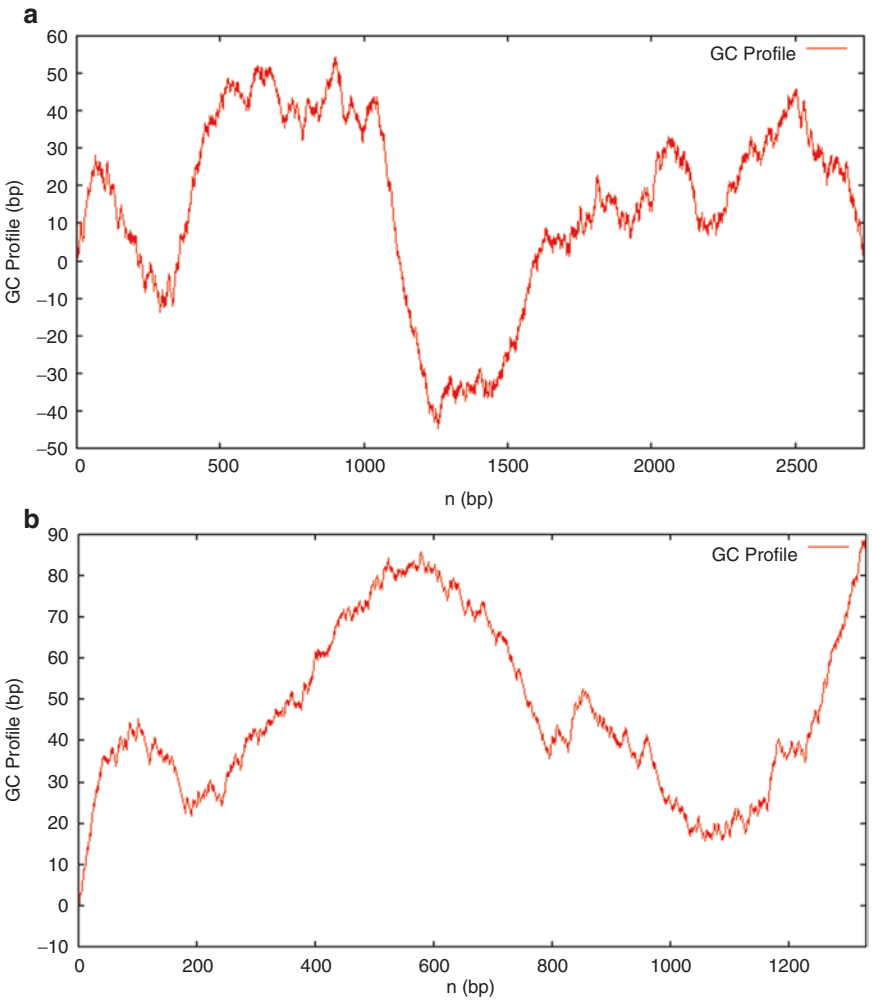

Fig. 9.3 GC profile of (**a**) *Rose leaf curl virus* (*RoLCV*: KF584008) and (**b**) *Rose leaf curl betasatellite* (RoLCB: KF584009) infecting Rose

overall GC content of the geminivirus sequence isolated from Rose was calculated as 44.64%.

Rose leaf curl betasatellite (RoLCB: KF584009) has a very good GC profile. Though two segmented boundaries were found, one spans from 150 to 250 bp and the second boundary ranges from 1000 to 1200 bp. None of the boundaries below zero were encountered (Fig. 9.3b). The complete GC content for betasatellite DNA isolated from Rose was intended to be 36.39%. The above results indicate the association of G + C content with rest of the genomic features, like allocation of genes, can be studied in a perceivable manner. It further highlights, in case of higher eukaryotic genomes for characterizing their isochore arrangement, GC profile is a

suitable preliminary approach and also a good source for recognizing genomic islands in prokaryotic genomes.

9.4 Binding Site Prediction of *Rose leaf curl virus* Protein Molecules

Relationships between proteins are an important constituent of a number of biological processes and attribute an assortment of functions comprising of regulating gene expression, transportation, signal transduction, etc. (Russell et al. 2004; Aloy and Russell 2004). While in-depth information regarding protein structure is a must for considering the fundamental mechanisms, such aspects are sometimes tricky to attain via conventional experimental experiments (i.e. X-ray crystallography, NMR). Finally understanding the 3D structure of a protein complex is quite important for studying the interaction mechanism (Perez et al. 2016; Taherzadeh et al. 2016; Shin et al. 2016). Though, the experimentation procedure to crack the protein structure is repeatedly established to be complicated (Salwinski and Eisenberg 2003; Szilagyi et al. 2005).

A similar kind of approach has been applied for *Rose leaf curl virus* and its associated *Rose leaf curl betasatellite* molecule infecting Rose plant. Examination of AC1 protein suggested that amino acids accountable for binding and their location were established by asparagine at 88th position, histidine at 90th position and glycine at 91st position was accountable. The ligand/heterogen residing at the binding site was found to be NAG (N-acetyl-D-glucosamine). When the PDB file of AC2 protein was subjected to analysis on the server, studies ascertained that residues cysteine, histidine, asparagine, histidine and glycine were present at 39th, 44th, 46th, 59th and 67th positions, respectively, for binding site activity, and the heterogen was revealed as MG (magnesium ion) (highlighted in red).

During the analysis of AC3 protein, it was remarkably found that 12 amino acid residues are responsible for the binding activity of AC3, which includes isoleucine at 50th, glutamine at 51st, phenylalanine at 52nd, histidine at 54th, alanine at 68th, phenylalanine again at 69th, arginine at 70th, isoleucine at 71st, tryptophan at 72nd, methionine at 73rd, threonine at 74th and phenylalanine once again at point 82 were accountable for binding site in AC3 protein and ligand/heterogen molecule in binding site were FRU (fructose). Leucine at location 42 and arginine at the 45th were established for binding site in AC4 protein particle. PO4 (phosphate ion) serves as a heterogen or as a ligand molecule which suitably resides in the locality of the protein (Fig. 9.4).

When the PDB file of AV1 protein was subjected to analysis on the server, studies ascertained that only one residue glycine at 189th position was responsible for binding site, and the ligand molecule/heterogen was identified as CA (calcium ion). Further, results revealed that two binding sites were predicted in AV2 protein at positions 59 and 63, conquered by arginine and histidine, respectively, having GOL (glycerol) as the ligand molecule. Considering the beta C1 protein of *Rose leaf curl betasatellite*, two residues are viable in the binding site that constitutes glycine at

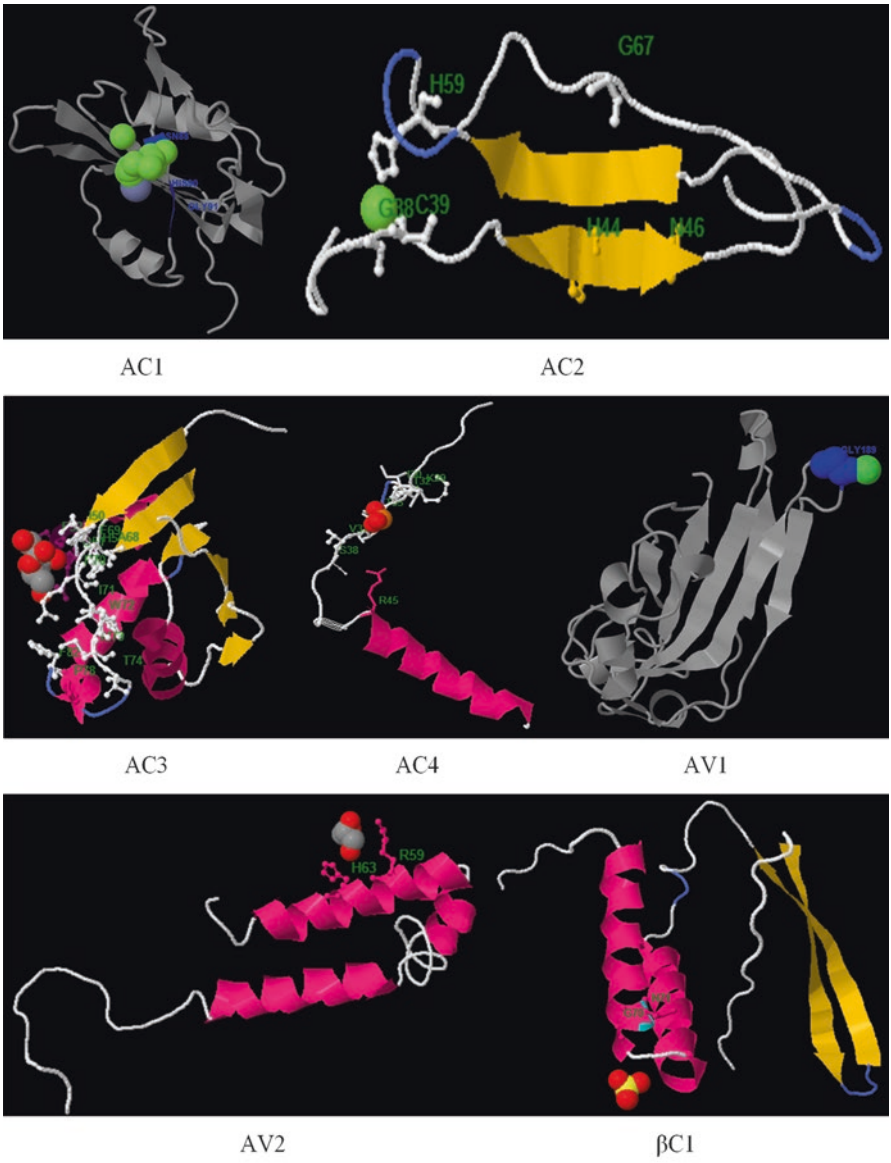

Fig. 9.4 Three-dimensional image produced by the server RaptorX and 3DLigandSite for the prediction of target proteins of *Rose leaf curl virus* (*AC1*, *AC2*, *AC3*, *AC4*, *AV1* and *AV2*) and *Rose leaf curl betasatellite* (*βC1*). The Jmol application exhibits the structure of protein molecule in association of predicted binding site as depicted in blue frame. The ligands in the cluster used to make the prediction are displayed with ions in spacefill and organic molecules in wireframe formats

position 70 and asparagine at 71st place, respectively. The heterogen particle was identified as a ZN (zinc ion) (highlighted in red).

Proteomes represent the strength of character for the various functions of cells by performing the responsibility encoded in the genes communicated for a particular type of cell. Acquaintance of the make-up of a newly identified protein is greatly precious in formatting the task it performs in the biological processes, thus acting as a crucial link in highlighting hypotheses or signifying research to additionally investigate the protein's character (Qin et al. 2016; Bairoch 2000; Cimermancic et al. 2016; Hannum et al. 2009).

9.5 Conclusions

Restriction mapping is an incredibly beneficial method employed in identifying the position of gene of interest ligated in the cloning vector, by mapping the location of restriction site in the transgene. Restriction maps were represented in a linear or circular fashion. The software employed analysed the geminivirus DNA sequence to discover large nonoverlapping open reading frames taking in account of *E. coli* genetic code. Further it also identifies the restriction sites which make a single cut in the genome using all type II and commercially available type III restriction enzymes. Stipulated that the predictions made are precise and sufficiently correct, might help in answering applicable biological questions, then predicting binding sites for proteins of *Rose leaf curl virus* and the betasatellite associated with it, has the impending for towering impact in begomovirus research. This job of predicting binding sites is of high significance varying from functional identification of new proteins to its relevance in antiviral drug designing against geminiviruses.

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Part II

Diversity and Status of Begomovirus: Asia and Africa

Begomoviruses in India

10

Savarni Tripathi and Raj Verma

Abstract

Begomoviruses, a group of whitefly-transmitted single-stranded DNA viruses that are widely spread, cause significant economic losses in several important crops in tropical and subtropical regions of India. Begomoviruses have been known to be associated with and cause many diseases in cucurbitaceous, solanaceous, malvaceous vegetable and legume crops in most parts of the country. However, these viruses have emerged as a major threat to vegetable and legume production in India. Tomato, chillies, cucurbits, cotton, okra, legumes, papaya, and cassava are the most seriously affected crops. In recent decades, the most dramatic emergence of begomoviruses has been observed in tomato, chilli, and cucurbits throughout the country specially in tropical areas. The major factors responsible for the emergence of new viruses and their spread in the ecosystem are introduction of viruses, introduction of susceptible crops or genotypes, change in vector population, recombination in viruses, weather factors, and new intensive agricultural practices. This article presents the current understanding of begomovirus diseases in India and the driving forces for their emergence.

10.1 Introduction

Whitefly (*Bemisia tabaci*)-transmitted geminiviruses are the major constraints specially to vegetable cultivation in tropical and subtropical regions of India. Geminiviruses have unique paired icosahedral capsids and are characterized by circular single-stranded DNA genomes. Geminiviruses are large and diverse

S. Tripathi (✉) • R. Verma
ICAR-Indian Agricultural Research Institute, Regional Station,
125 ITI Road, Aundh, Pune 411007, India
e-mail: savarni_pune@iari.res.in

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plant-infecting viruses and broadly consist of four genera (*Mastrevirus*, *Begomovirus*, *Curtovirus*, and *Topocuvirus*) based on vector type, host range and genome organization (Hull 2002). Among these four genera, begomoviruses are the most common and widely spread in India (Chakraborty et al. 2008). Based on the presence of one or two DNA components in its genome, begomoviruses can be grouped into either monopartite or bipartite. Begomovirus diseases have been present for a long time in India; however, in recent years, diseases caused by begomoviruses have been threatening in several important crops mainly due to the emergence and spread of new begomoviruses and their variants (Varma and Malathi 2003; Varma et al. 2011; Borah ad Dasgupta 2012). The ability to undergo recombination and pseudo-recombination of begomoviruses usually results in the evolution of new emerging viral strains. Begomoviruses are continuously evolving as evident by the frequent appearance of disease epidemics in many parts of tropical and subtropical regions of India. Occurrence of several severe disease epidemics caused by these viruses in recent years have seriously impacted the vegetable, legume, and fiber production. The consequences of emerging begomovirus diseases in various crops have been addressed earlier and their possible cause of transmission along with alternative hosts (Varma and Malathi 2003; Varma et al. 2012; Pandey et al. 2011; Khan et al. 2012, 2013). The annual yield losses caused by begomoviruses in legumes (black gram, *Vigna mungo*; mung bean, *V. radiata*, and soybean, *Glycine max*) have been estimated to be approximately $300 million (Varma et al. 1992; Varma and Malathi 2003). The losses caused by some of the begomovirus diseases have been estimated to be as high as 100% (Dasgupta et al. 2003). The economic losses caused by these diseases in different crops have been reported by others (Pun and Doraiswamy 1999; Saikia and Muniyappa 1989; Narula et al. 1999).

In this chapter, we briefly discuss the status of some of the major emerging diseases caused by begomoviruses in important crops and the factors responsible for their emergence and spread in India.

10.2 Major Emerging Diseases Caused by Begomoviruses

10.2.1 Vegetable Crops

10.2.1.1 Cucurbitaceous Vegetables

Cultivation of cucurbits in the country has been impacted by several begomovirus infections. Yellow vein mosaic of pumpkin (*Cucurbita pepo*) caused by a begomovirus has been known to occur in central-western India for over 60 years (Varma 1963; Tiwari and Rao 2014). Begomoviruses are major problems for the cucurbitaceous crops in eastern Uttar Pradesh (UP), India (Tiwari et al. 2008). Generally, diseases caused by begomoviruses in cucurbits spread in epidemic proportions in tropical and subtropical India coinciding with increase in whitefly population early in the growing season. The commercial crops of pumpkin, muskmelon (*Cucumis melo*), watermelon (C*itrullus lanatus*), and bottle gourd (*Lagenaria siceraria*) severely affected by begomoviruses causing more than 50% loss in northern India

have been reported (Varma and Malathi 2003), and later it was observed from UP (Tiwari and Rao 2014). *Squash leaf curl China virus* (from *C. maxima*) was recorded by Tiwari et al. (2012a). A similar situation was also observed in many cucurbits growing in Maharashtra and other tropical parts of India. An important cucurbit, bitter gourd (*Momordica charantia*), has been reported to be the natural host of begomovirus (Raj et al. 2005a). The yellow mosaic disease was responsible for great economic yield losses and lowering of several nutrients and antioxidant in bitter gourd (Raj et al. 2005a). The typical symptoms of the disease are yellow mosaic at early state followed by curling of leaves. Extensive chlorosis and vein banding were observed in severely infected plants. Based on most characteristic symptom, the disease was named as yellow mosaic of bitter gourd. The association of virus was confirmed based on molecular methods and the causal virus was named as *Bitter gourd yellow mosaic virus*. Further cp-gene sequence analysis showed 93-98% similarities with several isolates of *Tomato leaf curl New Delhi virus* (ToLCNDV). Based on highest sequence similarities and closest relationships with ToLCNDV, the virus isolated from bitter gourd was considered as an isolate of ToLCNDV (Tiwari et al. 2010). Later, *Pepper leaf curl Bangladesh virus* was found to be associated with the bitter gourd plants in UP with leaf curling and yellow mosaic (Raj et al. 2010b). Sponge gourd (*Luffa aegyptiaca*), widely grown in India, is affected by high incidence of ToLCNDV (Sohrab et al. 2003). *Luffa cylindrica* and *Luffa acutangula* were reported to be the hosts of ToLCNDV by Tiwari et al. (2012a) from eastern Uttar Pradesh, India, with symptoms of yellow mosaic and leaf curling, and *L. cylindrica* was also found to be the host of *Tomato leaf curl Palampur virus* (Tiwari et al. 2012b). ToLCNDV has also spread to a variety of other cucurbits causing serious limitations of cucurbit cultivation in many parts of the country (Ito et al. 2008; Mandal 2010; Varma et al. 2011; Raj et al. 2010a). A severe mosaic disease of pointed gourd (*Trichosanthes dioica* Roxb.) was observed with significant disease incidence in Gopalganj, India, during 2008, and an associated virus was confirmed as *Ageratum enationvirus*, which was first reported from the world (Raj et al. 2011); later, Tiwari et al. (2012c) reported the detection and elimination of begomoviruses on *T. dioica* plant.

10.2.1.2 Solanaceous Vegetables

Tomato (*Solanum lycopersicum*)

Tomato seems to be the most preferred host of begomoviruses (Varma et al. 2011), and hence, tomato leaf curl disease (ToLCD) is a common disease of tomato in the country. The occurrence of ToLCD has been reported in the 1950s from North India (Vasudeva and Samraj 1948) and subsequently from southern India (Govindu 1964; Sastry and Singh 1973). However, many different species of begomoviruses and their recombinants have emerged recently. Symptoms of ToLCD include leaf curling, severe reduction in leaf size, crinkling of interveinal areas, interveinal and marginal chlorosis, occasional development of enations, general yellowing of the leaves, shortening of internodes, development of small branches, stunting, and reduced fruiting due to sterility and poor flower formation (Varma et al. 2011; Saikia and

Muniyappa 1989). Plants are unable to bear fruit when they get infected at nursery or very early after transplanting. ToLCD is persistently transmitted by whitefly (*B. tabaci*). The host of ToLCD ranges over 23 plant species (Saikia and Muniyappa 1989). There was a sharp increase in the incidence of ToLCD (up to 100%) after the introduction of high-yielding tomato varieties in the 1960s. An epidemic of severe ToLCD in southern India in 1999 coincided with nearly 1000-fold increase in the whitefly population and appearance of the B biotype of *B. tabaci* (Banks et al. 2001). Tomato-infecting begomoviruses have also been detected in weeds like *Datura metel* and *Solanum miasum*, which may play an important role in the spread of these viruses (Sivalingam and Varma 2007). Typical symptoms caused by begomovirus on tomato plant in Bahraich district of UP were recorded, and association of ToLCNDV was reported by Khan et al. (2014). Later on, from the same place, Khan et al. (2015) reported the leaf curl symptoms accompanied with puckering, vein swelling, and stunting of the wholetomato plant with the incidence of 85%, and associated begomovirus was confirmed as *Croton yellow vein mosaic virus*.

The first conclusive evidence on the causal virus of ToLCD as geminivirus in India was reported by Muniyappa et al. (1991) and full-length sequencing of *tomato leaf curl virus* (ToLCV) by Srivastava et al. (1995). One mild and one severe isolate of ToLCV from New Delhi with bipartite genomes, sharing 94% identity in the DNA-A component with identical DNA-B components, were reported (Padidam et al. 1995). Four additional ToLCV isolates, three from Bangalore (Chatchawankanphanich et al. 1993; Hong and Harrison 1995) and one from Lucknow (Srivastava et al. 1995), were also reported. Later, many more sequences of begomoviruses associated with ToLCD have been reported from various parts of India (Muniyappa et al. 2000; Kirthi et al. 2002; Chakraborty et al. 2003; Reddy et al. 2005). A considerable level of variability and recombination in ToLCV genome has been reported from several regions of India (Kumar et al. 2008; Pandey et al. 2010; Kumari et al. 2011). Infectivity test of cloned ToLCV genomes showed the DNA-A of the ToLCNDV-severe virus enhance the replication of DNA-B of the *Tomato leaf curl Gujrat virus* (ToLCGV) and vice versa. This resulted in enhanced pathogenicity when DNA-A of ToLCNDV-severe was trans-complemented with ToLCGV DNA-B (Chakraborty et al. 2008). Diversity of β-satellites of ToLCV from various parts of India was investigated (Sivalingam et al. 2010).

Several efforts have been made in India to minimize the losses caused by ToLCD. Major efforts toward identification of sources of resistance (Banerjee and Kalloo 1987; Muniyappa et al. 2002; Tripathi and Varma 2003) and transgenic resistance development (Varma and Praveen, 2006; Raj et al. 2005b; Praveen et al. 2005a, b; Ramesh et al. 2007; Pandey et al. 2009; Singh et al. 2009) have been made in India to minimize the losses caused by this virus.

Chilli (*Capsicum annum*)

Although the chilli leaf curl disease was reported during the 1960s (Mishra et al. 1963; Dhanraj and Seth 1968), recently in the last decade, begomoviruses emerged as a serious threat to chilli cultivation in many chilli-growing areas. Partial genome sequence analysis revealed the virus associated with the chilli leaf curl in India was

a strain of the *Chilli leaf curl virus* from Multan (Mul-98) of Pakistan origin (Senanayake et al. 2006), which shared 95% of sequence identity. Further study on begomovirus associated with chilli from Punjab was found to be similar to the ToLCV from Joydebpur, Bangladesh (Shih et al. 2006). The whole genome sequence including DNA-β satellite of a *Chilli leaf curl virus* from Varanasi was analyzed with agro-infectivity demonstration (Chattopadhyay et al. 2008). *Indian cassava mosaic virus* was reported to be associated with chilli leaf curl disease of chilli from UP by Khan et al. (2011).

10.2.1.3 Malvaceous Vegetable

Okra (*Abelmoschus esculentus*)
The whitefly-transmitted begomoviruses are a serious problem for okra cultivation in many parts of India. The most common disease of okra caused by *Bhindi yellow vein mosaic virus* (BYVMV) was first reported by Kulkarni in 1924 from Bombay province of India, and later it was shown to belong to begomovirus (Harrison et al. 1991). It is characterized by different degrees of chlorosis and yellowing of veins and veinlets, smaller leaves, fewer and smaller fruits, and stunting (Venkataravanappa et al. 2012). Infection of 100% plants in a field is quite usual with yield loss ranging between 50 and 94% (Fajinmi and Fajinmi 2010). Apart from BYVMV, okra leaf curl and okra enation leaf curl diseases (OLCD and OELCD) result in huge losses in okra cultivation (Venkataravanappa et al. 2013). The loss in yield, due to YVMD and/or OELCD in okra, was found ranging from 30 to 100% depending on the age of the plant at the time of infection (Singh 1996). In India, OELCD was first reported from Bangalore (Karnataka) during the early 1980s, causing yield loss up to 80–90% (Singh 1996). The characteristic symptoms of OELCD include leaf curling, vein thickening, and a decrease in the leaf surface area. Moreover, the infected plants become severely stunted with fruits being small, deformed, and unfit for marketing (Sanwal et al. 2014). Mixed infection with the begomoviruses causing BYVMD and OLCD results in severe disease and complete loss in yield. The bhindi yellow vein mosaic disease is caused by a complex consisting of the monopartite begomovirus BYVMV and a small satellite DNA-β component. Alone BYVMV can infect bhindi and produces only mild leaf curling in the host. However, the typical symptom of disease is shown when BYVMV is agro-inoculated with DNA-β to bhindi (Jose and Usha 2003).

10.2.2 Legume Crops

Yellow or golden mosaic diseases (YMD) are major limitations in production of grain legumes in India. YMD was first reported in India by Nariani (1960). Since, then it has spread at alarming proportions and causes up to 85–100% yield loss (Nene 1973). The economic yield loss per annum in legumes (black gram, mung bean, and soybean) was estimated to be $300 million due to the YMD (Varma et al. 1992; Varma and Malathi 2003). YMD has emerged as a major threat to the

production of a variety of leguminous vegetables, including french bean (*Phaseolus vulgaris*), cluster bean (*Cyamopsis tetragonoloba*), hyacinth bean (*Lablab purpureus*) and mung bean (*V. radiata*) in Indian subcontinent (Varma et al. 2011).

The initial symptoms of YMD appear in the form of irregular yellow patches which coalesce to form larger patches of bright yellow or golden color. Generally, diseased plants are often stunted, and in severe cases, the entire leaves turn yellow or golden and plants bear few flowers and smaller pods with immature seeds. The disease is mostly limited to the family Leguminosae and a few plants in families Compositae, Gramineae, Cruciferae and Caricaceae (Nene 1973). The most serious diseases include the bean golden mosaic (BGMD), cowpea golden mosaic (CPGMD), and yellow mosaic of mung bean (MYMD). CPGMD was first detected in the Indian subcontinent in cowpea germplasm introduced from West Africa in 1978, and by 1984, it emerged as a major problem of cowpea cultivation in northern India. In the Indian subcontinent, the disease is caused by a minor variant of *Mung bean yellow mosaic India virus* (MYMIV), showing the association of different begomoviruses with CPGMD in Africa and Asia (Varma et al. 2011). Black gram plants were severely affected with *Ageratum enationvirus* in UP with the incidence of 68% (Khan et al. 2014).

In India, the whitefly-transmitted begomoviruses with bipartite genomes have been found associated with the YMD (Honda and Ikegami 1986; Vanitharani et al.1996; Mandal et al. 1997; Karthikeyan et al. 2004) which are classified into four major species: (1) *Mung bean yellow mosaic India virus* (MYMIV), (2) *Mung bean yellow mosaic virus* (MYMV), (3) *Dolichosyellow mosaic virus*, and (4) *Horse gram yellow mosaic virus* (Qazi et al. 2007). The MYMV and MYMIV are the most prevalent and infect several leguminous species posing a serious threat to the legume production in the country. Studies on MYMIV showed higher (>90%) nucleotide sequence identity between different isolates with their distinct host preferences (Varma et al. 1992; Mandal and Varma 1996). Both genomic components (DNA-A and DNA-B) of MYMIV from black gram were cloned (Varma et al. 1991), and the infectivity of cloned genome was confirmed (Mandal et al. 1997). A bipartite begomovirus associated with cowpea mosaic disease was characterized which has the DNA-A of MYMIV and DNA-B similar to MYMV (John et al. 2008). In contrast, another begomovirus isolated from black gram showed DNA-A, a variant of MYMV, and DNA-B, a variant of MYMIV (Haq et al. 2011).

10.2.3 Fiber Crop

10.2.3.1 Cotton (*Gossypium* spp.)

The cotton leaf curl disease is one of the most damaging diseases of cotton in India. Leaf curling, darkened veins, vein swelling, and enations on the undersides of leaves that frequently develop into cup-shaped, leaflike structures are the characteristic symptoms induced by the virus (Briddon and Markham 2001). Variation in symptoms exhibited by the virus in fields are commonly noticed (Sharma 2002). This disease was first recorded from Punjab and Rajasthan in the areas bordering Punjab

province in Pakistan (Varma et al. 1993; Rishi and Chauhan 1994). However, the association of a begomovirus with cotton leaf curl was established in the early 1990s (Varma et al. 1993). Since then, the disease has spread to almost the entire cotton-growing areas in Rajasthan, Punjab, and Haryana (Rishi and Chauhan 1994; Ahuja et al. 2007; Narula et al.1999) in North India and in Karnataka in South India (Nateshan et al. 1996). The disease incidence in some areas was reported up to 97%. Sharma (2002) reported 17.48% disease incidence causing reduction in boll weight (17.5%), in seed weight (32.67%), and in seed (33.8%).

The disease is transmissible by whitefly (*B. tabaci*) and grafting. The host range is confined to families Malvaceae, Solanaceae, Fabaceae, and Cucurbitaceae (Nateshan et al. 1996; Radhakrishnan 2002; Sharma and Rishi 2003). Several monopartite begomoviruses with a betasatellite have been reported to be associated with the disease (Kirthi et al. 2002). At least four begomoviruses are known to be associated with cotton leaf curl disease in India: (1) *Cotton leaf curl Rajasthan virus* (CLCuRV), (2) *Cotton leaf curl Multan virus*, (3) *Cotton leaf curl Kokhran virus* (CLCuKV), and (4) *Tomato leaf curl Bangalore virus* (Ahuja et al. 2007). Another isolate of cotton leaf curl (Hissar 2) from Haryana was reported with 97.3% amino acid sequence identity with *Pakistan cotton leaf curl virus* (Sharma et al. 2005). In 2010, two recombinant isolates with other begomoviruses (SG01 and VSG02) from Rajasthan were reported (Kumar et al. 2010). Genetic variability and infectivity in begomoviruses associated with CLCuD in India have been demonstrated (Kirthi et al. 2004; Radhakrishnan, 2002). Research work on cotton leaf curl is summarized by Sharma and Rishi (2007).

10.2.4 Other Crops

10.2.4.1 Cassava (*Manihot esculenta*)

Cassava mosaic disease (CMD) was reported by Alagianagalingam and Ramakrishnan in 1966 (Alagianagalingam and Ramakrishnan 1966) and has subsequently become prevalent in cassava-growing area of southern India (Calvert and Thresh 2002). Two bipartite begomoviruses, *Indian cassava mosaic virus* (ICMV) and *Sri Lankan cassava mosaic virus* (SLCMV), have been found associated with CMD (Hong et al. 1993; Patil et al. 2005). The efficiency of transmission by its vector whitefly in controlled experiment was 85% and took about 25 days for symptom expression (Duraisamy et al. 2012). Studies on biodiversity showed SLCMV was widespread in southern regions of India as compared to ICMV. The infectivity of cloned components of the virus was demonstrated on cassava (Dutt et al. 2005). Further studies on CMD showed high variability and occurrence of recombinants among cassava-infecting begomoviruses (Patil et al. 2005; Rothenstein et al. 2006).

Papaya (*Carica papaya*)

Papaya leaf curl disease has been reported from India by Nariani in 1956. The papaya leaf curl disease emerged as a serious threat to papaya growers in northern states of India. The causal virus of this disease, *Papaya leaf curl virus*, is a

whitefly-transmitted begomovirus (Saxena et al. 1998a, b; Raj et al. 2008). Further molecular characterization and sequence diversity in the virus from different parts of India have been reported (Krishna Reddy et al. 2010). Dubey et al. (2015a, b) reported the survey, symptomology, molecular characterization, and transmission of *Papaya leaf curl virus* on papaya plant from eastern UP, India. A mixed infection of *leaf curl virus* and *ringspot virus* is common in many regions and results in complete loss of the crop.

10.3 Factors Responsible for Emergence of Begomoviruses

In recent years, there has been surge in emergence of diseases caused by begomoviruses in various crops specially cucurbits and solanaceous vegetables. There can be (a) expansion of viruses and their insect vectors due to natural and human interference and (b) evolution of viruses making it more virulent. Several major factors leading to emergence of new begomovirus diseases include introduction of viruses, introduction of susceptible crops or genotypes, change in vector population, recombination, weather, and new agricultural practices.

10.3.1 Introduction of Viruses

Domestication of crop along with international trade of plant and plant products has enhanced the virus encounters between viruses and plants and has played an important role in the emergence of begomoviruses. In India, the movement of infected potato seed tubers resulted in the spread of ToLCNDV in potato in serious proportions.

10.3.2 Introduction of Susceptible Crop or Genotypes

Introduction of exotic germplasm as part of crop improvement program has also resulted into introduction of gene for susceptibility (Varma and Malathi 2003). Introduction of exotic accessions of cowpea from West Africa resulted in introduction of gene for susceptibility for cowpea golden mosaic disease to popular cowpea varieties. The causal virus of this disease was later confirmed as a variant of MYMIV (Roy and Malathi 2001). Similarly, leaf curl of okra emerged through accession introduction from West Africa. Mixed infection of yellow vein mosaic and leaf curl in okra resulted in severe losses to the farmers (Varma and Malathi 2003). In India, cassava crop, free from begomoviruses, was introduced from Latin America, however due to disease caused by endemic viruses, it becomes severely affected by cassava mosaic disease in the southern part of India (Varma et al. 2011).

10.3.3 Change in Vector Population

The international spread of B biotype of *B. tabaci* through transcontinental transport of ornamental and other host crops is one of the reasons for emergence of begomoviruses. The polyphagous nature of the B biotype is the cause of encounter between begomoviruses present in the indigenous flora and susceptible cultivated crops resulting into emergence of new begomoviruses (Ha et al. 2008). Also there has been a change in the biology of *B. tabaci* in India. Earlier in the 1970s, the peak of whitefly population was during rainy season; however, in the 1990s, the population buildup started before the start of rainy season and remained active throughout the year. This change in pattern of whitefly population resulted in infection of crops which were not infected earlier (Varma et al. 2011). The change in winter climate in northern India due to global warming resulted in increase in whitefly population during the early growth period of cucurbits (Varma and Malathi 2003). Movement of whiteflies which depends on the availability of the susceptible host, right stage, and microclimate of the crop also plays important role in the spread of begomoviruses. Emergence of cotton leaf curl in India is an example of the spread of the virus disease due to movement of whiteflies from Pakistan to neighboring Indian states growing their preferred host cotton.

10.3.4 Recombination

Genetic variations in plant viruses can be brought about through recombination. During mixed infection, genetic exchange either through reassortment or recombination provides begomoviruses with a tool to combine sequences from different origins which might help them to evolve quickly. Reassortment of DNA-B component with DNA-A molecules of different viruses has not only resulted in gain of virulence but also expanded host range (Idris et al. 2008; Seal et al. 2006). Recombination between the viral and subviral DNAs can also occur which might play an additional evolutionary role by generating component diversity (Nawaj-ul-Rehman and Fauquat 2009). Considerable molecular diversity occurs in the betasatellites associated with ToLCD in India (Sivalingam et al. 2010).

10.3.5 New Agricultural Practices

Farming techniques such as monoculture of crops and overlapping plantings result in exposure of potential host plants to high population of whiteflies and a diverse population of begomoviruses. Introduction of summer crop of mung bean under irrigated conditions resulted in the unseasonal appearance of MYMIV (Varma et al. 1992). Advancement of potato planting time in North India plains resulted in potato leaf curl disease caused by ToLCNDV (Varma and Malathi 2003) as it coincides with the period of increased population of *B. tabaci*. The use of excessive

insecticides has also resulted in the development of resistance in whitefly population leading to increase in vector population.

10.3.6 Weather

Increase in temperature due to global warming has affected the distribution of the viruses and vectors resulting into new virus-host combinations. The occurrence of begomoviruses is closely associated with its vector whitefly, whose distribution is affected by climatic conditions. The *B. tabaci* population is highest at high temperature and low rainfall (Morales and Jones 2004). A drastic change in whitefly population in different seasons has been observed in New Delhi conditions (Tripathi and Varma 2002) which can be correlated with weather parameters.

10.4 Concluding Remarks

Diseases caused by begomoviruses are a serious concern for growing various crops particularly cucurbits and solanaceous vegetables throughout India. In the recent couple of decades, a large number of occurrences and emergence of new strains have been reported from India. This increased emergence of begomoviruses in India is probably due to its tropical climatic condition supporting the year-round survival of the whitefly vector and intensive crop cultivation practiced including indiscriminate use of chemical pesticides. The polyphagous nature of the vector whitefly and the mixed cropping practices prevalent in India might be responsible factors for widening and overlapping the host range for begomoviruses. This situation is likely to result in an emergence of new or recombinant viruses which could be more virulent than already existing in the ecosystem. The emergence of a large number of β-satellites and α-satellites associated with begomoviruses in India makes the disease situation more complex. The changing scenario suggests higher occurrence of disease epidemics caused by begomovirus. Therefore, there is an immediate need to understand the major responsible factors leading to disease epidemics caused by begomoviruses in diverse agroecosystems. In addition, the comprehensive studies on epidemiology of begomoviruses and their interaction with whitefly vector in specific region are needed. Profiling of emerging and reemerging diseases caused by begomoviruses through regular surveillance in the ecosystem is required to take timely suitable measures to prevent or manage the recurrence of these diseases.

To manage begomoviruses, the host plant resistance, natural or transgenic, is being the best solution. However, this alone will not last longer due to emergence of new viruses or strains. Therefore, better crop management along with host resistance should be used for effective viral disease management as well as holding the host resistance for longer duration. A better awareness about diseases caused by begomoviruses, their identification, ecology, and management to the agricultural extension workers, and the proactive involvement of concerned national and state

departments and private companies at the farm level is required for effective and sustainable begomovirus disease management.

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Begomovirus in Taiwan

11

Wen-Shi Tsai and Chien-Jui Huang

Abstract

Begomoviruses (whitefly-transmitted geminiviruses) have been considered as the most important pathogen constraining crop productions in tropical and subtropical regions. In Taiwan, the earliest *Begomovirus*-like disease is reported in 1946. In 1981, *Begomovirus* disease was discovered and confirmed to damage cropping of tomato plants, constraining the production after the 1990s. Consequentially, tomato resistance breeding as well as transgenic resistance was conducted against the disease. Most *Begomovirus* diseases on crops and weeds were observed following the molecular identification in this century. Up to date, begomoviruses were detected in cucurbits, legumes, papaya, passion fruit, tomato, pepper, and weeds in Taiwan. As *Begomovirus* diseases cause significant yield losses in crops in Taiwan, net protection has been suggested to reduce the usage of pesticides. Combining the resistant cultivars and net protection has also revealed the best management of tomato *Begomovirus* disease. This strategy may be useful for controlling begomoviral diseases in Taiwan and worldwide.

Keywords

Ageratum • Cucurbits • Ceylon Pouzolzia • Legume • Papaya • Passion fruit • Pepper • Ornamental plant • Sweet potato • Taiwan *Begomovirus* • Tomato • Velvet bean

W.-S. Tsai (✉) • C.-J. Huang
Department of Plant Medicine, National Chiayi University,
Chiayi, Taiwan, Republic of China
e-mail: wenshi.tw@mail.ncyu.edu.tw

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11.1 Introduction

In the last decades, whitefly (*Bemisia tabaci*)-transmitted begomoviruses have emerged as a major disease in agricultural areas worldwide, due to the increasing population of virus transmission vector – whitefly (Jones 2003; Hsieh et al. 2006: Brown et al. 2012). They revealed major symptoms of leaf curling, yellow mosaic, and vein yellowing on virus-infected plants (Varma et al. 2011). So far, begomoviral diseases have been found to damage crop productions of cassava, cucurbits, legumes, malvaceous, solanaceous, papaya, and sweet potato, causing significant yield loss with up to 100% frequently (Makkouk et al. 1979; Picó et al. 1996; Polston and Anderson 1997; Tsai et al. 2002; Varma et al. 2003, 2011). *Begomovirus* contains either monopartite (DNA-A) or bipartite (DNA-A and DNA-B) ssDNA which encapsided in a twin particle (Brown et al. 2012). The DNA-A contains two virus-sense (AV1 and AV2) and four complementary-sense (AC1–AC4) open reading frames (ORFs), whereas DNA-B has one virus-sense (BV1) and one complementary-sense (BC1) ORF. The additional AV3 ORF has been also determined in some begomoviruses (Wang et al. 2014). Based on 91% DNA-A nucleotide sequence identity of the demarcation criteria for *Begomovirus* species, more than 300 *Begomovirus* species have been reported (Brown et al. 2015). Highly diverse begomoviruses were discovered in agricultural crops, such as more than 21 *Begomovirus* species in cucurbits, 30 in legumes, and 117 in solanaceous crops (Varma et al. 2011; Tsai et al. 2013b), resulting in the difficulty of the disease management.

As early as 1946 in Taiwan, tobacco leaf curl disease is reported to be a *Begomovirus*-like disease (Matsumoto 1946). In 1981, tomato leaf curl disease was observed and consequently identified *Begomovirus* as the causing agent by morphological, serological, and biological methods (Green et al. 1987). Consequentially, tomato breeding resistant to *Begomovirus* disease was also conducted in the 1990s. However, most *Begomovirus* diseases were observed in agricultural crops and weeds after year 2000, and molecular identification was also conducted following the discovery of the disease (Table 11.1).

11.2 Solanaceous Begomoviruses in Taiwan

A *Begomovirus*-like disease, tobacco leaf curl disease, has been reported in 1946 (Matsumoto 1946). However, no molecular data of *Begomovirus* is available afterward from natural tobacco plants. This may be due to the reduction of tobacco production in Taiwan or other reasons. So far, begomoviruses in four species, *Ageratum yellow vein virus* (AYVV), *Tomato leaf curl Hsinchu virus* (ToLCHsV), *Tomato leaf curl Taiwan virus* (ToLCTV), and *Tomato yellow leaf curl Thailand virus* (TYLCTHV), were detected in tomato crops in Taiwan (Tsai et al. 2011b). One (TYLCTHV) of them also infects pepper plant (Shih et al. 2010). The diseased tomato plant revealed symptoms of yellowing, leaf curling, and stunting (Fig. 11.1). The symptoms of yellow vein and spot were observed on the TYLCTHV infecting pepper plants (Fig. 11.2). No *Begomovirus* disease was observed in other *Solanaceae* crops in the natural field.

Table. 11.1 Full-length viral sequences of begomovirus isolates collected from Taiwan

Virus	Host	Location	Isolate	Year collected	Abbreviation	GenBank Accession No.		References
						DNA-A	DNA-B	
Ageratum yellow vein virus	*Ageratum* sp.	Pingtung	PD		AYVV-TW[TW:PT:PD]	AF327902		
		Taoyuan	Taoyuan3	2000	AYVV-SG[TW:TY:T:00]	DQ866134		
	A. conyzoides	Hualian	Hualien4	2000	AYVV-Hua[TW:HL:Hua4:00]	DQ866132		
		Nantou	NT	2007	AYVV-SG[TW:NT:07]	EF458639		
		Tainan	Tainan	1999	AYVV-TW[TW:TN:99]	AF307861		
	A. houstonianum	Hualian	Hualien2	2000	AYVV-Hua[TW:HL:Hua2:00]	DQ866133		
	Eustoma grandiflorum	Unknown	Lis 1	2010	AYVV-TW[TW:Lis:10]	JN703794		
	Solanum lycopersicum	Hsinchu	HS7	2003	AYVV-Hsi[TW:HC:Tom:03]	DQ866124		Tsai et al. (2011a)
Euphorbia leaf curl virus isolate	*Passiflora edulis* f. flavicarpa	Nantou	PF1	2011	EuLCuV-[TW:NT:Pas:PF1:11]	KC161185		Cheng et al. (2014)
Lisianthus enation leaf curl virus	*Eustoma grandiflorum*	Yunlin	BG-1	2015	TYLCTHV-[TW:YL:Lis:BG1:15]	LC091538		
			BG-9	2015	TYLCTHV-[TW:YL:Lis:BG9:15]	LC091539		
Papaya leaf curl Guangdong virus	*Euphorbia pulcherrima*	Unknown	Pt9	2005	PaLCuGdV-[TW:Eup:05]	JN703795		

(continued)

Table. 11.1 (continued)

Virus	Host	Location	Isolate	Year collected	Abbreviation	GenBank Accession No.		References
						DNA-A	DNA-B	
	E. grandiflorum	Yunlin	BG-2	2015	PaLCuGdV-[TW:YL:BG2:15]	LC089766		Chen et al. (2016a, b)
		Changhua	FY-0	2015	PaLCuGdV-[TW:CH:FY0:15]	LC089013		Chen et al. (2016a, b)
			FY-2	2015	PaLCuGdV-[TW:CH:FY2:15]	LC089014		Chen et al. (2016a, b)
	Carica papaya	Southern Taiwan		2002	PaLCuGdV-[TW:Pap:11]	AY183472[a]		
	P. edulis f. flavicarpa	Nantou	PF1	2011	PaLCuGdV-[TW:NT:Pas:PF1:11]	KC161184		Cheng et al. (2014)
	P. sp.	Unknown	BXG4	2014	PaLCuGdV-[TW:Pas:BXG4:11]	KP876482		Quarantined by mainland China
Pouzolzia golden mosaic Guangdong virus	*Pouzolzia zeylanica*	Hsinchu	CH14W1	2014	PouMGdV-[TW:HC:CH14W1:14]	KU358527		
		Miaoli	ML13W1	2013	PouMGdV-[TW:ML:ML13W1:13]	KF927128		
		Pingtung	PT14W1	2014	PouMGdV-[TW:HC:PT14W1:14]	KU358528		
		Yunlin	Yu14W1	2014	PouMGdV-[TW:YL:Yu14W1:14]	KU358529		
Squash leaf curl Philippines virus	*Benincasa hispida*	Chaunhua	Wg1	2005	SLCuPV-[TW:CH:Ben:05]	EU310406		Liao et al. (2007)
	Cucumis melo	Yunlin	YL	2007	SLCuPV-[TW:YL:07]	EU479710	EU479711	
	Cucurbita moschata	Tainan	PA1	2001	SLCuPV-[TW:TN:PA1:01]	DQ866135		Tsai et al. (2007)
			PK5	2005	SLCPV-[TW:TN:PK5:05]	EF199774		Tsai et al. (2007)
	Sechium edule	Hualien	1–1	2010	SLCuPV-[TW:HL:1–1:10]	JF746195	JF746196	Tsai et al. (2011b)

Tomato leaf curl Hsinchu virus	*S. lycopersicum*	Hsinchu	CT1	2000	ToLCHsV-[TW:HC:CT1:00]	DQ866131		Tsai et al. (2011a)
Tomato leaf curl New Delhi virus	*Cucumis melo* var. makuwa cv. Silver light	Yilan	OM	2007	ToLCNDV-[TW:YiL:Mel:07]	GU180095	GU180096	Chang et al. (2010)
Tomato leaf curl Taiwan virus	*S. lycopersicum*	Changhua	Ch1	1998	ToLCTV-A[TW:CH:Ch1:98]	DQ866125		Tsai et al. (2011a)
		Chiayi	LJC14-2	2004	ToLCTV-C[TW:CY:LJC14-2:04]	DQ866128		Tsai et al. (2011a)
		Hsinchu	C1	2000	ToLCTV-A[TW:HC:C1:00]	DQ866126		Tsai et al. (2011a)
		Hualien	GT6-4	2007	ToLCTV-B[TW:HL:GT6-4:07]	DQ866123		Tsai et al. (2011a)
			H4-2	2007	ToLCTV-A[TW:HL:HT4-2:07]	GU723726		Tsai et al. (2011a)
			H7-1	2007	ToLCTV-A[TW:HL:H7-1:07]	GU723721		Tsai et al. (2011a)
			H7-3	2007	TYLCTHV-B[TW:HL:H7-3:07]	GU723722		Tsai et al. (2011a)
			H8-1	2007	ToLCTV-A[TW:HL:H8-1:07]	GU723723		Tsai et al. (2011a)
			H8-5	2007	ToLCTV-A[TW:HL:H8-5:07]	GU723724		Tsai et al. (2011a)
			H9-1	2007	TYLCTHV-B[TW:HL:H9-1:07]	GU723725		Tsai et al. (2011a)
			HT7	2000	ToLCTV-A[TW:HL:HT7:00]	DQ866129		Tsai et al. (2011a)
		Nantou	NT1-1	2007	ToLCTV-A[TW:NT:NT1-1:07]	GU723708		Tsai et al. (2011a)
			NT1-4	2007	ToLCTV-A[TW:NT:NT1-4:07]	GU723709		Tsai et al. (2011a)
		Tainan		1998	ToLCTV-[TW:TN:98]	U88692		
			15–7	2007	ToLCTV-A[TW:TN:15-7:07]	GU723711		Tsai et al. (2011a)
			87-1	2007	ToLCTV-A[TW:TN:87-1:07]	GU723713		Tsai et al. (2011a)
			FD2-2	1999	ToLCTV-A[TW:TN:FD2-2:99]	DQ866122		Tsai et al. (2011a)
			J1-8	2007	ToLCTV-A[TW:TN:J1-8:07]	GU723712		Tsai et al. (2011a)
			LY5	2007	ToLCTV-A[TW:TN:LY5:07]	GU723710		Tsai et al. (2011a)

(continued)

Table. 11.1 (continued)

Virus	Host	Location	Isolate	Year collected	Abbreviation	GenBank Accession No.		References
						DNA-A	DNA-B	
		Taitung	KD	2000	ToLCTV-A[TW:TT:KD:00]	DQ866130		Tsai et al. (2011a)
			T2-1	2007	ToLCTV-A[TW:TT:T2-1:07]	GU723729		Tsai et al. (2011a)
			T2-4	2007	ToLCTV-B[TW:TT:T2-4:07]	GU723730		Tsai et al. (2011a)
			T3-3	2007	ToLCTV-A[TW:TT:T3-3:07]	GU723727		Tsai et al. (2011a)
			T3-6	2007	ToLCTV-A[TW:TT:T3-6:07]	GU723728		Tsai et al. (2011a)
		Taoyuan	T1	2000	ToLCTV-A[TW:TY:T1:00]	DQ866127		Tsai et al. (2011a)
		Yilan	LS5	2007	ToLCTV-A[TW:YiL:LS5:07]	GU723716		Tsai et al. (2011a)
			LS8	2007	TYLCTHV-B[TW:YiL:LS8:07]	GU723717		Tsai et al. (2011a)
			NH10-6-1	2007	ToLCTV-A[TW:YiL:NH10-6-1:07]	GU723718		Tsai et al. (2011a)
			NH10-6-6	2007	ToLCTV-A[TW:YiL:NH10-6-6:07]	GU723719		Tsai et al. (2011a)
			YL10-3-1	2007	ToLCTV-A[TW:YiL:YL10-3-1:07]	GU723720		Tsai et al. (2011a)
			YL7-1	2007	ToLCTV-A[TW:YiL:YL7-1:07]	GU723714		Tsai et al. (2011a)
			YL7-8	2007	TYLCTHV-B[TW:YiL:YL7-8:07]	GU723715		Tsai et al. (2011a)
Tomato yellow leaf curl Thailand virus	*Capsicum annuum*	Chiayi	SG4-3	2009	TYLCTHV-B[TW:CY:SG4-3:09]	GU208517	GU208521	Shih et al. (2010)
		Kaohsiung	LJ3-5	2009	TYLCTHV-B[TW:KH:LJ3-5:09]	GU208516	GU208520	Shih et al. (2010)
		Pingtung	LG6-2	2009	TYLCTHV-B[TW:PT:LG6-2:09]	GU208515	GU208519	Shih et al. (2010)
		Tainan	P2-4	2007	TYLCTHV-B[TW:TN:P2-4:07]	EU249457	EU249458	Shih et al. (2010)
		Yunlin	HW2-2	2009	TYLCTHV-B[TW:YL:HW2-2:09]	GU208518	GU208522	Shih et al. (2010)

	S. lycopersicum	Chiayi	J2-6	2007	TYLCTHV-B[TW:CY:J2-6:07]	GU723739		Tsai et al. (2011a)
			J8-2	2007	TYLCTHV-B[TW:CY:J8-2:07]	GU723740		Tsai et al. (2011a)
		Hsinchu	HS3-1	2007	TYLCTHV-B[TW:HC:HS3-1:07]	GU723734		Tsai et al. (2011a)
			HSG1-6	2007	TYLCTHV-B[TW:HC:HSG1-6:07]	GU723733		Tsai et al. (2011a)
			HSG5-3	2007	TYLCTHV-B[TW:HC:HSG5-3:07]	GU723732		Tsai et al. (2011a)
		Hualien	H5-5	2007	TYLCTHV-B[TW:HL:H5-5:07]	GU723748		Tsai et al. (2011a)
			H6-4	2007	TYLCTHV-B[TW:HL:H6-4:07]	GU723749		Tsai et al. (2011a)
		Nantou	NT1-3	2007	TYLCTHV-B[TW:NT:NT1-3:07]	GU723738		Tsai et al. (2011a)
			NT3-5	2007	TYLCTHV-B[TW:NT:NT3-5:07]	GU723735		Tsai et al. (2011a)
			NT4-1	2007	TYLCTHV-B[TW:NT:NT4-1:07]	GU723736		Tsai et al. (2011a)
			NT5-4	2007	TYLCTHV-B[TW:NT:NT5-4:07]	GU723737		Tsai et al. (2011a)
		Pingtung	PT3-7	2007	TYLCTHV-B[TW:PT:PT3-7:07]	GU723747		Tsai et al. (2011a)
			PT4-1	2007	TYLCTHV-B[TW:PT:PT4-1:07]	GU723745		Tsai et al. (2011a)
			PY2-7	2007	TYLCTHV-B[TW:PT:PY2-7:07]	GU723746		Tsai et al. (2011a)

(continued)

Table. 11.1 (continued)

Virus	Host	Location	Isolate	Year collected	Abbreviation	GenBank Accession No.		References
						DNA-A	DNA-B	
		Tainan	1–1	2007	TYLCTHV-B[TW:TN:1-1:07]	GU723744		Tsai et al. (2011a)
			CK18	2007	TYLCTHV-B[TwW:TN:CK18:07]	EF577264	EF577265	Jan et al. (2007)
			CK2	2006	TYLCTHV-B[TW:TN:CK18:06]	EF577264	EF577265	Tsai et al. (2011a)
			D8	2007	TYLCTHV-B[TW:TN:D8:07]		GU723752	Tsai et al. (2011a)
			LY2	2007	TYLCTHV-B[TW:TN:LY2:07]	GU723741	GU723753	Tsai et al. (2011a)
			LY3	2007	TYLCTHV-B[TW:TN:LY3:07]	EF577266	EF577267	Jan et al. (2007)
			LY5	2007	TYLCTHV-B[TW:TN:LY5:07]	GU723742	GU723754	Tsai et al. (2011a)
			TM32	2007	TYLCTHV-B[TW:TN:TM32:07]	GU723743	GU723755	Tsai et al. (2011a)
		Taipei	TP6-5	2007	TYLCTHV-B[TW:TP:TP6-5:07]	GU723731		Tsai et al. (2011a)
		Taitung	TC2	2007	TYLCTHV-B[TW:TT:TC2:07]	GU723750		Tsai et al. (2011a)
Velvet bean golden mosaic virus	*Mucuna pruriens*	Hualien	1–6	2014	VbGMV-[TW:HL:1-6:14]	KU569578		
			2–3	2014	VbGMV-[TW:HL:2-3:14]	KU569579		
			3–3	2014	VbGMV-[TW:HL:3-3:14]	KU569580		
			3–6	2014	VbGMV-[TW:HL:3-6:14]	KU569581		
			5–9	2014	VbGMV-[TW:HL:5-9:14]	KU569582		
			6–5	2014	VbGMV-[TW:HL:6-5:14]	KU569583		

[a]Only the sequence of viral coat protein gene is available

Fig. 11.1 Diseased tomato plants revealed typical begomovirus disease symptoms in the fields in Taiwan. The *left* picture is diseased tomato plant which revealed moderate symptoms of yellowing, leaf curling, puckering, and stunting. The *right* picture is field plants showing severe symptoms

Fig. 11.2 Yellow vein and spot symptoms on pepper plant inoculated by *Tomato yellow leaf curl Thailand virus* Taiwan isolate

As early as 1981, tomato leaf curl disease has been observed in Tainan County, southern Taiwan. The causal agent was confirmed to be a *Begomovirus* by observing geminate particles through electron microscope and an immunoassay with an antibody against tobacco leaf curl *Begomovirus* (Green et al. 1987). The disease had spread throughout the island of Taiwan in the 1990s, causing significant tomato yield loss (Shih et al. 1995, 2004). In 1997, a viral genomic sequence was completed and classified as a new *Begomovirus*, named as *Tomato leaf curl Taiwan virus* (ToLCTV) based on the ICTV classification criteria (Brown et al. 2012). This is the first molecular-identified tomato-infecting *Begomovirus* in Taiwan. Later on, ToLCTV isolates were confirmed as a monopartite *Begomovirus* and can be classified into three strains (Tsai et al. 2011b). Strain A is distributed throughout the country, whereas the distribution of strains B and C were restricted. So far, ToLCTV has been also distributed in Southeast China (Mugiira et al. 2008). The host range of ToLCTV has been tested to tomato (*Solanum* sp.), *Datura stramonium*, *Lonicera japonica*, *Nicotiana benthamiana*, *Petunia hybrida*, *Physalis floridana*, and *S. melongena* by grafting and whitefly transmission (Green et al. 1987). Consequentially, AVRDC, the World Vegetable Center, a nonprofit international organization in Taiwan, initiated the *Begomovirus*-resistant tomato breeding program and identified the ToLCTV resistance in accessions of *S. chilense*, *S. habrochaites* f. *glabratum*, *S. lycopersicum*, and *S. peruvianum* (Green et al. 2007). A single dominant gene responsible for the resistance in H24, derived from *S. habrochaites* B6013, was mapped to the bottom of chromosome 11 and named *Ty-2* (Hanson et al. 2006). *Ty-2* has been used in tomato breeding programs publicly and privately, and commercial cultivars carrying this resistance were also released for tomato production in Taiwan after 2003.

The second tomato-infecting *Begomovirus*, *Tomato leaf curl Hsinchu virus* (ToLCHsV), was first detected in samples collected in 2000 (Tsai et al. 2011b). The ToLCHsV was limited in Hsinchu area with low detected frequency; only in 4 of 14 *Begomovirus*-positive samples collected in 2000 and 2 of 39 samples in 2001, following not detectable in Taiwan after 2001. Interestingly, the *Ramie mosaic virus* (RamMV) detected in ramie (*Boehmeria nivea* L.) in China (Li et al. 2010) has high genetic identity to ToLCHsV and should be reconsidered as the same *Begomovirus* species. The ToLCHsV/RamMV also infected tobacco, but were not detected in tomato in China. Since RamMV was found to infect ramie in many provinces of China, it may be that ramie represents the original host of ToLCHsV (Li et al. 2010).

In 2003, the third tomato *Begomovirus*, *Ageratum yellow vein virus* Hsinchu strain (AYVV-His), originally named as *Ageratum yellow vein Hualien virus*, was also detected in Hsinchu area (Tsai et al. 2011b). However, the virus was only detected in two collected samples in the area and not detected in the samples collected after 2003. The tomato isolates of AYVV Hsinchu strain were considered as recombinants of ToLCTV and AYVV Hualien strain. The recombinant AYVV isolates were based on ageratum isolate of AYVV Hualien strain and the recombination region (607 nucleotides in length) corresponding to the complete AC4 ORF, the 5′ half of AC1ORF, and the left part of the intergenic region of the ToLCTV strain B tomato isolate GT6 with 92.8% nucleotide identity.

The *Tomato yellow leaf curl Thailand virus* (TYLCTHV) was the fourth tomato *Begomovirus* detected in Taiwan. TYLCTHV was originally distributed in the area of South China, Myanmar, and Thailand (Green et al. 2001; Li et al. 2004; Sawangjit et al. 2005; Guo et al. 2009). However, it was firstly detected in the sample collected in 2005 in Western Taiwan (Jan et al. 2007) and considered as a recently introduced pathogen (Tsai et al. 2011b). The genomic sequences of TYLCTHV Taiwan isolates were revealed to have high homology. Since TYLCTHV has been introduced in 2005, the virus was fast distributed in Western Taiwan and then in eastern Taiwan (Tsai et al. 2011b). Unlike ToLCTV, which did not naturally infect pepper plant in Taiwan, TYLCTHV Taiwan isolates can use pepper as a natural host and infect both tomato and pepper plants in the field (Shih et al. 2010). The disease incidence on pepper was up to 70% in a survey conducted in 2009 in southern Taiwan (Shih et al. 2010). TYLCTHV has been considered as a bipartite and mechanical transmissible *Begomovirus* (Tsai et al. 2011b). Although TYLCTHV DNA-A alone can also infect the host plants and cause symptoms, the symptom development is delayed, and it is tested as not mechanical transmissible (Tsai et al. 2011b).

Based on the short period of the appearance of AYVV and ToLCHsV, both of them were considered as viruses occasionally in Taiwan (Tsai et al. 2011b). ToLCTV is the predominant virus before the emergence of TYLCTHV and should be considered as an endemic tomato-infecting *Begomovirus* in Taiwan based on the higher genetic diversity (Tsai et al. 2011b). Since the TYLCTHV was first detected in western Taiwan in 2005, it has become prevalent across all tomato-growing regions of Taiwan. Consequentially, TYLCTHV is likely to displace ToLCTV in many parts of Taiwan (Tsai et al. 2013a). This phenomenon has been also observed in the introduction of *Tomato yellow leaf curl virus* into a new area (Sanchez-Campos et al. 1999; Davino et al. 2006; Gilbertson et al. 2007). Mixed infection of two begomoviruses in a tomato plant can be significantly detected in collected samples in Taiwan, including ToLCTV with the not common species, AYVV or ToLCHsV (Tsai et al. 2011b). When the TYLCTHV has been distributed in Taiwan, the mixed infection of ToLCTV and TYLCTHV continued to be detected in a significant portion in diseased tomato plants (Tsai et al. 2013a). This may promote the presence of recombinant *Begomovirus* in the area.

Unfortunately, ToLCTV-resistant sources including the *Ty-2* resistance were not efficient against TYLCTHV (Jan et al. 2007; Tsai et al. 2011b). To face the difficult situation of tomato leaf curl disease in Taiwan, including the virus dynamic changing and no resistance available for TYLCTHV, pyramiding of resistances may provide a solution (Joshi and Nayak 2010) and has been tested in tomato plant (Vidavski et al. 2008). So far, tomato resistances *Ty-1*/*Ty-3*, *Ty-4*, *Ty-5* and the newly discovered resistance from FLA456 are availed for developing tomato cultivars against *Begomovirus* (Zamir et al. 1994; Ji et al. 2007, 2009; Anbinder et al. 2009; Kadirvel et al. 2013). AVRDC, The World Vegetable Center, has been applying molecular markers to pyramid multiple *Ty* genes and develop tomato lines combining *Ty-2* and *Ty-1*/*Ty-3*. These tomato lines revealed great resistance to leaf curl disease in the field in Taiwan in the presence of both ToLCTV and TYLCTHV (Onozato et al. 2013). Lines containing multiple *Ty* genes were also released to public and private

sectors for commercial breeding purpose. Tomato resistances against whitefly vector have been discovered (Channarayappa et al. 1992; Baldin et al. 2005). In the future, those whitefly resistances may be used as another tool for the disease management and composed in the breeding program.

11.3 Cucurbit Begomoviruses in Taiwan

In Taiwan, the cucurbit *Begomovirus* was detected in the field squash samples collected as early as 2001 (Tsai et al. 2007). The squash plant has revealed symptoms of leaf curling, blistering, and yellowing (Fig. 11.3), and a bipartite *Squash leaf curl Philippines virus* (SLCuPV) was identified to be associated with the disease. The SLCuPV isolates were first identified in the Philippines (Kon et al. 2003) and then in Taiwan and were not reported to be identified in other areas as of now. In 2005, wax gourd (*Benincasa hispida*) revealed virus symptoms such as yellowing, vein enation, rugose mosaic, and leaf curling in the field in central Taiwan. Later on, it was also identified to be associated with the SLCuPV (Liao et al. 2007, 2010). In the cucurbit fields of southern Taiwan, severe leaf curl disease was revealed on the several cucurbit crops, and the incidence reached to 92.5% (Liao et al. 2010). The SLCuPV has also been considered as one of the causing agents responsible for the severe disease damage on melon production in 2008. And a ranged 0–100% with in average 28.6% of viruliferous whitefly in the melon field has been also investigated

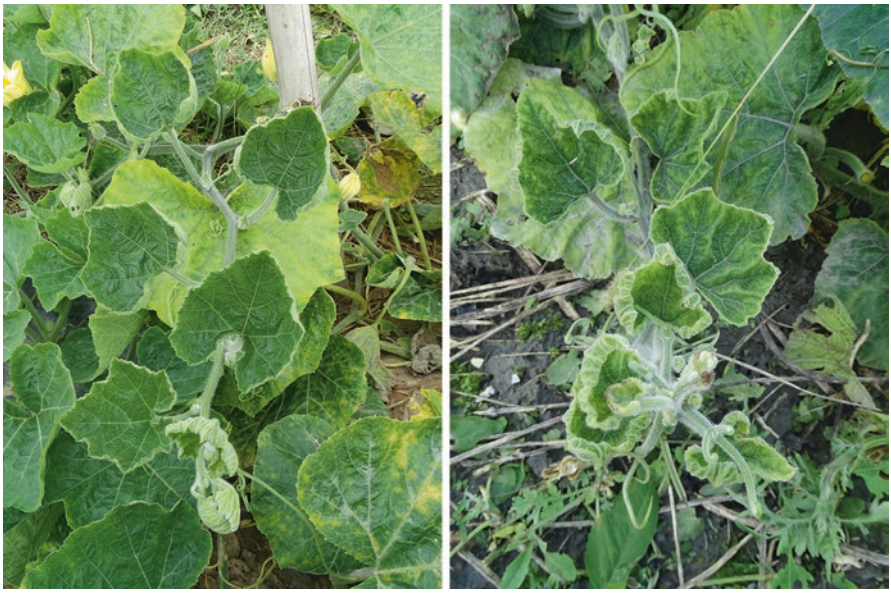

Fig. 11.3 Diseased squash plants reveal typical begomovirus disease symptoms in the fields in Taiwan. The field squash plants are showing symptoms of upward cupping and yellow mosaic, and additional stunting symptom is shown in right picture

(Peng and Tsai 2012). For the efficiency disease control on melon fields, it shows that to prevent the SLCuPV infection in early growing stage is the key role. Up to 50% of melon plants would be infected if there is presence of high population of viruliferous whitefly present in the early growing stage. In comparison to the presence of low population of viruliferous whitefly present in the early growing stage, the disease incidence is much low, even with high population of viruliferous whitefly presenting in later growing stage (Peng and Tsai 2012). Chayote (*Sechium edule*), its young shoots and leaves are used as a commercial vegetable in Taiwan, were also infected by the SLCuPV causing mosaic symptoms on the leaves, and result in about 15% yield loss in 2010 (Tsai et al. 2011a). The TaqMan real-time PCR technique has been developed for virus quantitation in the plant tissue of melon and squash (Kuan et al. 2012).

The second cucurbit-infecting *Begomovirus* identified in Taiwan is the bipartite *Tomato leaf curl New Delhi virus* (ToLCNDV) collected in eastern Taiwan in 2007 (Chang et al. 2010). The diseased melon plant showed mosaic, leaf curling, and puckering symptoms. The virus is mechanical transmissible and can infect at least five cucurbit species. The infected plants of oriental melon (*Cucumis melo* var. *makuwa*), pickling melon (*C. melo* var. *conomon*), bottle gourd (*Lagenaria siceraria*), and cucumber (*C. sativus*) revealed severe mosaic, leaf curling, and puckering symptoms. However, related mild symptoms of mosaic, leaf curling, and mild puckering were observed on the virus-infected zucchini squash (*Cucurbita pepo* var. *zucchini*) and loofah (*Luffa cylindrica*) (Chang et al. 2010). However, the ToLCNDV is rarely detectable in Taiwan.

11.4 Begomoviruses Infect Other Crops in Taiwan

As early as 1985, *Begomovirus* has been observed to cause symptoms of leaf curling and enation on sweet potato resulting in significant yield loss about 45–50% (Chung et al. 1985). The symptom development was more fast and severe in high temperature and became mild or symptomless when the temperature is going lower. The twin particle of virus was examined by electron microscope, but the viral genomic sequence was not available until now.

In 2002, the papaya *Begomovirus* was found in southern Taiwan (Chang et al. 2003). The diseased papaya plant revealed downward leaf curling, twisted petioles, vein enation, and stunting, resulting in small and distorted fruits. However, only partial genomic sequence of viral CP gene is available and has shown low (80%) nucleotide identity with other *Papaya leaf curl virus*. When re-blasting the sequences within the GenBank, the virus sequence revealed high (>92%) nucleotide identity with isolates of *Papaya leaf curl Guangdong virus* (PaLCuGdV) and should be reconsidered as a virus isolate of PaLCuGdV.

PaLCuGdV companied with *Euphorbia leaf curl virus* (EuLCuV) was found to infect passion fruit (*Passiflora edulis*) in Taiwan in 2011 (Cheng et al. 2014). The diseased plant revealed mild symptom, but the fruit was affected showing striped concave symptoms. Later on, a PaLCuGdV infecting passion fruit sample was inspected in the seedling exported to Mainland China (KP876482).

The poinsettia (*Euphorbia pulcherrima*), a common ornamental plant in Taiwan, was also reported to be infected by a *Begomovirus* in 1997 (Tsai et al. 1997). The virus can be transmitted by whitefly and grafting, causing disease symptoms of stunting, leaf curling, thickening and hardening, vein enations, and petiole twisting. Later on, a PaLCuGdV isolate was identified to respond to the disease (Cheng et al. 2006; Accession No.JN703795).

Lisianthus (*Eustoma grandiflorum*) is another important ornamental crop in Taiwan. The lisianthus *Begomovirus*, AYVV, was identified from the diseased samples collected in 2010 (Accession No. JN703793). Recently, the PaLCuGdV isolates were also found to infect lisianthus in central Taiwan in 2015, and the disease incidence is significantly up to 30% (Chen et al. 2016a). The disease symptoms include leaf curling and enation on cup-shaped upper leaves and flower petals. A new *Begomovirus*, tentatively named as *Lisianthus enation leaf curl virus*, was also found to associate with lisianthus plant disease in central Taiwan in 2015 (Accession No.LC091538 and LC091539).

The begomoviral diseases were also found to cause yellow mosaic symptom on velvet bean (*Mucuna pruriens*) and symptoms of leaf curling and enation on *Hibiscus* plants (Personal communication).

11.5 Weed Begomoviruses in Taiwan

Two Ageratum species, *Ageratum conyzoides* and *Ageratum houstonianum*, are common weeds in the crop field and in the wild land of Taiwan. The yellow vein disease caused by *Begomovirus* on both Ageratum species is frequently observed throughout of Taiwan island (Fig. 11.4). The disease incidence can be up to 30–80%. Three AYVV strains have been identified from the diseased *Ageratum* plants (Table 11.1). Although the diseased *Ageratum* plants were frequently observed in the *Begomovirus*-infected crop fields or nearby, *Ageratum* begomoviruses were found to be distinct from *Begomovirus* infecting crop plants. The exception is a tomato isolate of AYVV Hsinchu strain which was also temporarily detected in Hsinchu area, northern Taiwan, in 2003 (Tsai et al. 2011b). The function of AV3 promoter has been studied in AYVV-SG strain (Wang et al. 2014). The viral AV1, C2, C3, and C4 protein were confirmed to enhance the AV3 promoter activities, neither AV2 nor C1 proteins.

Begomovirus isolates, named as *Pouzolzia golden mosaic Guangdong virus*, were also detected in Ceylon Pouzolzia (*Pouzolzia zeylanica*) sample collected in central and southern Taiwan during 2013 and 2014 (Table 11.1).

11.6 Conclusion

Since 1946, the begomoviruses have been detected in bean, cucurbits, papaya, passion fruit, pepper, tomato, ornamental plants, and weeds in Taiwan (Table 11.1). Significant disease incidence and economical yield losses were observed frequently.

Fig. 11.4 An *Ageratum* plant revealed yellow vein disease symptom in the wild land in Taiwan

The host resistance against tomato *Begomovirus* was identified and used in the breeding program. The pyramiding of *Ty* genes can be efficient against both predominant tomato begomoviruses (ToLCTV and TYLCTHV) in Taiwan. The host resistance to cucurbit *Begomovirus* was also screened in squash germplasm but still needs to confirm the resistance again, to study the inheritance, and to identify the linkage molecular marker (ongoing project). The *Begomovirus* betasatellite has been detected in diseased tomato plants and found to influence the symptom development using AYVV as a helper virus (Bull et al. 2004). Both full-length and deficient mutants of betasatellite DNAs were found in Taiwan. In the future, the influence of betasatellites on virus infection should be tested on tomato lines carrying*Ty* genes to clarify the resistance efficiency to *Begomovirus* and its associated satellites.

The transgenic resistance provides alternate strategy controlling the virus disease and has been only tested, so far, against tomato begomoviruses in Taiwan. The viral whole genome has been screened to generate the transgenic resistance to ToLCTV, and the IR and C2 regions of the viral genome are found more efficient against the virus based on gene-silencing strategy (Lin et al. 2012). Later on, those virus regions were used to generate the broad resistance against multiple viruses including *Tospovirus* and *Begomovirus* by posttranscriptional gene silencing (PTGS) (Lin et al. 2011). The transgenic tomato lines generated by the PTGS construct containing viral C1, C2, and C3 region were also found to be efficient against ToLCTV and TYLCTHV, and the transgenic R_2 tomato plants were symptomless until at least 3 weeks after challenged by viruliferous whiteflies, and the viral DNA revealed low titer in the plants (Chen et al. 2016b).

Considering the virus transmission, mechanical transmission was only provided by the ToLCNDV-OM and TYLCTHV isolates of Taiwan begomoviruses (Chang et al. 2010; Tsai et al. 2011b). For the study of virus transmission by whitefly in Taiwan, the B-biotype whitefly has been tested to be more efficient than Q biotype for tomato begomoviruses (Weng et al. 2015). The combination of whitefly biotype B with TYLCTHV has the best efficiency of disease distribution and contributed one of the reasons for the replacement of ToLCTV by TYLCTHV in Taiwan (Weng et al. 2015). Surely, the releasing of tomato cultivars carrying *Ty-2* resistance gene after 2003 may also play a critical role for the virus replacement (Tsai et al. 2011b). The physical control measure to prevent *Begomovirus* and its whitefly vectors has been suggested in Taiwan. The net with more than 50 meshes revealed high efficiency to prevent the whitefly invasion and is widely used to reduce the using of pesticides for melon production in the field and tomato production in net house. The combination of tomato-resistant cultivars with physical control measure such as net protection has been tested and found to be an ideal strategy for begomoviral disease management. The study conducted in 2004 revealed very low virus infection in the treatment of resistant/tolerant cultivar protected by 60 mesh nets during the growing period (Tsai et al. 2013a). This indicates the use of resistant cultivars should be combined with prevention of virus/whitefly to generate the best control strategy for the *Begomovirus* diseases, and this may extend the standing period of resistant cultivars in the cropping fields.

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Status of Begomovirus in Oman

12

Adel Ali Mohammed Al Shihi

12.1 Introduction

The whitefly-transmitted begomoviruses (family: *Geminiviridae*) emerged, during the late twentieth century, as serious pathogens of many vegetable crops across tropical and subtropical regions of the world. This importance is due to the economic impact of the diseases they cause (Morales and Anderson 2001). *Tomato yellow leaf curl virus* (TYLCV) refers to a heterogenous complex of geminiviruses (Czosnek and Laterrot 1997) vectored by the whitefly, *Bemisia tabaci*, that infects tomato (*Solanum lycopersicum* L), pepper (*Capsicum annuum*), potato (*Solanum esculentum*), tobacco (*Nicotiana tobacum*), several dicot weed and ornamental species (Green and Kalloo 1994; Polston and Anderson 1997; Ramappa et al. 1998; Al-Shihi et al. 2014a). In tomato and pepper, disease symptoms including leaf curling and yellowing, reduction in leaf size, and plant stunting are most common if plants are infected in early growth stages (Green and Kalloo 1994; Pico et al. 1996). TYLCV is a serious tomato production constraint in tropical and subtropical Asia (Green and Kalloo 1994; Zeidan et al. 1998). In some ornamental plants like petunia when infected with begomovirus, mosaiclike symptoms can be seen (Al-Shihi et al. 2014a). Leaf curling is the most common symptom induced by begomovirus infection that can be seen in other crops such as radish (Al-Shihi et al. 2017b), papaya (Ammara et al. 2015), and cotton (Briddon et al. 2001).

Geminiviridae consist of seven genera, namely, *Becurtovirus*, *Begomovirus*, *Eragrovirus*, *Mastrevirus*, *Curtovirus*, *Topocuvirus*, and *Turncurtovirus*, based on the genome organization, host range, and insect vector (Brown et al. 2015). The begomovirus constitutes the largest percentage of viruses represented in this family. They are exclusively transmitted by whiteflies (*Bemisia tabaci*). Most

A.A.M. Al Shihi (✉)
Department of Plant Protection, Ministry of Agriculture and Fisheries Wealth, Al Khuwair 100, Muscat, Oman
e-mail: adelkhasab@gmail.com

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begomoviruses have a bipartite genome and are frequently found in tropical and subtropical Americas' new world, and few have monopartite genome which is associated sometimes with satellite virus and found in the Old World.

The presence of begomoviruses in Oman first surfaced in 1992–1993, when the Ministry of Agriculture and Fisheries observed problems with tomato leaf curl disease (ToLCD) in some crops such as okra (*Abelmoschus esculentus*) and papaya (*Carica papaya*). However, the disease was characterized and determined recently only on tomato crops in 2007. Begomovirus infection continues to increase, and many different isolates have now been identified (Polston and Anderson 1997). This factor is directly related to the presence of polyphagous whitefly biotype B, which has been identified recently to be present in all the regions of Oman (Al-Shihi and Khan 2013). Yield losses due to plant diseases can have a great economic impact, leading to a significant decline in income for tomato producers which in turn results in higher prices for consumers. In Oman, the farmers usually use some control methods, which are mainly directed to the vector such as spray of chemicals, physical barrier (Agryl cover), and partially resistant cultivars. However, the incidence of begomovirus can reach up to 100% in a number of farms (Khan et al. 2008). Mixed infection of begomoviruses is common in some farms. Five begomovirus strains were identified in tomato crops only from one field in Barka (Al-Shihi et al. 2014b). It was reported that most of these strains have their origins outside Oman but they find their way through human migration and transport of plant material (Khan et al. 2014a), especially since Oman has historical trade links with Africa and Asia and most of these viruses belong to these two continents. These plant products or materials can carry the viruses or the eggs of the whitefly which cannot be seen visually. Both export and import of viruses can be countered by strict phytosanitary precautions. In addition, there is the emergence of new geminiviruses through recombination or pseudo-recombination (Zhou et al. 1997; Navas-Castillo et al. 2000; Saunders et al. 2001).

12.2 Review of Literature

Geminiviruses are plant viruses infecting both monocots, such as wheat and maize, and dicots, such as tomato and cassava (Hanley-Bowdoin et al. 1999). There are more than 199 recognized species of geminivirus of which 181 belong to the genus *Begomovirus.* There are more than 670 complete sequences deposited in databases (Fauquet et al. 2008), which, in reality, reflect their economic importance and the higher diversity in different geographic locations and their adaptation to many host species.

The International Committee on Taxonomy of Viruses (ICTV) has approved 3 orders, 73 families, 9 subfamilies, 287 genera, and ~1950 species of viruses (Briddon and Mansoor 2008) on the classification and nomenclature of viruses. From these, the plant viruses constitute 20 families, 88 genera, and about 750 species. More than 90% of plant viruses have ssRNA genomes, while less than 10% have DNA genomes, including both single-stranded ssDNA and double-stranded

dsDNA. Caulimoviruses (family *Caulimoviridae*) are dsDNA viruses, whereas nanoviruses (*Nanoviridae*) and geminiviruses (*Geminiviridae*) are ssDNA viruses. The family *Geminiviridae* has a worldwide impact on agricultural production that is ongoing. The diseases caused by geminiviruses represent serious constraints to agriculture. The name was derived when virus particles, which have a unique twinned quasi-isometric morphology, were isolated from maize which had streak symptoms and beet which showed curly top symptoms (Bock et al. 1974; Mumford 1974). This attribute provided the name geminivirus, symbolizing twins (Harrison 1977). Because of the great losses caused by geminiviruses, such as those causing cotton leaf curl disease (CLCuD) in Pakistan, and tomato leaf curl disease (ToLCD) in Oman, they have become the subject of concern worldwide (Briddon et al. 2001).

Begomoviruses are whitefly-transmitted viruses that infect dicots (Stanley et al. 2005). The viruses in this group are economically very important due to their widespread distribution and the ability to cause excessive losses in the production of many crops. The genomic component DNA-A of bipartite begomoviruses and the genomes of monopartite begomoviruses have four ORFs (named as C1, C2, C3, and C4 in monopartite viruses), located on the complementary-sense strand. (A) C1 encodes for the Rep protein, A (C2) encodes for the transcriptional activator protein (TrAP), (A) C3 encodes for replication enhancer protein (REn), and (A) C4 (C4 protein) is involved in symptom severity (Jupin et al. 1994; Laufs et al. 1995). Begomoviruses encode two genes in the virion sense, the V1 (coat protein) and V2 (pre-coat protein). The DNA-B component encodes BV1 (the nuclear shuttle protein (NSP)) in the virion sense and BC1 (the movement protein (MP)) in the complementary sense. These two genes act together and help in virus movement from cell to cell within plants. Both components, DNA-A and DNA-B, share little sequence similarity with the exception of about 170 nt in the intergenic region (IR) (Hanley-Bowdoin et al. 1999). The monopartite begomoviruses are sometimes associated with a small DNA satellite of approximately half of their size.

A DNA satellite (DNA βs) is defined as a virus that has no sequence homology to its helper virus and is entirely dependent on the helper virus for replication (Mayo et al. 2005). The first satellite was found to be associated with *Tobacco ring spot virus* in 1969 (Schneider 1969). DNA βs are widely distributed in the Old World (OW) and absent in the New World (NW), and they are usually associated with monopartite begomoviruses (Briddon and Mansoor 2008). They are dependent on their helper virus for systemic movement in plants, virus encapsidation, and vector transmission (Saunders et al. 2008). Betasatellites have a genome of about 1350 nucleotide (nt) that consists of three conserved regions which include satellite conserved region (SCR), an adenine-rich region, and one ORF known as βC1 (Zhou et al. 2003; Briddon et al. 2003; Bull et al. 2004).

Begomoviruses have the ability to form new genetic variants which can result in variants with increased genetic diversity enabling them to adapt with changing selection pressure (Seal et al. 2006). Mixed infection is a prerequisite for recombination to occur in begomoviruses. Mixed infection has been reported to occur frequently in some crops such as tomato (Zhou et al. 2003; Jovel et al. 2004; Chowda Reddy et al. 2005). The mixed infection complexes can change according to the

growing season and location (Torres-Pacheco et al. 1996) and are also affected by the vector type and crop type (Sanchez-Campos et al. 1999). Genetic variation can arise in the genome of geminiviruses through mutation, recombination, and pseudo-recombination (Seal et al. 2006). Recombination is the process by which the segments of one nucleotide strand are incorporated into segments of other nucleotide strands during replication. Recombination is common under field conditions among geminiviruses (Zhou et al. 1997; Padidam et al. 1999; Al-Shihi et al. 2014b). Recombination has been reported to occur between DNA-A molecules of different geminiviruses. For example, Zhou et al. (1997) reported that *Cassava mosaic virus* (CMV) is a recombinant between *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV). In addition, Al-Shihi et al. (2014b) reported a recombinant between Tomato leaf curl Oman virus (ToLCOMV) and *Croton yellow vein mosaic virus* (CroYVMV) that has been given the name *Tomato leaf curl Barka virus* (ToLCBrV).

Yield losses due to infection by TYLCD have become the major threat of crop production in the Middle East, Southeast Asia, Africa, Europe (Moriones and Navas-Castillo 2000; Czosnek and Laterrot 1997), and South and North America (Zambrano et al. 2007). About 7 million hectares in 40 different countries are subjected to the attack by begomovirus (Fiallo-Olive et al. 2013).In the Caribbean basin and Florida, begomoviruses are responsible for high yield losses in tomato production (Polston and Anderson 1997). In Indonesia, losses of up to 100% were observed on pepper crops which led to an 80% reduction in fruit production (De Barro et al. 2010). Total tomato cultivated area was reduced by 50% due to losses caused by *Tomato yellow mosaic virus* (TYMV) in Venezuela (Romay et al. 2014). About 7 million hectares in 40 different countries are subjected to the attack by begomovirus (Fiallo-Olive et al. 2013). In Oman, begomovirus-like symptoms have been observed in different crops reflecting their wide host range (Fig. 12.1). The widespread occurrence of begomoviruses among different crops is due to the presence of the efficient whitefly vector.

Bemisia tabaci is a virus vector in all continents wherever agriculture is practiced. It has a wide host range colonizing more than 500 plant species including fiber, vegetable, and ornamental plants (Cock 1986). *Bemisia tabaci* and its various biotypes have extended their geographic distribution and host range as a result of introductions of *B. tabaci* transported on different crops by global trade. Some damage is caused by whitefly while feeding on the host plants. Direct damage is caused by feeding on the sap of the leaves which leads to reduced plant vigor, growth rate, and yield (Schuster et al. 1998). Indirect damage occurs following the secretion of honeydew which affects the functions of the leaves and promotes the growth of sooty mold which disrupts the photosynthetic process (Brown 2007). Damage also comes from whitefly through their ability to spread begomoviruses to other plants (Brown and Bird 1992). There are some variations in the symptoms that affect crops in relation to the time of infection. Plants become severely stunted with shoots becoming erect when plants are infected in early growth stages. Leaflets are reduced in size and pucker. Leaflets curl upward, become distorted, and have prominent yellowing along margins. Flowers wither and plants will set very few fruit after

Fig. 12.1 Different crops showing different types of begomovirus-like symptoms: (**a**) papaya crop showing severe *curling*, (**b**) tomato crop showing *yellowing* and bushy-like appearance, (**c**) sweet pepper showing *yellowing* with mosaiclike patterns, (**d**) squash crop showing stunting of newly developed leaves, (**e**) radish crop showing *curling* symptom, and (**f**) petunia plant showing curling and mosaiclike patterns (*Source*: Adel Al-Shihi)

infection occurs; therefore, any plants infected before the flowering stage will produce very low yields. In Oman, begomoviruses were isolated and characterized from different crops that have shown begomovirus-like symptoms such as tomato, squash, radish, hot pepper, petunia, and papaya (Fig. 12.1).

12.3 Begomoviruses in Oman

12.3.1 Agriculture in Oman

Oman has a long history of trade relations with different countries, mostly with African and Asian countries. Nowadays, Oman has agricultural trade with many countries and relies on international trade for income. Oman has depended on the import of agricultural products from countries such as Iran, India, Pakistan, and some countries of East Africa. The agricultural output of Oman is mostly for local consumption via local markets. The cultivation of crops in Oman is mostly possible in the Al Batinah region, Al Hajar Mountains, and the southern Dhofar region. The cultivated area is small (67,000 ha; Ministry of Agriculture and Fisheries 2015; www.maf.gov.om), and most planting material is imported. Many commercial farms in Oman rely on monoculture and as a social habit, with farmers sometimes exchanging planting materials between themselves. Returning Omanis, coming back from other countries, have brought their own planting materials with them to their own farms. A large number of expatriate workers, mostly from the Indian subcontinent, work in Oman, and they are growing herbs and vegetables in their gardens. All these aspects contribute to the risk of introducing and spreading of plant begomoviruses.

12.3.2 Begomovirus Emergence in Oman

As mentioned, the presence of begomoviruses in Oman first surfaced in 1993, when problems with tomato leaf curl disease (ToLCD) were detected by the Ministry of Agriculture and Fisheries (Government of Oman). Whitefly-transmitted begomoviruses constitute the largest and the most economically important group in *Geminiviridae*. They include viruses with either a monopartite or bipartite genome with each component being about 2.7 kb in size. Most monopartite begomoviruses are associated with DNA satellites that are half of their size. Most begomoviruses that have been identified in Oman are monopartite associated mainly with DNA satellite (Fig. 12.2a) and are not native to the region. In spite of the diversity of betasatellites in the Middle East and Africa, only two betasatellites have been identified in Oman: tomato leaf curl betasatellite (ToLCB) (Fig. 12.2b) and okra leaf curl Oman beta satellite (OLCOMB) (Akhtar et al. 2014). These interactions of begomoviruses and satellites in Oman show that the interference of geographically distinct viruses and satellites is activating the emergence of new viruses by recombination. Table 12.1 shows the begomoviruses that have been identified in Oman to date.

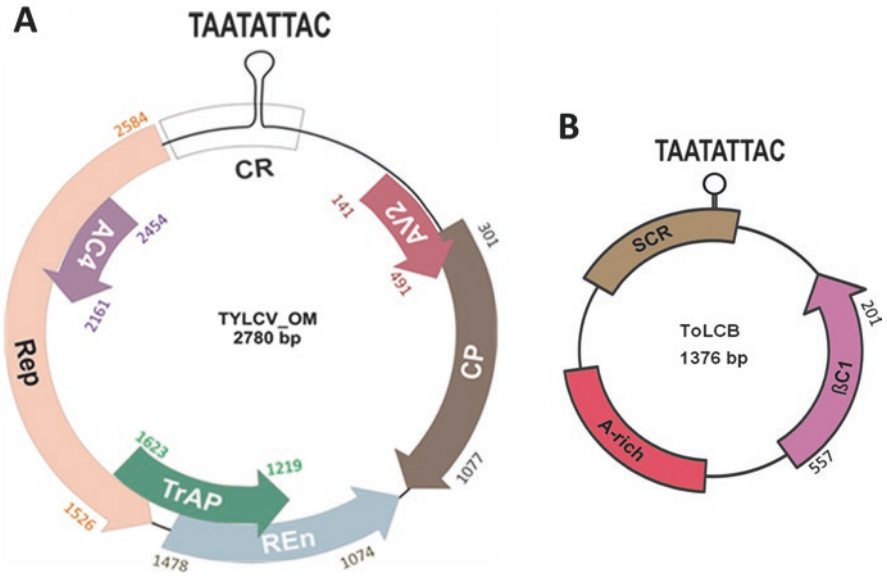

Fig. 12.2 Genome maps of (**a**) *Tomato yellow leaf curl virus-Oman* (TYLCV-OM) and (**b**) *Tomato leaf curl betasatellite* (ToLCB). The numbers belong to start and stop coordinates (nucleotide positions), and the orientations of the open reading frames (ORFs) are shown as an *arrow*. *CR* common region, *Rep* replication associated protein, *TrAP* transcriptional activator protein, *Ren* replication enhancer protein, *CP* coat protein, and *AV1* pre-coat protein. For betasatellite, genome consists of one gene, ßC1, and two regions, satellite conserved region (SCR) and *A-rich* adenine-rich region

Table 12.1 Begomoviruses identified in Oman and their origin

Virus name	Virus origin	Strain found in Oman	References
Tomato yellow leaf curl virus	Mediterranean and/or Middle East	TYLCV-OM	Khan et al. (2008)
Chili leaf curl virus	Indian subcontinent	ChLCV-Pet	Al-Shihi et al. (2014a)
Tomato leaf curl Al-Batinah virus	Middle East and/or Indian subcontinent	ToLCABV	Khan et al. (2014b)
Tomato leaf curl Barka virus	Middle East and/or Indian subcontinent	ToLCBrV	Al-Shihi et al. (2014b)
Cotton leaf curl Gezira virus	North Africa	CLCuGV	Al-Shihi et al. 2017a (in press)
Tomato leaf curl Sudan virus	North Africa	ToLCSDV-OM	Khan et al. (2013)

12.3.3 Human Migration and Geography in Oman

Begomoviruses are transmitted by insect vectors, which cannot travel for long distances. However, the ancient and modern trade either by sea or air may spread them over long distances. Oman has historical links of trade with Africa and Asia. Currently, Oman has agricultural trade links with multiple countries (Foreign Trade Statistics 2012, Directorate General of Customs, Oman; www.rop.gov.om), from which begomoviruses could spread to Oman. Human migration may play an important role in the distribution of begomoviruses in Oman. There are some factors that are believed to speed the emergence and spread of begomoviruses including virus recombination, the presence of polyphagous biotypes of whitefly, intensive agricultural practices (mainly monoculture of highly susceptible crop varieties), and the global movement of agricultural products. Begomoviruses form a serious threat to global food security, and strategies should be employed to stop ongoing crop losses due to begomoviruses and prevent the movement and introduction of further begomovirus strains into new locations. The distribution of different begomovirus strains in different Oman governorates is shown on the Oman map below which has their origin outside Oman (Fig. 12.3).

12.4 Conclusion

In spite of the presence of begomoviruses and satellites of diverse origins in Oman, there is no evidence to prove that begomoviruses have spread out from Oman. Both the import and export of begomoviruses carrying products can only be restricted by greater phytosanitary precautions. Currently, although wood containers arriving by sea at Sohar quarantine are usually sprayed to kill insects and other pests, all other imports that arrive by road are only inspected visually for the presence of pests and diseases. The inspection of live plant materials should be given further particular attention. In 2012, Oman imported nearly 1 million tons of live plant material that can harbor many pests and pathogens, including begomoviruses. It is believed that the import of ornamental plants has resulted in the recent introduction of cotton leaf curl disease in China (Sattar et al. 2013). In addition, there is concern about newly evolved begomoviruses or begomovirus satellite complexes that could spread outside using the same trade links that are believed to have brought viruses originally into the country. Therefore, to prevent further import and export of begomoviruses, Oman needs to establish a phytosanitary program using the most up-to-date molecular techniques for detecting and identifying plant viruses (Macdiarmid et al. 2013). Furthermore, applying stricter phytosanitary screening in the countries from where Oman receives imports, particularly those in the Middle East, East Africa, and Asia, would help to prevent the introduction of further viruses.

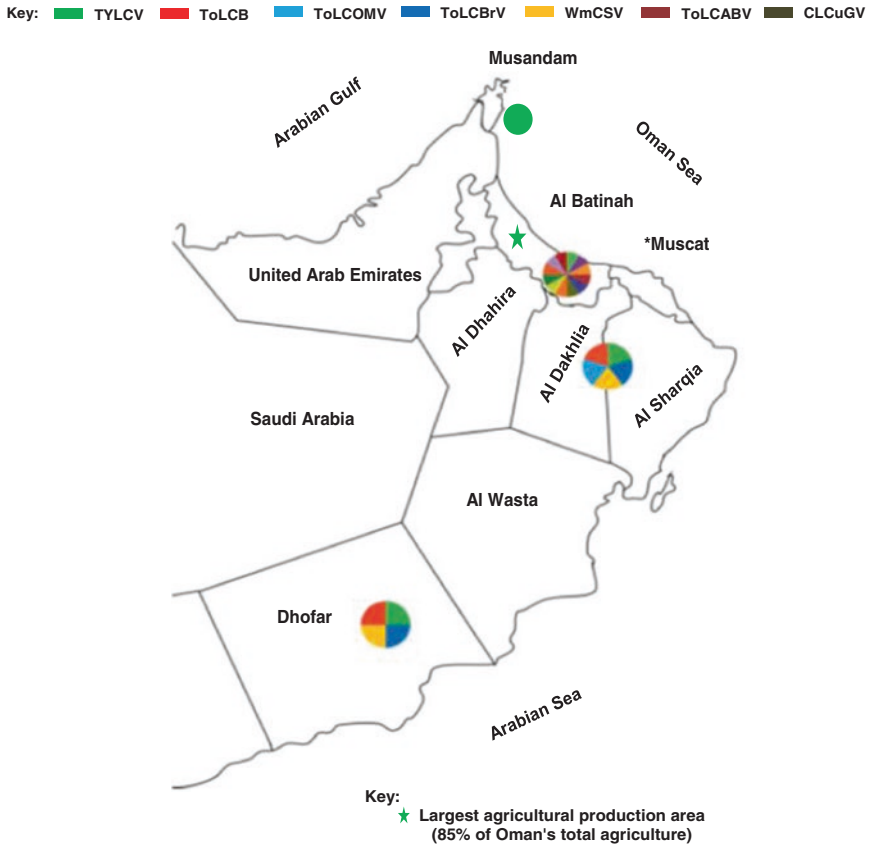

Fig. 12.3 Begomovirus and satellite diversity in Oman. (A) Oman map showing the regions in which begomoviruses have been identified. The fertile Al Batinah has the highest level of begomovirus diversity, whereas Musandam, the Al Dakhliya and Dhofar regions, has less intense agriculture with lower levels of begomovirus diversity. Begomoviruses are shown in different colors to highlight their distribution. *ChLCV* Chili leaf curl virus, *CLCuGV Cotton leaf curl Gezira virus, ToLCBrV Tomato leaf curl Barka virus, ToLCOMV Tomato leaf curl Oman virus*, *ToLCABV Tomato leaf curl Al-Batinah virus*, *ToLCB Tomato leaf curl betasatellite*, *TYLCV Tomato yellow leaf curl virus*.*Muscat is the capital of Oman

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Current Status of Begomoviruses Infecting Cultivated Crops and Weeds in Saudi Arabia

13

Sayed Sartaj Sohrab

Abstract

Begomoviruses are economically important viruses with ssDNA containing monopartite or bipartite genomes, encapsulated in twinned particles belonging to family *Geminiviridae*. Currently, a total seven genera are known in the family *Geminiviridae*, and they are designated as *Mastrevirus*, *Curtovirus*, *Begomovirus*, *Topocuvirus*, *Eragrovirus*, *Turncurtovirus*, and *Becurtovirus*. They are transmitted by the vector whitefly (*Bemisia tabaci*). Begomoviruses are known to have satellite molecules known as betasatellites and alphasatellites. The plant virology in Saudi Arabia is new and at infancy stage. Currently, very little information is available at the molecular level about the begomoviruses infecting cultivated and weed crops. The begomovirus infection and disease spread on new hosts as well as new geographic regions are significantly increasing in many new regions in the kingdom, and currently begomovirus-associated disease on multiple crops like *Amaranthus* plant, beans, *Corchorus* plant, cucumber, okra, ridge gourd, squash, and tomato has been reported from Saudi Arabia. There is an urgent need to perform more research on the disease spread, epidemiology, as well as molecular characterization of begomoviruses to identify the currently circulating virus isolates as well as possible emergence of new strains with their extended host characteristics in Saudi Arabia. This article provides the current status of major developments on the begomovirus research and future prospects in Saudi Arabia.

Keywords

Begomovirus infection • Saudi Arabia • Beans • Cucurbits • Tomato • Weeds

S.S. Sohrab (✉)
Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, No-80216, Jeddah 21589, Saudi Arabia
e-mail: ssohrab@kau.edu.sa; sayedsartaj@gmail.com

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13.1 Introduction

Begomoviruses belongs to the family *Geminiviridae* and currently divided into seven genera as *Mastrevirus*, *Curtovirus*, *Begomovirus*, *Topocuvirus*, *Eragrovirus*, *Turncurtovirus*, and *Becurtovirus* (Group 2014; Muhire et al. 2014; Varsani et al. 2014; Brown et al. 2015). Begomovirus contains monopartite or bipartite ssDNA genome. The bipartite has DNA-A and DNA-B, while monopartite has only DNA-A with either betasatellites (1.4 kb) or alphasatellites (Briddon et al. 2002) with single-stranded circular DNA with only one gene known as beta C1 (Sivalingam et al. 2010). Alphasatellites are earlier designated as DNA 1 with just half size of the genome of begomoviruses (~1375 nt) with a single ORF (Rep) and conserved genome organization (Xie et al. 2010). Helper virus is responsible for the replication and symptom attenuation for both alpha- and betasatellites (Idris et al. 2011). The DNA-A contains five and sometimes six ORFs known as AV1, AV2 and AC1, AC2, AC3, and AC4. The DNA-B has two ORFs (BV1 and BC1), 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 known as RNA silencing suppressor. In DNA-B, the BV1 codes for a nuclear shuttle protein (NSP) and the BC1 for a movement protein (MP). Whiteflies (*Bemisia tabaci*) are known as serious pathogens and can transmit begomoviruses efficiently to dicotyledonous plants in a persistent manner globally (Varma and Malathi 2003; Idris and Brown 2005; Kenyon et al. 2014). Virus-resistant transgenic plants have been developed by using multiple techniques (Vanderschuren et al. 2007).

The extent of yield loss caused by some geminiviruses has been estimated by Dasgupta et al. (2003) to be as high as 100%. In legumes, the yield losses have been estimated to be approximately $300 million/year taking black gram, mung bean, and soybean together (Varma and Malathi 2003). Keeping the urgent requirement and the importance of begomovirus infection to various crops in Saudi Arabian agriculture, this article was prepared to provide the status of begomovirus infection in Saudi Arabia. Currently, very less work has been on begomoviruses, and there is an urgent need to perform deeper research to produce valuable information especially about the begomovirus disease spread and incidence in the Kingdom of Saudi Arabia in the near future.

13.2 Natural Occurrence and Detection of Begomovirus Infection in Saudi Arabia

The status of begomovirus infection on various crops has been summarized in Table 12.1 with natural infection symptoms in Fig. 12.1. The first repost about the begomovirus infection was published in 1957 in Saudi Arabia causing mosaic disease in tomato based on symptom expression under field condition (Talhouk 1957). Plant virology received considerable attention and grew more rapidly at the

Table 12.1 Current status of begomovirus infection in Saudi Arabia

S. no.	Crops	Locations	References
Cultivated crops			
1	Beans	AL-Hassa, Hofuf, Saudi Arabia	Ghanem et al. (2003)
2	Cucumber	Jeddah, Saudi Arabia	Sohrab et al. (2016a)
3	Okra	Al-Hasa, Jizan, Saudi Arabia	Ghanem (2003), Al-Saleh et al. (2013), and Idris et al. (2014)
4	Ridge gourd	Jeddah, Saudi Arabia	Sohrab et al. (2016b)
5	Squash	Jeddah, Saudi Arabia	Al-Shahwan et al. (2002) and Sohrab et al. (2016e)
6.	Tomato	Jizan, Al-Qassim, Jeddah, Saudi Arabia	Alshahwan et al. (2001), Ajlan et al. (2007), Idris et al. (2012, 2014), Alhudiab et al. (2014) and Sohrab et al. (2016c, d)
Weed crops			
1	*Amaranthus*	Jeddah, Saudi Arabia	Sohrab (2016a)
2	*Corchorus*	Jeddah, Saudi Arabia	Sohrab (2016b)

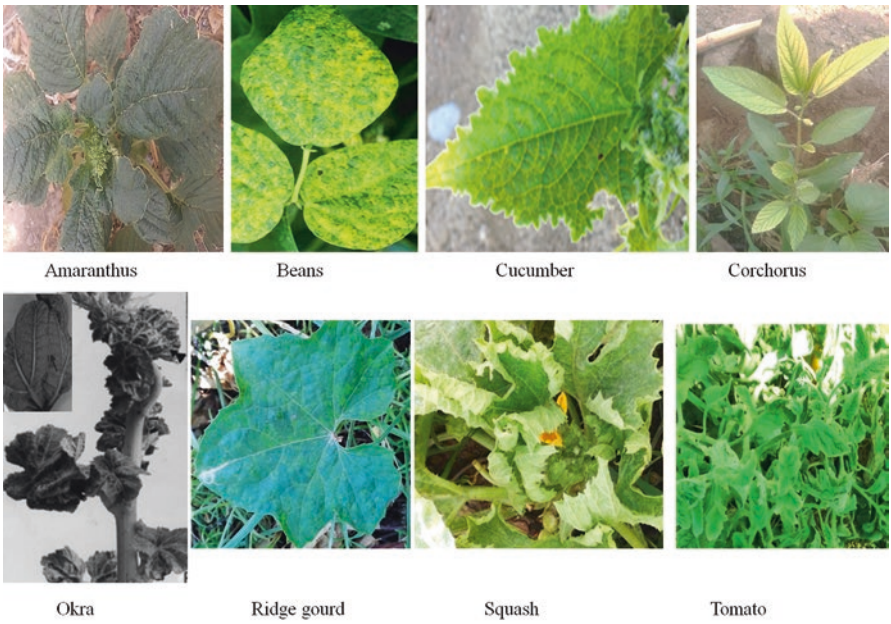

Fig. 12.1 Begomovirus infection on field collected samples

mid-1980s and afterward due to the active research and collaboration between native and nonnative plant virologists who were attempting to solve the unsolved problems that accompanied the intensive modern agriculture in Saudi Arabia. The little attention paid to the area of plant virology prior to that period was mainly due to the lack of professional plant virologists and/or to the lack of well-equipped plant virology laboratories. As far as the methods of investigations are concerned, plant virology research in Saudi Arabia seems to have gone through three main developmental stages. The first stage was based solely on symptom expression on their respective host plants. The second stage was based on symptoms on the host plant and symptoms that occur after mechanical inoculations to diagnostic hosts. The third stage started diagnostic techniques such as serological tests and/or electron microscopy. Most of what was achieved prior to the 1980s was based on stages 1 and 2, whereas most of what was achieved thereafter was based on stage 3. The indexing provided the most comprehensive study to have been achieved so far, the real situation in viruses and virus diseases and the plant virology in the Kingdom of Saudi Arabia. Currently, advanced molecular plant virology is in infancy stage in the Kingdom.

Till now, very few crops are known to be affected by begomovirus in Saudi Arabia, and less research work has been done on the begomovirus especially on the detection, sequencing, analysis, and phylogeny. Currently, the begomovirus infection in Saudi Arabia has been reported on various crops like *Amaranthus* plant, beans, cucumber, *Corchorus* plant, okra, ridge gourd, squash, and tomato (Al-Shahwan et al. 1997, 2001, 2002; Ghanem et al. 2003; Ghanem 2003; Ajlan et al. 2007; Idris et al. 2011, 2012, 2014; Al-Saleh et al. 2013; Alhudiab et al. 2014; Sohrab et al. 2016a, b, c, d, e; Sohrab 2016a, b). Begomoviruses cause important damage to tomato crops in Arabian Peninsula (Ajlan et al. 2007). The begomovirus and satellite molecule (1.4 kb and 0.7 kb) infection is endemic in the Eastern Hemisphere (Idris et al. 2012, 2014, 2011). The current status of begomoviruses infecting crops has been provided.

13.3 Cultivated Crops

13.3.1 Tomato

Tomato is a useful crop and leaf curl and yellow mosaic disease significantly reduce its cultivation around the world. In Saudi Arabia, the association of begomoviruses known as tomato leaf curl Sudan virus (ToLCSDV) and *tomato yellow leaf curl virus* (TYLCV) has been reported (Al-Shahwan et al. 2001; Ajlan et al. 2007; Idris et al. 2012, 2014; Alhudiab et al. 2014; Sohrab et al. 2016c, d). Very recently, the associations of *tomato leaf curl Sudan virus* as well as *tomato yellow leaf curl virus* causing leaf curling and yellow mosaic disease in Jeddah has been confirmed based on full genome sequencing and phylogenetic trees analysis in the Kingdom of Saudi Arabia (Sohrab et al. 2016c, d). The complete genome of *tomato leaf curl Sudan virus* associated with leaf curl disease in Arabian Peninsula has been reported

earlier (Idris et al. 2014). The TYLCV genome showed highest identity with *tomato yellow leaf curl virus*-Jizan 103 isolate (Jazan and Al-Qassim) (Sohrab et al. 2016d).

13.3.2 Okra

Okra (*Abelmoschus esculentus* L.) is an important crop, and begomovirus-associated diseases like leaf curl and yellow vein mosaic disease cause severe reduction of okra production at the global level. The okra leaf curl disease exhibiting symptoms like leaf curling, stunting, and vein enation has been reported in a greenhouse- as well as field-grown crop in Hofuf, Al-Hassa, King Faisal University, Saudi Arabia. The begomovirus infection was confirmed in okra by only serological detection tools like ELISA, blotting immunobinding assay (TBIA), and dot blot immunobinding assay (DBIA) (Ghanem 2003). But recently, the molecular detection of begomovirus from naturally infected okra leaf samples exhibiting leaf curl disease has been detected from Jazan, Saudi Arabia. The full genome was cloned and sequenced and found to have 2769 nucleotides and showed highest sequence identity (93%) with cotton leaf curl Gezira virus (CLCuGV) and 89% with CLCuGV-Egypt isolate that has been introduced recently in Jordan. The newly identified isolate was tentatively designated as CLCuGV-Jaz (Al-Saleh et al. 2013). The identified virus was observed to be CLCuGV with both alpha- and betasatellites showing the presence of multiple genomic components (Idris et al. 2014).

13.3.3 Beans

Bean (*Phaseolus vulgaris* L.) is a valuable crop grown in Saudi Arabia. The natural occurrence of begomovirus exhibiting symptoms like dwarfing, leaf malformation, vein yellowing, and leaf abnormalities was observed in field- and greenhouse-grown beans in Al-Hassa, Hofuf, Eastern Province, Saudi Arabia, during 2002–2003 (Ghanem et al. 2003), and the begomovirus infection was confirmed in bean (*Phaseolus vulgaris* L.) plants cv. Lolita based on serological techniques like double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and dot blotting immuno-printing assay (DBIA) and tissue blotting immuno-printing assay (TBIA) in the infected leaves. In serological relatedness studies, DBIA indicated the presence of a close relationship between BDMV-SA, bean golden mosaic virus (BGMV), and cotton leaf curl mosaic virus (CLCMV), while, no serological relationships were observed between BDMV-SA, *squash leaf curl virus* (SqLCV), and *tomato yellow leaf curl virus* (TYLCV). The associated virus was easily transmissible by sap as well as whiteflies to different species belonging to families *Cruciferae*, *Cucurbitaceae*, *Leguminosae* (*Fabaceae*), *Malvaceae*, and *Solanaceae*. This virus was identified and designated as bean dwarf mosaic virus-Saudi Arabian isolate (BDMV-SA) (Ghanem et al. 2003).

13.3.4 Cucumber

Cucumber (*Cucumis sativus*) is known as a valuable crop widely used as salad globally. The monopartite begomovirus with yellow mosaic disease in cucumber has been identified by PCR and characterized at the molecular level from Saudi Arabia. The complete DNA-A genome had 2784 nucleotides and showed highest identity with *tomato yellow leaf curl virus* earlier reported from Saudi Arabia. The newly identified virus could be a variant of tomato yellow leaf curl virus isolate circulating in the Kingdom (Sohrab et al. 2016a).

13.3.5 Squash

Begomovirus-associated disease was observed in squash crops with leaf curl symptoms under natural condition in Saudi Arabia. The association of begomovirus infection was confirmed in *Abelmoschus esculentus* L., *Cucurbita pepo* L., *Cucumis sativus L.*, and *Medicago sativa* L. by using monoclonal antibodies (mAbs) of African cassava mosaic virus (ACMV) in ELISA test. Begomovirus particles were also observed in partially purified extract of squash samples under immunosorbent electron microscopy, and the identified virus was tentatively designated as SqLCV (Al-Shahwan et al. 2002). Recently, leaf curl disease was observed in squash grown under natural field condition in Jeddah, Saudi Arabia, and begomovirus infection was confirmed by PCR and full genome sequencing and phylogenetic analysis (Sohrab et al. 2016e).

13.3.6 Ridge Gourd

Ridge gourd (*Luffa acutangula*) is used as vegetable crops and widely grown around the world. The monopartite begomovirus infection has been confirmed by PCR from naturally infected ridge gourd samples exhibiting yellow mosaic symptoms collected from farmer's field at Jeddah, Saudi Arabia. The full DNA-A genome of identified virus was found to have 2788 nucleotides, and the highest similarity was observed with TYLCV previously reported from Jizan and Al-Qassim, Saudi Arabia (Sohrab et al. 2016b).

13.4 Weed Crops

13.4.1 Amaranthus

Amaranthus plant is well known as amaranth and used as leafy vegetables, cereals, and ornamentals in many countries. In April 2014, *Amaranthus* plants were found to be associated with leaf curl disease at Jeddah, Saudi Arabia. The begomovirus was transmitted through whiteflies (*Bemisia tabaci*) from infected to healthy

Amaranthus seedlings and detected by PCR, and full genome was cloned and sequenced. The full genome showed maximum sequence identity and clustered with tomato leaf curl Sudan virus reported from the Arabian Peninsula (Sohrab 2016a).

13.4.2 *Corchorus*

Corchorus (*Corchorus capsularis* L. and *Corchorus olitorius* L.) is known as important crops for fiber and extensively grown in tropical and subtropical regions. During field survey, yellow mosaic symptoms were observed on *Corchorus* plants in the farmers' field at Jeddah, Saudi Arabia, and the association of begomovirus with yellow mosaic disease has been detected by PCR using specific primers. The complete DNA-A and betasatellite sequences showed highest identity, and closest cluster was found with tomato yellow leaf curl virus (TYLCV) from Jizan and Al-Qassim, Saudi Arabia isolates (Sohrab 2016b).

13.5 Future Prospects

Begomovirus infection is increasing at an alarming rate in the new geographic region in Saudi Arabia with their extended hosts. An open opportunity is available to identify any possible emergence of new recombinant virus strain or isolate which can be identified by analyzing the sequences by applying the bioinformatics tools. In Saudi Arabia, identification of other alternative hosts not only weed but also other crops harboring begomoviruses is urgently required. This opens a new platform to generate valuable information about the natural occurrence of both bi- and monopartite begomoviruses as well as mixed infections on important hosts and weed crops as well as the presence of different biotypes of whitefly vector and to produce complete genome information, presence of satellites molecules (alphasatellites and betasatellites), and emerging recombinant virus strains by using sequence analysis studies.

Based on field survey, it was observed that the presence of whiteflies is growing gradually in Saudi Arabia, and this will lead to emergence of disease spread in other new geographical regions with their extended new hosts. Hence, more work is required to search for plant with natural resistance characteristics against begomovirus. After generating this information, there is an extra urgent need to carefully investigate the interaction of begomoviruses with the vector whiteflies to control the spread of begomoviruses. Gathering these important and valuable information will open an exciting development on plant virus research in the near future to control huge loss incurred due to begomovirus infection in Saudi Arabia.

13.6 Discussion and Conclusion

Plant virology and plant virus research is comparatively new in Saudi Arabia. Very few begomoviruses have been reported to cause disease on limited crops like beans, cucurbits (cucumber, ridge gourd, and squash), okra, tomato, and weed (*Amaranthus* and *Corchorus*) from Saudi Arabia, but the infection will increase in the near future to other crops also, and this requires more research to generate valuable information about disease spread, and due to this, there is a strong reason to do extensive research about the begomoviruses-associated disease in many unknown crops in Saudi Arabia. The whiteflies can spread the disease on other crops due to overlapping host range. Weeds play an important role in disease spread by serving as an alternative for both viruses and whiteflies, and recently the natural occurrence of begomovirus on a weed called *Corchorus* have been reported from Saudi Arabia. The percentage of disease incidence caused by *tomato leaf curl Sudan virus* and *tomato yellow leaf curl virus* is currently very low, but this will increase in the near future with extended host and due to increase in whitefly population in the near future. So, finally, it is concluded that an extensive research work is urgently needed about the begomovirus infection and disease spread so that an effective disease management strategies can be designed and developed in Saudi Arabia.

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The Status of Begomoviruses in Iran

14

Sara Yazdani-Khameneh and Alireza Golnaraghi

Abstract

The diverse climatic conditions, high-grade and fertile soil, and potential access to water resources have provided favorable platform for the extensive cultivation of different crops in Iran and subsequently proper conditions for the activity of a variety of plant viruses, including members of the genus *Begomovirus*. Several mono- and bipartite begomoviruses have been reported to infect various crops in the country; some of them seem to be new species/strains. Widespread occurrence and high economic impacts of a number of *Begomovirus* species across the country have been well documented. In recent years, reports of the natural occurrence of these viruses from new regions/hosts in Iran have been significantly increased. This is mainly because of the suitable climate conditions (especially in the southern areas), presence of various host plants, high activity of whitefly vectors in climatically different regions, and emergence of pesticide-resistant whitefly populations. Moreover, neighboring with some countries where a broad range of genetically variable begomoviruses exist and the presence of common hosts to different begomoviruses, that favor mixed infections and recombination events, have made Iran as a center for diversification of these viruses. These aspects, coupled with global warming, which possibly provides appropriate envi-

S. Yazdani-Khameneh
Department of Plant Pathology, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University (IAU), Tehran, P.O. Box 14515-775, Iran

A. Golnaraghi (✉)
Department of Plant Protection, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University (IAU), Tehran, P.O. Box 14515-775, Iran
e-mail: agolnaraghi@yahoo.com

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ronmental conditions for both viruses and vectors in other regions of the country, show the serious and continuous threats of begomoviruses in the mid-Eurasia of Iran and indicate the necessity to develop new strategies for their efficient control.

14.1 General Geography of Iran

Iran, or as it is officially called the Islamic Republic of Iran, with an area of approximately 1,648,198 km^2 or 636,375 mi^2, is the eighteenth largest country in the world in terms of population and is the second largest country in the Middle East in terms of land size. Iran is a country of Western Asia and is located in the Middle East and Central Asia and the Caucasus. The country has common borders with Armenia, Azerbaijan (including the Nakhchivan Autonomous Republic), and Turkmenistan in the north, Afghanistan and Pakistan in the east, and Iraq and Turkey in the west. In addition, in the south, Iran has aquatic borders in the Persian Gulf to Kuwait, Iraq, Saudi Arabia, Bahrain, Oman, Qatar, and the United Arab Emirates. The largest lake of the world, the Caspian Sea, is situated at the north. By having two important mountain ranges, including Alborz Mountains in the north and Zagros Mountains in the west, Iran is considered as one of the most mountainous countries in the world. In contrast, the wide deserts of Dasht-e-Kavir (the Great Salt Desert) and Dasht-e-Lut (the Emptiness Desert) cover the central and eastern sections of the country. The presence of a range of natural resources, including seas, mountains, forests, and deserts, makes Iran as one of the few countries that possess all four seasons at the same time during a year; therefore, its climate is very variable. The temperature can vary widely; for example, in the summer it varies from 50 °C in the south to 1 °C in the northwest. Precipitation also differs greatly, ranging from less than 50 mm in the southeast to about 2000 mm in the Caspian region, compared to an annual average of about 250 mm. In general, the Iranian climate varies from semiarid to subtropical, due to its location between the Arabian Desert and the Eastern Mediterranean areas (Ghorbani 2013). Interestingly, researchers have recently found evidences indicating that Iran was one of the first regions where agriculture was developed (Riehl et al. 2015), confirming its long history and tradition in Iran. The diversity of climate plays a significant role in the development of agriculture in the country and has created favorable conditions for production of diversified crops. Among the various plants cultivated in Iran, particularly important are the vegetables.

14.2 The Importance of Vegetable Crops in Iran

Iran, possessing the fifth place in climate diversity in the world, is one of the main producers and exporters of all kinds of vegetables. According to the amount of total area harvested (811,616 Ha) and yield (264,367 Hg/Ha) of vegetables, Iran is considered one of the world's top ten producers of vegetable crops (Food and Agriculture Organization of the United Nations 2014). Since vegetables have a special value in

the Iranian diet, these crops are widely cultivated in diverse regions and climates. These products not only supply the needs of the domestic consumers but also are partly exported to the global markets. Therefore, Iran is one of the major centers for the production of vegetable crops, especially in the Middle East and mid-Eurasian regions. Due to their extensive cultivation in various climatic conditions, vegetables are exposed to the attacks of several biotic and abiotic factors.

14.3 Begomoviruses Reported from Iran

Among the biotic factors affecting vegetable crops, plant viruses cause significant losses every year. So far, many plant viruses belonging to different genera and families have been reported from vegetables in the world and Iran (Brunt et al. 1995; Farzadfar et al. 2002; Ayazpour 2014). The circular single-stranded DNA viruses of the genus *Begomovirus*, family *Geminiviridae*, are among the main limiting factors of a number of economically important crops throughout the world, especially in tropical and subtropical regions (Zerbini et al. 2005). In particular, members of this genus are among the most important viruses of vegetable crops worldwide (Brown and Bird 1992; Moriones and Navas-Castillo 2000). Several begomovirus species have been reported not only to cause severe damages to vegetable crops but also to infect plant species of other distinct families. Iran is one of the important centers of diseases resulting from the complex of begomoviruses. Several begomoviruses have been reported from vegetable crops and weeds in Iran. Some of them infect economically important crops such as potato, tomato, and cucurbits and can cause epidemics and significant yield losses, e.g., *Tomato yellow leaf curl virus* (TYLCV) (Moriones and Navas-Castillo 2000). In this chapter, a review is given of begomoviruses that occur in vegetables and other host plants in Iran.

14.3.1 Begomoviruses Associated with Leaf Curl Diseases (LCDs)

Leaf curl diseases (LCDs) are among the most widespread and damaging diseases affecting vegetable production throughout the tropical and subtropical regions in the world (Moriones and Navas-Castillo 2000). In particular, LCDs are considered as destructive diseases and limiting factors in tomato cultivations worldwide. The disease was first detected in 1990 and reported in 1993 from some tomato fields in the warm and dry conditions of southern Iran (Hormozgan and Sistan-va-Baluchestan provinces) (Hajimorad et al. 1993). In these areas, which are now considered as centers for the production of tomato and cucurbits (e.g., cucumber and melon), such crops are cultivated extensively in commercial greenhouses, plastic tunnels, and open farms. Since, the first report of LCDs, these diseases have increasingly spread throughout these regions and are becoming a major constraint to vegetable production in the country (Bananej et al. 2004; Behjatnia et al. 2004; Fazeli et al. 2009). LCDs in Iran are caused by a number of *Begomovirus* species as shown below.

14.3.1.1 *Tomato Yellow Leaf Curl Virus* (TYLCV)

Yellow leaf curl disease of tomato (*Solanum lycopersicum*), TYLCD, is one of the most devastating viral diseases worldwide, especially in tropical, subtropical, temperate, and even semiarid regions. The disease sometimes leads to the loss of a substantial part of the production (Czosnek and Laterrot 1997; Moriones and Navas-Castillo 2000). TYLCD is a complex disease caused by begomoviruses belonging to at least ten different species and related strains, all of which generally known as TYLCV-like viruses (Diaz-Pendon et al. 2010). The disease symptoms include stunting, yellowing, reducing number and size of fruits, leaf rolling, leaf cupping, and yellowing of the leaf margin. Symptoms are more severe when plants are infected in early growth stages (Fig. 14.1; Diaz-Pendon et al. 2010; Bananej 2016). TYLCV, a causal agent of the disease, is considered as one of the ten most economically important plant viruses in the world (Rybicki 2015).

In Iran, this whitefly-transmitted virus was first reported in 1996 from tomato fields in the southern provinces (Kerman, Khuzestan, Hormozgan, Sistan-va-Baluchestan, and Bushehr) (Hajimorad et al. 1996). Afterward, TYLCV spread toward the central (Esfahan, Markazi, Tehran, and Yazd), northern (Mazandaran), northeastern (Golestan, Khorasan-e-Razavi, and Khorasan-e-Shomali), and southern (Fars) provinces with various climate conditions. In addition to tomato, which is the most important host plant of TYLCV in Iran, the virus was detected on other plant species, including alfalfa (*Medicago sativa*), cowpea (*Vigna unguiculata*), cucumber (*Cucumis sativus*), cantaloupe (*Cucumis melo* var. *cantalupensis*), pepper (*Capsicum annuum*), red pepper (*Capsicum* sp.), and spinach (*Spinacia oleracea*). The virus was also found to infect weed species belonging to different plant families: *Chenopodium album*, *Daucus* sp., *Echinochloa crus-galli*, *Heliotropium* sp., *Malva sylvestris*, *Malva* sp., *Melilotus officinalis*, *Physalis alkekengi*, *Trigonella* sp., and *Solanum nigrum*. The infected host plants exhibited the typical TYLCV symptoms, although in some cases no symptom was present. The analysis on the virus isolates tested indicated the absence of DNA-B component or DNAβ (Shahriary and Bananej 1997; Bananej et al. 1998a, 2003a, 2009; Fazeli et al. 2009; Pakniat et al. 2010; Hosseinzadeh and Garivani 2014; Shirazi et al. 2014; Hosseinzadeh et al. 2014; Azadvar et al. 2016; Bananej 2016; Yazdani-Khameneh et al. 2016).

Due to the increasing spread of the TYLCV in Iran from the first report in 1996 and to the detection of new virus strains in recent years, extensive studies were carried out in order to analyze the features and genetic diversity of the Iranian isolates, also in comparison with isolates of the same species from other parts of the world. Based on these studies, five out of seven TYLCV strains so far described, i.e., TYLCV-IL, TYLCV-IR, TYLCV-Bou, TYLCV-Ker, and TYLCV-OM, are present in Iran. The presence of these strains was confirmed through phylogenetic analyses and pairwise genome-wide similarity comparisons of DNA-A sequences of different TYLCV isolates. This is the greatest number of TYLCV strains that have been found in a country (Fig. 14.1; Bananej et al. 2004; Lefeuvre et al. 2010; Pakniat et al. 2010). Among TYLCV strains, TYLCV-IL is considered as the most devastating and damaging strain in the world, and it is present in different parts of Iran. Phylogenetic analysis revealed a clustering of Iranian and worldwide isolates of

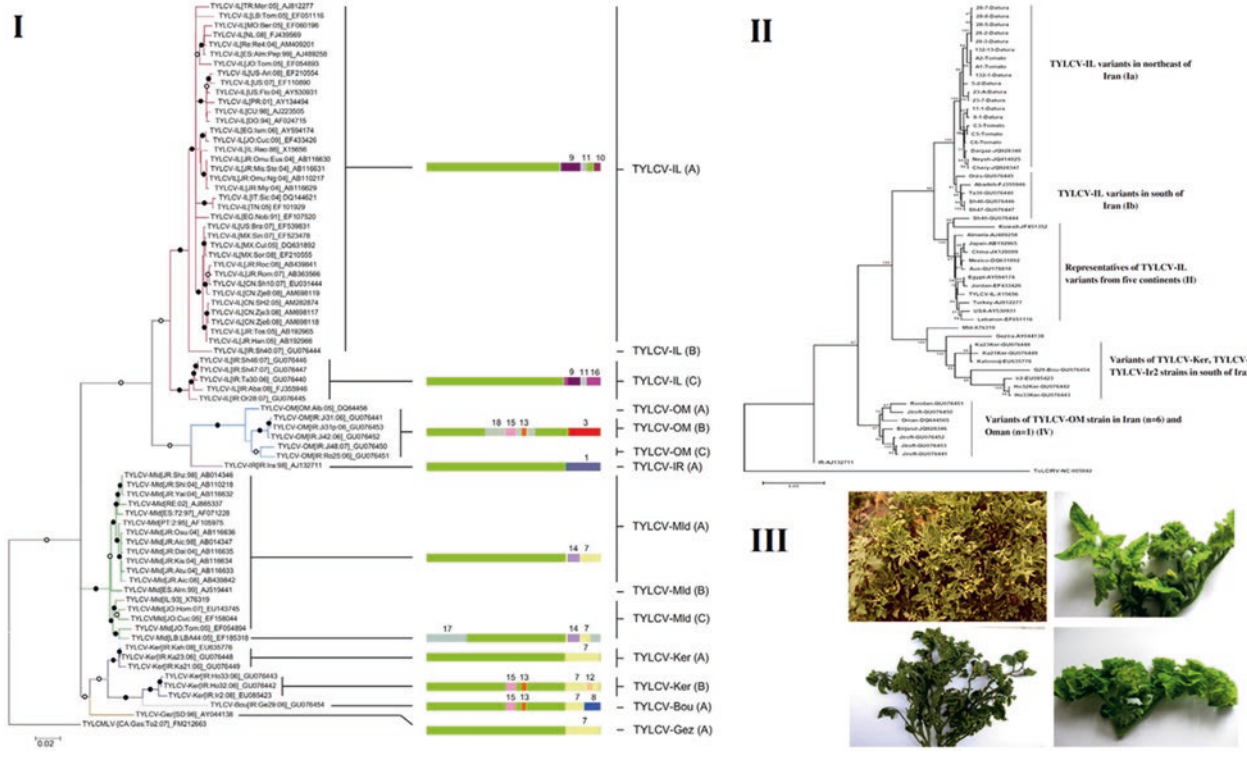

Fig. 14.1 **I** A maximum-likelihood (ML) tree indicating the relationships between the full-length genomes of Iranian and worldwide isolates of *Tomato yellow leaf curl virus* (TYLCV). Twelve recombination events resulting in ten recombination patterns are shown in the *right* of the tree; sequences derived from TYLCV and non-TYLCV sources are indicated with *green* color and other colors, respectively; **II** an ML tree showing the relationships between the full-length genomes of variants of the virus strains from Iran and nearby geographical locations and other parts of the world; **III** symptoms associated with TYLCV infections on tomato (Reproduced from Lefeuvre et al. 2010; Hosseinzadeh et al. 2014; Bananej 2016)

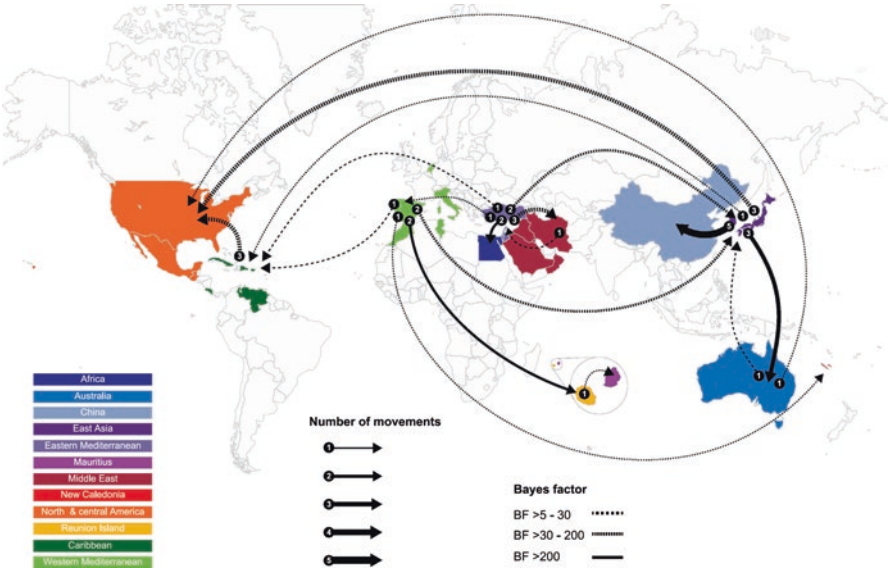

Fig. 14.2 A graph showing the inter- and intracontinental movements of *Tomato yellow leaf curl virus* (TYLCV) (Reproduced from Mabvakure et al. 2016)

TYLCV into four monophyletic clusters, which could be differentiated on the basis of geographical origin. Sequence analyses showed a higher genetic diversity in the TYLCV-IL isolates from the south than in those from the north of Iran. Iranian isolates of TYLCV-OM were genetically less diverse than those from Oman. Recombination analysis also indicated several inter- and intraspecies recombination events in the virus isolates studied. The results revealed that the breakpoints were mostly located in the internal (IR) and Rep regions. In other words, the recombination events mainly start at the C1, C1/C4, C2/C3, and V1 open reading frames (ORFs) and end at the noncoding region and the C1, C1/C2, and C3 ORFs (Fig. 14.1; Lefeuvre et al. 2010; Hosseinzadeh et al. 2014). These findings support the idea that Iran should be considered as a center for diversification of the virus and that new strains/variants of TYLCV are very likely present in the country.

In the latest studies, Mabvakure et al. (2016) considered the full-genome sequences of 414 non-recombinant isolates of TYLCV from 33 countries including Iran and showed their clustering in 12 distinct groups, reflecting their geographical origin, namely, Africa, North and Central America, Australia, China, East Asia, Eastern Mediterranean, Western Mediterranean, Mauritius, the Middle East, New Caledonia, Reunion Island, and the Caribbean. The results suggested an Eastern Mediterranean or Middle Eastern origin of the most recent ancestor of the virus, which may have occurred around 1946. In the same work, the authors also perused the distribution and movement patterns of TYLCV strains in the 12 regions. On this basis, some regions located in the East Asia and Eastern and Western Mediterranean are considered as crucial and outstanding areas of dispersion of the virus strains to other regions of the world (Fig. 14.2). It was also shown that the long-distance movement of TYLCV to some regions (i.e., East Asia) has come to an end but is

probably still in progress to other regions (i.e., Americas and Australia). According to these observations, the wide and easy dissemination of TYLCV in the world in recent years, either through short or long distances, represents serious shortcomings in controlling the dispersal of the virus. Considering the seed transmissibility of some TYLCV-IL variants (Kil et al. 2016), development of new strategies to limit the spread of the virus in contaminated areas and to prevent its introduction into non-contaminated regions is essential.

14.3.1.2 *Tomato Leaf Curl Palampur Virus* (ToLCPMV)

ToLCPMV, one of the LCD agents in Iran, was the first bipartite begomovirus on tomato reported from the southern region of the country (Hormozgan province) in 2006. The virus was subsequently detected in the southern and southeastern parts of Iran on tomato and weed species *Herniaria* sp. and *Chrozophora hierosolymitana* between 2006 and 2007. Then, the virus began to spread rapidly in other regions and to infect new plant hosts, so that in some cases, damages caused by ToLCPMV epidemics in cucurbit crops in Jiroft (Kerman, south Iran) reached up to 100% (Fazeli et al. 2009; Heydarnejad et al. 2009). The virus infection results in the decrease of plant growth and yields, especially if plants are infected in early growth stages. Occurrence and incidence of ToLCPMV in different regions in the south, southeast, northeast, and center of Iran were studied which indicated the occurrence of the virus in tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), zucchini squash (*Cucurbita pepo*), common bean (*Phaseolus vulgaris*), and some weed species such as *Chenopodium* sp. and *Heliotropium europaeum*. The infection rates were high (50–100%) in cucurbit crops and very low in watermelon and common bean (Heydarnejad et al. 2013). Full-length genome characterization showed high nucleotide sequence identities between Iranian isolates of ToLCPMV and indicated that they shared the highest identities with Indian isolates of the virus. Sequence analyses also predicted some recombination events in some isolates under study (Fig. 14.3; Heydarnejad et al. 2009, 2013).

Even though the occurrence of ToLCPMV was only recently reported from Iran, the wide distribution and rapid movement of the virus from one region to another and the increasing number of its hosts in recent years led to consider this virus as a serious threat to the production of tomato and cucurbits. Natural coinfection of the virus with *Watermelon chlorotic stunt virus* (WmCSV), another limiting factor for cucurbit production in southern and southeastern Iran (see Sect. 14.3.2), was recently reported in a watermelon sample (Heydarnejad et al. 2013). Although ToLCPMV failed to infect watermelon (Sabouri and Heydarnejad 2013), coinfection of the plant with both viruses using agroinoculation caused more severe symptoms in watermelon and zucchini as compared with those observed in single infections. This seems consequent to the ability of the replication-associated protein of WmCSV DNA-A to be bound to the suitable region of ToLCPMV DNA-B or *vice versa* to start rolling circle replication. Interestingly, agroinoculation of zucchini and tomato, but not watermelon, with WmCSV DNA-A and ToLCPMV DNA-B or *vice versa* resulted in the production of viable pseudo-recombinant particles (Esmaeili et al. 2015). Zucchini is an important host plant because it is a

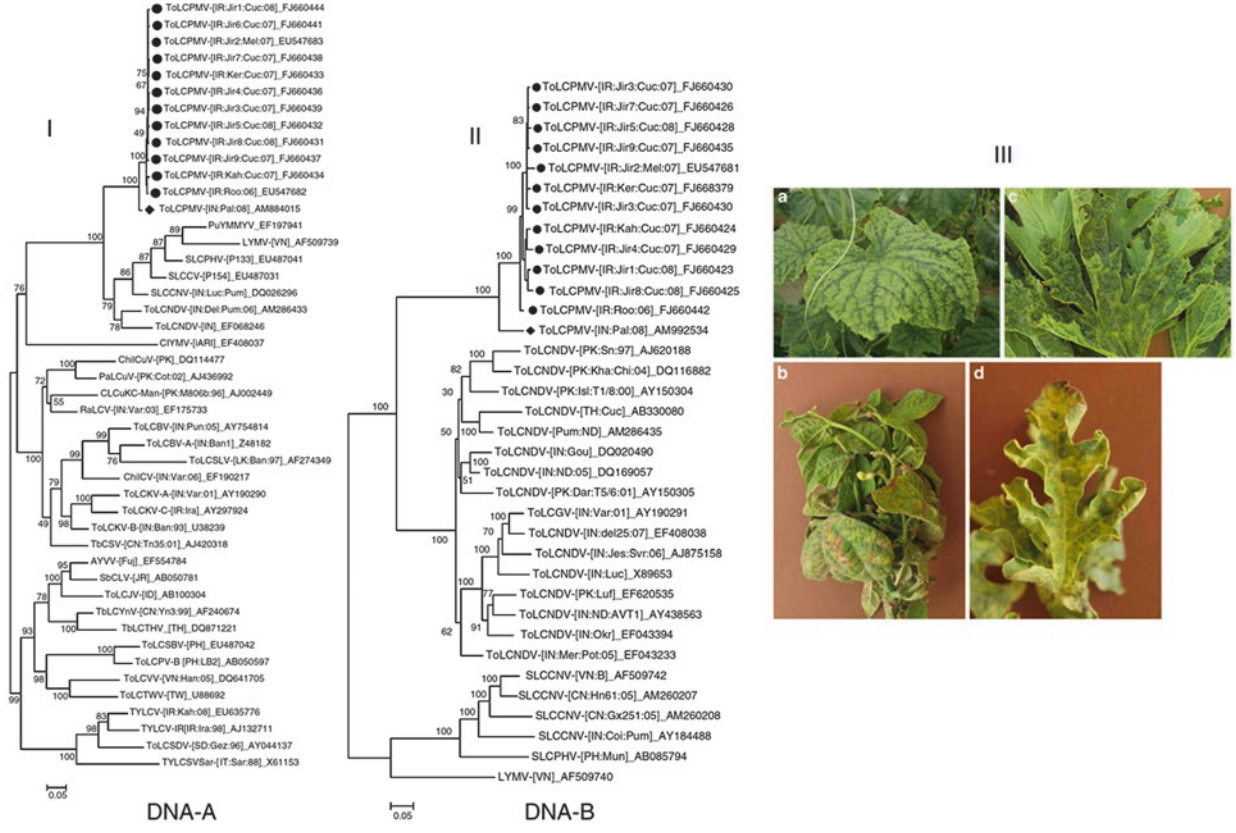

Fig. 14.3 **I**, **II** Maximum-likelihood (ML) trees indicating the relationships between the full-length genomes of DNA-A and DNA-B components of Iranian isolates (*filled circle*) and Indian isolates of *Tomato leaf curl Palampur virus* (ToLCPMV) (*filled diamond*) as compared with representative sequences of begomoviruses; **III** symptoms associated with ToLCPMV infections on (**a**) cucumber, (**b**) common bean, (**c**) zucchini squash, and (**d**) watermelon (Reproduced from Heydarnejad et al. 2009, 2013)

common natural and experimental host of both viruses (Bananej et al. 2002; Heydarnejad et al. 2013; Sabouri and Heydarnejad 2013). Similar results were previously obtained for ToLCPMV and *Tomato leaf curl New Delhi virus* (ToLCNDV) (Malik et al. 2011), another reported LCD agent from Iran (Yazdani-Khameneh et al. 2016; see Sect. 14.3.1.3). The presence of common susceptible hosts increases the possibility of the occurrence of mixed infections between two or more viruses and facilitates recombination events and subsequently the possible emergence of new strains/viruses. These findings are of great importance by an epidemiological point of view in cucurbit crops wherever both viruses are present (e.g., south parts of the country). On the other hand, phylogenetic analysis showed a close relationship of Iranian ToLCPMV isolates with those from Indian subcontinents (Heydarnejad et al. 2009, 2013), thus suggesting their possible origin from neighboring countries such as Pakistan. Therefore, the possibility of finding new variants of the virus in Iran in the future is highly probable.

14.3.1.3 *Tomato Leaf Curl New Delhi Virus* (ToLCNDV)

ToLCNDV is another destructive bipartite begomovirus species causing LCDs. In 2012, a melon sample having a weak reaction with broad-spectrum TYLCV-reacting antibodies was found in Khuzestan province in the southwest of Iran. Mosaic and leaf deformation symptoms were associated with this virus infection (Fig. 14.4; Yazdani-Khameneh et al. 2013). This was similar to the symptoms caused by several begomoviruses in many host plants (Zerbini et al. 2005). Preliminary results showed that the partial nucleotide sequence of DNA-A of the isolate had 100% identity with ToLCNDV (Yazdani-Khameneh et al. 2013). These results were subsequently confirmed by the nearly complete sequence determination of the DNA-A component (Fig. 14.4; Yazdani-Khameneh et al. 2016). The detection of ToLCNDV in Iranian melons added another begomovirus to the list of species reported to infect cucurbit crops in the country.

The virus has a wide host range, including pepper, potato, tomato, and cucurbit plants (Hussain et al. 2005). Despite the severe symptoms, i.e., yellow mosaic, reported in association with ToLCNDV infections on various cucurbit plants under natural conditions (Tiwari et al. 2012), the isolate Kz-Me198 induced different and milder symptoms on melon (Fig. 14.4; Yazdani-Khameneh et al. 2013). This may reflect a different pathogenicity of the Iranian isolate, which seems to be a new virus strain in the country. These mild symptoms also differ from the severe symptoms observed on cucurbit crops affected by other begomoviruses previously reported in Iran (Heydarnejad et al. 2009; Kheyr-Pour et al. 2000). Since mosaic and leaf deformation symptoms associated with the melon infection can be observed following the infection of cucurbit plants by several other viruses (Farzadfar et al. 2002; Ayazpour 2014), it makes difficult to diagnose ToLCNDV infections only on the basis of visual inspections in the field.

In addition to Khuzestan, ToLCNDV was also found to infect some solanaceous crops in the southeast of Iran, Sistan-va-Baluchestan province (accession nos. KJ778692 and KJ778694). Although the three Iranian isolates used in the phylogenetic analysis were clustered in two different branches, they were all clustered with

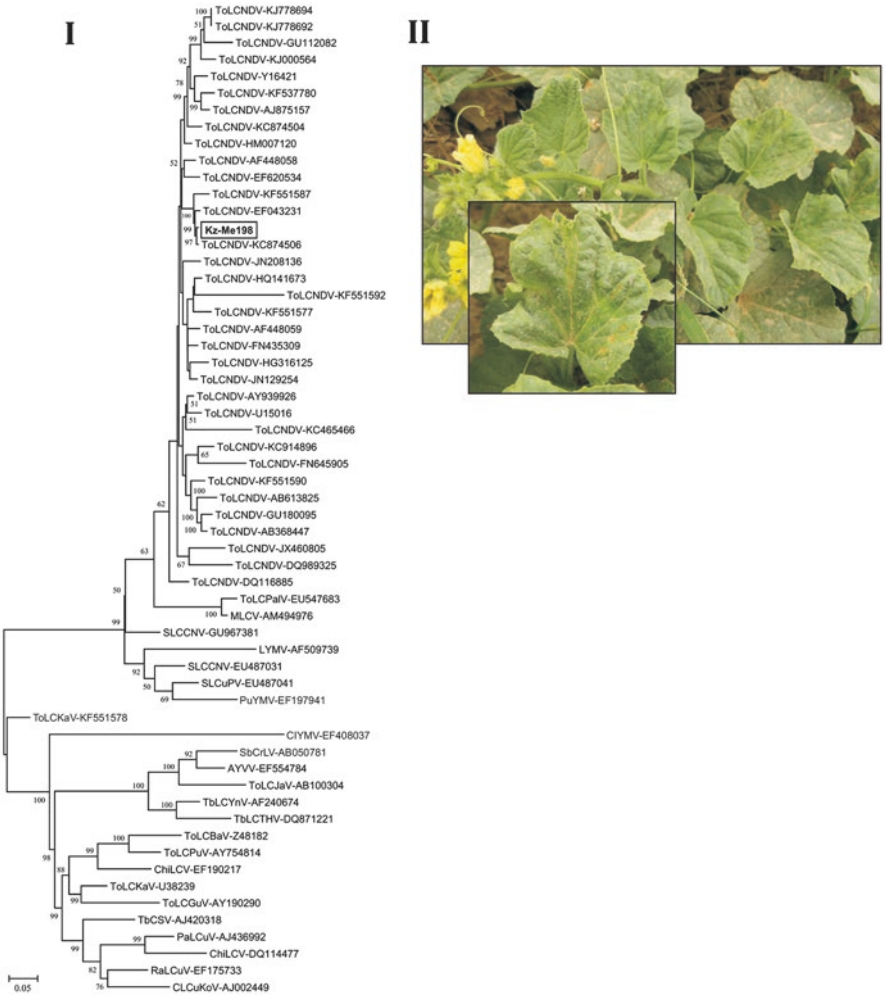

Fig. 14.4 **I** Mosaic and leaf deformation symptoms associated with the Iranian melon isolate (Kz-Me198) of *Tomato leaf curl New Delhi virus* (ToLCNDV) on melon; **II** a maximum-likelihood (ML) tree indicating the relationships between the nucleotide sequences of DNA-A of the melon isolate as well as two other Iranian isolates of the virus (accession nos. KJ778692 and KJ778694) compared to worldwide isolates of ToLCNDV and representative sequences of different begomoviruses (Reproduced from Yazdani-Khameneh et al. 2013, 2016)

different isolates from the Indian subcontinent (Fig. 14.4; Yazdani-Khameneh et al. 2016). Moreover, three virus isolates were reported from two symptomless weeds (*Chrozophora hierosolymitana* and *Herniaria* sp.) and one tomato plant in Kerman and Hormozgan provinces in the southeast and south of Iran, respectively, and their partial sequences shared high identities (78.0–80.5%) with an Indian isolate of ToLCNDV (Fazeli et al. 2009). These findings support the idea that the virus has been introduced from a neighboring country to Iran and is now spreading to the

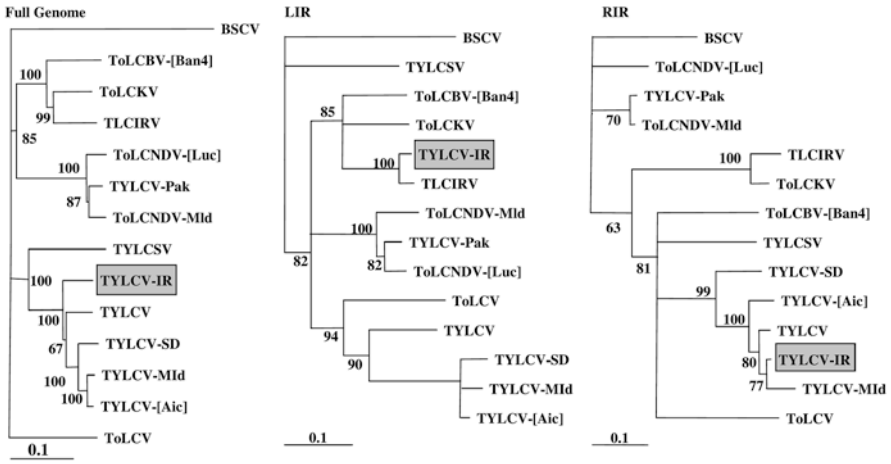

Fig. 14.5 Neighbor-joining (NJ) trees indicating the relationships between the nucleotide sequences [including full-length genome, left intergenic region (LIR), and right intergenic region (RIR)] of tomato yellow leaf Iran virus (TYLCIRV), also named as TYLCV-IR, and some other begomoviruses causing leaf curl diseases (LCDs) on tomato (Reproduced from Bananej et al. 2004)

other geographical areas, also thanks to the high number of the potential host plants in the country. This could represent a new emergence and serious threat to the agricultural production in the mid-Eurasian region of Iran.

14.3.1.4 *Tomato Yellow Leaf Curl Iran Virus* (TYLCIRV)

TYLCIRV is a tentative species in the genus *Begomovirus* and another monopartite begomovirus causing LCDs in Iran. The virus, originally named TYLCV-IR, was first reported from tomato plants showing typical yellow leaf curl symptoms in Iranshahr, Sistan-va-Baluchestan province. Studies revealed that the genome of TYLCIRV (accession no. AJ132711) contained a single DNA component, i.e., DNA-A, comprising six open reading frames (ORFs), two on the virion-sense strand (V1 and V2) and four on the complementary-sense strand (C1, C2, C3, and C4). The genome organization of TYLCIRV is similar to other whitefly-transmitted geminiviruses (WTGs). The virus does not have a DNA-B component or this component is not necessary for its infectivity. Comparison of the full-genome sequence of TYLCIRV with other TYLCV-like sequences in GenBank showed high identities (89–90%) with two isolates of TYLCV from the Middle East (accession nos. X15656 and X76319) (Bananej et al. 2004; King et al. 2012).

In phylogenetic trees, the TYLCIRV isolate was clustered in a separate branch when its complete genome sequence was compared with those of other begomoviruses causing LCDs or of different TYLCV isolates (Figs. 14.1 and 14.5; Bananej et al. 2004; Hosseinzadeh et al. 2014). Similar results were obtained by analyzing the coat protein (CP) or the intergenic region (IR) sequences (Fig. 14.6; Bananej et al. 2009). All these results unequivocally indicate that TYLCIRV should be considered as a distinct species in the genus *Begomovirus*. However, the virus was

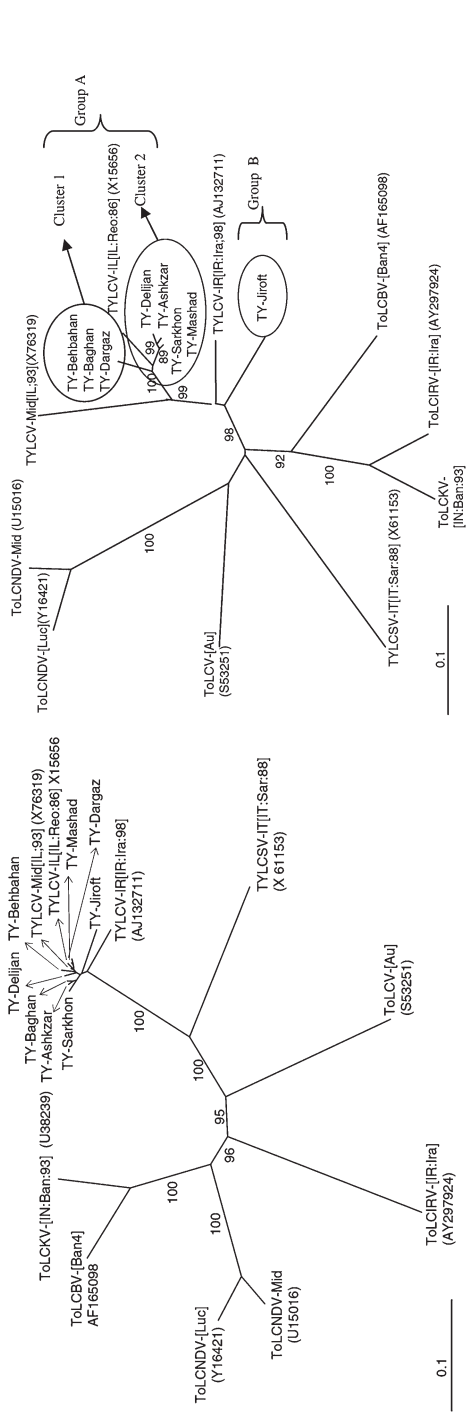

Fig. 14.6 Unrooted neighbor-joining (NJ) trees showing the relationships between the nucleotide sequences of the coat protein (CP) gene (*left*) and intergenic region (IR) (*right*) of tomato yellow leaf curl Iran virus (TYLCIRV), originally named as TYLCV-IR, and eight Iranian *Tomato yellow leaf curl virus* (TYLCV) isolates as well as some other begomoviruses (Reproduced from Bananej et al. 2009)

clustered in different places in phylogenetic trees when different regions of its genome were analyzed (Fig. 14.5), suggesting its possible recombinant origin. Indeed, the recombination analysis showed that TYLCIRV could have resulted from a recombination between TYLCV-MId and tomato leaf curl Iran virus (ToLCIRV) (Bananej et al. 2004), another proposed species in this genus (see Sect. 14.3.1.5). Similar observations have been reported previously between *Tomato leaf curl virus* (ToLCV) and TYLCV-Is (Navas-Castilo et al. 2000).

14.3.1.5 *Tomato Leaf Curl Iran Virus* (ToLCIRV)

ToLCIRV is also a recently proposed species in the genus *Begomovirus*. The virus is a whitefly-transmitted begomovirus causing LCDs and was reported for the first time on tomato plants in Iranshahr, Sistan-va-Baluchestan province of Iran. Similar to other monopartite ToLCV isolates, the genome of ToLCIRV (accession no. AY297924) contains a single DNA component, including six ORFs; the viral strand of the virus encodes two overlapping ORFs (V1 and V2) and the complementary strand encodes four ORFs (C1, C2, C3, and C4). Degenerate primers designed for the specific amplification of begomoviral DNA-B failed to amplify the component for the virus. Comparison of the full-length DNA-A of different begomoviruses showed that ToLCIRV was considerably different from TYLCIRV, with only 79% nucleotide sequence identity. Moreover, ToLCIRV had a closer phylogenetic relationship with southern Indian isolates of ToLCV and a distant relationship with ToLCVs and TYLCVs from the Middle East, America, Europe, and Australia (Behjatnia et al. 2004). Similar results were subsequently reported (Figs. 14.1, 14.5, and 14.6; Bananej et al. 2004, 2009; Hosseinzadeh et al. 2014), supporting the idea that ToLCIRV should be considered as a new *Begomovirus* species. Also, host range studies using agroinoculation showed that the virus was a mild isolate compared with a severe isolate of ToLCV or TYLCIRV (Behjatnia et al. 2009). The presence of the two tomato-infecting begomoviruses, i.e., ToLCIRV and TYLCIRV, in the same location may facilitate recombination events between these viruses and emerging new begomoviruses, similar to those reported previously (Navas-Castilo et al. 2000; Bananej et al. 2004).

14.3.1.6 *Okra Enation Leaf Curl Virus* (OELCuV)

Papaya (*Carica papaya*) is one of the economically important horticultural crops cultivated in tropical and subtropical regions of the world. This crop has been recently introduced into Iran where it is cultivated in some limited areas, mainly in Sistan-va-Baluchestan province (southeast of Iran). Papaya is susceptible to infection by different plant viruses, including some members of the genus *Begomovirus*. LCDs are considered as serious threats to papaya cultivation in many countries where this crop is grown. Several begomoviruses have been reported to cause LCDs on papaya (Singh 2006; King et al. 2012).

LCD symptoms on papaya in Iran were first observed in Bahu Kalat (near the border of Pakistan) and Zarabad in Sistan-va-Baluchestan. The affected plants showed these symptoms also in the following years. The begomoviral infection in these plants was ascertained by polymerase chain reaction (PCR) using

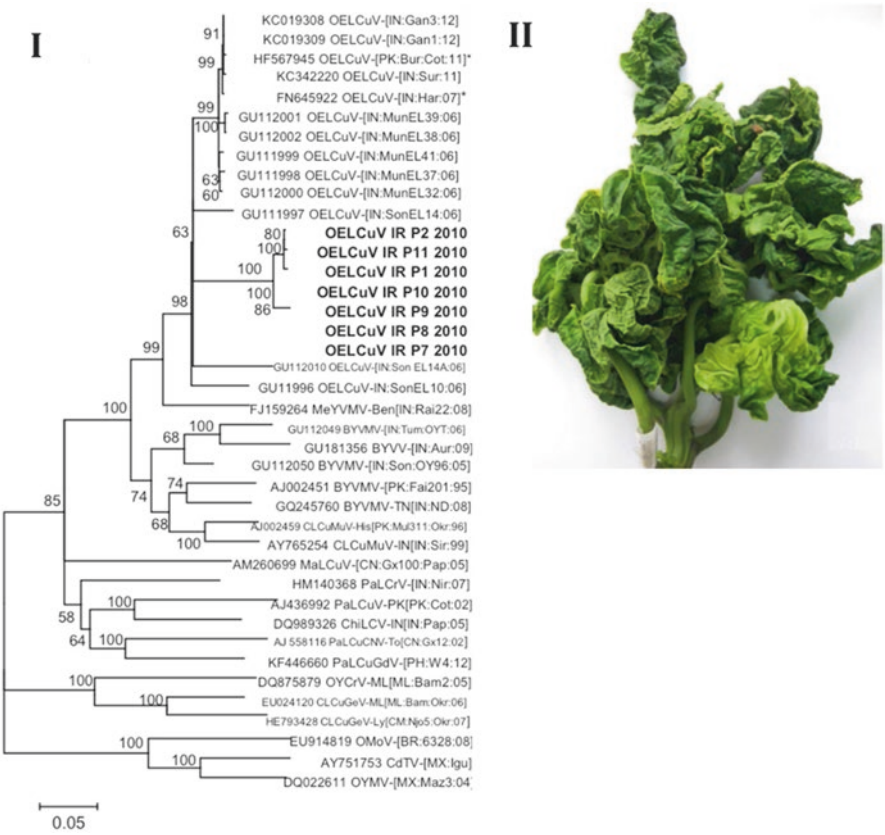

Fig. 14.7 **I** A neighbor-joining (NJ) tree indicating the relationships between the full-length genomes of Iranian and worldwide isolates of *Okra enation leaf curl virus* (OELCuV); **II** severe leaf curling and vein swelling symptoms associated with the virus infection on papaya (Reproduced from Bananej et al. 2016)

virus-specific primers. The complete nucleotide sequences of DNA-A of seven virus isolates were determined, clearly confirming the natural occurrence of OELCuV on papaya. Phylogenetic analysis also supported the virus species identification. The Iranian isolates shared more than 97.3% nucleotide sequence identities with each other, 85.5–91.6% with worldwide isolates of the virus, but less than 82% with other papaya-infecting begomoviruses. Based on the results, papaya was listed as a new species in the natural host range of the virus (Fig. 14.7; Bananej et al. 2016). Due to the presence of whitefly vectors and of favorable conditions for their activity and in consideration of the severity of symptoms induced, this emerging begomovirus should be considered as a serious threat for papaya cultivation in southern parts of Iran.

14.3.2 *Watermelon Chlorotic Stunt Virus* (WmCSV)

Cucurbits are among the main vegetable crops that are extensively grown in commercial greenhouses, plastic tunnels, and open farms in many areas of Iran. Many begomoviruses are known to cause serious damages in economically important cucurbit crops such as watermelon and melon. Among these viruses, WmCSV has been quoted as one of the major limiting factors for cucurbit production throughout the Middle East and North Africa. WmCSV was first identified and reported from Yemen and then from Sudan (Jones et al. 1988; Walkey et al. 1990; Bedford et al. 1994). In 1998, watermelon and melon cultures in the south of Iran were found to be severely infected with a whitefly-associated virus disease, and the affected plants showed similar symptoms to those described for WmCSV; the virus was subsequently identified in plants through biological and molecular studies (Bananej et al. 1998b; Kheyr-Pour et al. 2000). Infected watermelon plants display symptoms such as mosaic, vein yellowing, chlorotic mottling, stunting, deformation, and severe reduction of fruit size (Fig. 14.8; Bedford et al. 1994; Bananej et al. 2002). Natural occurrence of the virus was also detected on zucchini. Contrary to the symptoms on watermelon, the affected zucchini plants exhibit marginal yellowing, stunting, and leaf shape alternation (Esmaeili et al. 2015). Serological assays showed the natural occurrence of the virus on squash and cucumber plants as well (Bananej and Vahdat 2008). WmCSV has been reported from the south, southeast, and north parts of Iran, including Bushehr, Fars, Guilan, Hormozgan, Kerman, and Sistan-va-Baluchestan (Kheyr-Pour et al. 2000; Bananej et al. 2002; Gholamalizadeh et al. 2008; Heydarnejad et al. 2010; Esmaeili et al. 2015). Greenhouse studies performed by whitefly-mediated inoculation and agroinfection showed that most of plant species in the Cucurbitaceae and some species in the Fabaceae and Solanaceae were susceptible to the virus (Bananej et al. 2002). Also, several weeds, belonging to the Boraginaceae, Brassicaceae, Chenopodiaceae, Euphorbiaceae, Fabaceae, Malvaceae, Myrsinaceae, and Papilionaceae families, can be infected by the virus under natural conditions. These species may play – as alternate hosts – an important epidemiological role in the spread of the virus, also in consideration of the fact that most of them can be infected without any visible symptoms (Esmaeili and Heydarnajad 2014).

Phylogenetic analysis of the complete nucleotide sequences of DNA-A and DNA-B components of different WmCSV isolates showed a clustering of Iranian isolates into separate branches, which was supported by high bootstrap values. Similar results were obtained by comparing partial nucleotide sequences of different Iranian and worldwide isolates of the virus (Mohammed et al. 2014). Sequence analysis revealed that isolates from Iran and Saudi Arabia had the highest diversity (Fig. 14.8; Ali-Shtayeh et al. 2014; Esmaeili et al. 2015). These findings seem to indicate Iran as one of the possible origins of WmCSV, although further studies are necessary to confirm this hypothesis.

Aside from severe damages of WmCSV on cucurbits, the coinfection of the virus with other begomoviruses leads to a significant symptom enhancement on plants. For example, coinfection of some cucurbit plants with WmCSV and *Squash leaf*

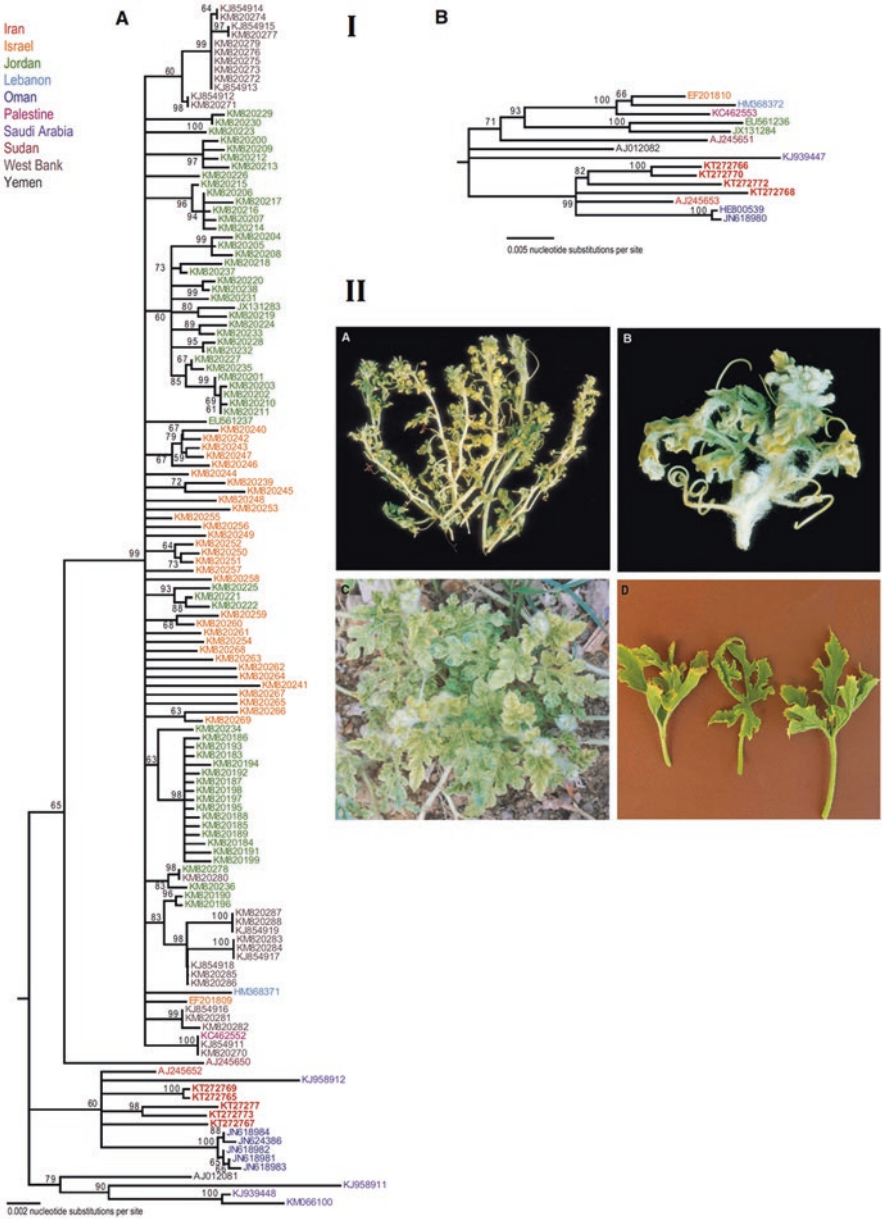

Fig. 14.8 **I** Neighbor-joining (NJ) trees of DNA-A (**a**) and DNA-B (**b**) of *Watermelon chlorotic stunt virus* (WmCSV) sequences. Sequence accession numbers are color coded with location of isolates; **II** symptoms associated with WmCSV on naturally infected watermelon (**a**), (**b**), (**c**) and zucchini (**d**) plants (Reproduced from Bananej et al. 2002; Esmaeili et al. 2015)

curl virus (SLCV) results in a synergistic reaction that induces more severe symptoms and the destruction of a major part of the production (Abudy et al. 2010). Similar results are obtained in mixed infections with ToLCPMV using agroinoculation; the coinfection also may result in producing pseudo-recombinant viruses (Esmaeili et al. 2015), which are of great importance epidemiologically (see Sect. 14.3.1.2). Based on the above evidence, WmCSV can be considered as a serious disease threatening cucurbit production, especially watermelon, either individually or in interaction with other begomoviruses.

14.3.3 *Bean Golden Mosaic Virus* (BGMV)

Common bean (*Phaseolus vulgaris*), also known as green bean or kidney bean, is a diploid annual plant of Fabaceae (legume or bean family) and native to southern and central regions of America. This plant is now cultivated in many parts of the world (the widest cultivated area among legumes) for its edible seeds and pods (Food and Agriculture Organization of the United Nations 2014). In Iran, several main bean-growing regions are located in south, north, northwest, and central parts of the country, in Markazi, Lorestan, Guilan, Azarbayejan-e-Sharghi, Azarbayejan-e-Gharbi, Esfahan, Khuzestan, Fars, and Zanjan provinces. Common bean is susceptible to infection by several plant pathogens, including viruses. Different viruses, belonging to various plant virus genera and families, have been reported to infect common bean crops in Iran (Farzadfar et al. 2002; Ayazpour 2014). Among them, BGMV is one of the most striking begomoviruses causing the golden mosaic of common bean in all tropical and subtropical regions where common bean is grown. This virus is widespread in these regions and represents the largest constraint to bean production in some growing areas around the world (Bird 2012). Although the damage caused by BGMV in bean production in some regions of the world (e.g., Latin America) is very severe (40–100% yield losses) (Bonfim et al. 2007), in Iran there is just one report on the presence of the virus, only based on serological assays (Ghorbani et al. 2010). However, considering the widespread cultivation of bean and the abundance of *Bemisia tabaci* (BGMV vector) in the country, it cannot be excluded that the virus, like other bean-infecting viruses, may cause significant damages in the future. Hence, to prevent this potential challenge, further researches are required to confirm the occurrence, incidence, and distribution of BGMV in Iran.

14.3.4 Begomoviruses Vector

Bemisia tabaci (Genn.) (Hemiptera: Aleyrodidae) is ubiquitous and has a wide host range (polyphagous), i.e., more than 700 species belonging to 86 plant families. The insect causes reduction of plant potency and growth through sucking the sap and honeydew production. However, the ability to transmit more than 110 plant viruses seems to be the most harmful effect of the insect. Because of the wide host range, rapid reproduction, worldwide distribution (except for Antarctica), efficacy of virus

transmission, and pesticide resistance, whiteflies are considered as very efficient vectors (Martin et al. 2000; Jones 2003; Navas-Castillo et al. 2011; Ghanim 2014). Members of the genus *Begomovirus* are naturally vectored by *B. tabaci* (Genn.) in a circulative and persistent manner. Although mechanical transmission has been also reported for some species, whitefly transmission is the most important way for the spread of these viruses in nature. Having a considerable genetic and behavioral variation between different haplotypes makes the vector as a sibling species group with various biotypes, including biotype B, which is widespread in many parts of the world. Interestingly, the origin of the biotype B seems to be in the Old World, probably the Middle East and eastern Africa (Brown 2007). A significant correlation was found between the spread of begomoviruses and the outbreaks of this biotype. Coadaptation and coevolution have been suggested to explain the efficacy of transmission for some *B. tabaci* begomovirus complexes. This relationship can range from weak to strong for different begomovirus-vector interactions. Some amino acid residues in the coat protein are possibly involved in their vector specificity (Moriones and Navas-Castillo 2000; King et al. 2012).

In Iran, *B. tabaci* was first found in Kerman province in 1944 (Kiriukhin 1947). Then it began to spread in the south (Fars), southeast (Bushehr, Hormozgan, and Sistan-va-Baluchestan), southwest (Khuzestan), north (Mazandaran), northeast (Golestan), and central (Esfahan and Yazd) parts of the country (Habibi 1975; Javanmoghaddam 1993). Currently, this species is widely dispersed in Iran and on diverse host plants. Although there are no published reports on the economic impact of this species in the country, their importance seems to be very high because of their ability to transmit different viruses, including begomoviruses (see Sects. 14.3.1, 14.3.2, and 14.3.3). The *B. tabaci* biotype B was identified in the country based on the analysis of ITS1 region in the ribosomal DNA. However, the fragments amplified by RAPD-PCR using the primer H16 showed the presence of other biotypes, e.g., the biotype Cv. The biotype B was the most prevalent biotype in Iran (Rajaei Shoorcheh et al. 2008; Shahbazi et al. 2010). Due to Iran's favorable climatic conditions, *B. tabaci* seems to be rapidly expanding throughout the country. On the other hand, owing to the indiscriminate use of pesticides, the species has become resistant to many of them. For this reason, the outbreaks of *B. tabaci* are very frequent in many parts of the country, even in Tehran province, where it has become a factor of environmental crisis in recent years (personal observations). Hence, permanent monitoring and detailed identification of *B. tabaci* are essential to prevent direct and indirect damages caused by this pest.

14.3.5 Management and Disease Control

Finding approaches to manage plant pathogens, particularly those responsible for imposing irreparable damages on economically important crops, has always been the main challenge for plant pathologists. As mentioned above, whitefly-transmitted begomoviruses are the most important plant viruses responsible for destructive diseases in vegetables and crops throughout the world. Their ability to produce new

strains/species through recombination or pseudo-recombination events occurring in various crops has increased their interest at global level, and it is clear why so many attempts have been performed for their control and management. Adopting more efficient management strategies requires profound knowledge on the virus, its vectors, host diversity, and evolution, and a deep understanding of host-virus-vector interactions as well as the virus interactions with other viruses in coinfections (Seal et al. 2006). We are currently trying to diminish the begomoviral disease impact by the employment of some chemical and nonchemical methods, including avoidance and elimination of sources of infection and vectors, accurate identification of the spreading centers, understanding the life history and activities of vectors and methods of their survival in the fields from one year to another, planting trap crops, use of physical and optical barriers to prevent vectors to access target plants, adoption of particular agricultural practices, utilization of virus-resistant transgenic plants, and use of virus-free materials for host plants for which grafting is routinely used (e.g., cucurbits). In addition to agricultural crops, some begomoviruses have been reported from weeds that may serve as their alternate hosts. Therefore, elimination of weeds is another effective approach to reduce begomovirus populations (Al-Musa 1982; Berlinger et al. 1991; Dobson 1994; Czosnek 2007; Polstone and Lapidot 2007).

In Iran, like other regions of the world where begomoviruses are present, separated or integrated disease management practices are applied to control these viruses, depending on various factors such as the type of cultivated crops, the planting location (i.e., open fields or greenhouses), the type of species/strains of the virus, etc. Employment of control measures such as selecting optimum cultivation date, planting trap crops, elimination of infection sources, and use of physical barriers (e.g., nets for TYLCV) had significant impacts on the control of these viruses. Studies showed some less susceptible cultivars to WmCSV among commercially cultivated watermelon cultivars. Moreover, the screening of Iranian and non-Iranian germplasm collections revealed some tolerant and resistant accessions to TYLCV (Bananej et al. 2003b; Azizi et al. 2008; Jafari et al. 2010; Azadvar et al. 2016; Esmaeili and Heydarnejad 2016). Due to the numerous reports and evidences of the possible introduction of some begomoviruses from the neighboring countries, more attempts to optimize quarantine programs in the country seem justified.

14.3.6 Discussion

Due to the widespread occurrence and continuing identification of new species, begomoviruses are considered as emerging plant viruses whose number of confirmed and tentative species reported from the world and Iran are increasing annually. Iran, because of the coexistence of multiple factors suitable for both viruses and whitefly vectors, provides outstanding conditions for outbreaks of these viruses. However, in some cases the infected plants show either no typical symptoms (Fazeli et al. 2009) or unrelated symptoms to begomoviruses, e.g., mosaic (Yazdani-Khameneh et al. 2016); consequently, the farmers are unable to detect and prevent

the spread of the begomoviral diseases at early stages. Weeds are of great importance in the epidemiology of different viruses, including begomoviruses. The high diversity and abundance of such plants in different geographical regions make them as a remarkable factor for the spread of begomoviral infections. They can host various species of begomoviruses and, therefore, are considered as sources for the variation and emergence of possible new *Begomovirus* species or strains. Interestingly, these viruses may infect weeds asymptomatically (Fazeli et al. 2009; Esmaeili and Heydarnajad 2014), thus suggesting the idea that numerous new virus species are yet to be discovered in wild plants (Roossinck 2012; Wylie et al. 2012; Valouzi et al. 2017). In this respect, it seems justifiable to place increasing emphasis on studying viruses on weeds and wild plants in the country.

The presence and abundance of *B. tabaci* in various parts of Iran with different climatic conditions (from cool to hot and from humid to dry) seem to support the potential spread of these viruses in most of the areas of the country. It is worth noting that the distribution of whitefly-transmitted begomoviruses may expand in the future as a consequence of the global warming. Moreover, the diversity of *B. tabaci* biotypes (Shahbazi et al. 2010) and the emergence of insecticide-resistant populations of the insect as a result of the excessive use of pesticides, which can in turn result in the emerging of secondary pests, may help begomoviral epidemics in the future.

Possessing the common features including overlapping host ranges and transmission by the same vectors provides ideal conditions for mixed infections in different hosts, as it has been well documented for different host plants and distinct viruses in Iran (Farzadfar et al. 2006; Pourrahim et al. 2007; Aghazadeh et al. 2014). Similarly, coinfections of mono- and/or bipartite begomoviruses have been previously reported (Fazeli et al. 2009; Heydarnejad et al. 2013). The main reason for such phenomena is the attraction of insects to the yellow color of infected plants (Eastop and Raccah 1988). Coinfections by two or more begomoviruses not only may have synergistic effects on the host plants (Abudy et al. 2010), but may also facilitate the exchanging of viral genetic materials through recombination or pseudo-recombination (reassortment of begomoviral components) (Lefeuvre et al. 2010; Hosseinzadeh et al. 2014), thus favoring the emergence of new begomovirus species/strains (Bananej et al. 2004). The viable pseudo-recombinants which occurred experimentally between some begomoviruses reported from Iran, especially in the locations where the viruses, whitefly vectors, and host plants exist (Esmaeili et al. 2015), may be a signal for new and more devastating diseases in the future.

Given the ancient history of farming, presence of diversified host plants, high genetic variability among some begomovirus species (e.g., TYLCV), diversity and possible origin of some *B. tabaci* biotypes, and detection of previously non-described viruses, at least some members of the genus *Begomovirus* might have originated from Iran (Brown 2007; Bananej et al. 2009; Fazeli et al. 2009; Hosseinzadeh et al. 2014; Riehl et al. 2015). This idea seems to be supported by the finding of many TYLCV-positive samples in serological tests which failed to be PCR-amplified using different specific and universal primers (Aghazadeh et al.

2014; Yazdani-Khameneh et al. 2016). In contrast, it seems that some begomoviruses might have been introduced to Iran from neighboring countries from the Indian subcontinent (Fazeli et al. 2009; Heydarnejad et al. 2009; Yazdani-Khameneh et al. 2016). In this respect, Iran could be considered as a center for begomovirus diversification in the world.

Crucially, the wide and rapid spread of begomoviruses over the past two decades in Iran strongly indicates that the current management strategies utilized to control such important pathogens are either not enough or performing imperfectly. Thus, application of new strategies, including biological controls and the use of Iranian natural enemies for *B. tabaci* (Al-e-Mansour and Ahmadi 1994), finding new sources of begomoviral resistance or tolerance among Iranian and non-Iranian germplasm collections (Azizi et al. 2008), using more effective control strategies especially in the outbreak regions, improvement of quarantine programs, usage of virus-free plant materials, development of more efficient virus-detection systems, and more importantly performing collaborative studies with neighboring countries, seems necessary. Also, further studies on Iranian isolates of begomoviruses that occurred on either cultivated or wild plants are essential to have a better understanding of the viral epidemics in the country.

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Status and Diversity of Begomoviruses in Pakistan

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Muhammad Shah Nawaz-ul-Rehman, Sara Shakir, Sehrish Ijaz, Muhammad Mubin, and Nazia Nahid

Abstract

Begomoviruses are plant pathogenic viruses belonging to family *Geminiviridae*. Based upon their host ranges, genome organizations, and insect vectors, geminiviruses are classified into nine genera: *Begomovirus*, *Mastrevirus*, *Curtovirus*, *Becurtovirus*, *Topocuvirus*, *Turncurtovirus*, *Capulavirus*, *Grablovirus*, and *Eragrovirus*. Most of the economically important geminiviruses belongs to genus *Begomovirus* and infect important cash crops of several families including Malvaceae (cotton, okra), Cucurbitaceae (melon, watermelon, squash, and gourds), Euphorbiaceae (cassava), Solanaceae (tobacco, tomato, potato, petunia, and pepper), and Fabaceae (soybean, cowpea, common bean, mung bean, and lima bean) in different regions of the world.

Keywords

Begomovirus • Diversity • Pakistan

Begomoviruses are plant pathogenic viruses belonging to family *Geminiviridae*. Based upon their host ranges, genome organizations, and insect vectors, geminiviruses are classified into nine genera: *Begomovirus*, *Mastrevirus*, *Curtovirus*, *Becurtovirus*, *Topocuvirus*, *Turncurtovirus*, *Capulavirus*, *Grablovirus*, and *Eragrovirus* (Varsani et al. 2014, 2017; Brown et al. 2015). Most of the economically important geminiviruses belongs to genus *Begomovirus* and infect important cash crops of several families including Malvaceae (cotton, okra), Cucurbitaceae

M.S. Nawaz-ul-Rehman (✉) • S. Shakir • S. Ijaz • M. Mubin
Virology Lab, Center of Agricultural Biochemistry and Biotechnology, University of Agriculture Faisalabad, Faisalabad, Pakistan
e-mail: msnawazulrehmanz@uaf.edu.pk

N. Nahid
Department of Bioinformatics and Biotechnology, GC University, Faisalabad, Pakistan

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(melon, watermelon, squash, and gourds), Euphorbiaceae (cassava), Solanaceae (tobacco, tomato, potato, petunia, and pepper), and Fabaceae (soybean, cowpea, common bean, mung bean, and lima bean) in different regions of the world (Seal et al. 2006).

Begomoviruses are transmitted by the whitefly, *Bemisia tabaci* (Gennadius) (order, *Hemiptera*; family, *Aleyrodidae*), move in a circulative persistent manner, and are mostly limited to the phloem of infected plants (Gilbertson et al. 2015). The genus, *Begomovirus*, includes 320 species (www.ictvonline.org) classified in two groups, based on their genome organization: monopartite (which have a single genomic component) and bipartite (with two genomic components, DNA-A and DNA-B). DNA-A and DNA-B are 2.7–2.8 kb, and each component encodes its own ORFs, which transcribes in bidirectional manner. Monopartite begomoviruses (or DNA-A of bipartite begomoviruses) encode six ORFs, four in complementary sense orientation (AC1/C1 – AC4/C4) and two in virion sense orientation (AV1/V1 and AV2/V2). The ORF AV2 is missing in begomoviruses from the New World. Begomoviruses encode multifunctional proteins and are given names according to their functions. AC1/C1 encodes replication-associated protein (Rep), AC2/C2 encodes replication enhancer protein (REn), AC3/C3 encodes transcriptional activator protein (TrAP), and AC4/C4 encodes AC4/C4 protein. The ORF AV1 encodes for the coat protein, while AV2 encodes another protein called pre-coat protein. The DNA-B component of bipartite begomoviruses encodes nuclear shuttle protein (NSP) and movement protein (MP) from the BC1 and BV1 ORFs, respectively (Fondong 2013).

The genes in the virion and complementary sense orientations on DNA-A and DNA-B are separated by an intergenic region (IR) containing a common region (CR) of sequences that are conserved between DNA-A and DNA-B. The main topological feature of the CR is a hairpin structure with a conserved nonanucleotide (TAATATT/AC) that spans the virion strand origin of replication (v-ori, indicated by the "/") (Padidam et al. 1996). Iterons (~5–7 nt long sequences) present at 5′ of the hairpin form binding sites for the virus replication-associated protein, Rep (encoded by AC1). Begomoviruses in the Old World are mostly associated with symptom/pathogenicity determinant betasatellites and self-replicating alphasatellites. Betasatellites encode βC1 gene and play a critical role in important diseases like cotton leaf curl disease in the Indian subcontinent (Briddon et al. 2014).

15.1 Diversity of Begomoviruses in Pakistan

The agriculture-based economy of Pakistan is threatened by abiotic (heat, drought, frost, salinity, etc.) and biotic stresses (insect pests, fungi, bacteria, viruses, etc.). Among biotic stresses, diseases caused by begomoviruses pose a major threat to crops in Pakistan. Several cash crops of Pakistan like tomato, cotton, chilies, soybean, and mung bean are severely infected by begomoviruses. In this chapter, we will highlight some major begomoviruses infecting crop plants and weeds in Pakistan.

Fig. 15.1 Representative begomoviruses on plants. *Panel A* represents tomato plant infected with *tomato leaf curl New Delhi virus*, *panel B* represents eclipta plants infected by *alternanthera yellow vein virus*, *panel C* represents the croton plants infected with papaya leaf curl virus, and *panel D* shows cotton plants infected with cotton leaf curl Kokhran virus in Pakistan

15.2 Cotton Leaf Curl-Associated Begomoviruses in Pakistan

In Pakistan, cotton crop is facing a real threat due to cotton leaf curl disease (CLCuD), the most important disease and limiting factor of cotton production in Pakistan (Briddon and Markham 2000; Sattar et al. 2013). During the year 1991–1992, Pakistan achieved a record cotton production of 12.82 million bales. But during 1992–1993, a CLCuD epidemic caused a severe reduction in yield, and only 9.05 million bales were produced. A further reduction to 7.9 million bales occurred during 1994–1995. Since then, the yield losses have become a constant phenomenon every year as a result of this disease.

Several monopartite begomoviruses have been reported to cause CLCuD on cotton in Pakistan. In 1990s, at least four begomovirus species, *cotton leaf curl Kokhran virus* (CLCuKoV), *cotton leaf curl Multan virus* (CLCuMuV), *cotton leaf curl Alabad virus* (CLCuAlV), and *papaya leaf curl virus* (PaLCuV), were involved in cotton leaf curl disease, either as single or mix infections (Zhou et al. 1998; Mansoor et al. 2003b; Tahir et al. 2011). In contrast, only single betasatellite named as cotton leaf curl Multan betasatellite (CLCuMuB) was found to be associated with cotton leaf curl disease (Briddon et al. 2004).

In 2001–2002, while CLCuD-resistant varieties of cotton were grown throughout the country, a resistance breaking recombinant strain of CLCuKoV (cotton leaf curl Kokhran virus-Burewala) derived some of its portion from CLCuMuV was diagnosed in the Burewala territory of the Punjab province, which quickly became the dominant strain throughout the Punjab province of Pakistan and neighboring states of northern India (Mansoor et al. 2003a). Cotton plants showing severe leaf curling and vein swelling accompanied by leaf enation are representative of CLCuKoV infection in Pakistan (Fig. 15.1: Panel D). However, other monopartite begomoviruses, including *cotton leaf curl Shahdadpur virus* (CLCuShV) (Amrao et al. 2010a) and *cotton leaf curl Gezira virus* (CLCuGeV) in Sindh province (Tahir et al. 2011), while some other such as *cotton leaf curl Bangalore virus* (CLCuBaV), *okra enation leaf curl virus* (OELCuV), *African cassava mosaic virus* (ACMV), and *chickpea chlorotic dwarf virus* (CpCDV), have also been found associated with CLCuD in Pakistan. Recently bipartite begomoviruses, *tomato leaf curl Gujarat virus* (Zaidi et al. 2015) and *tomato leaf curl New Delhi virus* (ToLCNDV) (Zaidi et al. 2016c), have been reported to be involved in cotton leaf curl disease in

Table 15.1 Comprehensive list of ToLCNDV infecting host species along with their reference accession number in Pakistan

Host		Year	Reference accession no.
Common name	Scientific name		
Black nightshade	*Solanum nigrum*	1997	AJ620187
Tomato	*Solanum lycopersicum*	1997	DQ116883
Bitter gourd	*Momordica charantia*	2004	AM491590
Chili	*Capsicum annuum*	2004	Hussain et al. (2004)
Luffa	*Luffa cylindrical*	2004	AM292302
False daisy	*Eclipta prostrata*	2006	AJ889185
Goosefoot	*Chenopodium album*	2012	KC914896
Cotton	*Gossypium hirsutum*	2013	LN845962
Field bindweed	*Convolvulus arvensis*	2013	LN845964
Night-blooming jasmine	*Cestrum nocturnum*	2013	LM645011
Santa Maria	*Parthenium hysterophorus*	2013	KF002409
Toothed dock	*Rumex dentatus*	2013	HG316125

One accession number has been given as reference, where multiple sequences have been reported from the same host but from different districts and years

Pakistan. This increasing list of begomoviruses in cotton indicates rapidly evolving CLCuD complex in Pakistan and poses a challenge for breeders to develop CLCuD-resistant varieties.

15.3 *Tomato Leaf Curl New Delhi Virus* in Pakistan

Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite begomovirus that is mostly associated with tomato leaf curl disease (ToLCD) of tomatoes (Fig. 15.1: Panel A). ToLCD was reported from India in 1948 (Vasudeva and Sam Raj 1948) and is now known to occur in Asia, North Africa, and Europe. ToLCD is a major disease of Tomato in sub-Saharan Africa and also associated with a complex of monopartite begomoviruses (Abhary et al. 2007; Osei et al. 2008; Zhou et al. 2008; Leke et al. 2011; Kon and Gilbertson 2012). In addition to tomato, ToLCNDV infects 43 dicotyledonous plants including weeds, vegetables, and ornamental plants throughout the world (Zaidi et al. 2016d). A comprehensive list of ToLCNDV infecting hosts in different districts of Pakistan is given in Table 15.1.

These reports indicate that ToLCNDV has been the major constraint in not only for tomato production but for many other economically important crops such as chili, tomato, and cotton. The virus has not only increased its host range, but it has also increased its spread over vast geographical regions in Pakistan. The increasing host range of ToLCNDV, from weeds and vegetables to other economically important agricultural crops, indicates that ToLCNDV are capable of infecting a wide variety of crop plants especially in the Pakistan.

15.4 Mixed Infection of Begomoviruses and Pseudo-Recombination of *Tomato Leaf Curl New Delhi Virus*

Replication event between genomic components of two different viruses is called trans-replication and also called pseudo-recombination, and the ability of a virus to interact with one or more satellites/component of a different virus is called component capture. ToLCNDV is reported to trans-replicate with both monopartite and bipartite begomoviruses. DNA-B component of ToLCNDV has an ability to pseudo-recombine with DNA-A component of bipartite *tomato leaf curl Gujarat virus* (ToLCGUV) in cotton (Zaidi et al. 2015) and *tomato leaf curl Palampur virus* (ToLCPalV) in melon causing systemic infection in both field and experimentally grown plants. Similarly, ToLCNDV has also shown to be associated with monopartite pathogenic viruses, cotton leaf curl Kokhran virus-Burewala strain (CLCuKoV-Bur) and cotton leaf curl Multan betasatellite (CLCuMuB), hence causing severe leaf curl disease in cotton (Zaidi et al. 2016d).

ToLCNDV also infects chilies, and doing so, it also trans-replicates with *chili leaf curl virus* (ChiLCV) and *pepper leaf curl Lahore virus* (PepLCLaV) and found associated with betasatellite (chili leaf curl betasatellite (ChLCuB)).

Mix infection of monopartite begomoviruses is a common event, but the presence of both mono- and bipartite begomoviruses is a recent event as well as an alarming sign for plant virologist to control virus evolution via trans-replication and component capture.

15.5 Legumoviruses in Pakistan

Grain legumes are prime sources of dietary protein in southern Asia but suffer from extensive yield losses due to different viruses of family *Geminiviridae*, which are collectively called legume yellow mosaic viruses (LYMVs).

LYMV disease is caused by two main pathogenic viruses: mung bean yellow mosaic India virus (MYMIV) and mung bean yellow mosaic virus (MYMV). MYMIV, a bipartite begomovirus, has significant importance, infecting different leguminous crops and weeds in Pakistan. MYMIV was first time reported from mung bean in 2004 (Hameed and Robinson 2004). Later on, it was identified from leguminous weeds and grains from Pakistan (Table 15.2). Moreover, a distinct legumovirus was found in leguminous weed, *Rhynchosia minima*, which was named afterward as *Rhynchosia* yellow mosaic virus (RYMV). Some monopartite begomoviruses such as *Pedilanthus* leaf curl virus (PeLCV) and papaya leaf curl virus (PaLCuV) has been shown to participate in disease development, but less virus titer and diversity was a good sign (Ilyas et al. 2009). However, two betasatellites, tobacco leaf curl betasatellite TbLCB and CLCuMuB, have so far identified to be associated with legumoviruses in legumes (Ilyas et al. 2009, 2010).

Tobacco leaf curl betasatellite (TbLCB) and cotton leaf curl Multan betasatellite (CLCuMB) have been identified from soybean and cowpea, respectively (Rouhibakhsh and Malathi 2005; Ilyas et al. 2009).

According to the reports showing bipartite begomoviruses and betasatellite role in infection on non-leguminous crops in Pakistan, characterization of betasatellite from legumes is also of grave concern.

Table 15.2 Complete list of legumoviruses in Pakistan

Host		Virus name	Year	Reference or GenBank accession no.	
Common name	Scientific name			DNA-A	DNA-B
Mungbean	*Vignaradiate*	MYMIV	2004	Hameed and Robinson (2004)	
Cowpea	*Vigna unguiculata*	MYMIV	2006	FM208840	FM202446
Black gram	*Vigna mungo*	MYMIV	2005	FM208835	
Black gram	*Vigna mungo*	MYMIV	2006	FM208844	FM202447
Snout bean	*Rhynchosia capitata*	MYMV	2007	FM242701	FM242702
Soybean	*Glycine max*	MYMIV	2006	AM992618	FM161881
Snout bean	*Rhynchosia minima*	RhYMV	2009	AM999981	AM999982
Snout bean	*Rhynchosia capitata*	PaLCuV	2007	FM955601	
Soybean	*Glycine max*	PeLCV	2006	AM948961	

One accession number is given for multiple sequences reported from different location and year from the same host

Mung bean yellow mosaic India virus (MYMIV), *Rhynchosia* yellow mosaic virus (RhYMV), papaya leaf curl virus (PaLCuV), *Pedilanthus* leaf curl virus (PeLCV)

15.6 Weeds Harbor Different Begomoviruses in Pakistan

Weeds act as a reservoir for different begomoviruses at a same time, creating the chances of new virus emergence by providing space for viral recombination and exchange. Many potential weed hosts were studied after the finding of associated betasatellite along with monopartite begomoviruses in cotton and ageratum (Table 15.3). Now various weeds are reported in Pakistan that are infected with different begomoviruses.

15.6.1 *Eclipta prostrata*

Eclipta locally called as bhangra or false daisy is a common weed in Pakistan which is extensively found across the water channels. The most common virus of this weed is alternanthera yellow vein virus (AlYVV) causing severe vein yellowing in the field (Fig. 15.1: Panel B). Tomato leaf curl New Delhi virus was reported from Eclipta in 2002 (Haider et al. 2006), and recently it is found that it is also infected by AlYVV that was previously present in China. This AlYVV sequence showed 96% nucleotide identity with previous AlYVV (FN432361) reported on *Sonchus arvensis* from Faisalabad, Pakistan (Zaidi et al. 2016a).

15.6.2 *Croton bonplandianus*

Croton belongs to family Euphorbiaceae and is found in Asia. This weed commonly shows the vein yellowing, potentially an indication of begomoviruses (Fig. 15.1: Panel C). A begomovirus similar to cotton leaf curl virus was amplified from croton along with a subgenomic DNA (betasatellite). The presence of betasatellite with

Table 15.3 Monopartite begomoviruses infecting weeds in Pakistan

Sr. No.	Accession No. DNA-A	Species name	Acronym	Isolate
1	FN432361	Alternanthera yellow vein virus	AlYVV-A[PK:FAI:SON:08]	Alternanthera yellow vein virus – A [Pakistan:Faisalabad:Sonchus:2008]
2	LN713273	*Pedilanthus* leaf curl virus	PeLCV[PK:Sesbania:13]	*Pedilanthus* leaf curl virus – Sesbania [Pakistan:Sesbania:2013]
3	AM261836	Ageratum enation virus	AEV-NP[PK:Lah:04]	Ageratum enation virus – Nepal [Pakistan:Lahore:2004]
4	AM698011	Ageratum enation virus	AEV-NP[PK:Lah:06]	Ageratum enation virus – Nepal [Pakistan:Lahore:2006]
5	FN543112	Papaya leaf curl virus	PaLCuV-Pun[PK:Pun:Cro:06]	Papaya leaf curl virus – Punjab [Pakistan:Punjab:Cro:2006]

DNA-A strengthened the monopartite nature of these begomoviruses in Pakistan (Amin et al. 2002). Croton yellow vein virus was identified from croton plants from a cotton field in Faisalabad, Pakistan. It is a monopartite begomovirus associated with betasatellite. Sequence analysis of virus showed that it is a recombinant virus showing the sequence homology with papaya leaf curl virus (PaLCuV) and croton yellow vein mosaic virus (CYVMV). Associated betasatellite was found to be an isolate of croton yellow vein mosaic betasatellite (CroYVMB) (Hussain et al. 2011).

15.6.3 *Sonchus arvensis*

Sonchus is a common weed that is also infected by begomoviruses. A monopartite begomovirus associated with different beta and alphasatellites was isolated from sonchus. The isolated begomovirus showed the 95–99% identity with alternanthera yellow vein virus which was previously reported from India, China, and Vietnam. Two types of alphasatellites were also identified with virus component, one of them showing the similarity with Potato leaf curl alphasatellite (PotLCuA) and the other was similar to hibiscus leaf curl alphasatellite (HLCuA). Two types of betasatellite were also associated with the complex. One of them was showing the sequence homology of 95–99% with ageratum yellow leaf curl betasatellite (AYLCB) and the other was closely related to cotton leaf curl Multan betasatellite (CLCuMuB) isolates (Mubin et al. 2010). In another study, ageratum enation virus was also found in *Sonchus oleraceus* collected from Pakistan and Nepal (Tahir et al. 2015).

15.6.4 *Xanthium strumarium*

Xanthium strumarium is another weed locally called as Puthkanda in Pakistan. It often shows the typical symptoms of begomovirus. Two different types of begomoviruses were found along with a betasatellite and an alphasatellite. One of them was an isolate of *tomato leaf curl Gujarat virus* (ToLCGUV) and the other was an isolate of *cotton leaf curl Burewala virus*; a recombinant virus responsible for breaking resistance in cotton varieties against cotton leaf curl disease. The potato leaf curl alphasatellite (PotLCuA) was found in this complex, previously identified in various weeds and potato. Similarly, an isolate of tomato yellow leaf curl Thailand betasatellite was also found with this virus complex (Mubin et al. 2012).

15.6.5 *Sesbania bispinosa*

Sesbania bispinosa is a weed which is found commonly in cotton fields in Pakistan. Typical symptoms of begomoviruses were found in sesbania. After analysis, it was found infected by a monopartite begomovirus and associated betasatellite. This DNA-A was named as *Pedilanthus* leaf curl virus (PeLCV) as it showed the 98% similarity with a begomovirus previously identified from soybean. Amplified betasatellite shared the sequence similarity (96–97%) with tobacco leaf curl betasatellite (TbLCB) (Zaidi et al. 2016b).

15.6.6 *Ageratum conyzoides*

Ageratum is also a common perennial weed in Pakistan, usually growing along irrigation channels. In 2000, a begomovirus similar to a virus involved in cotton leaf curl disease was isolated from ageratum (Mansoor et al. 2000). In another study, symptomatic samples were collected from Pakistan and Nepal. The begomovirus isolated from ageratum showed the 89.1% similarity with *ageratum enation virus* (AEV). Betasatellite was also found with begomovirus and it was an isolate of ageratum yellow leaf curl betasatellite (AYLCB) (Tahir et al. 2015).

15.6.7 *Urtica dioica*

Urtica dioica (nettle weed) belongs from the family Urticaceae. It is a herbaceous, flowering plant which also showed the suspected symptoms of begomoviruses in Pakistan. *Chili leaf curl virus* was isolated from nettle weed along with two alphasatellites and one betasatellite. The betasatellite showed 94.4% identity with ageratum yellow leaf curl betasatellite (AYVB), while one of the alphasatellite was an isolate of ageratum yellow vein Pakistan alphasatellite (AYVPKA), and the other alphasatellite was found to be an isolate of Bhendi yellow vein alphasatellite (BYVA) (Iqbal et al. 2016).

15.7 Betasatellites Associated with Begomoviruses in Pakistan

In the Old World, most of the monopartite begomoviruses are associated with a class of single-stranded DNA satellites collectively known as betasatellites (Briddon and Stanley 2006). The most important betasatellite in Pakistan is cotton leaf curl Multan betasatellite (CLCuMuB).

CLCuD across central Pakistan during the 1990s was associated with at least six monopartite begomoviruses, but only a single betasatellite "Multan" strain of cotton leaf curl Multan betasatellite ($CLCuMuB^{Mul}$) was identified to be responsible for CLCuD (Mansoor et al. 2003b). Following resistance breaking in cotton, CLCuD across central Pakistan was reported to be associated with only single recombinant "Burewala" strain of *cotton leaf curl Kokhran virus* (CLCuKoV-Bur), having sequence combined from two virus species (CLCuMuV and CLCuKoV). The betasatellite associated with CLCuKoV-Bur was also recombinant, with some sequence replacement within the SCR that was derived from a different betasatellite, tomato leaf curl betasatellite (ToLCuB). This is now known as the "Burewala" strain of CLCuMuB ($CLCuMB^{Bur}$) (Amrao et al. 2010b).

In 2005, there was an upsurge of CLCuD in Sindh, which was reported to be associated with a virus specie in cotton during pre-resistant breaking in the Punjab and a new recombinant strain of CLCuMuB containing a smaller nucleotide

fragment from tobacco leaf curl betasatellite (ToLCuB), now characterized as the "Shahdadpur" strain of CLCuMuB (CLCuMuB^{Sha}) (Amrao et al. 2010a).

Several other species of betasatellites are also found associated with begomoviruses in Pakistan. A recombinant CLCuMuB was associated with a begomovirus disease complex of *Digera arvensis*, a weed host in Pakistan (Mubin et al. 2009). CLCuBuV variant and its associate CLCuMuB caused yellow mosaic disease on eggplant (*Solanum melongena*) in Pakistan (Ullah et al. 2015). The same complex (CLCuBuV and cognate CLCuMuB) was associated with symptomatic *Luffa cylindrica* in Pakistan (Zia-ur-Rehman et al. 2013).

Prominently, a single species of betasatellite, chili leaf curl betasatellite (ChLCB), is prevalent in chili-growing areas across north central Pakistan (Hussain et al. 2009). ChLCB is usually found associated with its helper virus *chili leaf curl virus* (ChiLCV). A specific species of tobacco leaf curl betasatellite (TbLCB) is usually found associated with a specific species of begomovirus *Pedilanthus* leaf curl virus (PeLCV) and has also been widely reported in Pakistan on hosts like *Pedilanthus tithymaloides* (Tahir et al. 2009) and *Sesbania bispinosa* (Zaidi et al. 2016b). A newly identified begomovirus rose leaf curl virus (RoLCuV) with its associated *Digera arvensis* yellow vein betasatellite (DiAYVB) infects ornamental rose (*Rosa chinensis*) in Pakistan (Khatri et al. 2014). Tomato yellow leaf curl Thailand betasatellite (TYLCTHB) with its helper virus *tomato leaf curl Gujrat virus* (ToLCGUV) was reported on a weed host *Xanthium strumarium* in Pakistan (Mubin et al. 2012). *Croton yellow vein virus* (CYVV) with its associated croton yellow vein mosaic betasatellite (CroYVMB) infects croton (*Croton bonplandianus*) in Pakistan (Hussain et al. 2011). Other reported betasatellites are ageratum yellow leaf curl betasatellite (AYLCB) from *Ageratum conyzoides*, tomato leaf curl betasatellite (ToLCB) from tomato, Bhendi yellow vein betasatellite (BYVB), and okra leaf curl betasatellite (OLCuB) from *Hibiscus esculentus* (Briddon et al. 2003).

15.8 Alphasatellites Associated with Begomoviruses in Pakistan

Alphasatellites (~1380 bp) are self-replicating molecules having circular ssDNA genome which are dependent on their helper virus for transmission and encapsidation. Earlier they were named as DNA 1 (Mansoor et al. 1999; Briddon et al. 2004). Exact function and reason for frequent occurrence of alphasatellites with begomoviruses yet need to be elucidated. However, a few available reports regarding alphasatellites suggest that they have a key role in suppression of posttranscriptional gene silencing (PTGS) (host defense mechanism) and symptom attenuation (Nawaz-ul-Rehman et al. 2010; Idris et al. 2011). The origin of alphasatellites is thought to be the nanoviruses because of their genome structure. They contain three conserved regions: (1) a stem-loop like structure with a nonanucleotide (TAGTATT/AC) sequence similar to the members of family *Nanoviridae*. This region has origin of replication (ori), where Rep create a nick to start the rolling circle replication (RCR),

(2) an ORF coding the Rep protein up to 315 amino acids, and (3) an Adenine-rich region of ~200 nt, referred as a stuffer sequence (Briddon et al. 2004).

Alphasatellites were first identified to be associated with CLCuD complex in 1999 (Mansoor et al. 1999) but, due to no apparent role in disease development and no previous detection with CLCuD complex over the period of 2001–2008, may lost their importance (Shahid 2009). In 2009–2010, alphasatellites reappeared with CLCuD complexes. For two alphasatellites, GDarSLA and GMusSLA, the Rep was reported to interfere in PTGS, a defense mechanism by plants (Nawaz-ul-Rehman et al. 2010), and other alphasatellites were shown to attenuate symptoms (Wu and Zhou 2005; Idris et al. 2011). Nevertheless, a more precise role of alphasatellites yet needs to be determined.

In Pakistan, alphasatellites are found to be associated with many begomovirus/betasatellite complexes, such as those causing CLCuD and okra leaf curl disease (OLCuD) (Mansoor et al. 2003c; Mansoor et al. 2006). Since the emergence of a resistance breaking species of begomovirus cotton leaf curl Burewala virus (CLCuBuV), many studies have been made on the etiology of the disease (Amin et al. 2006; Amrao et al. 2010b). In the period of 2009–2010, alphasatellites started to appear with CLCuD complexes. In a recent study, the sequence diversity of alphasatellite components was assessed in cotton, covering the central Punjab region of Pakistan. Many of the plant samples were found with the presence of more than one type of alphasatellite (Siddiqui et al. 2016). Previously, CLCuD in Pakistan has been reported to be associated with five species of alphasatellites CLCuMuA, GDarSLA, GDavSLA, GMusSLA, and CLCuShA. In a recent survey, six alphasatellite species were found: GDarSLA, GuLCuA, OLCuA, ToLCuPKA, CLCuMuA, and CLCuBuA, where CLCuBuA is the most prevalent (Siddiqui et al. 2016).

Alphasatellites have also been identified on several plant species in Pakistan. *Gossypium darwinii* symptomless alphasatellite have been identified from an asymptomatic weed plants, *X. strumarium* in Pakistan (Akram et al. 2013). Same alphasatellite have also been reported from cultivated cotton (Zia-ur-Rehman et al. 2013) and wild cotton (Nawaz-ul-Rehman et al. 2012) in Pakistan.

15.9 Diversity of *Bemisia tabaci* in Pakistan

Begomoviruses are transmitted from one plant to another by an insect vector, *Bemisia tabaci* (whitefly), in circulative and persistent manner that is restricted to humid and warmer climates of subtropical and tropical countries. Whitefly is a complex of species consisting on more than 24 different biotypes and 12 genetic groups that have been classified on the basis of biological features like reproductive rate, dispersal, and host plant colonizing efficiency (Dinsdale et al. 2010; De Barro et al. 2011). Commonly, 16 biotypes of whitefly have been reported throughout the world, namely, B, Q, M, L, H, B2, A, N, C, P, R, K, J, S, E, and AN. Using Bayesian phylogenetic analysis, global whitefly specie complex was divided into 12 major genetic groups (Indian Ocean, Mediterranean, sub-Saharan Africa silver leafing,

Mediterranean/Africa/Asia minor, Asia I, Asia II, Italy, Australia, China, New World) (Boykin et al. 2007).

Recent studies based on sequence analysis of 3′ and 5′ end of gene, cytochrome c oxidase 1 (COI), revealed that six species of whitefly are present in Pakistan including Asia 1, Asia II 1, Asia II 5, Asia II 7, MEAM 1, and Pakistan. Relative distribution of whitefly species over Punjab and Sindh district of Pakistan revealed that Asia II 1 is a dominant species in Sindh and Punjab, while Asia 1, Asia II 5, and Asia II 7 are restricted to central/southern Punjab, central/northern Punjab, and central/northern Punjab, respectively. MEAM 1 was detected from southern Punjab and three regions of Sindh (central, northern, and southern) (Ashfaq et al. 2014).

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Begomoviruses in Nigeria

16

Boniface David Kashina

Abstract

The family *Geminiviridae* primarily constitutes an important family of circular single-stranded DNA (ssDNA) plant-infecting viruses, which pose severe constraints in agricultural production globally and serious threat to food security in sub-Saharan Africa. There are seven known genera, *Mastrevirus*, *Begomovirus*, *Curtovirus*, *Becurtovirus*, *Eragrovirus*, and *Turncurtovirus* of viruses belonging to the family *Geminiviridae*. Of these, the best characterized economically important species belong to the genus *Begomovirus*. *Begomovirus* species are either monopartite (possessing only DNA-A) or bipartite (having both DNA-A and DNA-B components). Majority of the monopartite begomoviruses also have subviral ssDNA satellite components, called DNA α or DNA β. In some cases, defective interfering DNAs can be found in the helper virus due to deletions of some genomic parts associated with bipartite and monopartite begomoviruses. New begomoviral species and their associated subviral components continue to emerge globally, thereby, constituting a formidable challenge to the profitable production of vegetables and other crops. Key begomoviruses associated with major crops in Nigeria are documented in this review.

Keywords

Geminiviridae • *Begomovirus* • Crops • Diversity • Nigeria

B.D. Kashina (✉)
Institute for Agricultural Research, Ahmadu Bello University, P. M. B. 1044, Zaria, Nigeria
e-mail: bkashina@gmail.com

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16.1 Introduction

Geminiviruses have single-stranded DNA (ssDNA) and circular genomes with twin-like particles (Fauquet et al. 2008). They are grouped into the genera *Becurtovirus*, *Begomovirus*, *Eragrovirus*, *Mastrevirus*, *Curtovirus*, *Topocuvirus*, and *Turncurtovirus* based on genome organization, similarities in nucleotide sequences, vectors, host range, and biological properties (Rey et al. 2012).

Virus species in the genus *Begomovirus* have genome organization with either two particles (DNA-A and DNA-B) or one particle (DNA-A). *Begomovirus* species are among the causes of huge losses on many economically important crops globally (Navas-Castillo et al. 2011). There are many species of begomoviruses demarcated based on sequence similarity (<89% nucleotide identity) with the DNA-A component of already recognized species (Fauquet et al. 2008). Begomoviruses have been classified into the New World (NW) and Old World (OW), with the former having bipartite genomes that are necessary for infectivity (Hamilton et al. 1983). Many Old World begomovirus group have monopartite genomes and ssDNA satellite molecules referred to as alpha- and beta-satellites (Zhou et al. 2003). Phylogenetically distinct and diverse groups of bipartite begomoviruses ("legumoviruses") and monopartite begomoviruses ("sweepoviruses") have evolved from both OW and NW begomoviruses and are found on legumes and sweet potatoes, respectively.

The whitefly, *Bemisia tabaci* (Gennadius), is the vector of all begomoviruses, including the "legumoviruses" and "sweepoviruses" (Ghanim et al. 2000). The emergence of various begomovirus species worldwide constitutes a major threat to agriculture. This growing threat is a function of the global evolution and spread of invasive species, as well as the sibling complex of whitefly species and possible combination of many factors such as the evolutionary adaptability of begomoviruses to diversity of hosts from differing ecologies, the intensification of agropractices with attendant consequences of expansion of whitefly population, and the inadvertent spread of host species, viruses, and whitefly biotypes outside their natural habitats via international trade in horticultural products (Seal et al. 2006; Rey et al. 2012).

Food security in sub-Saharan Africa is threatened by these begomoviruses. In Nigeria, they constitute a formidable challenge that requires urgent critical focus on strategies to both stop ongoing losses to these viruses and prevent the future emergence of any further begomoviral diseases. Considering the likely roles played by diversity of factors ranging from viral/vector evolution and population dynamics of emerging begomoviral diseases, it is highly imperative to tackle this problem frontally (Fondong et al. 2000; Esterhuizen et al. 2012). The current understanding and efforts put into this in Nigeria have not yielded the expected results in matching up with the scale of emerging geminiviral diseases. This document on begomoviruses in Nigeria is intended to synthesize available information that will further provide the needed impetus and direction for further study of these significant agricultural pests in Nigeria.

16.2 Begomoviruses of Crops in Nigeria

16.2.1 Cassava Mosaic Begomoviruses

Cassava, like most other crops, is prone to attack by viruses. The cassava mosaic disease (CMD) is the most severe, widespread, and the major limiting factor to the production of cassava in sub-Saharan Africa (SSA). Typical symptoms of CMD range from mosaic, mottling, and curled leaflets to reduced leaf size and stunting of plants. The causal agent of CMD was determined and identified by molecular characterization and Koch's postulates (Harrison et al. 1997; Stanley and Gay 1983; Bock and Woods 1983) to be the African cassava mosaic virus (ACMV). As a follow-up to this discovery, many variant species of ACMV have been reported from SSA (Hong et al. 1993; Berrie et al. 1998; Zhou et al. 1998; Fondong et al. 2000; Maruthi et al. 2004; Bull et al. 2006; Alabi et al. 2011) and from the Indian subcontinent (Matthew and Muniyappa 1992; Saunders et al. 2002). The ACMV and its variants are placed in the genus *Begomovirus* and family *Geminiviridae*.

Morphological and physiological alterations arising from various distortions suffered by cassava plants infected with viruses result in significant tuber yield and storage losses (Seif 1982). Cassava viruses and their satellites are transmitted via infected stem cuttings, experimentally by grafting (Atiri et al. 2004), biolistic inoculation (Briddon et al. 1998), and primarily, by whiteflies, *Bemisia tabaci*, for spread from plant to plant.

Diagnostically, polyclonal antibodies, immunosorbent electron microscopy, and polymerase chain reaction techniques have been employed to detect cassava viruses in infected cassava plants (Sequeira and Harrison 1982; Roberts et al. 1984; Ogbe et al. 1997; Fondong et al. 2000; Berry and Rey 2001; Pita et al. 2001; Harrison et al. 2002; Thottappilly et al. 2003; Ndunguru et al. 2005; Alabi et al. 2008; Monde et al. 2010). Using these techniques, several plants belonging to different plant families have been reported as hosts of cassava viruses (Bock and Woods 1983; Thottappilly et al. 2003; Ogbe et al. 2006; Alabi et al. 2007; Mgbechi-Ezeri et al. 2008; Monde et al. 2010).

16.2.2 *Jatropha Mosaic Nigerian Virus* (JMNV)

Jatropha curcas L. is a plant of considerable potentials to produce biofuel, fertilizer, and biogas and reclaim degraded and eroded soils (Singh et al. 2010). These potentials threatened the prevalence of a disease associated with severe mosaic, mottling, and blistering symptoms on the leaves, as well as stunting and eventual death of severely diseased plants. Elsewhere, several viruses have been reported on jatropha (Brown et al. 2001; Narayana et al. 2006; Raj et al. 2008; Ramkat et al. 2011; Snehi et al. 2011). In a study conducted to characterize the agent causing the disease in Nigeria, Kashina et al. (2013) identified it as a monopartite begomovirus named jatropha mosaic Nigeria virus (JMNV) (Brown et al. 2015). Jatropha mosaic Nigeria virus, the only begomovirus reported on jatropha in Nigeria so far, is transmitted by

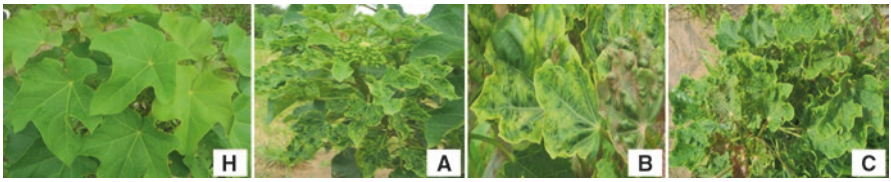

Fig. 16.1 Healthy (*H*) and infected jatropha plants showing different shades and combinations of symptoms: mosaic, leaf blistering, and mottling (**a**–**c**)

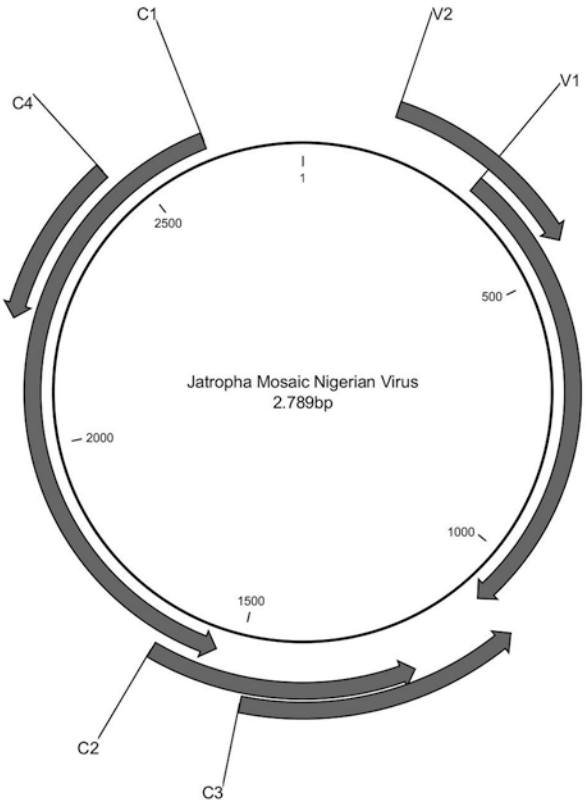

Fig. 16.2 Genomic map of DNA-A of jatropha mosaic Nigerian virus with complementary-sense (C1–C4) and virus-sense (V1 and V2) open reading frames and their direction of transcription

whiteflies, *Bemisia tabaci*, and by cuttings infecting up to 100 % of plants. The study was conducted from a sampled collection of young symptomatic leaves (Fig. 16.1) maintained in a plantation of *Jatropha curcas* accessions collected from different locations in Nigeria. The genome of JMNV has four complementary-sense open reading frames and two of the virus-sense strands (Fig. 16.2), which is typical of begomoviruses from the Old World (Rybicki 1994). The virus formed a cluster with other African Old World viruses (Fig. 16.3).

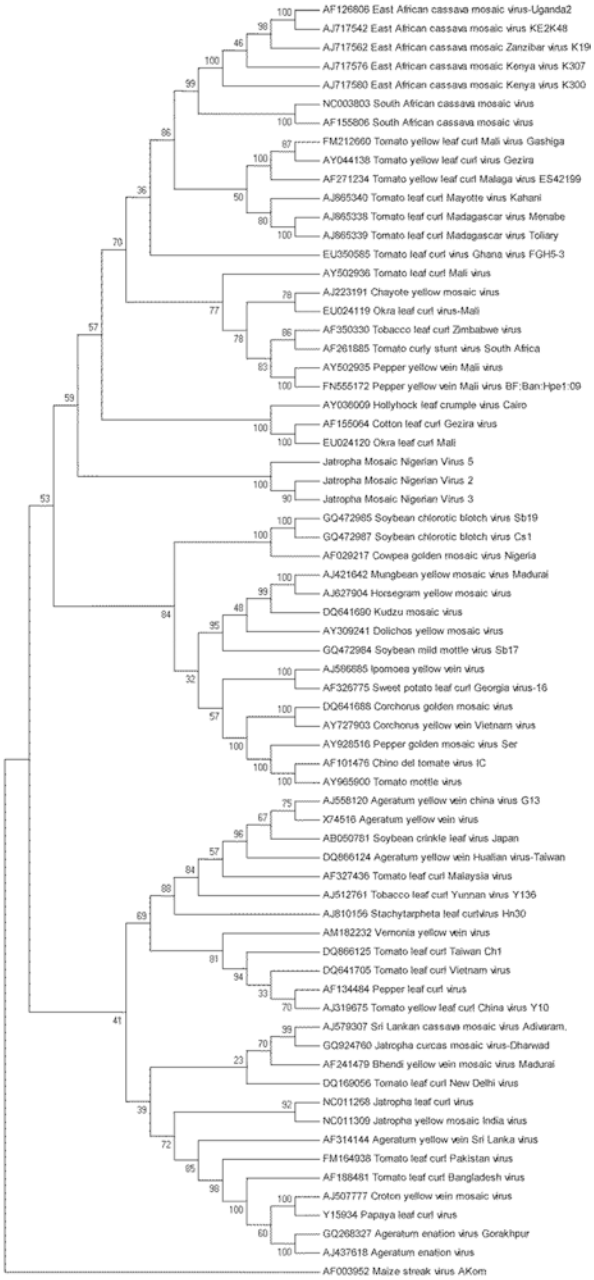

Fig. 16.3 Phylogenetic tree showing the clustering of jatropha mosaic Nigerian virus with other begomoviruses constructed using the neighbor-joining algorithm of MEGA 5 (30) and 500 replicates of bootstrap values. The maize streak *Mastrevirus* was used as an outgroup (Culled from Kashina et al. 2013)

16.2.3 Potato Leaf Curl Virus

Sweet potato (*Ipomoea batatas*) is an important food crop in sub-Saharan Africa. The African continent, led by Uganda, is the second largest producer of sweet potatoes after Asia (FAOSTAT 2008). The crop can be grown all through the year in the tropics.

Over 20 viruses from different genera are known to infect sweet potato (Valverde et al. 2007), causing yield losses between 80 and 90% (Aritua et al. 2000; Mukasa et al. 2006). The most economically important of these viruses is the sweet potato virus disease (SPVD) complex arising from two viruses, namely, sweet potato chlorotic stunt virus (SPCSV) and sweet potato feathery mottle virus [SPFMV], which are transmitted by whiteflies and aphids, respectively (Valverde et al. 2007). Through the successful transmission of a filamentous virus by aphids from sweet potato to *Ipomoea setosa* and a whitefly-vectored component which they named sweet potato vein clearing virus (SPVC) and sweet potato chlorotic stunt virus (SPCS), respectively, Schaefers and Terry (1976) reported, for the first time, the occurrence of SPVD complex in Nigeria.

Begomoviruses have received much less mention in most surveys of sweet potato viruses in the past. However, scientists are delving into the study of a phylogenetically divergent group of monopartite begomoviruses, named sweepoviruses (Fauquet and Stanley 2003). These viruses frequently infect sweet potatoes and related *Ipomoea* species. In sub-Saharan Africa, the first reported sweepovirus was detected in sweet potato crops in Uganda (Wasswa et al. 2011). Rossel and Thottappilly (1988) suggested that the first probable detection of a sweepovirus in Nigeria was in 1984 when an agent causing upward rolling of sweet potato leaves was transmitted by whiteflies. Basically, research on sweepoviruses in Nigeria is a fallow area waiting to be explored.

16.2.4 *Cotton Leaf Curl Virus* and *Cotton Yellow Mosaic Virus*

Cotton (*Gossypium hirsutum* L.) is an important cash crop grown in Nigeria by approximately 0.8 million farmers second to groundnuts as a cash crop in the north (Ogungbile and Kyari 1989). Many pathogens attack cotton plant and induce different diseases that cause severe losses in cotton production. The cotton leaf curl disease (CLCuD) caused by association of several begomoviruses transmitted by the whitefly, *Bemisia tabaci*, is a major threat to cotton production (Briddon et al. 2000; Khan and Ahmad 2005). The disease was first reported in Nigeria during 1912 (Farquarson 1912) followed by reports from Tanzania and Sudan in 1926 and 1934, respectively (Bailey 1934). Geographically, the virus had been reported from Nigeria, Sudan, Tanzania, the Philippines, and Pakistan. Most studies published between 1912 and the mid-1980s centered on leaf curl isolates from Nigeria and Sudan, while other reports involve isolates from Pakistan and India (Brown 1994).

Estimates of cotton yield losses range up to 20% when infection occurs early in the growing season and/or with highly susceptible cultivars. In Pakistan, up to 80%

of the plants in some fields have become infected early in the season, and entire plantings have been lost to the disease (Brown 1994).

Natural hosts of the virus isolates studied from various locations on the African continent are relatively few and primarily confined to species within the Malvaceae. The natural and experimental host range of the virus from Africa are reported to include several species within the genera *Althaea*, *Gossypium*, *Hibiscus*, *Malva*, and *Sida* and *Corchorus fascicularis*, *Lavatera cretica*, *Malvaviscus arboreus*, and *Pavonia hastata* (Brown and Bird 1992).

Different symptom types are visible on virus-infected cotton plants depending upon the severity of infection. Typical symptoms include curling of leaf, thickening and swelling of veins, and production of foliar outgrowth at the underside of the leaf, called enations (Mansoor et al. 1997). Characteristically, there are two types of vein thickening, major and minor vein thickening, which are associated with the disease. Cotton plants affected by the disease appear stunted due to reduced intermodal distance. Significant reduction in number of bolls per plant, branches per plant, boll weight, and seed cotton yield and deterioration in lint quality have been reported on CLCuD-infected plants (Tanveer and Mirza 1996).

Another virus, cotton yellow mosaic virus also referred to as African cotton mosaic virus (Malathi et al. 2003), had been reported on cotton in Nigeria. The virus, which is responsible for losses in cotton yield between 30 and 50 %, is transmitted persistently by whiteflies and, experimentally, by grafting (Malathi et al. 2003), but neither by seed nor sap (Cauquil and Follin 1983).

The disease is distributed in West Indies and Venezuela (Malathi et al. 2003). It occurs irregularly in Benin Republic, Cameroon, Central African Republic, Cote d' Ivoire, Ghana, Mali, Nigeria, Tanzania, Chad, and Togo (Cauquil and Follin 1983; Brunt et al. 1996; Alegbejo et al. 2008). Primary hosts of the virus are *Gossypium* spp. and *Abelmoschus esculentus.* Generally, only a limited research had been conducted on cotton begomoviruses in Nigeria.

16.2.5 *Maize Streak Virus*

Maize (*Zea mays* L.) production in tropical Africa is constrained by a complex of pests and diseases causing economic losses of quantity and quality of produce. In Nigeria, *maize streak virus* (MSV) disease, transmitted by the leafhoppers (*Cicadulina* species), reduces maize grain yield. The virus is one of many reported worldwide (Redinbaugh et al. 2004). First reported in South Africa in 1901, the disease has now spread to at least 20 African countries (Wambugu and Wafula 2000; Lagat et al. 2008; Karavina 2014). The disease reached epiphytotic level in Nigeria in the 1970s, causing significant yield losses (Fajemisin 2003). Alegbejo et al. (2002) reported up to 100 % yield losses due to the MSD.

The *maize streak virus* has 11 strains (MSV-A to MSV-K) with the A strain, which has five variants (MSV-A_1 to MSV-A_4 and MSV-A_6), being the most severe in maize, while the others (B to K) infect crops like barley, wheat, oats, rye, sugarcane, millet, and most wild annual grass species (Martin et al. 2001; Varsani et al.

2008; Shepherd et al. 2010; Monjane et al. 2011; Oluwafemi et al. 2014; Karavina 2014). In West African countries, such as Nigeria, where maize is not cultivated year-round, this MSV strain is forced to overwinter in non-maize hosts (Oluwafemi et al. 2014).

Collaborative research efforts by maize scientists at both international and national agricultural research center systems had led to the release of varieties that are resistant to the streak virus from different gene sources for cultivation in MSD endemic areas (Fakorede et al. 2001).

16.2.6 *Soybean Chlorotic Blotch Virus* and *Soybean Mild Mottle Virus*

Soybean [*Glycine max* (L.) Merr.] is an important leguminous crop cultivated worldwide for its oil and protein. Nigeria is reputed to be the largest producer of the crop in Africa with over 719,300 ha of arable land committed to its production (FAOSTAT 2014). The performance of soybean in Nigeria is threatened by pests and diseases from fungal, bacterial, and viral pathogens (Dugje et al.2009). Hughes and Shoyinka (2004) documented the occurrence of many viruses of economic significance on soybean in Nigeria and other countries of sub-Saharan Africa. Despite the documentation of several begomoviruses on soybean in other regions of the world, relatively fewer reports of the occurrence of these in soybean in Nigeria and other African countries of south of the Sahara have been made (Samretwanich et al. 2001; Quazi et al. 2007; Fernandes et al. 2009). These include the *African cassava mosaic virus* (Mgbechi-Ezeri et al. 2008) and a few others, such as *mung bean yellow mosaic India virus*, *mung bean yellow mosaic virus*, *soybean crinkle leaf virus* (Samretwanich et al. 2001), *soybean blistering mosaic virus* (SbBMV) [Unpublished], *sida micrantha mosaic virus* (Jovel et al. 2004), *bean golden mosaic virus* (Gilbertson et al. 1991), and *okra mottle virus* (Fernandes et al. 2009) reported elsewhere. Ilyas et al. (2009) demonstrated by agroinoculation that RhYMV is capable of infecting soybean. A study was conducted to characterize two begomoviruses naturally infecting soybean in Nigeria. This led to the first report of *soybean chlorotic blotch virus* (SbCBV) and *soybean mild mottle virus* (SbMMV) on soybean in Nigeria (Alabi et al. 2010). Also, SbCBV was found in a wild legume host (*Centrosema pubescens* (DC.) Benth.) (Alabi et al. 2010). Studies by Kashina (2015 unpublished data) confirmed the occurrence of SbCBV on lima beans, *Phaseolus lunatus* L., in Nigeria.

16.2.7 *Okra Leaf Curl Virus*

Okra (*Abelmoschus esculentus* (L.) Moench) is believed to have originated from Northeast Africa, but now widely grown all over Africa especially, Nigeria, Sudan, and Egypt. India is the world's largest producer of okra followed by Nigeria and Sudan. It is grown on about 2 million hectares annually in Nigeria. It is a popular

fruit vegetable which is grown during the wet and dry seasons under irrigation in Nigeria. The crop is prone to attack by pests and diseases, causing yield losses of economic proportion (Anaso and Lale 2001). One of the key insect pests is the whitefly (*Bemisia tabaci* Genn.), which transmits the okra leaf curl virus in a persistent manner. This geminivirus causes severe disease of okra with yield losses of up to 80% (Basu 1995). Typical symptoms on infected okra plants include leaf wrinkling, curling, vein distortion, leaf yellowing, stunted growth, and reduced yields. The incidence of the disease has been reported in Nigeria (Askira 2012).

16.2.8 *Tomato Leaf Curl Virus* and *Tomato Yellow Leaf Curl Virus*

Tomato yellow leaf curl virus (TYLCV) and *Tomato leaf curl virus* are two devastating begomoviruses causing economic losses on cultivated tomatoes (Brown 1994; Lapidot et al. 2001). They are distributed widely in the Middle East; North, Central, East, and West Africa; Southeast Asia; and Southern Europe (Czosnek et al. 1990; Alegbejo 1995; Navas-Castillo et al. 1999; Ladipot et al. 2001; Kashina et al. 2002). Hong and Harrison (1995) reported 84–86 % in the coat protein sequences between *tomato yellow leaf curl virus*-Nigeria and the species from Israel and Saudi Arabia. In a study to characterize begomoviruses associated with tomato grown in Togo and Nigeria, Kon and Gilbertson (2012) reported that the begomovirus from Nigeria has a recombinant genome and sequence identity of less than 89 % when compared with sequences of previously characterized begomoviruses. Hence, it was designated as a new species named tomato leaf curl Nigeria virus-Nigeria (ToLCNGV).

Both viruses are persistently vectored by *Bemisia tabaci*. Similar symptoms caused by both viruses on infected plants include stunting, flower shedding, fruit fall, reduced fruit size, leaves rolling upward and inward, and proliferation of lateral branches (Kisha 1981; Moustafa 1991). Yield losses of 23%, 50%, 63%, and 100% have been reported in Nigeria, Sudan, Lebanon, and the Mediterranean, respectively (Yassin and Abu 1972; Makkouk et al. 1976; Alegbejo and Ogunlana 1995; Lapidot et al. 2001).

In Nigeria, Alegbejo (1995) evaluated 16 tomato cultivars, some of which are commonly grown by farmers in northern Nigeria and possessing some desirable agronomic characteristics for resistance to TLCV. None of the cultivars was resistant to TLCV, except five cultivars with moderate resistance. The rest were either moderately or highly susceptible to the virus.

16.3 Conclusion

Begomoviruses are diverse and new species which continue to emerge. Their impact on the performance and yield of crops cannot be underestimated. Some countries have developed advanced technologies to study the plethora of begomoviruses in existence and the ones evolving, while there is a lack of the personnel, capacity, and

facilities to do same in other countries. The twin problem of emerging/evolving begomoviruses and cryptic species of their vector, *Bemisia tabaci*, continues to present a challenge to phytovirologists and vector entomologists. This is also not being helped by the harmful agricultural practices that could negatively tilt the desired balance. The picture and environment of research available for researchers in the developing nations, including Nigeria, are gloomy, but not hopeless. A seeming remedy to the prevailing challenge will require targeted research collaboration in knowledge sharing and skill acquisition between and among scientists from endowed and not-too-endowed nations. A refocusing of priorities to favor research in key areas, such as the understanding and mitigation of the impact of pathogens, such as viruses on crop production and food security, will most certainly be a step in the right direction.

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Status of *Begomovirus* Research and Management in Kenya

17

Douglas W. Miano and Paul K. Kuria

Abstract

Viruses belonging to the genus *Begomovirus* (family: *Geminiviridae*) infect different crops in different parts of the world, resulting in great economic losses. In Kenya, begomoviruses have been reported to infect important cultivated crops such as cassava, sweet potatoes, and tomatoes and noncultivated plant *Deinbollia borbonica*, a perennial weed. Apart from begomoviruses infecting cassava, those infecting other crops have not been fully characterized, and their distribution within the country has not been established. This paper describes the current status of begomoviruses in Kenya, management strategies employed, and research gaps that need to be addressed.

17.1 Introduction

Begomoviruses belong to the family *Geminiviridae*, infect different crops and have been devastating all over the world. The threat to world agriculture by *Begomovirus* species is generally recognized as emergent, increasing in the last three decades (Rey et al. 2012). Begomoviruses are transmitted by the whitefly *Bemisia tabaci* (*Gennadius*) in a persistent, circulative manner (Czosnek et al. 2002) and through planting materials for clonally propagated crops such as cassava and sweet potato. The global emergence of begomoviruses and associated disease outbreaks have

D.W. Miano (✉)
Department of Plant Science and Crop Protection, University of Nairobi, 29053 00625, Nairobi, Kenya
e-mail: dwatuku@yahoo.com

P.K. Kuria
Biotechnology Centre, Institute of Biotechnology Research, Kenya Agricultural and Livestock Research Organization, 57811 00100, Nairobi, Kenya

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been associated with the global spread of *Bemisia tabaci* whitefly complex, ability of begomoviruses to adopt to new hosts in geographical locations, agricultural intensification that favors rapid whitefly population expansion, and international trade in horticultural products that has spread host species, viruses, and whitefly biotypes outside their natural geographical ranges (Rybicki and Pietersen 1999; Seal et al. 2006; Rey et al. 2012).

In Kenya, studies on begomoviruses have been limited to only a few crops including cassava, sweet potato, tomato, and, recently, a weedy perennial host *Deinbollia borbonica*. There is almost no information available on begomoviruses infecting other crops, probably due to poor documentation because of low publications in scientific journals, which makes it difficult to capture all of what may have been done.

17.2 Sweet Potato Begomoviruses

Sweet potato [*Ipomoea batatas* Lam (L.)] is a dicotyledonous plant, in the family Convolvulaceae (morning glory). It is ranked as the seventh most important food crop worldwide with more 130 million metric tons of fresh storage roots produced annually. Among root and tuber crops, sweet potato is the third most popular worldwide and is an important food security crop in Kenya (FAOSTAT 2014). Sweet potato is highly adaptable to areas with seasonal rainfalls or long drought periods and persists well in marginal soils; thus, it is a suited crop in western regions, areas around Lake Victoria, eastern regions, and central and coastal areas of Kenya. Sweet potato is vegetatively propagated through storage roots, shoot tips, and stem cuttings. This mode of propagation results in the accumulation of viruses over subsequent generations, consequently suppressing yields and storage root quality. Viruses have been reported to cause over 90% yield reductions in East Africa including Kenya (Gibson et al. 1997).

Over 30 viruses have been described as pathogens of sweet potato, half of them belonging to the families *Geminiviridae* and *Caulimoviridae* (Clark et al. 2012). *Sweet potato leaf curl virus* (SPLCV, genus *Begomovirus*) is transmitted through vegetative propagation and persistently by the insect vector whitefly (*Bemisia tabaci*). SPLCV has been isolated from sweet potato fields in different parts of the world including the United States, South America, the Middle East, Southeast Asia, and East Africa (Briddon et al. 2006; Luan et al. 2006; Miano et al. 2006; Prasanth and Hegde 2008; Lozano et al. 2009; Paprotka et al. 2010; Albuquerque et al. 2011; Wasswa et al. 2011). Sweet potato plants infected with SPLCV exhibit upward curling and/or rolling of leaves, vein swelling, and vein mottle in young sweet potato plants (Fig. 17.1). However, symptom remission is observed in mature plants, and most plants become symptomless. Despite the lack of characteristic foliar symptoms, SPLCV can cause between 10 and 80% yield loss for different sweet potato cultivars (Clark and Hoy 2006; Ling et al. 2011; Gibson and Kreuze 2015).

Sweet potato leaf curl virus was first reported in Kenya in 2006 (Miano et al. 2006). A countrywide survey conducted in 2011 (Maina 2014) revealed that the

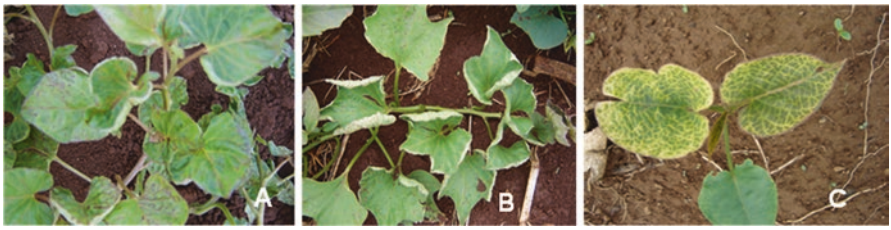

Fig. 17.1 Different sweet potato genotypes showing typical leaf curl symptoms associated with *Sweet potato leaf curl virus*. Leaf curling (**a**), leaf rolling (**b**), and vein mottle (**c**) symptoms observed on sweet potato in Kenyan fields (Adopted from Miano (2008))

virus was present in all the major sweet potato-growing regions, with the highest incidence being reported in the western region at 33%, while the coastal region had an incidence of 17.0% and central 2.6%. Coat protein gene AV1 from isolates collected from each region was sequenced, and their comparison revealed 88.4–100% nucleotide identity and that Kenyan SPLCV isolates have a clear diversity and also are closely related with other isolates from different parts of the world.

Though several reports indicate the presence of SPLCV in major sweet potato-growing regions of Kenya, characterization of complete genome sequences of SPLCV isolates in Kenya and their phylogenetic relationships with other isolates from other parts of the world has not been fully elucidated.

17.2.1 Management of *Sweet Potato Leaf Curl Virus*

The distribution of sweet potato planting materials in Kenya is through the informal seed system where there is no seed certification and no virus indexing. Lack of streamlined virus-indexing techniques for sweet potato limits distribution of high-quality planting materials and can heighten SPLCV when asymptomatic but systemically infected vines are distributed to farmers. The wide distribution of the virus within the country means that urgent measures are needed to clean the farmers' planting material and reduce possible losses incurred due to the presence of the virus.

17.3 Cassava Begomoviruses

The most important *Begomovirus* disease of cassava in Kenya is cassava mosaic disease (CMD), caused by a complex of viruses. These viruses are transmitted by whitefly, *Bemisia tabaci*, (*Gennadius*) and vegetative propagules. Several different *Begomovirus* species associated with CMD including *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), *East African cassava mosaic Malawi virus* (EACMMV), *East African cassava mosaic Cameroon virus* (EACMCV), *East African cassava mosaic Zanzibar virus* (EACMZV), and *East*

African cassava mosaic Kenya virus (EACMKV) have been reported in different cassava-growing regions of Kenya (Patil and Fauquet 2009; Legg et al. 2015; Were et al. 2016). Additionally, numerous strains of these viruses have been recognized. Under natural environments, mixed infection is a common feature of different species and/or strains of cassava-infecting begomoviruses resulting into increased overall virus titer leading to more severe symptoms. Furthermore, virulent recombinant strains arise from mixed infection; a remarkable example of these is *East African cassava mosaic virus*-Uganda (EACMV-UG) also known as the "Uganda variant" that was ascribed to CMD pandemics in East Africa, including Western Kenya (Zhou et al. 1997).

17.3.1 Symptoms of Cassava-Infecting Begomoviruses Infection

The most typical symptoms exhibited by plants infected with begomoviruses include yellow or pale green chlorotic mosaic of leaves, commonly accompanied by distortion and crumpling developing in an asymmetrical manner about the midrib. Where CMD symptoms are severe, plants are generally stunted, while petioles immediately below the shoot tip are necrotic, shrivel, and abscise resulting in a characteristic "candlestick symptom" (Alabi et al. 2011). Where the virus or virus strain is mild, or the cassava variety is tolerant, leaf chlorosis may be patchy and absent on some leaves, with little or no leaf distortion or malformation and little effect on overall plant vigor.

17.3.2 Economic Impact of Cassava-Infecting Begomoviruses

Yield loss estimates associated with cassava-infecting begomoviruses are largely a function of cassava varieties' susceptibility and virulence of the virus. For example, in Uganda yield losses of 66% were reported in the susceptible landrace Ebwanatereka (Byabakama et al. 1999). Similar studies in Tanzania documented yield losses in locally grown cultivars as 72% for Msitu Zanzibar, 85% for Rushura, and 90% for Bukalasa Ndogo (Legg et al. 2006). In Western Kenya, yield loss estimates of up to 72% have been reported in susceptible local cultivars (Legg et al. 2006). However, none of these studies accounted for the nature of infection or the virus species associated with the symptoms. Mixed infections with CMGs induce severe symptoms that compromise plant vigor and ultimately yield. Yield reductions of 82% were recorded in Ebwanatereka plants carrying a dual infection with ACMV and EACMV-UG compared with 68% with a single infection of EACMV-UG and 42% when infected with ACMV alone (Owor et al. 2004). Accurate country and regional yield losses are therefore hard to accurately quantify and depend on many variables such as cassava genotype, infectious geminivirus, environmental conditions, and market value for the roots.

In Western Kenya, cassava production and financial losses associated with the disease is well over 140,000 tons which is close to half the total production figure of

430,000 tons (FAO 1997), equivalent to US $ 14 million. This is based on the assumption that overall CMD incidence is over 70% (Legg and Okoa-Okuja 1999) and yield losses attributed to severe CMD is 40%. Such losses mean that CMD is one of the most globally damaging plant virus disease and continues to pose a great threat to all those with a stake in cassava production in Africa.

17.3.3 Management of Cassava-Infecting Begomoviruses

In Kenya, cassava is grown mainly by small-scale farmers with limited resources. Successful management of these viruses must therefore be inexpensive, sustainable, and involve little or no inputs. Two major approaches have been used in attempts to control CMD, the maintenance of a CMD-free crop through phytosanitation and the development and deployment of host plant resistance (Thresh and Otim-Nape 1994; Thresh and Cooter 2005). Other techniques which are also being used and/or tested include control of whitefly vectors and use of transgenic cassava with resistance to cassava mosaic geminiviruses (Taylor et al. 2012).

17.3.3.1 Phytosanitation

Phytosanitation involves the removal of diseased plants (roguing) from within a crop stand to prevent further spread and/or the selection of symptom-free cassava stems at the end of each growing cycle in order to plant new fields with "clean" material (selection) (Thresh et al. 1998). However, both techniques are difficult for farmers to apply since farmers have small plots of land, and even if they try to maintain a "clean" crop, it may become infected from external inoculum sources in neighboring fields. Farmers are also unwilling to remove growing plants that might contribute to some yield. The reluctance is even greater when there is a high rate of disease spread leading to the infection of a substantial proportion, if not all, of the plants. Selection or planting of virus-infected cuttings translates into establishment of diseased crop and elevated viral loads within fields. Planting high-quality disease-free stem cuttings results in faster crop establishment and avoids, or delays, initial infection translating into higher yields. This method is limited due to insufficient number of disease-free plants remaining at the end of the growing season from which to select, or, if there are sufficient plants, the conditions at harvest time may be unfavorable for symptom development, as can occur during hot, dry periods when many of the symptom-bearing leaves abscise. In Kenya, formal seed systems designed to deliver virus-free planting materials have been rare for cassava. Cassava stakeholders have recently been implementing quality management protocol (QMP) through the support from various donor agencies. These programs provide a system for assuring the quality and health of varieties promoted through multiplication schemes. This is achieved through the development of "clean seed site" for the production of "prebasic seed" of new improved cassava varieties (Legg et al. 2014b). The benefits of phytosanitation has not been fully realized as plants are readily infected by whiteflies with inoculum from various sources and are therefore only fully effective if the germplasm carries inherent resistance to begomoviruses.

17.3.3.2 Host Plant Resistance to CMD

Breeding programs were established in Madagascar and Tanzania to tackle the threat of CMD in the East African region as early as the 1930s, where interspecific crosses were made between cassava cultivars of diverse origins and accessions of the wild relative of cassava, *Manihot glaziovii* (Jennings 1976; Nichols 1947). The progenies designated Tropical Manioc Selection (TMS) series were reported to carry CMD1 locus that harbor multiple recessive resistance genes against begomoviruses. The most notable of these are cultivars TMS 30337, TMS 91934, TMS 30001, TMS 60142, TMS 30572, and TMS 4(2) 1425 which were deployed in CMD hotspots in Africa in the 1980s and 1990s (Hahn et al. 1980). Other sources of CMD resistance have been described in closely related West African cassava landraces designated TME 3–TME 7 and TME 14 and two phenotypically distinct genotypes TME 204 and TME 419 (Rabbi et al. 2014; Legg et al. 2006). The landraces were collected, designated as the Tropical *Manihot esculenta* (TME) series, and are currently preserved in the IITA germplasm collection (Rabbi et al. 2014). They display high resistance to all species of CMGs in diverse environments. Under high disease pressure, they develop typical CMD symptoms followed by complete recovery (Okogbenin et al. 2013). Based on molecular marker diversity studies, the CMD2-type landraces are genetically very similar and in some cases may be identical (Rabbi et al. 2014). CMD2 genotypes were heavily disseminated in East and Central Africa as a counter measure to CMD epidemics of the 1990s and early 2000s (Legg and Thresh 2000). They remain an essential component of farmer-preferred planting materials and parent lines within breeding programs. Cassava cultivars TMS 97/2205 and TMS 98/0505 were released from cassava breeding program of IITA to tackle CMD (Okogbenin et al. 2012; Dixon et al. 2010). Both genotypes are popular among West African cassava farmers and confer high resistance to CMD (Okogbenin et al. 2012). These genotypes are among the ones targeted for deployment in Kenya to mitigate the problems of cassava-infecting begomoviruses.

17.3.3.3 Vector Management

The most applicable vector control strategy is insecticide spray (Legg et al. 2014b). However, this is seldom practiced by cassava farmers due to the high costs of pesticides. It has been demonstrated that whiteflies develop resistance to pesticides upon repeated application, necessitating frequent rotation and change of pesticide types (Crowder et al. 2008). Farmers in Kenya are unlikely to purchase pesticides to control whiteflies in cassava unless they access subsidies or their farming practices shift from subsistence to commercial production, whereby production will be concentrated in large-scale profitable units.

17.3.3.4 Transgenic Approaches

Development of plant transformation systems offers an alternative method for generation of CMD-resistant plants (Chauhan et al. 2015; Ntui et al 2015). Various research groups have exploited pathogen-derived resistance techniques to develop transgenic cassava with resistance to cassava-infecting begomoviruses (Beyene

et al. 2017; Bull et al. 2009; Ntui et al. 2015; Vanderschuren et al. 2009). The advantage of the method is the possibility to keep traits that are considered of primary importance to cassava producers and consumers such as processing and taste qualities of the roots or to combine the virus resistance phenotype with high-yielding qualities of some of the inbred lines. Work is ongoing to develop transgenic cassava resistant to CMD in Kenya and Uganda. Varying levels of resistance to cassava-infecting begomoviruses have been reported in model cultivar TMS 60444 utilizing RNAi-based constructs (Taylor et al. 2012). However, these constructs have not been transformed into susceptible farmer-preferred varieties. More intriguingly, CMD resistance is lost in CMD2-type cassava genotypes after regeneration through embryogenesis (Beyene et al. 2015). Additional challenges also exist, most importantly demonstration of long-term durability of transgenic resistance to CMD under field conditions, where, as described above, the begomoviruses continue to evolve and undergo genetic change due to recombination and pseudo-recombination.

The challenge of developing a comprehensive integrated pest management (IPM) approach for cassava viruses remains unmet. New concerns about whitefly populations and emerging virus isolates therefore means that efforts are needed to extend and improve the control options available for farmers and, together with the incorporation of genetic transformation-based control methods, will give conditions under which each is most appropriate and on how best to combine them into an integrated strategy.

17.4 Begomoviruses Infecting Tomato

Tomato (*Solanum lycopersicum*) is an important vegetable in Kenya and is grown in almost all agroecological zones, either in the fields or greenhouses. The crop is affected by different pathogens including viruses. Among the viral pathogens, whitefly-transmitted geminiviruses have become the most important in the tropics and subtropics. Tomato yellow leaf curl disease (TYLCD) is one of the most devastating diseases in tomato. The disease is spread all over the world and is reported in over 30 countries in all continents. The disease is caused by a group of viral species of the genus *Begomovirus*, family *Geminiviridae* (geminiviruses), referred to as *Tomato yellow leaf curl virus* (TYLCV). These are transmitted by an insect vector, the whitefly *Bemisia tabaci*, classified in the family Aleyrodidae. TYLCV causes severe stunting of young leaves and shoots, resulting in bushy growth of infected tomato seedlings. Plants infected early in the season are normally stunted and excessively branched. Such plants have terminal and axillary shoots erect, while leaflets are reduced in size and abnormal in shape. Affected leaves are curled upward or inward. Flower drop is common, and therefore infected plants have a reduced number of flowers and fruit. If infection takes place at a later stage of growth, fruits already present develop normally. There are no noticeable symptoms on fruits derived from infected plants. Generally, table tomatoes are severely affected by the disease, especially when infection occurs before the flowering stage.

TYLCV was first reported in Kenya in 1996 and was associated with losses estimated at 50% (Nono-Womdim 2004; Nono-Wondmin et al. 1999). However, little has been done to determine the diversity, distribution, and current levels of damage caused by the disease in the recent years.

17.5 Begomoviruses in Noncultivated Plants

Begomoviruses have been reported to infect noncultivated plants in Africa (Sseruwagi et al. 2006). Kyallo et al. (2016) reported on a bipartite *Begomovirus* naturally infecting *Deinbollia borbonica* plants in Kenya and Tanzania. The DNA-A of the virus isolates was closely related to that of *Tomato leaf curl Mayotte virus* (82%), while the nucleotide sequence of DNA-B was highly identical to that of *East African cassava mosaic virus* at 65%. *Deinbollia borbonica* is a perennial tropical shrub that grows as a weed within mixed cropping farming systems where crops such as cassava, tomato, and beans are grown. Weed-infecting begomoviruses are important in the epidemiology of crop diseases, especially in the tropics where the crops are present throughout the year and therefore act as reservoirs. However, little is known about the role played by different noncultivated plants in *Begomovirus* epidemiology.

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Status of Begomoviruses in Ghana: The Case of Vegetables and Root and Tuber Crops

18

Michael Kwabena Osei, Eric Cornelius, Elvis Asare-Bediako, Allen Oppong, and Marian Dorcas Quain

Abstract

In developing countries, including Ghana, a greater proportion of the population depends on small-scale farming for their income and livelihood. Crops are frequently affected by a wide array of virus diseases showing varying degrees and kinds of symptoms including leaf curling and distortion, green or yellow foliar mosaic, stunting of plants, and reduced yields. An emerging and economically important group of plant viruses belonging to the genus *Begomovirus*, family *Geminiviridae*, are known to cause extreme yield reduction in a number of economically important crops in Ghana. These begomoviruses have a very wide host range, infecting dicotyledonous plants, and are transmitted by the whitefly vector, *Bemisia tabaci* (Genn.). Vegetable crops such as tomato, okra, and pepper and root and tuber crops such as cassava and sweet potato are greatly affected by begomoviruses resulting in substantial yield losses. Begomoviruses associated with tomato yellow leaf curl disease in Ghana include tomato leaf curl Kumasi virus (ToLCKuV), tomato leaf curl Ghana virus (ToLCGHV), tomato leaf curl Mali virus (ToLCMLV), and tomato leaf curl virus (TYLCuV). Pepper leaf curl diseases have been observed in pepper fields in several parts of Ghana, but begomoviruses associated with these diseases have not been characterized even though pepper veinal mottle virus (PVMV) and tomato yellow leaf curl virus (TYLCV) are the most widespread in the Western Africa subregion. The coat protein gene of cotton leaf curl Gezira virus (CLCuGV) and the DNA-β of satellite DNA have been amplified from diseased okra leaf samples collected from the

M.K. Osei (✉) • A. Oppong • M.D. Quain
CSIR-Crops Research Institute, P.O.BOX 3785, Kumasi, Ghana
e-mail: oranigh@hotmail.com

E. Cornelius
Department of Crop Science, University of Ghana, Legon, Accra, Ghana

E. Asare-Bediako
Department of Crop Science, University of Cape Coast, Cape Coast, Ghana

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Central Region of Ghana with characteristic leaf curl disease symptoms. Viruses are the second most important constraint to the production of sweet potato (*Ipomoea batatas*) after weevils, resulting yield losses by up to 90%. Sweet potato leaf curl virus has been detected in Ghana using both PCR and disease symptoms. Cassava mosaic disease (CMD), caused by cassava mosaic geminiviruses mainly African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV), is the most important constraint to the production of cassava in Ghana. In view of the increasing economic importance of begomoviruses in Ghana and worldwide, there is an urgent need for their effective management in order to improve yields and quality of crop plants. This requires accurate detection and identification procedures, stimulating intensive research efforts focused on virus biology, diversity, and epidemiology to develop successful control strategies. Inadequate plant virologists with requisite skills and knowledge in plant virology together with inadequate logistical and financial supports coupled with low institutional and governmental supports hinder the efforts in developing effective management strategies against the numerous viral diseases confronting the country.

Keywords
Plant viruses • Begomoviruses • Geminiviruses • Okra leaf curl disease • Tomato yellow leaf curl disease • Cassava mosaic disease • Sweet potato leaf curl disease

18.1 Introduction

The global expansion of agriculture has likewise resulted in the emergence and spread of numerous diseases and insect pests. Insect-transmitted viruses are of particular importance, especially in tropical and subtropical regions. Insect-transmitted viruses cause some of the most damaging and economically important diseases of crop plants. Geminiviruses are insect-transmitted viruses that have emerged over the past 20 years. These viruses cause extremely damaging diseases in a wide range of crops throughout the world. Viruses in the family *Geminiviridae* have small twinned icosahedral virions and a circular single-stranded (ss) DNA genome. Geminiviruses are subdivided into seven genera, *Mastrevirus*, *Begomovirus*, *Curtovirus*, *Topocuvirus*, *Becurtovirus*, *Eragrovirus*, and *Turncurtovirus* based on genome structure, while *Turncurtovirus* is based on genome structure and phylogenetic relationships, host range, and type of insect vector. The geminiviruses transmitted by the whitefly *Bemisia tabaci* are in the genus *Begomovirus*, which has the largest number (322) of species in the family. Begomoviruses cause economically important diseases of vegetables and root and tuber crops, mostly in Ghana and Africa regions of the world. In Ghana, vegetable crops such as tomato, okra, and pepper and root and tuber crops such as cassava, wild (tree) cassava, and sweet potato are the most heavily or greatly affected by begomoviruses resulting in

Fig. 18.1 Tomato shoot leaves showing marginal yellowing symptoms associated with TYLCV (www.avrdc.org)

substantial yield losses. This chapter presents prevalence and economic importance, incidences, and severity of begomoviruses on certain vegetables and root and tuber crops in Ghana. In addition, strategies for begomovirus disease management in Ghana including prospects and challenges are discussed.

18.2 Prevalence and Economic Importance of Begomoviruses in Ghana

18.2.1 Vegetables (Tomato, Okra, and Pepper)

18.2.1.1 Tomato

Begomoviruses are predominant and prominent to Ghana's tomato production. The major tomato virus in this group of viruses having monopartite single-stranded DNA is tomato yellow leaf curl virus (TYLCV). TYLCV was first observed in Israel in 1939–1940 associated with outbreaks of *Bemisia tabaci*. It has also, for many years, caused economically significant yield losses in tomato in the East Mediterranean region, the Middle East, Asia, Australia, and Africa. In Ghana, the disease is known to cause severe yield losses leading to the importation of tomatoes from Burkina Faso especially during dry seasons. Devastating losses to TYLCV in the Upper East Region of Ghana since 2002 have had major consequences for farmers, with market repercussions that have lasted up to the present. The increasing economic importance of TYLCV has also resulted in the need for accurate detection and identification procedures, stimulating intensive research efforts focused on virus biology, diversity, and epidemiology to develop successful control strategies (Fig. 18.1).

18.2.1.2 Okra

Okra leaf curl disease (OLCD) is commonly observed among okra crops in Ghana and is widespread in Africa. Affected plants show symptoms of leaf wrinkle, upward or downward curling of apical leaves, vein distortion and thickening, leaf yellowing, stunted growth, and reduced yields. Yield losses due to OLCD reported in Ghana

Fig. 18.2 Okra plants showing leaf curl disease symptoms (note the upward curling of leaves) (Picture courtesy of Elvis Asare-Bediako)

range from trace to over 50%. Elsewhere OLCD has been reported to cause yield losses of up to 80%. OLCD in Africa is associated with a complex of begomoviruses: cotton leaf curl Gezira virus (CLCuGV), okra yellow crinkle virus (OYCrV), hollyhock leaf crumple virus (HoLCrV), and okra leaf curl virus. These begomoviruses of the family *Geminiviridae* are transmitted by the whitefly *Bemisia tabaci* (Genn.) (Hemiptera, Aleyrodidae) (Fig. 18.2).

18.2.1.3 Pepper

Pepper production in Ghana is reported to attain only 50% of potential yields, and the low productivity has been attributed to pest and disease pressure, unavailability and high cost of irrigation, low soil fertility, and lack of improved varieties. Microbial pests, particularly viruses and prominently begomoviruses, affect pepper production in Ghana. Typically in pepper, the following viral diseases are associated with the *Begomovirus*: pepper golden mosaic complex (previously Texas pepper, Serrano golden mosaic, and pepper mild tigre viruses), Serrano golden mosaic virus (SGMV), pepper mild tigre virus (PMTV), pepper Huasteco (genus *Begomovirus*), pepper Huasteco virus (PHV), and others. The leaf curl disease in chili pepper is also caused by *Begomovirus* of the family *Geminiviridae*. Susceptibility to begomovirus infections in pepper however varies (Fig. 18.3).

18.2.2 Root and Tuber Crops (Cassava, Wild/Tree Cassava, and Sweet Potato)

18.2.2.1 Cassava and Wild/Tree Cassava

Cassava is the number one staple food crop for majority of Ghanaians, with per capita consumption of 152.9 kg/head/year, and has played a key role in food security in Ghana. It contributes 22% of agricultural gross domestic product and is fastly becoming an important crop for industries because of its high starch content. Cassava mosaic disease (CMD), caused by cassava mosaic geminiviruses of the family *Geminiviridae*, is undoubtedly the most important constraint to the

Fig. 18.3 Pepper plants showing virus symptoms

production of cassava in Ghana. The characteristic severe distortion and stunting of leaf and entire plant associated with the disease, especially on local genotypes, indicates how serious yields could be affected. The spread of mosaic virus is highly linked with its whitefly (*Bemisia tabaci*) vector. The disease spreads easily from one field to another in most cassava-growing areas as farmers continue to use infected stem cuttings as planting materials. The symptoms of CMD were first reported in Tanzania in 1894. At that time, it was particularly prevalent in the Gold Coast (now Ghana) and other African countries. A wild-growing species of *Manihot* in Ghana is the tree cassava or Ceará rubber tree (*Manihot glaziovii*). It is a species of deciduous flowering plant in the family Euphorbiaceae that is native to eastern Brazil. It is a glabrous shrub or tree 3–6 m high, occasionally taller (10–20 m), often with several weak branches near the base. Both the ACMV and the EACMV-Ug2 have been detected in symptomatic and symptomless *M. glaziovii* plants in Ghana during a nationwide survey of CMD in Ghana (Fig. 18.4).

18.2.2.2 Sweet Potato

Production of sweet potato (*Ipomoea batatas*), despite its high potential for food security, is constrained by viruses which reduce yield by up to 90%. Viruses pose the second most important biotic constraint after weevils. Occurrence of begomoviruses in sweet potato is widespread and associated with most, if not all, geographic regions where sweet potatoes are grown. A study in Ghana identified begomoviruses in the coastal savanna and the forest-transition zones of Ghana. While these reports are contemporary, there is evidence that there is considerable variability among the strains of begomovirus in Ghana. For instance, the sweet potato leaf curl virus (SPLCV) symptoms suggest that these viruses were present long before they were reported. Some of the strains either do not induce symptoms or induce very mild, transient symptoms in the standard indicator host *Ipomoea setosa*. Besides, every 10–20% of sweet potato accessions originating from different parts of the world tested positive at Centro Internacional de la Papa for begomoviruses. The differences among sweet potato begomoviruses may also indicate that these viruses

Fig. 18.4 Symptoms of cassava mosaic disease (CMD) on cassava. An infected plant showing severe stunting and distortion of leaves (**a**) compared to a healthy plant (**b**). Leaves of CMD-affected plants produce misshapen and twisted leaflets with mosaic and mottling symptoms (**c** and **d**) (Source: Alabi et al. 2011)

undergo a high rate of recombination, similar to reports for other geminiviruses (Fig. 18.5).

18.3 Molecular Identification and Characterization of Begomoviruses in Ghana

18.3.1 Vegetables (Tomato, Okra, and Pepper)

18.3.1.1 Tomato

In 2008, the CSIR-Crops Research Institute of Ghana in collaboration with AVRDC-The World Vegetable Center, Taiwan, identified three new distinct begomovirus associated with tomato yellow leaf curl disease in Ghana. These were found in the Ashanti region of Ghana where 33 tomato samples with symptoms such as curling, yellowing, small leaves, and stunting were collected. Three of these samples were from Akumadan and 30 from Kumasi and were assembled in 2007 and 2008, respectively. Incidence of the disease was approximately 75%. The collected samples were tested for the presence of begomoviral DNA-A, DNA-B, and associated satellite DNA by PCR using previously described primers. Whereas DNA-B and DNA-β were not detected by PCR, an expected 1.4-kb DNA-A begomovirus fragment was obtained from Akumadan and Kumasi giving 1 sample and 25 samples,

Fig. 18.5 Symptom of begomovirus on sweet potato (Source: J. Kreuze and S. Fuentes (CIP, Lima))

respectively. These products (1.4 kb) were further cloned and sequenced. Using the MegAlign software (DNASTAR, Inc., Madison, WI) for sequence comparison, three distinct virus groups were shown. One isolate from each group was chosen, and DNA-A sequence was completed using specific designed primers. All the DNA-As of GH5-3 (group 1), GOTB2-2 (group 2), and GHK2 (group 3) isolates consisted of 2803 (GenBank Accession No. EU350585), 2794 (GenBank Accession No. EU847739), and 2792 nt (GenBank Accession No. EU847740), respectively. They contained the geminiviral conserved nonanucleotide sequence TAATATTAC in the intergenic region and the six predicted open reading frames (ORFs V1, V2, C1, C2, C3, and C4). Geminivirus sequences in the GenBank database (National Center for Biotechnology Information) were used in conducting BLASTn analysis. This was followed by a further sequence comparison using Clustal V algorithm of MegAlign software. The highest sequence identity of 96.5% was found between the isolate GHK2 from Kumasi and tomato yellow leaf curl Mali virus (TYLCMLV; GenBank Accession No. AY502934). The DNA-A sequence of GH5-3 and GOTB2-2 isolates, however, had 87.5% sequence identity with each other. Nonetheless both had the highest sequence identities of 76.7% and 77.6%, respectively, with tomato leaf curl Antsiranana virus, Madagascar (GenBank Accession No. AM701764). The International Committee on Taxonomy of Viruses (ICTV) proposed species demarcation of 89% sequence identity. Based on DNA-A sequence comparisons and the ICTV proposal, two distinct begomovirus species were constituted. This was the first report of molecular characterization of begomoviruses associated with tomato leaf curl disease in Ghana.

The names *Tomato leaf curl Ghana virus* for isolate GH5-3 and *Tomato leaf curl Kumasi virus* for isolate BOTB2-2 were proposed, respectively.

In 2011, the University of Ghana conducted similar survey in Akatsi and North Tongu districts of the Volta Region to establish the causal agent for leaf curl and

yellowing diseases affecting tomato in the Volta Region of Ghana. Prevalent symptoms on the field were similar to symptoms of tomato yellow leaf curl disease, described by CSIR-Crops Research Institute in 2008, and caused by the *Tomato yellow leaf curl virus* (TYLCV).

In 2012, the CSIR-Crops Research Institute of Ghana collaborated with UC-Davis under Tomato IPM CRSP Project with funds from USAID to characterize begomoviruses and betasatellites associated with ToLCD in Ghana. A survey of tomato fields in three major tomato areas of Ghana (Akumadan, Agogo, and Tuobodom) was conducted in October–November 2012. Leaf samples were collected from plants with ToLCD symptoms (e.g., stunting; erect, upright, and distorted growth; and leaf curl and yellow leaf curl). Leaf disks were squashed onto nylon membranes (Nytran), and leaf sap was applied to absorption strips (Agdia) in Ghana and then transported to the University of California, Davis, USA. Membranes were hybridized with a general begomovirus probe. Total genomic DNA was extracted from absorption strips with a modified Dellaporta method. The survey of tomato fields in Akumadan, Agogo, and Tuobodom tomato areas of Ghana conducted in October–November 2012 revealed relatively low incidences (~5–10%) of ToLCD in all fields as well as low population of whiteflies (<5 adults/plant). This survey coincided with the end of the rainy season, when whitefly populations are typically low. However, the potential for the disease to cause losses to tomato production in Ghana under favorable conditions (i.e., in the dry season) is shown by an outbreak in 2014 in the Agotime-Ziope District of the Volta Region in which over 1000 ha and 600 farmers were affected and 100% yield loss was experienced in some fields. Most of the samples from tomato plants with ToLCD symptoms (24/28) were positive for begomovirus infection based on squash blot (SB) hybridization and/or PCR analyses (i.e., amplification of the expected size ~1.2-kb fragment). This is consistent with a begomovirus etiology for ToLCD in Ghana. The results revealed additional genetic diversity in the begomoviruses causing the disease, as well as the presence of betasatellites in Ghana for the first time, including a new species. Agroinoculation of infectious clones of these viruses and betasatellites was used to fulfill Koch's postulates, determine host range properties, and investigate the begomovirus-betasatellite interaction.

The study revealed additional complexity in the etiology of ToLCD in Ghana, and provided further evidence that the disease is caused by a complex of monopartite begomoviruses, which have evolved via recombination. The tomato-infecting begomoviruses from Ghana were then placed in a distinct phylogenetic clade (Ashanti clade), which includes viruses that evolved in the geographic area that includes Cameroon, Ghana, Nigeria, and Togo. We demonstrated the association of betasatellites with ToLCD in Ghana for the first time describing a new betasatellite species (ToLCGHB) and demonstrating specificity in the begomovirus-betasatellite interaction. Finally, the agroinoculation systems developed for the tomato-infecting begomoviruses from Ghana allowed for the completion of Koch's postulates, revealed differences in host range properties, and should be useful for screening tomato germplasm for resistance to ToLCD in Ghana. This information is

fundamental in the development of stable begomovirus-resistant tomato cultivars for Ghana.

18.3.1.2 Okra

OLCD in Africa is associated with a complex of begomoviruses: cotton leaf curl Gezira virus (CLCuGV), okra yellow crinkle virus (OYCrV), hollyhock leaf crumple virus (HoLCrV), and okra leaf curl virus. These begomoviruses of the family *Geminiviridae* are transmitted by the whitefly *Bemisia tabaci* (Genn.) (Hemiptera, Aleyrodidae). Information on begomoviruses associated with OLCD in Ghana is limited. The coat protein gene of cotton leaf curl Gezira virus (CLCuGV) and the DNA-β of cotton leaf curl Gezira betasatellite (CLCuGB) were amplified from disease samples using polymerase chain reaction. Molecular characterization of begomoviruses associated with the OLCD in Ghana is nonetheless not done. In the neighboring Burkina Faso, OLCD is mainly caused by a single monopartite begomovirus species, *Cotton leaf curl Gezira virus* (CLCuGV), and satellite DNA complexes, cotton leaf curl Gezira betasatellite (CLCuGB) and cotton leaf curl Gezira alpha satellite (CLCuGA), whereas CLCuGeV and OYCrV were implicated in OLCD in the neighboring Côte d'Ivoire.

18.3.1.3 Pepper

Knowledge on the prevalence and impact of begomoviruses on pepper in Ghana and West Africa has been scanty. Identification of the virus had until recently been based only on serology and hybridization, with emphasis on other vegetable virus diseases such as okra leaf curl disease (OLCD) and tomato leaf curl disease (ToLCD). However, currently advanced molecular techniques in the study of begomoviruses, such as polymerase chain reaction (PCR), rolling cycle amplification (RCA)/restriction fragment length polymorphism (RFLP), and sequencing, have improved the identification of all strains targeting the begomovirus/satellite complexes infecting tomato, okra, and pepper such as *Tomato leaf curl Cameroon virus* (ToLCCMV), *Tomato leaf curl Nigeria virus* (ToLCNGV), and *Tomato leaf curl Ghana virus* (ToLCGHV). *Pepper yellow vein Mali virus* (PepYVMLV) has been identified infecting tomato and pepper in the West and Central Africa region; in pepper, okra, and tomato, putative recombination events of begomovirus genomes have been detected, and this is indicative that recombination is an important mechanism for their evolution. It is, however, not clear which strains exist in Ghana. More work is needed to characterize the begomovirus associated with pepper in Ghana.

18.3.2 Root and Tuber Crops (Cassava, Wild/Tree Cassava, and Sweet Potato)

18.3.2.1 Cassava and Wild/Tree Cassava

Nine distinct cassava mosaic viruses have been characterized worldwide from CMD-affected cassava plants, and seven of them are from sub-Saharan Africa. These viruses are *African cassava mosaic virus* (ACMV), *East African cassava*

mosaic virus (EACMV), *East African cassava mosaic Cameroon virus* (EACMCV), *East African cassava mosaic Kenya virus* (EACMKV), *East African cassava mosaic Malawi virus* (EACMMV), *East African cassava mosaic Zanzibar virus* (EACMZV), and *South African cassava mosaic virus* (SACMV). Two other viruses, *Indian cassava mosaic virus* (ICMV) and *Sri Lankan cassava mosaic virus* (SLCMV), were reported from the Indian subcontinent. Cassava mosaic geminivirus (CMG) strains reported so far in Ghana are ACMV and EACMV. ACMD was first observed near Accra in 1926, and its spread was more significant in the coastal areas of the country around 1930. At present, ACMD is widespread and found in all the agroecological zones in Ghana. Cassava grown in Ghana is attacked by two species of geminivirus in either single or mixed infections. These are the *African cassava mosaic virus* (ACMV) and the *East African cassava mosaic virus* (EACMV). In 2012, some scientists used rolling cycle amplification and reported that cassava mosaic virus disease (CMV) is caused by one or a combination of cassava mosaic geminiviruses, ACMV and EACMV-like, which were named *African cassava mosaic virus*-Ghana and *East African cassava mosaic Cameroon virus*-Ghana. This is because computer analysis revealed that their genome arrangement follows the typical Old World bipartite begomovirus genome. They suggested that the association between the two species and their interaction might account for the severe symptoms observed on infected plants in the field and screenhouse. A wild-growing species of *Manihot* in Ghana is the tree cassava or Ceará rubber tree (*Manihot glaziovii*). It is a species of deciduous flowering plant in the family *Euphorbiaceae* that is native to eastern Brazil. It is a glabrous shrub or tree 3–6 m high, occasionally taller (10–20 m), often with several weak branches near the base. Both the ACMV and the EACMV-Ug2 have been detected in symptomatic and symptomless *M. glaziovii* plants in Ghana during a nationwide survey of CMD in Ghana.

18.3.2.2 Sweet Potato

Sweet potato begomoviruses are different from all other begomoviruses and are termed sweepoviruses. In Africa, SPCSV have been identified. They include two serologically distinguishable and geographically separated strains of SPCSV. The strain from West Africa is referred as $SPCSV_{WA}$. It was first isolated from Nigeria. The severity of virus symptoms varies with the number of different viruses infecting synergistically. The detection and identification of sweet potato viruses are a difficult procedure. They are intricate by frequent occurrence of mixed infections and synergistic complexes as in SPVD. Despite this, several virus detection methods including symptomatology, use of indicator plants, and serology such as enzyme-linked immunosorbent assay (ELISA), DNA-based polymerase chain reaction (PCR), and reverse-transcription polymerase chain reaction (RT-PCR) are used for virus indexing in sweet potato.

The CSIR-Savanna Agricultural Research Institute and the Biotechnology, Nuclear Agriculture Research Institute (BNARI), and Ghana Atomic Energy Commission carried out a study to detect sweet potato (*Ipomoea batatas* L.) leaf curl virus (SPLCV) in Ghana using visual symptomatology and PCR Technique. Virus-associated symptoms comprising vein clearing, interveinal chlorosis,

chlorotic spots, upward curling on leaf edges, leaf narrowing and distortion, purpling, blistering, and general leaf yellowing were visually revealed in all 22 accessions grown on the field. At the end of the study, disease incidence (DI) significantly ($p \leq$ #0.05) varied between accessions. The accession US003 gave the lowest (20%), while ten other accessions had the highest DI (90%). The viral disease symptom severity ranged from mild to moderate (1.70–2.19 mean severity score) in the accessions. However, the index of symptom severity of all plants (ISSap) ranged from 1.08 ± 0.09 to 3.67 ± 0.11 with VOTCR003 having the lowest. This suggests that it is a mildly susceptible accession, while VOTCR002 had the highest, thus suggesting that it is moderately susceptible to viral diseases. Contrarily, the index of symptom severity of diseased plants (ISSdp) ranged from 2.00 ± 0.25 to 3.75 ± 0.32. Visual symptomatology showed that VOTCR002 had the highest DI, ISSap, and ISSdp, suggesting that it is highly susceptible to viral diseases. Of the ten severely infected accessions that were tested for sweet potato leaf curl virus (SPLCV) using PCR technique, 30% of the accessions were detected for SPLCV.

18.4 Incidences and Severities of Begomoviruses in Ghana

18.4.1 Vegetables (Tomato, Okra, and Pepper)

18.4.1.1 Tomato

From the time when TYLCV disease was discovered, it has become the most important constraint to tomato production in many countries where tomato is widely cultivated. The disease can cause up to 100% yield losses. It can also affect the functioning of the leaves, stems, and roots ultimately reducing yield. For instance, in Ghana, the recorded average fruit yield of 10 t/ha is partially attributed to the incidence of TYLCV disease. The disease is prevalent in Ghana causing severe yield losses. The disease became popular in 2002 when high incidences of the disease led to devastating losses in the Upper East Region of Ghana, a major tomato-growing area. This had major consequences for farmers, with market repercussions that have lasted to the present. It was so severe that most farmers resigned tomato farming which ensued Burkina Faso to take advantage and has since then made Ghana an importer of tomato instead of exporter. Later, several tomato farms also recorded similar symptoms believed to be TYLCV. The causal agent was not known in Ghana until 2008 when three new distinct viruses associated with the disease were found in some major tomato-growing areas in Ghana by the CSIR-Crops Research Institute and the observed leaf curl disease incidence in farmers' fields was approximately 75%. Nonetheless, incidence of the disease across most farms was as high as 100%. Plants affected with the disease appear to be stunted at an early stage of the disease development. The terminal and auxiliary shoots of infected plants are erect, and the leaflets they produce are small in size, and leaves are cupped downward giving it a deformed orientation. Cupped leaves may or may not have yellowing leaf margins. Infected plants may produce either no fruits or very small fruits. Flower symptoms of infected plants have not been observed. The disease

generally inhibits plant growth. Yield loss is greatest when tomato leaf curl virus infection occurs during the early stages of plant growth. Survey by the CSIR-Crops Research Institute indicated that farmers had some knowledge of the disease but were handicapped in the management of the disease. The findings showed that farmers were familiar with the disease and regarded it as a serious constraint upon production, particularly in the dry season where it is hot. Widespread incidence of the TYLCV disease causing severe yield losses in Ghana was reported by IFPRI in 2006. Again an outbreak in 2014 in the Agotime-Ziope District of the Volta Region in Ghana in which over 1000 ha and 600 farmers were affected and resulted in 100% yield loss was experienced in some fields. This necessitates massive efforts toward developing resistant cultivars.

18.4.1.2 Okra

Incidence of OLCD in okra in Ghana has been reported. A severe leaf curl disease of okra with high disease incidence and loss of production was observed during field survey of various locations of the Central Region of Ghana. During the 2013 major cropping season, a field survey conducted in the farmers' okra fields in the Komenda-Edina-Eguafo-Abirem (KEEA) municipality of the Central Region of Ghana revealed incidence of OLCD ranging from 63 to 70%, and the disease severity indices range between 41 and 46%. Further disease assessment conducted by the University of Cape Coast, Ghana, during the 2015 major cropping season at KEEA, Ajumako-Enyan-Essiam, and Assin South municipalities representing coastal savanna and transition and forest agroecological zones of the Central Region of Ghana (Fig.18.6) also revealed widespread incidence of OLCD in the region. They reported mean OLCD incidences of 69.7%, 64.2%, and 36.4% and corresponding severity scores of 2.023, 1.811, and 0.675, respectively (0, no symptom and 5, very severe symptom). Severe leaf curl disease of okra has also been observed at different locations in the Eastern, Volta, Ashanti, and Northern regions of Ghana, indicating widespread incidence of the disease in Ghana. Incidence of OLCD has also been reported in several other African countries including Ivory Coast, Niger, Mali, Burkina Faso, Nigeria, Cameroon, and Sudan.

18.4.1.3 Pepper

Virus diseases have become the most limiting factor affecting pepper production. They cause various forms of mosaic and distortions in plants with consequent reductions in crop growth and yield. Of the 68 viruses known to infect peppers worldwide, 11 are reported in Africa. *Pepper veinal mottle virus* (PVMV) and tomato yellow leaf curl virus (TYLCV) are the most widespread in the Western Africa subregion. Nevertheless, in Ghana, the incidence of TYLCV on pepper is low. Following the enormous role of pepper, it is important to assess the status of some viruses on the crop. This will offer suitable mechanisms for enhanced pepper production, reduce malnutrition, and poverty. Further studies are ongoing to actually determine the incidences of the two viruses across major pepper-growing areas in Ghana.

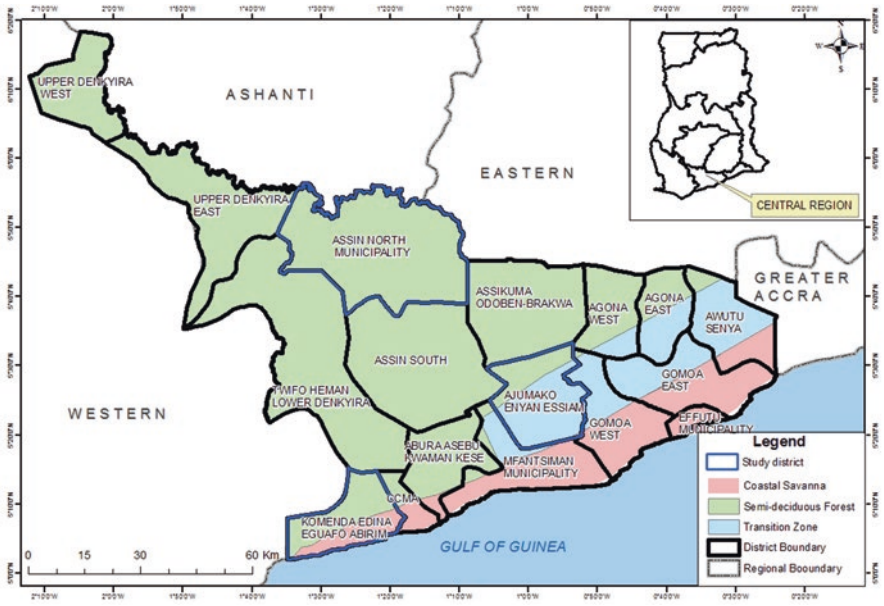

Fig. 18.6 A map of the Central Region of Ghana showing the districts where field survey was conducted

18.4.2 Root and Tuber Crops (Cassava, Wild/Tree Cassava, and Sweet Potato)

18.4.2.1 Cassava and Wild/Tree Cassava

The survey of CMD in all cassava-growing regions in Ghana showed that most cassava in farms are established with local landraces. Dokuduade, Bankyepampro, Dorkpan, and Efiakofie landraces were not infected by CMD. Worzeawonyi was found to be asymptomatic in seven fields, and no virus was detected in PCR assays. Low CMD incidence was recorded in landrace Dabor and Fetor gborze. The tolerance of Afisiafi to CMD appeared to be breaking down. Farmers rarely considered the health status of planting material prior to planting and usually established their cassava farms with planting materials from their own farms or neighbors. The dominant cassava mosaic resistance (CMD 2) gene was not detected in Ghanaian cassava landrace genotypes. CMD was prevalent in the country with field incidence greater than 70% in all the seven cassava-producing regions in the country and mean severity of 3.0 with a range score of 1–5. Cutting-borne infection was the main source of CMD incidence in Ghana. Cassava mixtures and intercropping did not result in reduction in CMD incidence and severity. Mixed infections of cassava by the two CMBs were widespread in all the cassava-growing regions in the country. These infections were usually characterized by severe symptoms. PCR-RELF of Ghanaian CMB isolates revealed limited diversity among the isolates.

18.4.2.2 Sweet Potato

Despite its high potential for food security in Ghana, production of sweet potato (*Ipomoea batatas* L.) is also constrained by viruses which reduce yield by 90 %. Visual symptoms including vein clearing, interveinal chlorosis, chlorotic spots, upward leaf curling, leaf narrowing, purpling, blistering, and leaf yellowing in most accessions grown on the field have been associated with the begomovirus *Sweet potato leaf curl virus* (SPLCV). The disease incidence associated with sweet potato leaf curl virus (SPLCV) in the country has been from 20 % to 90 %. Plants that tested positive to PCR assay were grown in the chamber at 35 °C for 4 weeks followed by meristem culture. The regenerates were indexed for SPLCV. Fifty-two percent (52.4 %) of the regenerates were successfully cleaned of the virus. Virus indexing and elimination will enhance the dissemination of disease-free planting materials to farmers.

18.5 Strategies for Begomovirus Disease Management in Ghana

18.5.1 Vegetables (Tomato, Okra, and Pepper)

18.5.1.1 Tomato

The use of resistant crop varieties is the most convenient and cost-effective control measure of TYLCV in tomato. However, this is not available in Ghana. Cultural and biological pest management tactics also provide a good option for controlling *B. tabaci* and overcoming the problem of insecticide resistance. As such, various cost-effective measures have been reported for controlling tomato virus diseases. They include cultural practices, vector manipulation, inoculum source elimination or phytosanitation, cross-protection, use of resistant varieties or even transgenic plants, and virus or vector exclusion. In Ghana, control of TYLCV has been based almost exclusively on insecticide treatments against *B. tabaci*. However, control of *B. tabaci* populations to an uneconomic level of virus transmission is difficult. Moreover, frequent pesticide applications have a detrimental environmental impact. Repeated insecticide use has resulted in the development of resistant *B. tabaci* populations, and treatments have become less effective. Under Tomato IPM CRSP Project (now IPM Innovation Lab), IPM package on tomato to manage TYLCV in Ghana was deployed in the tomato-growing areas in the Upper East, Brong-Ahafo, and Ashanti regions of Ghana. The technology was later extended to the Greater Accra region (tomato-growing areas at Ada) and Volta Region (tomato-growing areas at Adidome) (Fig. 18.7).

18.5.1.2 Okra

Management of the OLCD by the smallholder farmers in West and Central Africa is very difficult, due to the many potential alternative and crop hosts for the viruses and/or *B. tabaci* vector. It is also not desirable to manage the disease with insecticides because of its high cost and environmental and health hazards. *B. tabaci*

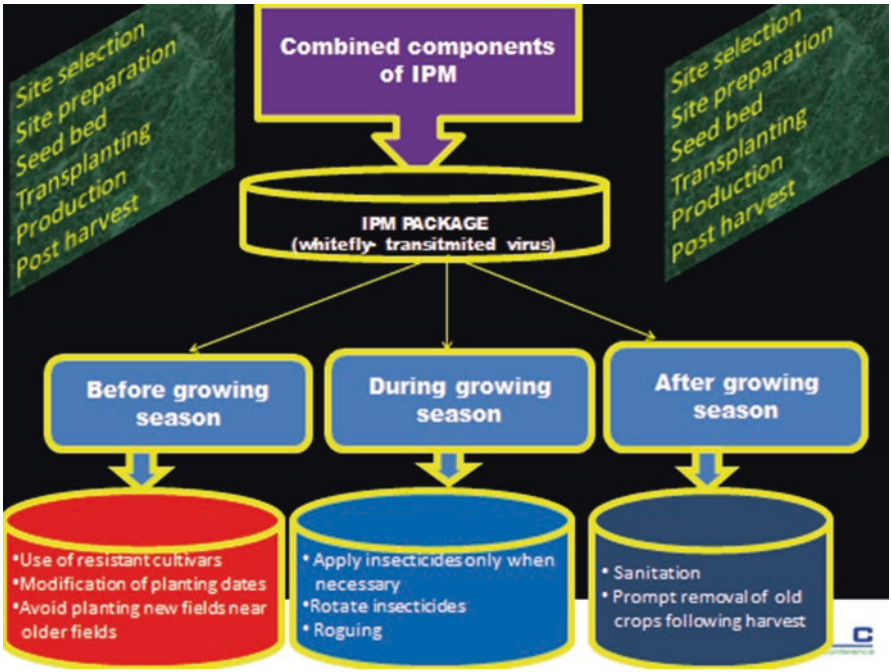

Fig. 18.7 Tomato IPM Package employed in Ghana in the management of TYLCV

vector has also developed resistance against insecticides in recent years. Host plant resistance is the most effective way of managing viral diseases. There is, however, limited information of host plant resistance against begomoviruses associated with okra leaf curl disease in Ghana. In screening 21 *A. esculentus* accessions over two cropping seasons against okra leaf curl disease under natural infection, the University of Cape Coast in 2016 reported that none of them was immune to the disease. They, however, identified five accessions (GH2052, GH5332, UCCC6, GH2063, and GH6105) showing mild disease symptoms and thus described them as being resistant to the OLCD. Elsewhere in Nigeria, in their screening studies to identify sources of resistance to okra leaf curl disease in Nigeria over two cropping seasons, they demonstrated that none of the *A. esculentus* accessions was resistant to the disease. They also reported that among the *A. caillei* cultivars, *Ebiogwu*, *Ojoogwu*, *Tongolo*, *VLO*, *Oruufie*, and *Ogolo* were found to be resistant based on yield decline of less than 10%.

Virologists and plant breeders need to double up in identifying okra cultivars that are resistant or tolerant to the whitefly vector *B. tabaci*. There is lack of information on okra cultivar resistance to the whitefly vector. Recently, 21 okra cultivars were screened by the University of Cape Coast for resistance to the vector and reported mild infestation of five accessions (GH2052, GH5332, UCCC6, GH2063, and GH6105) to the whitefly. Efforts are needed to introgress the resistant genes from these accessions in breeding programs as well developing more okra lines that are

resistant or tolerant to the insect vector. In this way, usage of insecticides will be minimal in okra production.

18.5.1.3 Pepper

Reports have it that the decline in productivity of pepper can be associated with the *Begomovirus*. Management of begomovirus diseases in a sustainable way will demand the use of an integrated pest management (IPM) approach. Resistant/tolerant cultivars have to be identified and utilized in improvement programs. Efforts also have be to put in place to limit the spread begomovirus as caused by the vector, whitefly. Breeding for resistance to begomoviruses is complex due to their high diversity, ability to form new genotypes through recombination, and the occurrence of DNA satellites. However, research groups have used both conventional breeding and different transgenic approaches for achieving resistance or tolerance against begomoviruses infecting pepper.

In Ghana, most farmers often use insecticides to fight the whiteflies which transmit the virus. Nevertheless this is to no avail as most of the whiteflies has built resistance to insecticides. Others also observe sanitation such as clean fields, thus ensuring no weeds to avoid alternate host for the whiteflies.

18.5.2 Root and Tuber Crops (Cassava, Wild/Tree Cassava, and Sweet Potato)

18.5.2.1 Cassava and Wild/Tree Cassava

The main strategies for management of CMD have been on the development and deployment of virus-resistant varieties. Six cassava varieties, namely, Otuhia, Bronibankye, Sikabankye, Ampong (recently released), Afisiafi, and Debor (local), were evaluated in replicated trials at Fumesua and Ejura. The recently released varieties were expected to show severe field symptoms; however, all of them showed high field tolerance which is quite significant. Only limited use has been made of phytosanitation involving CMD-free planting materials and the rouging of diseased plants. Cultural methods of control using varietal mixtures, intercrops, or other cropping practices have also been neglected.

18.5.2.2 Sweet Potato

SPLCV in sweet potato has been eliminated using thermotherapy-meristem tip culture (Arkorful et al.2015). The regenerates were indexed for SPLCV, and 52.4% of the regenerates were successfully cleaned of the virus. Virus indexing and elimination will enhance the dissemination of disease-free sweet potato planting materials to farmers.

18.6 Prospects and Challenges of Begomoviruses in Ghana

18.6.1 Financial Resources

Adequate financial resources must be in place if plant virus problems including begomoviruses in Ghana are to be addressed. Unfortunately, however, there is no direct financial support from the government of Ghana to address the numerous plant viral diseases confronting the country. The country depends on the donor support to conduct research. Most of these foreign agencies do not support basic research which can adequately address the various viral disease problems. The funding organization normally decides on the type of crop and the type of research (mainly applied research) to be conducted which most of the time spans between 1 and 3 years. Interestingly, the donor support for agricultural research in sub-Saharan Africa has been increasing in recent years. Government and scientist should therefore be well positioned to attract these funds.

18.6.2 Human Resources

Adequate and competent and skillful human resources are a key in addressing the challenge of plant viruses in Ghana. Currently, the numbers of plant virologists in Ghana are few and also lack the requisite equipment and financial resources coupled with poor institutional support. As a result, many are poorly motivated and sometimes compelled to abandon their profession for more lucrative jobs or take up administrative positions. Conscious efforts should be made by the government of Ghana and other stakeholders in training more plant virologists, as well as establishing and equipping modern laboratories and providing financial resources required in solving the plant virus disease problems.

18.6.3 Collaborations/Partnership

There is the need for coordinated measures by the Ministry of Food and Agriculture, universities, research institutes (CSIR), farmers, nongovernmental organizations, and donors to safeguard the production of cassava and sweet potato in Ghana so that farmers do not abandon their farms in the near future as was the case in Uganda.

18.6.4 Training/Education

There is the need to train and educate farmers on the selection of clean planting materials, early rouging of infected plants (when infection is not extensive), and removal of volunteer plants which are usually infected. Incorporate screening of planting material multiplication programs.

18.6.5 Periodic Surveys

There is the need for periodic surveys to monitor the changes in the incidence and severity of the disease, varieties grown, and disease status to ensure that research and extension efforts are well and effectively focused. It is also important to develop infectious clones of representative isolates to be used in screening planting materials in cassava breeding programs.

18.7 Conclusion

Begomoviruses constitute as an important constraint to the production of vegetables (tomato, pepper, and okra), sweet potato, and cassava/wild tree in Ghana. Yield losses attributed to begomoviruses have been enumerated which demand urgent research intervention to mitigate their effects on agricultural productivity and farmers' income. Farmers are encouraged to use virus-free planting materials/resistant varieties and other best viral disease management practices to manage the incidence and severity of viral diseases on their fields. Research institutions in the country should also be adequately resourced to provide the necessary technical support (both human and financial) for the identification of begomoviruses and breeding of begomovirus-resistant crop varieties for rapid adoption use.

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Begomoviruses in Cuba: Brief History and Current Status

19

Yamila Martínez-Zubiaur, Lidia Chang-Sidorchuk, and Heidy González-Álvarez

Abstract

The geminiviruses comprise a biologically and genetically diverse family (*Geminiviridae*) of viruses that are causal agents of extremely damaging diseases in a wide range of crops throughout the world. The genus *Begomovirus* can be subdivided into two large groups: (a) viruses with monopartite genome, prevalent in the Old World, and (b) viruses with bipartite genome, prevalent in the New World. They are plant pathogens, which have caused important losses to crops of economic interest in the last decade. This review presents a brief history about the presence of genus *Begomovirus* in Cuba, the impact of the introduction of *Tomato yellow leaf curl virus-Bemisia tabaci* biotype B complex into tomato crops, the research work developed for understanding the severe outbreaks and the current situation of begomoviruses in cultivable and noncultivable ecosystems, and their phylogenetic relationships. The research done supports the integrated management programs of these pathogens with a more far-reaching conception and integrating strategies and tactics to prevent and control them with lesser effects on the environment.

Keywords

Cuban begomovirus • Whitefly

Y. Martínez-Zubiaur (✉) • L. Chang-Sidorchuk • H. González-Álvarez
Plant Pathology Department, National Center for Animal and Plant Heath, CENSA, Apdo 10, San José de las Lajas, Mayabeque, Cuba
e-mail: yamila@censa.edu.cu

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19.1 Overview

The genus *Begomovirus*, family *Geminiviridae*, includes viruses with high dissemination and geographical distribution, exclusively transmitted by the whitefly *Bemisia tabaci* (*Gennadius*), and infects a long list of dicotyledonous plants in tropical and subtropical regions of the world. The begomoviruses, showing high diversity of species, cause severe losses in many economically important crops with damages to the agricultural production (Morales 2011; Brown et al. 2015).

In the global context, Africa, the Middle East, Southeast Asia, Europe, and Latin America are the most affected regions. In countries of the old continent, crops like tomato, cassava, crucifers, tobacco, and leguminous plants are the most affected ones, while the same is true for crops like tomato, soybean, beans, cotton, and tobacco in the Western Hemisphere (Morales 2011).

In the New World, these viruses were first studied in Brazil, due to their impact on agriculture particularly attributed to the presence of the *Bean golden mosaic virus* (BGMV) that caused the loss of million hectares of crops (Morales 2011; Costa 1975 cited by Morales 2011). The *Bean golden mosaic* diseases (BGMD) rapidly spread to the Caribbean and Central American countries, caused by the new species, *Bean golden yellow mosaic virus* (BGYMV), with biological and genomic properties different to BGMV (Faria et al. 1994). The widespread pandemic of BGYMV in Latin American and the Caribbean regions was associated with the expansion of the vector and produced the unprecedented emergence of *B. tabaci*-BGMD complex, mainly because of the losses and the severe economic, social, and environmental impact on the regional agriculture. Tomato was the second affected crop of the region, particularly in Mexico and the Caribbean islands, followed by pepper (*Capsicum* spp.) according to the severe yield losses reported (Morales 2011).

Cuba did not escape the effect of begomoviruses in the region, and since the early 1970s, yellowing symptoms in common beans were associated with BGMD (Fig. 19.1), presumably caused by the *Bean golden mosaic virus* (BGMV) (Blanco

Fig. 19.1 Common beans and tomato plots with high incidence of begomovirus diseases. (**a**) a common bean plot with *Bean golden yellow mosaic virus* (BGYMV), and (**b**) a tomato plot with *Tomato yellow leaf curl virus* (TYLCV-IL(CU))

and Bencomo 1978). This situation was associated with high population levels of whiteflies and the destruction of thousands of hectares in several growing regions (Blanco and Faure 1994).

Begomovirus symptoms showed up in tomato-growing areas (Fig. 19.1) that led to the destruction of seedbeds and production areas, with the consequent yield reduction of 40–100% depending on the location, until 42% of losses in the area nationally planted and in some localities was necessary eliminated fields with 100% of damages (Murguido 1993). These productive losses were followed by environmental damages caused by the use of chemical insecticides to control the vector and increases of the production costs by the purchase of tomato commercial resistant hybrids in the international market (Murguido et al. 2001).

An IPM was started which included the measures for before, during, and after the growing season, all of them aiming at controlling the vector *B. tabaci* and reducing the disease incidence. Legal regulations were issued concerning planting dates, seedling production, elimination of the cultivar "Campbell 28" (highly sensitive), introduction of commercial hybrids and new varieties from Cuban breeding programs to abiotic stress, and the elimination of harvest wastes. It was also included the organization of educational programs that stimulated knowledge spread and adoption of all the strategies proposed. These management measures allowed mitigating the epidemiological situation in certain growing areas and improving productive indicators (Murguido and Elizondo 2007).

19.2 Emergence of the Tomato Yellow Leaf Curl Diseases (TYLCD)

The early studies done in Cuba by electronic microscopy, indicator plants, and molecular hybridization showed the first evidences of the tomato yellow leaf curl disease (TYLCD) introduction (González 1995), conditioned by the detection of high levels of infestation of *Bemisia argentifolii* Bellows and Perring (formerly referred to as "B biotype" or Middle East–Asia Minor 1 (MEAM1)), with better ecological plasticity and higher aggressiveness and efficiency as pest and vector than the native biotypes, being difficult to control with insecticides (Vázquez et al. 1995).

The preliminary diagnostic results led to the need for further molecular characterization studies. The molecular studies evidenced the presence of a monopartite begomovirus (Martínez et al. 1996) with an identity of 98.2% with *Tomato yellow leaf curl virus* (TYLCV-[IL-Reo-86]) specie type from Israel (Navot et al. 1992). Both viruses showed high sequence identity, coincidence in the intergenic region (IR) located within nucleotides 1 and 300, and in the repeated sequences or iterons of nine nucleotides (AATCGGTGT) adjacent to the TATA sequence. The Cuban isolate of TYLCV was recognized as TYLCV-IL(CU) (Fauquet et al. 2008).

The TYLCV introduction was in early 1990s coinciding with the first report in the Caribbean region (Nakhla et al. 1994; McGlashan et al. 1994). Although its introduction is not well known, it could have been caused by anthropological

introductions due to the exchange of germplasm infected by the virus or *B. tabaci*, similarly to what was discussed for the Dominican Republic (Polston et al. 1999; Morales 2011). However, another possible way could have been meteorological events like the hurricanes. It must be considered the hurricane *Gilbert*, one of the most devastating hurricanes in the past century, which with winds of high intensity hit the Caribbean island in 1988 and could introduced and spread infected whitefly populations into Cuba.

The rapid spread of TYLCV was later confirmed in south of Florida, Puerto Rico, west coast of Mexico, and Lesser Antilles (Polston et al. 1999; Bird and Brown 2001; Brown and Idris 2006; Urbino and Dalmon 2007), and it is one of the events with the largest economic impact of the genus *Begomovirus* in the New World, mainly for the devastating losses caused in the affected countries. Recently, a new introduction of TYLCV from Asia into the Central American region was detected (Barboza et al. 2014) and considered as an example of the second introduction of TYLCV into the New World in the 2000s (Pèrèfarres et al. 2012).

Two bipartite begomoviruses were also detected in tomato crops in the national prospections. DNA-A and DNA-B from each isolate were sequenced, and the viruses were named *Tomato mosaic Havana virus* (ToMHaV) (Martínez-Zubiaur et al. 1998) and *Tomato mottle Taino virus* (ToMoTaV) (Ramos et al. 1997). According to the criteria proposed to separate species (Fauquet et al. 2008; Brown et al. 2015), both viruses are new species of bipartite begomoviruses that, together with TYLCV-IL(CU), infected tomato in Cuba in the early years of the disease outbreak.

ToMoTaV did not show a wide distribution in tomato; however, it was indeed reported in potato (*Solanum tuberosum*) (Cordero et al. 2003) and showed the possibility to form pseudorecombinants with ToYMV (Ramos et al. 1997).

ToMHV was early detected in mixed infections with TYLCV-IL(CU), which displaced it becoming the predominant virus. Cuban isolates of TYLCV-IL from different locations and years showed a low sequence variation with an average from 0% to 4% (Martínez et al. 2003; Quiñones et al. 2007), which was considered within the divergence range accepted for the same specie (Brown et al. 2015) and consistent with the low levels of recombination detected.

The recombination has not been the cause that conditioned the aggressiveness and distribution of TYLCV in the country, mainly because no introduction of other species of TYLCV has occurred for a genetic exchange of genomic segments in the replication process, which could have caused the emergence of new species with biological changes and major epidemiological consequences. It was the situation observed in other countries, where the introduction of TYLCV-IL in the presence of other TYLCV species, that set out the emergence of new recombinant variants (Moriones and Navas-Castillo 2008; Davino et al. 2009).

These results allowed concluding that, despite the adaptation and expansion process to new ecological niches, there was only one variant of TYLCV genetically stable in the country and confirmed that, through the high potential of the plant viruses for genetic variability, the populations could be genetically stable always that no process of genetic exchange intervenes (Garcia-Arenal et al. 2001).

In 2012, a new bipartite begomovirus was identified. It was a new species for the taxonomy of this genus named as *Tomato yellow leaf distortion virus* (ToYLDV). This virus was inoculated into *S. lycopersicum*, *Nicotiana benthamiana*, and *Nicotiana tabacum* by biolistic methods confirming its infectivity (Fiallo-Olivé et al. 2012a).

In spite of the identification of three bipartite begomoviruses in tomato, TYLCV-IL(CU) is still the most distributed and prevailing one, even in growing areas where it has been found in mixed infections with virus species of other genera such as *Tomato chlorotic virus* (ToCV) (Martínez-Zubiaur et al. 2008) and *Tomato chlorotic spot virus* (TCSV) (Martínez-Zubiaur et al. 2016a).

19.2.1 Other Cultivated Hosts of TYLCV-IL(CU)

TYLCV-IL(CU) was identified affecting also common beans (*Phaseolus vulgaris*), pepper (*Capsicum annum*), and squash (*Cucurbita pepo*) (Martínez et al. 2002, 2004; Quiñones et al. 2002). The sequencing and phylogenetic comparison, of the 5′ end of the *Rep* gen, the core region of the CP gene, and the intergenic region of several DNA fragments, showed homologies from 96% to 99% among them and with the TYLCV-IL(CU) isolate previously identified in tomato making evident the wide adaptation of this species to the productive agroecosystems of the country.

Until now, the studies done in Cuba do not permit the identification of TYLCV-IL(CU) as a begomovirus of severe effect or widely distributed in common beans, squash, or pepper crops, similar to other authors' reports (Navas-Castillo et al. 1999; Reina et al. 1999; Polston et al. 2006). Nonetheless, the current monitoring and surveillance in these crops are very important because they are usually bordering tomato plots and are grown in the same season, and they may act as reservoirs of the virus and the infecting vector.

19.2.2 Factors Driving TYLCV-IL(CU) Emergence in Tomato

The begomoviruses, and in particular TYLCV-IL(CU), have been studied in Cuba for more than 25 years. Several surveys have been done in the main tomato-growing areas emphasizing on the factors contributing to TYLCV-IL(CU) severity and incidence.

The results indicated that the highest incidence and severity values of the disease are in the tomato grown in the eastern regions of Cuba, while the population density of the whitefly (*B. tabaci*) did not show significant differences in any of the regions examined (Martínez et al. 2009). In most regions a combination of factors influenced the high incidence and severity of the disease. These factors were associated with the previous planting of host crops for the whitefly, continuous planting of tomato or other crops harboring either whiteflies or begomoviruses, high densities of *Bemisia tabaci* population, delays in planting and transplanting dates, and planting of susceptible cultivars. The low disease incidence and severity was associated

with factors such as better management practices, geographical location of the fields, planting and transplanting in appropriate dates, high altitude, and tomato seedling with protection measures.

The evaluation of the detection frequency of TYLCV-IL(CU) at the different regions of the country allowed knowing the differentiated situation relative to local ecosystems and taking actions regarding the use and regionalization of resistant or tolerant cultivars to TYLCV-IL(CU) (Martínez et al. 2009).

The continuous use of areas with susceptible crops and at the same time of the year is a cause favoring the high incidence values of the disease and the emergence of new recombinant species in other countries (García-Andrés et al. 2006).

These epidemiological elements in tomato-growing systems play an important role as external factors driving the incidence and severity of TYLCV-IL(CU), as well as increasing the populations of the biotype B of *B. tabaci*. These conditions favor the mutualistic virus-vector relationships and justify a regional individual program for the management of viruses and whiteflies according to the incidence of the main elements.

19.2.3 Role of the Genetic Breeding in the Control of TYLCV-IL(CU)

Predominance and adaptation of TYLCV-IL(CU) to horticultural agroecosystems; ban on planting the cv. "Campbell 28," for its high susceptibility to TYLCD (Murguido et al. 2001); and unavailability of TYLCV-resistant tomato cultivars supported the need for developing a tomato breeding program in search of resistance to this pathogen.

The program was initiated with the evaluation and introduction of cultivars tolerant to abiotic stress and hybrids with resistant genes to TYLCV imported from the commercial market (Gomez et al. 1988; Alvarez et al. 2003).

Even when the introduction of these cultivars along with management measures improved the severe phytosanitary situation of the crop with a consequent production recovery, introduced Cuban varieties started to show light to strong symptoms in the subsequent years. Likewise, the imported hybrids behaved symptomless but with high concentrations of the virus detected by molecular hybridization. It was confirmed that several cultivars performed as natural reservoirs of the virus, and TYLCV-IL(CU) was able to replicate efficiently, thus turning into sources of viral propagation (Martínez et al. 2003).

Plant pathologists and geneticists worked together in order to improve the breeding programs with the introduced diagnostic tools to detect TYLCV-IL(CU) in the genotypes evaluated since the beginning of the breeding programs. Tomato lines introgressed with loci *Ty-1* from *Lycopersicon chilense* were obtained and compared with the F_1 hybrids "ARO 8479" and "HA 3108," which are tolerant to *Tomato yellow leaf curl virus*, and the cv. "Campbell 28" as a susceptible control. The results permitted selecting new promising lines symptomless for TYLCV infection and with good agronomic values (Gomez et al. 2004; Piñon et al. 2005).

The tomato breeding program is continuously developing with the simultaneous and early evaluation of the promising lines through diagnostic tools and using the DNA marker technology. These technologies, helpful for the rapid and efficient transfer of loci Ty-2 and Ty-3 into agronomic desirable varieties and hybrids, reduce time and space in the process of genotype selection and produce hybrids and varieties with pyrimidized resistance genes (Dueñas et al. 2009).

New genotypes are being evaluated to improve the resistance level in high-yielding genotypes by pyramiding genes for TYLCV resistance to enhance a sustainable production with the efficient reduction of the selection time and space (Gomez-Consuegra et al. 2015). Mainly the genotype obtained with Ty-1/Ty-3 gen broadens the perspective of the Cuban breeding programs, though the resistance mechanism is being discussed at present (Caro et al. 2015).

National varieties, hybrids of determined and undetermined growth, have been registered by agriculture regulatory authorities (e.g., cvs. "Vyta," "Elbita," "Arturo," "Daniel") and permitted the diversification of a varietal strategy with better tolerance values to TYLCV-IL(CU), in varieties adapted to the Cuban climatic conditions. The conventional plant breeding in Cuba has a significant impact on improving tomato for resistance to TYLCV and is an important useful tactic for disease management.

19.3 Characterization of Whitefly Populations

Morphological studies, phytotoxicity tests, and esterase patterns showed the presence of *Bemisia tabaci* (*Gennadius*) (Hemiptera, Aleyrodidae) biotype B in most tomato-producing regions of the country, presumably introduced in the early 1990s (Vazquez et al. 1995).

The presence and wide distribution of this biotype, currently considered as the specie *Middle East–Asia Minor 1* (MEAM1) (Muñiz et al. 2011), were detected by using microsatellite markers (De Barro et al. 2003; Delatte et al. 2006) and sequencing the gene mtCOI (Dinsdale et al. 2010) in individuals collected in both productive and nonproductive ecosystems.

In different surveys done, the MEAM1 species showed its ability to use multiple hosts in productive and nonproductive ecosystems with low molecular divergence and how the prevalent species displaced the native species in productive ecosystems, while in nonproductive ecosystems, it still shares hosts with the native species of the *New World* (NW), previously biotype A, in some weeds like *Salvia officinalis* (Martínez-Zubiaur et al. 2016a, b). Also, have been detected coinfections with *Trialeurodes abutiloneus* species (Gonzalez-Alvarez and Martínez-Zubiaur 2015).

The *Mediterranean* whitefly species (previously biotype Q), widely distributed in several countries including Latin America (Morales 2011; McKenzie et al. 2012), has not been found in Cuban populations of *B. tabaci*. This specie is under surveillance because of its potential to cause significant crop damage and losses. Therefore, monitoring of *B. tabaci* distribution and identification of present species are crucial for an effective management and control.

19.4 Outbreak of Bipartite Begomoviruses in Recent Years

19.4.1 Common Bean (*Phaseolus vulgaris*) and Soybean (*Glycine max*) Damages

The devastating situation occurred in the major common bean-growing regions and associated with the presence of BGMD was probably due to the introduction of the most efficient vector *B. tabaci* biotype B (Vazquez et al. 1995).

Initially, the disease was attributed to the specie *Bean golden mosaic virus*, but studies by biological, serological, and molecular tools allowed identifying the Mesoamerican specie *Bean golden yellow mosaic virus* (BGYMV) as responsible for the disease (Echemendia et al. 2001). Several diagnostic methods were used in plant breeding programs for detecting this virus (Echemendía et al. 2010).

The introduction of cultivars with a better behavior to this disease and the implementation of preventive and control measures into an integrated management program allowed reducing productive losses and improving the sustainability of the crop (Echemendía et al. 2010).

However, in the last 3 years, increasing symptoms different from those caused by BGYMV have been evident and prospections carried out to find molecular variability with BGYMV, presence of new viral species, or other associated pathogens. In 2014, mixed infections of BGYMV and phytoplasms ('*Candidatus* Phytoplasm asteris' subgroup 16SrI) were found in production areas of Mayabeque Province (Zamora-Gutierrez 2014).

The incidence of other bipartite begomovirus species on this crop is marked by the identification in symptomatic plants of an isolate of the specie *Tobacco leaf curl Cuba virus* in the provinces of Sancti Spíritus (Leyva et al. 2016) and recently in Mayabeque. The finding of this virus, previously reported in tobacco by Moran et al. (2006), confirms the adaptation capability of this specie to more than one host, both of economic importance, and suggests the need for further epidemiological studies to know its impact on the production of both crops.

Two new species associated with severe mottling and mosaic symptoms were identified recently in Mayabeque Province and proposed to be named *Common bean mottle virus* (CBMV) and *Common bean severe mosaic virus* (CBSMV). The CBMV showed the highest percentages of identity with *Rhynchosia mild mosaic virus*, a begomovirus found infecting Fabaceae weeds like *Rhynchosia minima* in Puerto Rico (Brown and Idris 2009), while CBSMV is phylogenetically more related to the *Rhynchosia golden mosaic Havana virus*, a begomovirus that infects *R. minima* in Cuba.

It is timely to stand out that, despite the diversity of species identified in this crop in Mayabeque Province in 2015, 59.26% of the collected plants showed infection by BGYMV, thus confirming that it was the most distributed begomovirus in common beans, at least in this highly productive location.

Soybean (*Glycine max*) is an important leguminous plant for both human and animal consumption, and it is grown in many bordering areas of common bean crops, so the study of the begomoviruses occurring in soybeans is doubly important

for both their impact on the crop and the epidemiological interest. In 2015, an isolate of *Rhynchosia golden mosaic Yucatan virus* (RhGMYuV) (Chang-Sidorchuk et al. 2016) was identified that was similar to the species previously found in weeds like *R. minima* and *Desmodium* sp. in Mexico (Hernandez-Zepeda et al. 2010). It is a clear example of the geographical dispersion capacity of this species as reported for begomoviruses (Morales 2011).

19.4.2 Begomovirus in Tobacco (*Nicotiana tabacum*)

Tobacco is a crop of economic importance for the country mainly because it represents the exportable products in the international market. Fungal diseases and insects affect this crop, but *B. tabaci* has not been reported damaging tobacco production significantly (Rivas et al. 2012).

However, symptoms similar to those associated with begomoviruses, such as foliar yellowing, crinkling, curling, roughness, yellow mottle, and downward leaves with reduction in plant height, have been reported in the last two decades.

Four species were identified as *Tobacco leaf rugose virus* (TbLRV-[CU-Hav-01]) (Domínguez et al. 2002), *Tobacco leaf curl Cuba virus* (TbLCuCV-[CU-Tag-05]) (Morán et al. 2006), *Tobacco mottle leaf curl virus* (TbMoLCV-[CU-SS-03]) (Dominguez et al. 2008), and *Tobacco yellow crinkle virus* (TbYCV-[CU–07]) (Fiallo-Olivé et al. 2009). Also reported for the first time in the country was the specie *Euphorbia mosaic virus* (EuMV-[CU-Tb-07]) (Fiallo-Olivé et al. 2010a).

Besides the diversity of bipartite begomoviruses found in tobacco, ToYLDV was detected infecting this crop and sharing iterons with TbLRV (Fiallo-Olivé et al. 2012a). To date, no distribution studies of the identified species have been carried out, although it is known that begomoviruses are not within the key diseases affecting the crops and show a low economic impact. Possibly, among the main elements that have permitted this behavior are the seedlings grown under protected conditions, the high phytosanitary surveillance, and the strict program of disease management.

19.4.3 Bipartite Begomovirus Identified in Weeds (Noncultivated Hosts)

In order to detect and establish a provisional identity of begomoviruses, viral variants were identified by partial cloning and sequencing of DNA-A and DNA-B genome, which has been marked as regions of taxonomic value (Rojas et al. 1993; Brown et al. 2001).

In weeds present on common bean fields, partial sequences were identified indicating the presence of the specie *Sida golden yellow vein virus* (SiGYVV-[CU-Hav]). In *Sida* sp. and *Dicliptera vahliana* plants; an isolate of Dicliptera yellow mottle virus (DiYMoV) was found, also *Macroptilium yellow mosaic virus* (MacYMV-CU) was isolated from *Macroptilium lathyroides* (Echemendia et al. 2004).

Two previously identified viral species were detected in nonproductive ecosystems (Martínez et al. 2006); they were *Tobacco leaf rugose virus* (Domínguez et al. 2002) infecting *Jatropha gossypiifolia* and *Macroptilium yellow mottle virus* (MaYMV) infecting *Pseudelephantopus spicatus*. Also during a survey in 2005, the occurrence of the bipartite begomovirus in pepper crops (*Capsicum annum*) from the eastern region was detected (Martínez-Zubiaur et al. 2006).

Though no complete sequences able to provide reliable taxonomic information were achieved, these were indeed the first evidences of the diversity of bipartite begomoviruses and the role of weeds as reservoirs of several viruses.

The implementation of the rolling circle amplification (RCA) technique (Inoue-Nagata et al. 2004) allowed progress in studies of complete genomes in our country. Complete DNA-A and DNA-B genomes from weeds collected in surveys conducted from 2007 to 2011 were sequenced, and several species of bipartite begomoviruses were identified. They were *Sida yellow mottle virus* (SiYMoV-[CU-SSp159-1-09]), *Sida golden mosaic Florida virus* (SiGMFlV-Malv[CU-Hav-Mal-111-09]), *Sida golden yellow vein virus* (SiGYVV-[CU-Hav]), *Rhynchosia golden mosaic Havana virus* (RhGMHaV-[CU-Hav-07]), and *Rhynchosia rugose golden mosaic virus* (RhRGMV-[CU-Cam-09]) (Fiallo-Olivé et al. 2010b, 2012b). The species were designed according to the new species demarcation criterion for the begomoviruses, which is 91%, for the genus and 94% for the isolates (Brown et al. 2015).

Viral symptoms in weeds transmitted by whiteflies were already reported many years ago indicating the possible occurrence of begomoviruses (Kvicala 1978). It is now that this evidence is confirmed according to the diversity of begomovirus species found infecting weeds, which is an important result for the epidemiological monitoring, mainly because hosts like *Rhynchosia* sp., *Euphorbia* sp., *Sida* sp., and *Malvastrum* sp. are frequently found accompanying economical important crops such as common bean, tomato, soybean, and tobacco.

In studies conducted by Fiallo-Olivé et al. (2012c), in different geographical regions of the country, a new molecule of DNA satellites was found which is associated with the species *Sida golden yellow vein virus* in *Malvastrum coromandelianum* (L.) Garcke and *Sidastrum micranthum* with phylogenetic differences among the *M. coromandelianum* isolates from the western and eastern regions.

The new DNA satellites showed several features of geminivirus origin and genetic structure similar to betasatellites, to satellite associated with Tomato leaf curl virus from the Old World, and with circular DNA molecules amplified from adults of *B. tabaci* in Florida (Ng et al. 2011). These DNA satellites have recently been included into deltasatellites, one group proposed by Lozano et al. (2016).

19.5 Phylogenetic Relationships Found in Cuban Begomoviruses

A phylogenetic tree constructed from an alignment of the DNA-A component sequences of 21 begomoviruses isolated in Cuba compared with species type from the New World is shown in Fig. 19.2. This confirmed the diversity of bipartite

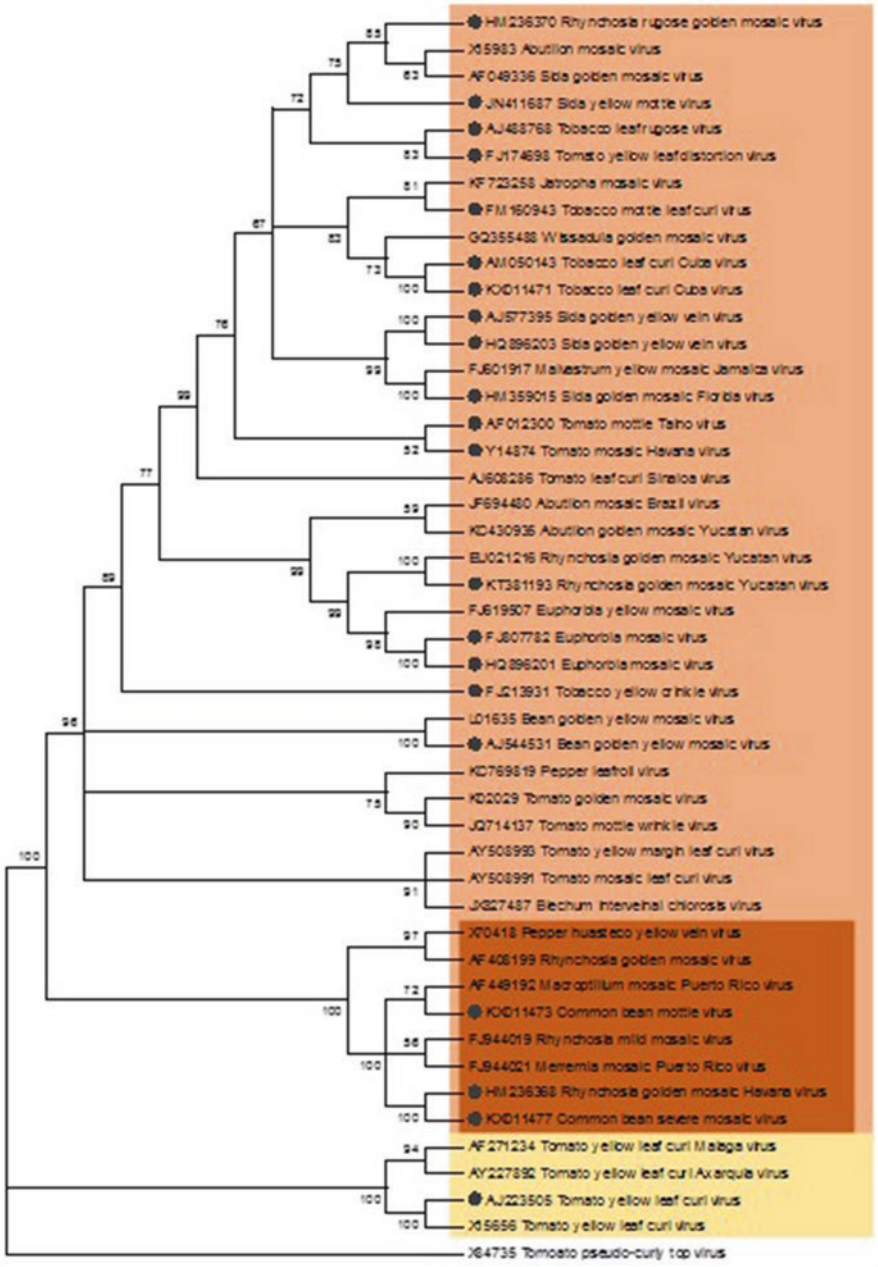

Fig. 19.2 Phylogenetic tree illustrating the relationships of complete DNA-As of Cuban begomoviruses, with selected sequence of the New World and TYLCV clade (51 nucleotide sequence). The tree was constructed by the neighbour-joining method with the MEG A6 program and condensed to show only clusters with >50% bootstrap (1000 replicates) support. GenBank accession numbers are shown in the trees, and the names of the viruses are according Brown et al. 2015. The tree is rooted with Tomato pseudo-curly top virus as outgroup

begomoviruses characterized in crops of economic interest and weeds where most species are more closely related to New World begomovirus clade, except TYLCV-IL(CU) that was separated on TYLCV clade as representative of the only monopartite species present in our country.

The New World (NW) Cuban begomovirus cluster shows two subgroups with 100% of bootstrap support. The minor subgroup, different to the rest of bipartite Cuban begomoviruses, was integrated by the new species identified from common bean (CBSMV and CBMV), *Rhynchosia Golden mosaic Havana virus* and *Macroptilium mosaic Puerto Rico virus*. Similar results were attained when all isolated species of *Rhynchosia minima* reported in the region were compared with RhGMHaV and RhRGMV, and they were grouped into different clades evidencing different phylogenetic lineages (Fiallo-Olivé et al. 2010b).

The detection of this minor subgroup indicated the variability in the NW–Cuban begomoviruses, consistent with the diversity referred to for the New World cluster where current evidences indicate minor subpopulations within the New World subpopulation (Prassana et al. 2010).

The majority subgroup showed a greater dispersion pattern. Species identified in plants of tomato, tobacco (except TbYCV), *Sida* sp. and the species RhRGMV were grouped in the same branch with 98% bootstrap support, while the EuMV isolates and RhGMYuV were grouped in a different branch. At the same time, TbYCV and BGYMV were separated in different branches with high bootstrap support.

The Recombination Detection Program v.4.55 (RPD4, Martin et al. 2015) was implemented for recombination analysis using different algorithm. Recombination events, likely parental isolates, and recombination break points were analyzed, and seven potential recombination events were detected in the full-length sequences of the NW–Cuban begomovirus. Table 19.1 shows the recombination fragments identified by more than five of the nine methods included in the RDP4 program and with good phylogenetic support (p-value < 0.001).

This analysis showed the recombinant nature of the DNA-A from *Tobacco yellow crinkle virus* (FJ213931), *Sida yellow mottle virus* (JN411687), *Tomato yellow leaf distortion virus* (FJ174698), and *Tobacco leaf curl Cuba virus* (KX011471). These species and *Sida golden mosaic Florida virus* (HM359015) and *Rhynchosia golden mosaic Yucatan virus* (KT381193) were detected as major and minor parentlike.

The studies done regarding the epidemiological and ecological factors impacting the distribution of the begomoviruses and their vectors, the recombination observed between species of the major subgroup of the NW–Cuban begomoviruses confirming the genetic exchange occurred, the evidence of the distinct evolutionary history undergone by the DNA-A and DNA-B genomes from RhRGMV (Fiallo-Olivé et al. 2010b), and the presence of the DNA satellite molecules are evidences of the favorable scenery for the begomovirus evolution in Cuba, with impact on the emergence of new species, or for providing greater opportunities to the present species for host range adaptation and diversification.

Table 19.1 Recombination events within the DNA-A sequences from species detected in Cuba by at least five methods included in the RDP4 package

Recombinant DNA	Recombination breakpoints	Parentlike sequences		*p-value*	Methods that detected recombination
		Major	Minor		
FJ213931 *Tobacco yellow crinkle virus*	1926–21	**KT381193** *Rhynchosia golden mosaic Yucatan virus*	?	2.276×10^{-85}	R,G, B, M, C, S, T
KC430935 *Abutilon golden mosaic Yucatan virus*	1984–87	**JN411687** *Sida yellow mottle virus*	**KT381193** *Rhynchosia golden mosaic Yucatan virus*	2.916×10^{-45}	R,G, B, M, C, S, T
GQ357649 *Sida golden mosaic virus*	1958–2616	**JN411687** *Sida yellow mottle virus*	**FJ174698** *Tomato yellow leaf distortion virus*	2.196×10^{-12}	R,G, B, M, C, S, T
JN411687 *Sida yellow mottle virus*	2305–2486	**HM236370** *Rhynchosia rugose golden mosaic virus*	?	3.608×10^{-17}	R,G, B, M, C, S, T
FJ174698 *Tomato yellow leaf distortion virus*	1968–2616	**AM050143** *Tobacco leaf curl Cuba virus*	**AJ488768** *Tobacco leaf rugose virus*	2.200×10^{-16}	R,G, B, M, C, S, T
KX011471 *Tobacco leaf curl Cuba virus*	1943–2133	**KF723258** *Jatropha mosaic virus*	?	2.234×10^{-09}	R,G, B, M, C, S
FJ944021 *Merremia mosaic Puerto Rico virus*	2880–253	**HM359015** *Sida golden mosaic Florida virus*	?	8.642×10^{-05}	R,M, C, S, T

The method with the lower p-value obtained for each species is underlined. *R* RDP, *G* GENCONV, *B* BootScan, *M* MaxChi, *C* Chimera, *S* SiScan, *T* 3Seq. Bold font for species detected in Cuba

19.6 General Considerations

The Cuban flora has a great diversity of plant species with the highest percentage of endemism in the Antilles. The diversity of the Cuban flora is concentrated in two regions, east and west, which means that there is a high influence of noncultivable ecosystems on productive ecosystems; therefore, the alterations in these ecosystems are the main factors driving emergence of begomoviruses and may increase the epidemic severity in the interface between cultivated and noncultivated ecosystems (Jones 2009).

The intensification, extensions, and diversification of agriculture in the last year have impacted the transformation of the noncultivated to cultivated ecosystems, and the production areas of crops such as common bean, soybean, tomato, tobacco, potato, sweet potato, and vegetables and hosts of *Bemisia tabaci* have been increased.

Cuba has made progress in the transfer of pest management, and programs have been developed in tobacco, tomato, and common bean crops, among others, with validation in different regions of the country. However, their sustainable implementation in agricultural practice is still insufficient, and actions are required to promote the optimal use of the local resources, according to their socioeconomic and agroecological characteristics, by combining the traditional experiences and skills of the producers within the context of the new technology (Murguido and Elizondo 2007).

In the case of tomato, the management of begomoviruses shows a better situation due to the existence of improved TYLCV-resistant cultivars in production and the next introduction of new cultivars with pyrimidized genes, favoring the continuous progress of the breeding programs. However, a more comprehensive vision is needed for tomato management because of the current introduction of new viruses in the production.

The popularization of common bean cultivation in the last years, based on the sustainable territorial self-sufficiency for consumption and obtaining of seed, has also led to an increase in the need to apply the integrated pest management because the whitefly-transmitted geminivirus are beginning to become a threat to production again, possibly due to the non-sustainable compliance with the management measures. The high spread of efficient vector population in local production areas and low diversity of varieties with sources of resistance to begomoviruses are aspects considered as a favorable scenario for the development of vector-borne viral diseases (Jones 2009).

New challenges for the management of pests are imposed on common bean crops in order to maintain the productive increases sustained in recent years in the production of this important component of the Cuban diet.

In spite of the low incidence of begomoviruses in tobacco, it is very important to consider that this crop could be a biologically active reservoir due to the coincidence that tobacco is grown in the same season when other crops of economic importance are grown, that is, the period of the country's largest production campaign, known as the winter campaign. The diversification in agriculture, adaptation to new environments and virus-host interactions could be advantageous epidemiological forces that are favoring the pass or jump later to crops of economic interest mediated by the high transmission capacity of the vector, such has been as observed for TbLCCuV.

In Cuba the use of rational identification and diagnostic systems and the implementation of agroecosystem management measures, including the introduction of resistant varieties, are the least cost, feasible, and environmentally friendly measures used for the control of losses caused by whitefly-transmitted geminivirus.

19.7 Concluding Remarks

- TYLCV-IL(CU) is the begomovirus that has caused the largest impact on Cuban agriculture, mainly on tomato production.
- The development of management strategies and particularly the introduction of improved resistant Cuban cultivars against TYLCV-IL(CU) have contributed to reduce the incidence of the pathogen and improve the productive indicators of tomato.
- The specie MEAM1 of *B. tabaci* was introduced in the country, and it is widely distributed.
- In tobacco, beans, and weeds, a wide diversity of bipartite begomoviruses has been observed, even sharing hosts.
- A new class of DNA satellite molecules associated with the specie *Sida golden yellow vein virus*-[Cuba:Havana] was detected.
- Phylogenetic analysis showed molecular diversity in the New World–Cuban begomovirus cluster.
- The presence of new species and epidemiological forces shows favorable scenery for the evolution and emergence of begomoviruses with non-predictable impacts on the agricultural output of the country.

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