Rajarshi Kumar Gaur · Nikolay Manchev Petrov
Basavaprabhu L. Patil
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Plant Viruses: Evolution and Management

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Preface

The current trends of environmental diversity and emerging virus species are becoming an increasing threat to our way of life economically and physically. Plant viruses are particularly significant as they affect our food supply and are capable of rapidly spreading to new plant species. In basic research, plant viruses have become useful models to analyze the molecular biology of plant gene regulation and cell-cell communication. The small size of DNA genome of viruses possesses minimal coding capacity and replicates in the host cell nucleus with the help of host plant cellular machinery. Thus, studying virus cellular processes also forms the best system in understanding the DNA replication, transcription, mRNA processing, protein expression and gene silencing in plants. A better knowledge of these cellular processes will help us in designing the antiviral strategies in plants.

This book will focus on the plant virus evolution, their molecular classification, epidemics and management. It covers topics on evolutionary mechanisms, viral ecology and emergence, appropriate methods for analysis and the role of evolution in taxonomy. This edited book also provides the in-depth knowledge of plant virus gene interaction with host, localization and expression. This book is expected to provide the most recent information regarding advances in plant virus evolution, their responses and crop improvement.

This book will be beneficial for molecular biologist and plant virologist because it combines characterization of plant viruses and disease managements. When these topics are present together, it is easy to compare all aspects of resistance, tolerance and management strategies.

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Plant Viruses: History and Taxonomy

1

Surabhi Awasthi, Reshu Chauhan, and Raghvendra P. Narayan

Abstract

Viruses are very small pathogenic particles made up of nucleoprotein (nucleic acid and protein). The study of plant viruses is so important because they cause diseases to the economically important crops. They cause a great loss to the quality and quantity of the crops. Plant viruses show various types of symptoms such as colour breaking, chlorosis, mottling, vein clearing, vein bending, leaf curl, decrease in size, distorted growth, etc. The plant viruses are very simple and are very host specific.

Keywords

History • Plant virus • Taxonomy • Nomenclature • ICTV

1.1 Introduction

Viruses are very small (submicroscopic) pathogenic particles (virions) composed of a protein which forms covering (coat) and a nucleic acid core. The nucleic acid, which is DNA or RNA, carries all genetic information required for sustaining. All viruses are obligate parasites and require cellular machinery of hosts for the multiplication. Replication and transcription of viruses to produce more nucleic acid and formation of proteins takes place within the host cell using some of the host's machinery by reprogramming hosts gene expression (Hanley-Bowdoin et al. 2004). Viruses are not functional outside their host. Therefore all the viruses are obligate parasites. All types of living organisms are hosts for viruses, but most of the viruses are host specific and infect only one type of host. Viruses are usually named on the basis of their host, for example, viruses that infect bacteria are known as bacteriophages, whereas others, those that infect algae, are phycoviruses, protozoa, fungi that are known as mycoviruses.

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1.2 Definition

'Viruses are obligate intracellular parasites that are capable of infecting eukaryotes, bacteria and archaea, as well as other organisms' (Desnues and Raoult 2012; Desnues et al. 2012; Raoult and Forterre 2008)

According to Roger Hull (2009), a virus is a set of one or more nucleic acid template molecules normally encased in a protective coat or coats of protein or lipoproteins that is able to organise its own replication only within suitable host cells. Within such cells, virus replication is (1) dependent on the host's protein-synthesising machinery, (2) organised from pools of the required materials rather than by binary fission and (3) located at sites that are not separated from the host cell contents by a lipoprotein bilayer membrane and (4) continually gives rise to variants through several kinds of change in the viral nucleic acid.

1.3 Plant Viruses

Plant viruses are also obligate intracellular parasites as the other viruses that use the molecular machinery of the host for their replication (Ahlquist et al. 2003). These viruses are widely distributed and economically important (Wren et al. 2006). The plant viruses cause many harmful plant diseases and they are responsible for a tremendous loss in crop production and crop quality worldwide. Virus-infected plants show several kinds of symptoms depending on the disease type and host but leaf yellowing is common. Some of the other symptoms of virus infection are whole leaf or in a pattern of stripes or blotches; leaf distortion, like leaf curling, mottling and other growth distortions like stunting of the whole plant; and abnormalities in flower or fruit formation (Giampetruzzi et al. 2012).

1.4 History

Tobacco mosaic virus (TMV) was the first virus to be discovered and studied, which causes mosaic disease in tobacco plants (Soosaar et al. 2005). In 1882, Adolf Mayer (1843–1942) while studying tobacco plant described a condition, which he called 'mosaic disease' (*Mosaaikkrankheit*), and now it is well known to be caused by the tobacco mosaic virus (TMV). The diseased plants had variegated leaves that were mottled (Mayer 1882). He excluded the possibility of a fungal infection and could not detect any bacterium and speculated that a 'soluble, enzyme-like infectious principle was involved' (van der Want and Dijkstra 2006). He did not pursue his idea any further and a major observation was made in 1892 by Iwanowski who showed that sap from tobacco plants displaying the disease described by Mayer was still infective after it had been passed through a bacteria-proof filter candle (Roger Hull 2009). However, based on previous studies, it was thought that this agent was a toxin. Iwanowski's experiment was repeated in 1898 by Beijerinck, who showed that the agent multiplied in infected tissue and called it contagium vivum fluidum (Latin for 'contagious living fluid') to distinguish it from contagious corpuscular agents (Beijerinck 1898). Beijerinck and other scientists used the term **virus** to describe the causative agents of such transmissible diseases to contrast them with bacteria (Roger Hull 2009). Earlier workers used the term 'virus' for both bacteria and viruses, but later on with more discoveries, the term 'filterable viruses' was used (Roger Hull 2009). With further discoveries the word filterable was dropped and term virus was adopted (Roger Hull 2009).

In the history of plant viruses, the importance of tobacco mosaic virus cannot be underestimated. TMV was the first virus to be studied and also to be crystallised. It was the very first virus to be studied in detail. In 1941 the first X-ray diffraction pictures of TMV was obtained by Bernal and Fankuchen. On the basis of her pictures, Rosalind Franklin discovered the full structure of the virus in 1955 (Creager and Morgan 2008). In the year 1941, Heinz Fraenkel-Conrat and Robley Williams showed that purified tobacco mosaic virus RNA and its coat protein can assemble by themselves to form functional viruses, suggesting that this simple mechanism was probably the means through which viruses were created within

their host cells. Replication of TMV involves -sRNA using + strand RNA as template (Buck 1999; Ishikawa and Okada 2004).

Nowadays at least 3705 viruses are known of which about 1000 are plant viruses. The plant viruses are studied because they have negative impact on crop production. The viruses were considered as a health threat to humans, livestock and crop plants. In recent few decades, research and development in virology has made it possible in understanding virus-host interactions and has transformed viruses into important tools of biomedicine and biotechnology (Rajamaki et al. 2004). For example, many plant viruses are used to produce proteins useful for plants and animals (Pogue et al. 2002), and many animal viruses are used for the development of vaccines against human and animal viruses such as chicken pox, rabies, foot and mouth disease, measles, etc. (Walmsley and Arntzen 2000).

The development of plant virology can be categorised into five major (overlapping) ages as follows according to Roger Hull 2009.

Prehistory age	752 AD Plant virus in Japanese poem written by the Empress Koken and translated by T. Inouye:
	In this village
	It looks as if frosting continuously
	For, the plant I saw
	In the field of summer
	The colour of the leaves were yellowing
	1600–1637 Tulipomania
Recognition of viral entity	1886 Mayer transmission of TMV
	1892 Iwanowski filterability of TMV
	1898 Beijerink viruses as an entity
Biological age	1900–1935 Descriptions of many viruses
Biochemical/ Physical age	1935 Purification of TMV
	1936 TMV contains pentose nucleic acid
	1939 EM TMV rod-shaped particles
	1951 TYMV RNA in protein shell
	1956 Virus particles made of identical protein subunit
	1955/56 Infectious nature of TMV RNA
	1962 Structure of isometric particles
	1983 Structure of TBSV to 2.9 Å
Molecular age	1960 Sequence of TMV coat protein
	1980 Sequence of CaMV DNA genome
	1982 Sequence of TMV RNA genome
	1984 Infectious transcripts of multicomponent BMV
	1986 Transgenic protection of plants against TMV
	1996 Recognition of RNA silencing
	1997 Recognition of virus suppressors of silencing

The transmissions of animal and plant viruses use different strategies to move from one host to other host and from one cell to other. The movements of plant viruses from one plant to the other need some vector, i.e., means of transmission such as insects, mites, flies, etc. The movement of viruses from one plant cell to other occurs through the plasmodesmata because viruses cannot pass through the thick cell wall. Plants probably have specialised mechanisms for transporting mRNAs through plasmodesmata, and these mechanisms are thought to be used by RNA viruses to spread from one cell to another (Ivanovski 1892).

1.5 Classification and Nomenclature of Viruses

The arrangement of different living organisms into different taxonomic categories (taxa) on the basis of their similarities and/ or relationships is called as *classification*, while assigning a particular name to them is called as *nomenclature.* The classification and nomenclature are studied under broader terminology known as taxonomy. The taxonomy of viruses is somewhat recent exercise. Johnson (1927) was the first virologist for emphasising the importance of the viral taxonomy. The earliest classification of virus was based on only few properties which include ecological and biological properties, basically the pathological property which was given greater emphasis. In 1939, Holmes published his system of classification of viruses, which was based on interaction of host with its pathogen using binomial and

trinomial system of nomenclature. With the discovery of electron microscope and biochemical studies, the classification of viruses as a group was done by different virologists such as herpesvirus group, myxovirus group, poxvirus group, etc. During this period several attempts were made to classify viruses but none were perfect. There was a need to develop a universal system of viral classification.

Earlier viruses were classified on the basis of the two developed system, the Linnaean system and Adansonian system (Roger Hull 2009). The Linnaean system was based on monothetic hierarchical system which was developed by Linnaeus for plant and animal taxonomy. The classification based on Linnaean system was not suitable for the classification of viruses due to several shortcomings. The second system, i.e., Adansonian system, was more suitable for the viral classification because this system considers several criteria at once. The Adansonian system used in viral taxonomy is polythetic hierarchical classification system published by Adanson in 1763. A polythetic class can be defined as the class in which all the members share the several properties in common (Adanson 1763). According to this system, the virus species are defined by several common properties which they share. In other words the members of a virus species are defined collectively by a consensus group of properties. Earlier this system was not so feasible due to its complexity of several characters. The problems of Adansonian system were sort out by use of computers and now it is used universally. At present more than sixty characters are used for classifying viruses. Various discoveries in cell and molecular biology have provided many tools and techniques, which helped in comparing nucleic acid sequences. The sequencing of DNA or RNA has helped in creating phylogenetic trees for the viruses (Hull 2009).

Several criteria are used for the classification of viruses. Some of the criterians are virion properties, which include shape, size, presence or absence of envelope and peplomers, molecular mass, buoyant density, sedimentation coefficient, pH stability, thermal stability, cation stability (Mg^{2+}, Mn^{2+}), solvent stability, detergent stability, radiation stability, properties of proteins, genome organisation and replication such as type of nucleic acid, DNA or RNA, single or double stranded, linear or circular, positive or negative sense or ambisense, number of segments, size of genome or genome sequence, presence or absence of 5Đ terminal cap, presence or absence of 5Đ terminal polypeptide, presence or absence of 3Đ terminal poly A tract; nucleotide sequence comparison; number of proteins, size of proteins, functional activities of proteins, presence or absence of lipid nature of lipids, presence or absence of carbohydrate, nature of carbohydrate, genome organisation, strategy of replication of nucleic acids, characteristics of translation and post-translational processing, site of accumulation of virion protein, site of assembly, site of maturation and release, cytopathology, inclusion body formation, antigenic properties such as serological relationship, mapping epitopes and biological properties; host range, natural and experimental, pathogenicity, association with disease, tissue tropisms, pathology, histopathology, mode of transmission in nature, vector relationship, geographic distribution (Roger Hull 2009; Leppard et al. 2007).

The nature (molecular and genetic composition) of the virus genome packaged into the virion particle is one of the major factors in classification of viruses. Possible genome types are:

- dsDNA
- ssDNA
- ssDNA(−)
- ssDNA(+)
- ssDNA(+/−)
- dsDNA-RT
- ssRNA-RT
- dsRNA
- ssRNA(−)
- ssRNA(+)
- ssRNA(+/−)
- Viroid

1.6 Baltimore System of Virus Classification

Developed by David Baltimore (1971). The Baltimore classification has+RNA as its central point. This system of virus classification is based upon the relationship between viral genome and messenger RNA. All viruses must produce mRNA, or (+) sense RNA and a complementary strand of mRNA, or nucleic acid is called (–) sense (strand) (Voyles 2002). According to Baltimore viruses can be grouped into seven classes on the basis of mRNA synthesis:

1. Class 1: dsDNA viruses; mRNA is synthesised normally using negative strand as template.
2. Class 2: ssDNA viruses ; mRNA is synthesised by double stranded DNA intermediate.
3. Class 3: dsRNA viruses; mRNA is synthesised by complementary strand(template strand).
4. Class 4: ssRNA viruses; RNA directly functions as mRNA.
5. Class 5: (–) sense ssRNA viruses; mRNA is synthesised by synthesis of positive strand.
6. Class 6: genome (+) strand RNA viruses; genome is synthesised by reverse transcription.
7. Class 7: DNA reverse transcribing viruses with RNA intermediates.

The international committee on nomenclature of virus was established by a group of 43 virologists from all over the world in 1966 at International Congress for Microbiology held in Moscow to develop a uniform system of classification and nomenclature (Fauquet et al. 2005). The name of ICNV was changed to International Committee on Taxonomy of Viruses in 1974. The ICTV is the main governing body for all matters related to viral taxonomy. At present, International Committee on Taxonomy of Viruses (ICTV) is a committee of the Virology Division of the International Union of Microbiological Societies. The ICTV is made up of an executive committee (EC) with officers of the ICTV, subcommittee chairs and elected members. The officers manage ICTV activities, while the subcommittee chairs are responsible for managing a series of study groups that assess the current virus taxonomy and recommend updates. Elected members assist the subcommittee chairs in managing the process of making taxonomic assignments.

At present the ICTV is composed of six subcommittees. The responsibilities of subcommittee are to classify fungal and algal viruses, plant viruses, invertebrate viruses, prokaryotic viruses and vertebrate viruses. These subcommittees discuss the classification of newly discovered viruses and manage rules accordingly. The last committee, i.e., the sixth subcommittee, is responsible for managing ICTV data and maintaining the ICTV database and websites. There are 76 international study groups (SGs) functioning under ICTV for the study of families and genera. Each SGs is headed by the chairman. The chairman is appointed by the relevant subcommittee chair. Chairman of the SGs is responsible for (1) organising discussions among SG members of emerging taxonomic issues in their field, (2) for overseeing the submission of proposals for new taxonomy and (3) for the preparation, or revision, of relevant chapter(s) in ICTV Reports. Since its inception ICTV has published nine reports. The first report was published in 1971, 2nd in 1976, 3rd in 1979, 4th in 1982, 5th in 1991, 6th in 1995, 7th in 2000, 8th in 2005 and 9th in 2011. In 2015 ICTV has published its virus taxonomy release. According to this taxonomic release, viruses are divided into seven orders, 111 families, 30 subfamilies, 610 genera and 3705 species.

ICTV activities are governed by statutes agreed with the virology division. The statutes define the objectives of the ICTV. These are:

1. To develop an internationally agreed taxonomy for viruses;
2. To develop internationally agreed names for virus taxa
3. To communicate taxonomic decisions to the international community of virologists;
4. To maintain an index of agreed names of virus taxa.

The present universal system of viral taxonomy given by ICTV follows the hierarchical system which includes order, family, subfamily in some, genus and species. Lower hierarchical system is

also developed by ICTV. According to ICTV the hierarchical system is as follows:

Order: An 'order' is the highest taxonomic level of virus classification into which virus species can be categorised. In the present taxonomic system, use of order is optional. Some of the viruses are unassigned during classification. If 'unassigned' has been entered, the taxon has not been assigned to an order. The first order to be established was *Mononegavirales* in 1990. This order comprises non-segmented ssRNA negative-sense viruses, namely the families *Filoviridae*, *Paramyxoviridae* and *Rhabdoviridae* (Fauquet et al. 2005). According to current taxonomic release of ICTV (2015), seven orders have been assigned, while 78 virus families have not been assigned to any orders. The orders are *Caudovirales* (3 families), *Herpesvirales* (3 families), *Ligamenvirales* (2 families), *Mononegavirales* (5 families), *Nidovirales* (4 families), *Picornavirales* (5 families) and *Tymovirales* (4 families), and 78 virus families have not been assigned to orders.

Family: A 'family' is a level in the taxonomic hierarchy into which virus species can be classified. If marked 'unassigned' (which is rare), the lower taxonomic level of 'genus' has not been assigned to a family. A total of 104 families have been described by ICTV 2015.

Subfamily: A 'subfamily' is a level in the taxonomic hierarchy into which virus species can be classified. Use of the taxonomic level subfamily is optional. If left blank, the lower taxonomic levels of genus and/or species have not been assigned to a subfamily

Genus: A 'genus' is a level in the taxonomic hierarchy into which virus species can be classified. Viral genus may be defined as 'a population of virus species that share common characteristics and are different from other population of species' (Fauquet et al. 2005). If 'unassigned' (which is rare), that species has not been assigned to a genus.

Species: The 7th ICTV Report formalised for the first time the concept of the virus species as the lowest taxon (group) in a branching hierarchy of viral taxa. As defined therein, 'a virus species is a polythetic class of viruses that constitute a replicating lineage and occupy a particular ecological niche' (Van Regenmortel 1990). A polythetic class can be defined as the class in which all the members share the several properties in common. According to this system, the virus species are defined by several common properties which they share. In other words the members of a virus species are defined collectively by a consensus group of properties. Virus species thus differ from the higher viral taxa, which are 'universal' classes and as such are defined by properties that are necessary for membership.

One 'type of species' is chosen for each genus to serve as an example of a well-characterised species for that genus. If the value in this column is '1', this indicates that this species has been chosen as the type species for its genus.

1.7 Nomenclature of Viruses

The guide line for naming of viruses by ICTV (9th Report) are as follows:

The genus name ends in '-virus', subfamily name ends in '-virinae', family name ends with '-viridae' and order name ends with '-virales' universally in formal taxonomy. In viral taxonomy, the finalised names of virus orders (e.g., *Caudovirales*), families (e.g., *Myoviridae*), subfamilies (e.g., Pseudovirineae *Peduovirineae*) and genera (e.g., *Hpunalikevirus*) are printed in italics, and the first letters of the names are written in capitals. The names of species are printed in italics with first letter of first word in capital (e.g., *Mumps virus*). The rest of the words is not capitalised unless they are proper nouns (e.g., *West Nile virus*), parts of proper nouns (*Enterobacteria phage MS2*) or alphabetical identifiers (e.g., *Enterovirus A*). Names of virus strains, on the other hand, are not italicised. The first letter of the first word is not capitalised (e.g., herpes simplex virus) unless it is a proper noun, typically based on the binomial name of the species it infects (Van Regenmortel 1999; Mayo 2000).

The outline of present, (ICTV taxonomic release, 2014) taxonomy of viruses is as follows:

	Order		Family	Genus	Subfamily	Genus
1	*Caudovirales*	1	*Myoviridae*	*Bcep78likevirus*	*Eucampyvirinae*	*Cp220likevirus*
				Bcepmulikevirus		*Cp8unalikevirus*
				Felixounalikevirus	*Peduovirinae*	*Hpunalikevirus*
				Hapunalikevirus		*P2likevirus*
				I3likevirus	*Spounavirinae*	*Spounalikevirus*
				Mulikevirus		*Twortlikevirus*
				Pbunalikevirus		Unassigned
				Phicd119likevirus	*Tevenvirinae*	*Schizot4likevirus*
				Phihlikevirus		*T4likevirus*
				Phikzlikevirus		Unassigned
				Punalikevirus		
				Viunalikevirus		
				Unassigned		
		2	*Podoviridae*	*Bcep22likevirus*	*Autographivirinae*	*Phikmvlikevirus*
				Bppunalikevirus		*Sp6likevirus*
				Epsilon15likevirus		*T7likevirus*
				F116likevirus		Unassigned
				Luz24likevirus	*Picovirinae*	*Ahjdlikevirus*
				N4likevirus		*Phi29likevirus*
				P22likevirus		Unassigned
				Phieco32likevirus		
				Unassigned		
		3	*Siphoviridae*	*3alikevirus*		
				77likevirus		
				Andromedalikevirus		
				Barnyardlikevirus		
				Bignuzlikevirus		
				Bronlikevirus		
				C2likevirus		
				C5likevirus		
				Charlielikevirus		

(continued)

	Order		Family	Genus	Subfamily	Genus
				Che8likevirus		
				Che9clikevirus		
				Chilikevirus		
				Cjwunalikevirus		
				Corndoglikevirus		
				D3112likevirus		
				D3likevirus		
				Halolikevirus		
				Hk578likevirus		
				Iebhlikevirus		
				Jerseylikevirus		
				L5likevirus		
				Lambdalikevirus		
				N15likevirus		
				Omegalikevirus		
				P23likevirus		
				Pbiunalikevirus		
				Pgonelikevirus		
				Phic3unalikevirus		
				Phicbklikevirus		
				Phie125likevirus		
				Phietalikevirus		
				Phifllikevirus		
				Phijlunalikevirus		
				Psimunalikevirus		
				Reylikevirus		
				Sap6likevirus		
				Sfi1unalikevirus		
				Sfi21dtunalikevirus		
				Skunalikevirus		
				Spbetalikevirus		

				T5likevirus		
				Tm4likevirus		
				Tp2unalikevirus		
				Tunalikevirus		
				Wbetalikevirus		
				Xp10likevirus		
				Yualikevirus		
2	*Herpesvirales*	1	*Alloherpesviridae*	*Batrachovirus*		
				Cyprinivirus		
				Ictalurivirus		
				Salmonivirus		
		2	*Herpesviridae*	–	*Alphaherpesvirinae*	*Iltovirus*
						Mardivirus
						Scutavirus
						Simplexvirus
						Unassigned
						Varicellovirus
					Betaherpesvirinae	*Cytomegalovirus*
						Muromegalovirus
						Proboscivirus
						Roseolovirus
						Unassigned
					Gammaherpesvirinae	*Lymphocryptovirus*
						Macavirus
						Percavirus
						Rhadinovirus
						Unassigned
						Unassigned
		3	*Malacoherpesviridae*	*Aurivirus*		
				Ostreavirus		

(continued)

	Order		Family	Genus	Subfamily	Genus
3	*Ligamenvirales*	1	*Lipothrixviridae*	*Alphalipothrixvirus*		
				Betalipothrixvirus		
				Deltalipothrixvirus		
				Gammalipothrixvirus		
			Rudiviridae	*Rudivirus*		
4	*Mononegavirales*	1	*Bornaviridae*	*Bornavirus*		
		2	*Filoviridae*	*Cuevavirus*		
				Ebolavirus		
				Marburgvirus		
		3	*Nyamiviridae*	*Nyavirus*		
				Unassigned		
		4	*Paramyxoviridae*	–	*Paramyxovirinae*	*Aquaparamyxovirus*
						Avulavirus
						Ferlavirus
						Henipavirus
						Morbillivirus
						Respirovirus
						Rubulavirus
					Pneumovirinae	*Metapneumovirus*
						Pneumovirus
		5	*Rhabdoviridae*	*Cytorhabdovirus*		
				Ephemerovirus		
				Lyssavirus		
				Novirhabdovirus		
				Nucleorhabdovirus		
				Perhabdovirus		
				Sigmavirus		
				Sprivivirus		
				Tibrovirus		
				Tupavirus		
				Unassigned		
				Vesiculovirus		

5	*Nidovirales*	1	*Arteriviridae*	*Arterivirus*		
		2	*Coronaviridae*	–	*Coronavirinae*	*Alphacoronavirus*
						Betacoronavirus
						Deltacoronavirus
						Gammacoronavirus
					Torovirinae	*Bafinivirus*
						Torovirus
		3	*Mesoniviridae*	*Alphamesonivirus*		
		4	*Roniviridae*	*Okavirus*		
6	*Picornavirales*	1	*Dicistroviridae*	*Aparavirus*		
				Cripavirus		
		2	*Iflaviridae*	*Iflavirus*		
		3	*Marnaviridae*	*Marnavirus*		
		4	*Picornaviridae*	*Aphthovirus*		
				Aquamavirus		
				Avihepatovirus		
				Avisivirus		
				Cardiovirus		
				Cosavirus		
				Dicipivirus		
				Enterovirus		
				Erbovirus		
				Gallivirus		
				Hepatovirus		
				Hunnivirus		
				Kobuvirus		
				Kunsagivirus		
				Mischivirus		
				Mosavirus		
				Oscivirus		
				Parechovirus		

(continued)

	Order		Family	Genus	Subfamily	Genus
				Pasivirus		
				Passerivirus		
				Rosavirus		
				Sakobuvirus		
				Salivirus		
				Sapelovirus		
				Senecavirus		
				Sicinivirus		
				Teschovirus		
				Tremovirus		
		5	*Secoviridae*		*Comovirinae*	*Cheravirus*
						Sadwavirus
						Sequivirus
						Torradovirus
						Unassigned
						Waikavirus
		6	Unassigned	*Bacillarnavirus*	–	
				Labyrnavirus		

7	*Tymovirales*	1	*Alphaflexiviridae*	*Allexivirus*		
				Botrexvirus		
				Lolavirus		
				Mandarivirus		
				Potexvirus		
				Sclerodarnavirus		
				Unassigned		
		2	*Betaflexiviridae*	*Capillovirus*		
				Carlavirus		
				Citrivirus		
				Foveavirus		
				Tepovirus		
				Trichovirus		
				Unassigned		
				Vitivirus		
		3	*Gammaflexiviridae*	*Mycoflexivirus*		
		4	*Tymoviridae*	Unassigned		
				Maculavirus		
				Marafivirus		
				Tymovirus		

(continued)

	Order		Family	Genus	Subfamily	Genus
8	**Virus families not assigned to an order**	1	*Adenoviridae*	5 genera		
		2	*Alphatetraviridae*	2 genera		
		3	*Alvernaviridae*	1 genus		
		4	*Amalgaviridae*	1 genus		
		5	*Ampullaviridae*	1 genus		
		6	*Anelloviridae*	11 genera		
		7	*Arenaviridae*	2 genera		
		8	*Ascoviridae*	1 genus		
		9	*Asfarviridae*	1 genus		
		10	*Astroviridae*	2 genera		
		11	*Avsunviroidae*	3 genera		
		12	*Baculoviridae*	4 genera		
		13	*Barnaviridae*	1 genus		
		14	*Benyviridae*	1 genus		
		15	*Bicaudaviridae*	1 genus		
		16	*Bidnaviridae*	1 genus		
		17	*Birnaviridae*	4 genera		
		18	*Bromoviridae*	6 genera		
		19	*Bunyaviridae*	5 genera		
		20	*Caliciviridae*	5 genera		
		21	*Carmotetraviridae*	1 genus		
		22	*Caulimoviridae*	8 genera		
		23	*Chrysoviridae*	1 genus		
		24	*Circoviridae*	2 genera		
		25	*Clavaviridae*	1 genus		
		26	*Closteroviridae*	4 genera		
		27	*Corticoviridae*	1 genus		
		28	*Cystoviridae*	1 genus		
		29	*Endornaviridae*	1 genus		
		30	*Flaviviridae*	4 genera		

		31	*Fuselloviridae*	2 genera		
		32	*Geminiviridae*	7 genera		
		33	*Globuloviridae*	1 genus		
		34	*Guttaviridae*	2 genera		
		35	*Hepadnaviridae*	2 genera		
		36	*Hepeviridae*	2 genera		
		37	*Hypoviridae*	1 genus		
		38	*Hytrosaviridae*	2 genera		
		39	*Inoviridae*	2 genera		
		40	*Iridoviridae*	5 genera		
		41	*Leviviridae*	2 genera		
		42	*Luteoviridae*	3 genera		
		43	*Marseilleviridae*	1 genus		
		44	*Megabirnaviridae*	1 genus		
		45	*Metaviridae*	3 genera		
		46	*Microviridae*	1 subfamily	1 subfamily	
		47	*Mimiviridae*	2 genera		
		48	*Nanoviridae*	2 genera		
		49	*Narnaviridae*	2 genera		
		50	*Nimaviridae*	1 genera		
		51	*Nodaviridae*	2 genera		
		52	*Nudiviridae*	2 genera		
		53	*Ophioviridae*	1 genera		
		54	*Orthomyxoviridae*	6 genera		
		55	*Papillomaviridae*	39 genera		

(continued)

	Order		Family	Genus	Subfamily	Genus
		56	*Partitiviridae*	5 genera		
		57	*Parvoviridae*		2 subfamilies	
		58	*Permutotetraviridae*	1 genus		
		59	*Phycodnaviridae*	6 genera		
		60	*Picobirnaviridae*	1 genus		
		61	*Plasmaviridae*	1 genus		
		62	*Polydnaviridae*	2 genera		
		63	*Polyomaviridae*	1 genus		
		64	*Pospiviroidae*	5 genera		
		65	*Potyviridae*	8 genera		
		66	*Poxviridae*	2 genera		
		67	*Pseudoviridae*	3 genera		
		68	*Quadriviridae*	1 genera		
		69	*Reoviridae*		2 subfamilies	
		70	*Retroviridae*		2 subfamilies	
		71	*Sphaerolipoviridae*	3 genera		
		72	*Spiraviridae*	1 genus		
		73	*Tectiviridae*	1 genus		
		74	*Togaviridae*	2 genera		
		75	*Tombusviridae*	13 genera		
		76	*Totiviridae*	5 genera		
		77	*Turriviridae*	1 genus		
		78	Unassigned	14 genera		
		79	*Virgaviridae*	6 genera		

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Transmission and Movement of Plant Viruses

2

Nikolay Manchev Petrov

Abstract

Plant viruses are obligate parasites and their survival depend on being able to spread from one susceptible organism to another. Viruses cannot penetrate the intact plant cuticle and the cellulose cell wall. Therefore penetration is made trough wounds in the surface layers, such as in mechanical inoculation and transmission by vectors. There is specificity in the mechanism by which the plant viruses are naturally transmitted. They are important economically only if they can spread from plant to plant rapidly. They are contagious agents that differ in their transmissibility. No transmission of virus occurred when the virus titer in the inoculum was too low and there is no susceptibility between virus, vector, and host. Also the presence of some substances in the inoculum, which inhibited the infection process, hampered the transmission of viruses. Knowledge of the ways in which plant viruses spread is essential for the development of control measures.

Keywords

Transmission • Plant viruses

2.1 Introduction

Plant viruses must go through two stages during their infection cycle. First, they must replicate inside host cells, employing cellular systems; they have to move to adjacent cells (short-distance movement) and, through the vascular system, reach other tissues and organs (long-distance movement). Second, viruses must spread to new hosts; to do that, they have to cross cellular barriers to enter cells. For most plant viruses this process is assisted by vector organisms (Matthews 1991). Transmission from plant to plant is an essential process for virus survival. Plant viruses have developed several strategies to perform this task efficiently, in many cases involving the existence of specific viral gene

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products known to facilitate the transmission process (Hull 1994; Gray 1996; Van den Heuvel et al. 1999).

Plant viruses are transmitted in different ways in nature-mechanical transmission, through the soil, by grafting, by planting material, through pollen, by seed, and by animal and vegetable vectors.

2.2 Mechanical and Contact Transmission

Mechanical transmission is when the viral particles contained in the juice of the diseased plant penetrate trough fresh wounds and infect the living cells of the healthy plant. Shortly after injury the cells die and cannot be the starting points for the penetration of viruses in plants. Most often, these wounds are obtained by touch and rubbing the leaves and stems of diseased plants that grow nearby. Not all viruses, however, infect in this way, but only highly infectious as TMV and PVX. Mechanical infections in tobacco, tomatoes, and other plants whose leaves are covered with plant trichomes are frequent. Touching the trichomes they break and juice of diseased plants is mixed with the juice of the healthy. For this contributes planting, breaking off sprouts, wringing, and other operations by which only one sick plant can contaminate the hands of the workers and the tools and infect many other healthy plants.

Grafting is an old established method to propagate the plants vegetatively. This is the easy way of transmission of virus from the scion or bud to rootstock through sap. The effectiveness of inoculation of sap-transmissible viruses can be increased by dusting the leaves by fine carborundum powder prior to inoculation (Rawlins and Tompkins 1936). The reported sap-transmitted virus includes cucumber mosaic virus (CMV), tobacco mosaic virus (TMV), potato virus X (PVX), and some geminiviruses. It implies direct transfer of sap from wounded plant to healthy plant on tools, hands, clothes, or machinery. PVX and Pepino mosaic virus can easily be spread by farm implements. The ability of these viruses to be spread by sap in the field is due to their extreme stability.

2.3 Transmission by Soil, Drainage, and River Water

By its nature, transmission by soil is also a mechanical transmission in which are grown diseased plants. In this case the source of infection is the remains of diseased plants in which certain viruses such as TMV, cucumber green mottle mosaic virus (CGMMV), and PVX retain their infectivity continuously. In very rare cases, viruses released from the roots of diseased plants and adsorbed to soil particles cause infections. (Smith et al. 1969). Highly infectious viruses in tomatoes – tomato mosaic virus (ToMV) and cucumber (CGMMV) – are being widely disseminated in greenhouses where the plants are grown hydroponic. In this case the nutrient solution acts as a carrier of these viruses. Viruses are isolated from the rivers passing through major cities such as the Thames, which flow into the city's canals (Tomlinson et al. 1982, 1984).

2.4 Transmission by Grafting

The safest way to transmit viruses is through the tissues from diseased to healthy plants. In vegetatively propagated crops, this transfer plays a big role because through it people transmit those viruses that do not carry mechanically or by vectors. Typically, in order to ensure the infection is necessary to obtain the bond between the graft and the substrate. Transmission by grafting is practiced for identifying viruses that infect not mechanically or were transmitted hardly by juice. In the natural conditions infections by grafting are possible not only in the vegetative propagation of plants, but spontaneously – in coalescence of roots of growing adjacent sick and healthy plants. They are particularly important for viruses that are found primarily in the roots, as in the prune dwarf virus in peach. Transmission by grafting is typical for *Potyviruses* like plum pox virus (PPV) in plums and PVY in pepper and tomato.

2.5 Transmission by Planting Material

With few exceptions, the viruses are in varying concentrations in nearly all tissues of the diseased plants. Therefore planting material obtained from such plants as cuttings, seedlings, buds, tubers, bulbs, etc., carries viral infection. That is why this mode of transmission and spread of viruses is essential in vegetatively propagating crops such as fruits, vine, berries, hops, potatoes, bulbs, and flowers. Regular transmission of the viruses in the generation of vegetatively propagated crops leads to the so-called degeneration. Plants received from infected propagating material are source of infection for neighboring plants. Thus from generation to generation the percentage of diseased plants is increasing and the yield is decreasing. This degeneration is quickly and typically for crops with a short growing season, such as potatoes. Therefore identification of potato viruses (PVY, PLRV, PVM, and PVS) in time is in great importance to stop the spread of disease and degeneration of potato cultivar. More often for 2–3 years, diseased tubers reach 100 %, so that its further cultivation is unprofitable and inappropriate.

2.6 Transmission Through Pollen

The virus transmitted by pollen may infect the seed and the seedlings which grow from it or it can also infect the plant through the fertilized flower. The pollen transmission is known to occur mainly in fruit trees like sour cherry. The ILAR (PPV, prune dwarf virus, prunus necrotic spot virus) viruses are known to be transmitted through pollen.

2.7 Transmission Through Seeds

Viruses that are transmitted trough seeds have some common properties. Most of them are mechanically transmitted easily; in infected plants mainly produce symptoms of mosaic and necrosis due to changes in the parenchymatous tissue. Aphids carry viruses transmissible through seeds but these are mainly nonpersistent viruses. Most cases of transmission of viruses trough seeds was by nematodes. Especially easy they carry the seed of annual weeds (Lister and Murant 1967).

Many important virus diseases are known to be transmitted by seeds. Bean common mosaic virus (BCMV) and CMV were among the first reported to be transmitted through seeds (Reddick and Stewart 1919). Pea seed-borne mosaic virus has been dispersed throughout the world in infected seeds. Seed-borne virus transmission involves virus-host interaction, a floral-infection stage, and the influence of the environment. Infection of an embryo with a virus is the most important factor of plant virus transmission through seed. TMV is a very stable virus that remains infectious on the surface of the seed coat. During germination or planting, seedlings get infected with TMV as a result of mechanical infection (Taylor et al. 1961; Broadbent 1965). Southern bean mosaic virus is found in the seed coat. The transmission frequency is, however, very low, and the virus is inactivated during the process of seed transmission (Crowley 1959; McDonald and Hamilton; 1972; Uyemoto and Grogan 1977). Melon necrotic spot virus is also seed transmitted, but no infection occurs when seeds containing the virus are sown in soil without the fungal vector *Olpidium bornovanus* (Hibi and Furuki 1985).

In general, plants infected after or shortly before the onset of flowering escape virus transmission. Seed transmission depends upon the ability of the virus to infect micro- and mega gametophyte tissues that give rise to infected pollen and ovaries. Ovule-based virus transmission is quite common, and few seed-transmissible viruses infect their progeny through pollen (Carroll and Mayhew 1976a, b; Carroll 1981; Hunter and Bowyer 1997). In ovule-based transmission, the virus infects floral parts early in their development. In pollen transmission, on the other hand, the virus is able to infect the floral meristems and pollen mother cells at an early stage, before the appearance of the callose layer (Hunter and Bowyer 1997). The virus-host interaction

plays a significant role in determining the frequency of seed transmission. Different isolates of the same virus show differences in frequency in the same or different cultivars of the same host (Timian 1974; Wang et al. 1993; Johansen et al. 1996, details in later part). Age of plant and environmental factors such as temperature also affect transmission rate (Hanada and Harrison 1977; Xu et al. 1991; Wang and Maule 1997).

2.8 Transmission by Vectors

2.8.1 Virus Transmission by Insects

In nature, most of the viruses are transmitted by vectors. These are organisms able to carry-over the virus from one plant to another over a short or long distance. The majority of plant virus vectors belong to the Arthropoda, in the classes Arachnida and Insecta (Harris 1981). Bennett first reported transmission of virus by insect (Bennett 1940).

Insects transfer viruses in persistent and nonpersistent manner (Watson and Roberts 1939). Persistently transmitted viruses are acquired from a diseased plant and the vector cannot transfer it to healthy plant immediately. First, the virus has to circulate within the midgut of the insect and later reach to the salivary system. The period between the acquisition of virus by vector and transmission to healthy plant is called latent period. Nonpersistent viruses are acquired by the vector and transmitted in a few seconds. The potato virus Y (PVY) is transmitted in nonpersistent manner, while potato leaf roll virus (PLRV) is persistent in its vector, *Myzus persicae*.

Aphids are the most important group of vectors because of their abundance and feeding behavior (Harris 1991). Leafhoppers and plant hoppers also are important vectors of many viruses, and they have a similar feeding mechanism (Nault and Ammar 1989). Treehoppers, thrips, whiteflies, mealybugs, mites, beetles, and other insects are also vectors of different viruses (Matthews 1991). From all known plant viruses, around 70 % are insect transmitted, and more than 50 % of those are transmitted by homopteran vectors (Francki et al. 1991). In some cases, the virus is able to replicate in vector cells. Specificity and selectivity of the transmission process influence the epidemic spread of diseases caused by plant viruses (Ferris and Berger 1993). Therefore, it is of great importance to study the transmission process with the ultimate practical purpose of designing effective strategies of controlling the spread of many economically important diseases.

2.8.1.1 Classification of Transmission Modes

Relationships of plant viruses and their insect vectors can be differentiated according to the duration of retention inside the vector. Acquisition of the virus from the vector spans from initiation of probing in the plant until the vector becomes able to transmit the virus. Period of latency is the time required after acquisition before the virus can be readily inoculated while the retention is the period for which the vector remains virulent.

Noncirculative and circulative transmission can be differentiated based on the sites of retention and the routes of movement through the vector (Matthews 1991). Noncirculative viruses are associated temporally with the surfaces of the digestive tract of the vector. These viruses have no latency period, and they are lost after molting. Noncirculative viruses can be either nonpersistent or semipersistent. Nonpersistent viruses are acquired in brief periods like seconds, and they can be inoculated immediately after acquisition, and retention is limited to short periods. Transmission is considered semipersistent when its efficiency increases directly with duration of acquisition and inoculation periods.

Circulative viruses need translocation inside the vector to be transmitted. Most of these viruses are found in vascular tissues of plants, and some cannot be inoculated mechanically. They need a latent period after acquisition. Circulative transmission can be classified into non-propagative and propagative. Non-propagative transmission occurs when the virus does not replicate in the vector, although it needs to cross barriers in the digestive tract of the vector to reach the hemolymph and, from there, the salivary glands to be inoculated during subsequent feeding. In propagative transmission, the virus is

able to replicate inside cells of the vector during its circulation; thus, the virus is a parasite of both plants and insects. In some cases, the virus can even be passed on transovarially to the vector progeny.

2.8.1.2 Nonpersistent and Semipersistent Transmission

Most of plant viruses are circulative (nonpersistent and semipersistent). In most cases, the number of virions needed for transmission may be too low (Walker and Pirone 1972), and extremely sensitive and specific methods of detection are needed to identify the presence of virus within the vector (Plumb 1989). Although retention time is generally considered short, its duration may depend on specific conditions, and, in practice, nonpersistent viruses have been shown to be retained for sufficient time to travel rather long distances in their vectors (Zeyen and Berger 1990). As is typical of piercing-sucking insects, aphids make brief insertions of their stylets to probe the adequacy of the plant as a food source, sucking sap and injecting saliva in the process. As a result, acquisition and inoculation of nonpersistent viruses occur during these probes (Lopez-Abella et al. 1988).

The acquisition of noncirculative viruses is related to intracellular ingestion by the vector, and the inoculation of the virus occurs during salivation (Martin et al. 1997). The transmissibility of viruses belonging to the genus *Cucumovirus*, on the other hand, depends on characteristics of only the capsid protein (CP) of the virions. For *Potyvirus* and *Caulimovirus*, vector transmission depends on characteristics of both the CP and the helper component (Pirone and Blanc 1996; Pirone 1977).

2.8.1.3 Circulative Non-propagative Transmission

Circulative non-propagative plant viruses are transmitted across vector membranes, and they have to survive inside the vector during circulation until they are inoculated in the host plant. The digestive system of insects can be divided into foregut, midgut, and hindgut. Entry of circulative plant viruses into the hemolymph may occur during their passage along the digestive tract through the midgut or hindgut. Once in the hemolymph, the virus moves to the salivary glands and passes into the saliva to be excreted later through the salivary duct (Gray 1996). For chewing insects such as beetles, the actual route of circulation could be different, with the viruses being transported across salivary gut membranes to the hemolymph. However, this process might not be totally essential, and the virus might be directly inoculated from the regurgitant (Wang et al. 1992).

2.8.1.4 Circulative Propagative Transmission

Some virus genera consist of plant viruses with complex infection cycles. They can replicate in the cells of their insect vectors, being parasites of both plants and animals. Propagative relationships include a long-term association with the vector that may have adverse effects on the insect host, for instance, in longevity and fecundity. In some cases, propagation includes transovarial transmission of the plant virus to the vector progeny. Propagative viruses encode genes that are differentially expressed in their infection cycle (Falk et al. 1987). Propagative plant viruses belong to families including viruses that also infect animal hosts (*Bunyaviridae*, *Reoviridae*, and *Rhabdoviridae*) and to the genera *Marafivirus* and *Tenuivirus*.

2.8.2 Virus Transmission by Nematodes

Many viruses are transmitted by soilborne nematodes. The three genera of nematode – *Xiphinema*, *Longidorus*, and *Trichodorus* – of the order Dorylaimida are known to transmit plant viruses. Nematode's vectors feed on cells of root tips with their stylet, acquiring viruses. The virus is retained within the gut or esophagus and transmitted to plants during feeding of nematodes. There are 38 *Nepoviruses* and 3 *Tobraviruses* already have been reported to be transmitted by soilborne nematodes (Williamson and Gleason 2003).

The stylets of Longidorids consist of an odontostyle, surrounded by a stylet guide sheath, for penetration of root tip cells as deep as the vascular cylinder, and a stylet extension, the odontophore, with nerve tissues and protractor muscles. The odontophore passes into the esophagus and the esophageal bulb, containing large gland cells that secrete saliva (Brown et al. 1995). During the feeding process, the stylet is inserted and after salivation the cytoplasm of penetrated cells is ingested. Trichodorids usually feed on epidermal cells by pressing their lips against the cell wall that is torn by the stylet so that the cell contents can be sucked in. Subsequently, the food passes through the pharynx and esophagus into the gut (Brown et al. 1995).

The natural distribution of *Longidorus* and *Trichodorus* spp. depends mainly on climate. Most *Xiphinema* spp. are found in the tropics and the Mediterranean. In contrast, the number of *Trichodorus* and *Paratrichodorus* spp. tends to decrease from north to south (Dijkstra and De Jager 1998). Soil type is another important factor that plays a role in the distribution of some longidorids and trichodorids. The vertical distribution of *Longidorus* and *Trichodorus* spp. shows great variation. *Longidorus* spp. prefer surface- rooted hosts; hence, most of them live in the upper soil layers, about 20 cm deep (Taylor 1967). In contrast, *Xiphinema* spp. are present in large numbers around deep-rooted host plants at depths varying from 20 cm to a couple of meters, depending on the type of soil (Taylor 1972). Usually, nematodes move to deeper layers in the soil during dry or very cold periods.

2.8.3 Virus Transmission by Fungal Vectors

The fungi are obligate endoparasites of plants that form zoospores. They belong to the *Chytridiomycota* (*Olpidium* spp.) or the Plasmodiophoromycota (*Polymyxa* spp. and *Spongospora* spp.). Two species of *Olpidium (O. bornovanus* and *O. brassicae)*, two species of *Polymyxa (P. betae* and *P. graminis)*, and one species of *Spongospora (S. subterranea)* are natural vectors of viruses (Campbell and Sim 1994; Campbell 1996). The life cycles of the two categories of fungal vectors have much in common (Adams 1991; Campbell 1996). Thick-walled resting spores are formed inside roots or young tubers of the host plant. With the plasmodiophorids the resting spores are formed in clusters, whereas the chytrids have single resting spores. When the infected roots or tubers decay in the soil, the spores are released. Depending on the conditions in the soil, resting spores germinate and release motile primary zoospores that move to roots. The zoospores attach to the root hairs or epidermal cells, often in the zone of elongation (Campbell and Fry 1966; Temmink 1971). In this process, the flagella are withdrawn and a cyst wall is secreted. Upon encystment of the zoospore, the axonema with its axonemal sheath is withdrawn inside the zoospore body (Temmink and Campbell 1969a, b; Temmink 1971).

The two types of fungal vectors use different mechanisms for penetration of the host cell. With *Olpidium* spp., belonging to the chytrids, the protoplast of the cyst enters the host through a minute pore dissolved in the wall of the host cell. With the plasmodiophorid fungi, *Polymyxa* spp. and *Spongospora* spp., the wall of the host cell is penetrated by a stylet. As soon as the cyst has settled down on root hairs or epidermal cells of the roots it forms a tube, the end of it being pointed at the surface of the host. The tube contains the stachel. Infection proceeds rapidly by evagination of the tube, resulting in a firm attachment to the host with an adhesorium and, subsequently, in puncturing the host wall with the stachel. The stachel is released into the host cell, where after the protoplast of the cyst follows. With both types of vectors, the protoplast of the fungus moves into the cytoplasm of the host, where the young thallus evolves into a multinucleate primary plasmodium that is enveloped in a thin thallus membrane. The thallus develops into zoosporangia from which the secondary zoospores are released into soil water. With *Olpidium* spp. the zoospores escape from the sporangia through a distinct exit tube penetrating the outer wall of the host cell. In the later part of the cycle, the thallus, now enveloped in a thicker membrane, develops into resting

spores or resting sporangia that may remain viable in root debris for a long time. The fungal vectors exhibit considerable host specificity.

According to the current classification of viruses (Pringle 1999), fungus-borne viruses are found in the genera *Tombusvirus*, *Carmovirus*, *Necrovirus*, and *Dianthovirus* of the family *Tombusviridae;* *Furovirus*, *Pomovirus*, *Pecluvirus*, and *Benyvirus*; and the genus *Bymovirus* of the family *Potyviridae* (Mayo 1995).

2.9 Movement of Plant Viruses

Plant virus movement is divided into two phases: (1) cell to cell, or short distance, and (2) long distance. Cell to cell movement is when an invading virus is transported from initially infected epidermal cells through the mesophyll and phloem parenchyma in the susceptible host (Carrington et al. 1996). In the absence of such cell-to-cell movement, the infection is confined to the initially infected cell and said to be subliminal (Cheo 1970; Schmitz and Rao 1996). The majority of plant viruses encode a nonstructural protein, referred to as a movement protein (MP) for promoting viral movement between cells. In some viral systems, in addition to MP, the structural or coat protein (CP) is also required to mediate this process. Thus, the overall movement process can either be coat protein independent or coat protein dependent.

2.9.1 Coat Protein-Independent Movement

In those viral systems which do not require the CP for cell-to-cell movement, the MP alone is sufficient. The best-understood example is TMV. The first two genes from its genome encode replicase proteins and the fourth encodes the structural CP (Dawson and Lehto 1990). The third gene specifies the production of a 30 kDa protein that is not required for replication or encapsidation. A TMV mutant with deletions in this gene replicates and encapsidates in protoplasts but does not move systemically in plants (Meshi et al. 1987). This suggests that the 30 kDa protein is involved in viral spread. The Lsl mutant strain of TMV does not infect tobacco at 32 °C, whereas the parental L strain remains infectious (Nishiguchi et al. 1978). Lsl infect tobacco in the presence of L at 32 °C. This implies that L can complement the movement function of Lsl (Taliansky et al. 1982b). The virus moves from cell to cell via plasmodesmata, which are, however, too small to allow free passage of virions or viral genomes (the gateway capacity or size exclusion limit (SEL) is not sufficient). To test this, fluorescent molecules of different sizes were injected into mesophyll cells of transgenic and nontransgenic plants. Molecules no larger than 0.7 kDa moved from cell to cell in nontransgenic plants, whereas 9.4 kDa molecules moved from cell to cell in the transgenic plants that accumulate the TMV-MP (Wolf et al. 1989). Although the plasmodesmata could accommodate the passage of these large molecules, which were predicted to have diameters between 2.4 and 3.1 nm, the modified plasmodesmatal SEL was still not large enough for the passage of virions or free-folded viral RNA. The modified plasmodesmata could allow the passage of viral RNA as a single-strand complex. Since TMV mutants unable to encapsidate can move from cell to cell (Saito et al. 1990), the virus must be able to move from cell to cell either as a naked RNA or as a virus-specific ribonucleoprotein complex (Dorokhov et al. 1983).

2.9.2 Tubule-Guided Mechanism

Cells infected with cowpea mosaic virus (CPMV) have distinct tubules that penetrate the plasmodesmata (Van Lent et al. 1990). When penetrated by tubules, plasmodesmata lose their characteristic desmotubules. Since the tubules penetrate the plasma membranes of protoplasts, the tubules are not modified desmotubules (Van Lent et al. 1991). Such tubular structures are involved in cell-to-cell movement of CPMV (Kasteel et al. 1996). Two overlapping genes that produce peptides 58 kDa/48 kDa in size are needed along with the viral CP gene to establish a successful

CPMV infection (Wellink and Van Kammen 1989). The 58 kDa/48 kDa proteins are not necessary for replication, but they do localize to the tubular structures (Van Lent et al. 1990). The 48 kDa protein is involved in tubule formation (Kasteel 1999). *Nepovirus* infection also induces the formation of movement-associated tubules. An antibody raised against the 45 kDa protein of tomato ringspot virus, analogous to the CPMV 48 kDa protein, recognizes the tubules (Wieczorek and Sanfacon 1993). Spherical objects appear to move through the tubules induced by both *Nepo-* and *Comoviruses* (Deom et al. 1992).

2.9.3 Non-tubule-Guided Mechanism

The cell-to-cell movement of CMV is also dependent on both the MP and the CP proteins (Taliansky and Garcia-Arenal 1995; Canto et al. 1997). CP required to support CMV movement is distinct from that of BMV CP. CMV variants lacking a CP, similar to BMV failed to move from cell to cell (Canto et al. 1997). Unlike BMV, virion assembly is not a prerequisite for CMV movement, since assembly-defective CMV variants were able to induce local lesions due to efficient cell-to-cell spread (Kaplan et al. 1998; Schmitz and Rao 1998). CMV also induces tubules in transfected protoplasts. However, tubules do not contribute to viral movement, since mutant CMV RNA3 defective in tubule production is competent for cell-to-cell and systemic spread (Canto and Palukaitis 1999).

2.9.4 Movement Complementation by Heterologous Movement Proteins and Other Virus Genes

A virus normally unable to move from cell to cell in a particular plant may be able to move with the help of a second virus of heterologous origin. Despite extensive variation in morphology, host range, and genome organization, many taxonomically distinct plant viruses exhibit complementary movement functions that may be a result of MP cross-compatibility (Atabekov et al. 1990). For example, whereas TMV-L can complement the movement of TMV-Lsl under high temperatures, PVX can complement the movement of TMV in Tm-2 gene tomato plants that normally resist TMV infection (Taliansky et al. 1982a). TMV and RCNMV are functionally homologous, since the cell-to-cell spread of movement-defective variants of TMV and RCNMV can be complemented in transgenic *Nicotiana benthamiana* plants expressing heterologous MPs (Giesman-Cookmeyer et al. 1995). However, while examining the cross-compatibility between MPs of TMV and CMV, it was observed that transgenic *N. tabacum* cv. Xanthi (tobacco) plants expressing the TMV-MP gene supported cell-to-cell movement, but not the systemic movement, of a movement-defective CMV (Cooper et al. 1996). Transgenic plants accumulating CMV MP can complement the movement of a movement-defective CMV and a wild type of BMV in inoculated leaves but cannot support the movement of TMV-Lsl, RCNMV, or *potato leafroll virus* (Kaplan et al. 1995). MPs share only a few identical amino acids (Melcher 1990). Based on amino acid and structural similarities in a nontaxonomic sense, an attempt was made to group the 30 kDa MPs. Eighteen groups are identified as "30 K" superfamilies: the MPs of *Alfamo-/ILAR-*, *Badna-*, *Bromo-*, *Capillo-/Tricho-*, *Caulimo-*, *Cucumo-*, *Diantho-*, *Furo-*, *Gemini-*, *Idaeo-*, *Nepo(A)-*, *Nepo(B)-*, *Tobamo-*, *Tobra-*, *Tombus-*, and *Umbraviruses*. Five groups of possible candidates are the MPs of *Clostero-*, *Rhabdo-*, *Tenui-*, and *Waikaviruses* and the phloem proteins. These groups can be subgrouped into four different sub-superfamilies (Melcher 2000).

Virus movement is regulated by either the MP alone or the MP in combination with the CP. Other gene products, such as replicase, also appear to influence the movement process. For example, several BMV replicase mutants capable of efficient replication and packaging in protoplasts failed to systemically infect barley plants (Traynor et al. 1991). Replicase genes of BSMV

(Weiland and Edwards 1994), CMV (Gal-On et al. 1995), and TMV (Nelson et al. 1993), as well as nonstructural protein p19 of *Tomato bushy stunt virus* (Scholthof et al. 1995) and a helper component proteinase of potyviruses (Cronin et al. 1995) have demonstrated specific roles in movement.

2.9.5 Role of Host Plant in Viral Movement

Viral movement in a given host plant is regulated also by the type of host itself. An unidentified host factor is also involved in potentiating the cell-to-cell movement of progeny viruses (Deom et al. 1992). *Nicotiana benthamiana* is susceptible to many viruses. For example, BMV has a very narrow host range. However, *N. benthamiana* is susceptible to BMV infection and the virus accumulates to very high concentrations (Rao and Grantham 1995). Following viral infections such as TMV, the MP increases the plasmodesmatal SEL in the previous plant species, permitting cell-to-cell movement of progeny virus (Lucas and Gilbertson 1994). It is possible that the plasmodesmatal SEL at the bundle sheath/phloem parenchyma cell barrier is inherently higher in *N. benthamiana* than in *N. tabacum.* This can explain why *N. benthamiana* is susceptible to a heterologous MP-mediated systemic infection by CMV and also to BMV (Rao et al. 1998). Likewise, the behavior in several hosts of a hybrid virus constructed between BSMV and RCNMV suggests that host-specific factors are involved in virus transport function (Solovyev et al. 1997).

2.10 Conclusion

Plant viruses are transmitted in nature in different ways from which the most common, with most economic importance, and widespread way is transmission by insects. There are different transmission modes. Virus movement is a complex process which involves virus and host factors. All these fundamental investigations about plant virus transmission and movement are essential for epidemiology to develop controlling strategies to stop virus spread.

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Infection, Replication, and Expression of Plant Viruses in Filamentous Fungi

3

Tiziana Mascia and Donato Gallitelli

Abstract

The recent demonstration that the plant virus *tobacco mosaic virus* replicates and expresses in the plant pathogenic fungus *Colletotrichum* spp. provides opportunities for examining fundamental aspects of the biology of plant pathogenic fungi and of their interaction with the host. The small genome size and the ability in colonizing systemically the host have implemented the use of plant viruses to carry segments of host genes that can then promote the silencing of the RNAs expressed from the corresponding endogenous genes in a process called virus-induced gene silencing (VIGS). This chapter presents support for the view that VIGS with a direct transfection of a plant virus vector in fungal cells can be used for functional genomics also in fungi that activate an antiviral defense based on RNA interference (RNAi). The silencing of genes in filamentous fungi is technically more problematic and labor intensive than in plants, especially if transgenic plants need to be generated first. Compared to current strategies to employ RNAi to investigate the basis of fungal pathogenesis, the VIGS approach described here is more direct, easy to do, and feasible. Future perspectives of both basic and practical aspects of this technology are discussed.

Keywords

Tobacco mosaic virus • Plant pathogenic fungi • VIGS

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3.1 Introduction

The notion that soil-inhabiting fungi can vector plant viruses dates back from 1958 with the association of species of the chytrid genus *Olpidium* with the lettuce big-vein disease (Fry 1958; Grogan et al. 1958). There are currently five

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species of zoosporic organisms that are known vectors of plant viruses (Rochon et al. 2004), and there are two modes of transmission recognized in virus–fungal vector relationships, denoted in vitro and in vivo transmission (Campbell 1996). In vitro transmission occurs when virus particles are adsorbed from soil onto the surface of zoospore membrane being able to enter zoospore cytoplasm only when the flagellum is withdrawn in. Thus, in this type of transmission, which is associated mostly to viruses with spherical particles, virions are not present in the spores but enter the plant root cells following zoospore encystment, through a mechanism that is not known. The so-called in vivo transmission is characteristic of viruses with rod-shaped particles, which are retained inside resting spores or zoospores where they might replicate. Driskel et al. (2004) demonstrated that *soilborne wheat mosaic virus* (SBWMV) and *wheat spindle streak mosaic virus* (WSSMV) were internalized in their vector *Polymyxa graminis* where the detection of movement protein (MP) of SBWMV and of the coat protein (CP) of WSSMV suggested viral replication and expression. In particular, since SBWMV MP is translated from a subgenomic RNA, this mode of expression would require production of minus strand RNAs and subsequent transcription of subgenomic RNAs. This synthesis would endorse the SBWMV replication in *P. betae* but such minus strand RNAs have not been detected (Driskel et al. 2004).

Similarly, Verchot-Lubicz et al. (2007) provided evidence for replication of *beet necrotic yellow vein virus* (BNYVV) and association of its MP with resting spores of *P. betae* suggesting that it might be a host other than a vector for BNYVV. This hypothesis was supported by the detection of viral replicase inside resting spores and zoospores and by the accumulation of BNYVV P42, P13, P15, and P14 proteins, which are translated from subgenomic RNAs derived from its RNA2.

Thus recent evidences demonstrate that some rod-shaped RNA plant viruses replicate and express in their fungal vectors. From the early studies of Brants (1969) and Nienhaus and Mack (1974), it was also known that both *tobacco mosaic virus* (TMV) and *tobacco necrosis virus* could infect the plant pathogen *Pythium* sp. although direct proof for viral replication in this organism was not provided. Further attempts to infect other fungal species like *Gaeumannomyces graminis*, *Aureobasidium bolleyi*, and *Pythium ultimum* with *barley stripe mosaic virus* (BSMV) were unsuccessful (Lange 1977) so this line of research did not receive further attention, probably also because the interest for viruses of fungi focused on true mycoviruses (Pearson et al. 2009; Ghabrial and Suzuki 2009).

Recently, Mascia et al. (2014) in the attempt to use virus-induced gene silencing (VIGS) for functional studies in filamentous fungi, through a direct virus infection, showed that TMV could indeed infect three species of *Colletotrichum*, *C. acutatum*, *C. clavatum*, and *C. theobromicola* and replicate therein. Here we report on how this evidence was provided and highlight basic and practical implications of the results.

3.2 Infection and Expression of TMV in *C. acutatum*

Two approaches were conducted to produce a TMV infection in *C. acutatum*. One involved addition of a purified virus suspension to a liquid culture of the microorganism placed on a rotatory shaker, while the second method involved adding sap extracted from infected plants in phosphate buffer and filtered or centrifuged to remove plant debris. In both instances it was compulsory to set up a liquid culture of the fungus 6 h before virus inoculation, starting from an inoculum of approx 10^6 conidia/ml. This gap was necessary to the fungus to reach a phase of germinating conidia prior to be exposed to viral inoculum. Attempts to produce a TMV infection in liquid cultures established by using, as starter, mycelia collected from agar plates were unsuccessful.

Infection of *C. acutatum* by TMV was a process as efficient as in plants. The virus was present already in 100 % of the mycelia samples collected after 24 h incubation in liquid medium following the addition of the viral preparation but how the virus entered the fungus is not known. In

plants TMV is transmitted readily by contact through wounds produced by rubbing between leaves of infected and healthy plants, during root growth in soil contaminated by plant debris or during germination of seeds contaminated externally by virus particles in infected fruits (Hull 2014). Similarly, it is possible that TMV entered *C. acutatum* hyphae via light damage to cell membrane generated during the liquid culture shaking or through natural openings produced in sporulation/growing processes. This might occur especially at the growing tip, where the old cell wall is broken down to accommodate the growth of the new hypha. This hypothesis is supported by the observation that to get a successful infection, liquid culture should start from germinating conidia rather than from mycelia. Interestingly Yu et al. (2013) demonstrated that a purified preparation of the mycovirus *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1 (SsHADV-1) could infect directly the fungal host when applied to mycelia growing on potato dextrose agar, i.e., apparently, without any injury to hyphae, and Brants (1969) reported that addition of carborundum to wound shaking cultures of *Pythium* sp. did not have any effect of the ability of TMV to enter mycelia. Thus it is still controversial whether TMV needs or not artificial wounding to enter mycelia of fungi. A third entry option is pinocytosis through plasma membrane, after damage of cell wall that might occur during shaking.

Observations with a transmission electron microscope (TEM) on dips of *C. acutatum* treated with sodium hypochlorite to eliminate virus particles adhering to hyphae externally showed masses of TMV-like rods inside fungal cells and extensive membrane vesiculation. The presence of TMV particles was confirmed by in situ immunogold labeling (IGL), as abundant IGL signals were seen scattered in the cytoplasm and/or associated with vesicles. Despite this, infection by TMV did not alter the growth rate, morphology, or pathogenicity of *C. acutatum*, nor vice versa replication in fungal mycelia altered TMV characteristics as the virus re-isolated or purified from infected cultures retained typical particle morphology and high infectivity to plants.

Persistence of TMV particles up to 20 days post-inoculation (dpi) and up to 2 months after seven subcultures in agar plates suggested viral replication inside fungal cells. This was confirmed by quantitative estimates of viral RNA by dot-blot hybridization with a TMV-specific probe, which showed a sixfold increase of TMV RNA accumulation from 5 to 20 dpi and by the detection of the replication-specific, negative-sense strand of the viral RNA and of the subgenomic RNA for the translation of viral CP (Mascia et al. 2014). TMV subgenomic RNAs are not encapsidated in virus particles but transcribed from negative-sense strand of the viral RNA synthesized during replication. Thus their detection provides strong evidence for viral replication and expression in *C. acutatum*.

Replication and expression of TMV in cells of *C. acutatum* was confirmed also by using the recombinant vector TMV-GFP in which the ORF of a gene encoding the green fluorescent protein (GFP) was transcribed in fungal cells from a duplicate of the TMV CP subgenomic mRNA promoter. The experiment demonstrated that the recombinant viral vector could be a promising strategy to obtain foreign protein expression in fungi. One major constraint to the ectopic expression of proteins in fungi is the instability as they are lost usually during subculture. The GFP-derived fluorescence was observed in both fungal hyphae and conidia and was maintained for six subcultures.

3.3 Why Produce a Plant Virus Infection in Fungi?

Due to the small-size genome, plant viruses have been implemented as expression vectors to study fundamental processes in plant biology. Recent applications in functional studies include expression of ectopic proteins and of fragments of plant genes to silence endogenes (Senthil-Kumar and Mysore 2011). The latter process, termed VIGS, delivers in plant cell sequences homologous to a target gene via a recombinant virus and exploits the natural plant defense mechanism based on RNA interference (RNAi). RNAi is a process

conserved in animals, plants, and fungi to play fundamental roles like regulation of mRNA accumulation and translation, chromatin silencing, programmed DNA rearrangements, genome surveillance, and host defense against invasive nucleic acids and viruses. The pathway entails the synthesis of a double-stranded RNA (dsRNA), which is recognized and diced into 21- to 25-long dsRNA fragments by ribonucleases of the Dicer-like protein (DCL) family. The small fragments produced—denoted small interfering RNAs (siRNA)—are then loaded onto members of the Argonaute protein (AGO) family to form an RNA-induced silencing complex that uses one of the two strands of the siRNA to direct RNA degradation, translational repression, or DNA methylation of sequence homologous target genes (Melnyk et al. 2011; Wang et al. 2012). In VIGS, the plant RNAi system generates siRNAs from and against both the viral RNA and the expressed gene sequence and since plant viruses move systemically, the silencing process continues in newly formed leaves allowing VIGS to be used for high throughput screening in functional genomics. Thus, in principle, VIGS could be applied to any (micro)organism in which a virus is able to replicate and move systemically.

The defensive role of the RNAi pathway also operates in fungi (Nuss 2011; Chang et al. 2012; Nicolás et al. 2013). It was first demonstrated in the chestnut blight fungus *Cryphonectria parasitica*, in which a Dicer-like gene, dcl2, and an Argonaute-like gene, agl2, were involved in the defense response against a mycovirus (Segers et al. 2007; Sun et al. 2009). Since then central components of the RNAi pathway have been discovered and tested in a number of plant pathogenic fungi as means for functional studies or for the development of fungal-derived resistance through the expression of silencing constructs in host plants. An exhaustive list of RNAi target genes and constructs tested in *Ascomycota*, *Basidiomycota*, *Zygomycota*, and oomycetes *Phytophthora* spp. can be found in the review of Nunes and Dean (2012). As a consequence, new viral vectors and VIGS protocols have been implemented also in fungi. For example, the *barley stripe mosaic virus* (BSMV)-VIGS system has been used successfully for RNAi of specific pathogenicity genes in *Puccinia triticina* by siRNA generated *in planta* through infection of the BSMV vector expressing dsRNAs from pathogen's genes involved in the induction of the disease in wheat (Panwar et al. 2013a). The ectopic expression of such genes in wheat using BSMV-VIGS constructs resulted in the generation of complementary siRNA molecules in systemic leaves which were transferred from the host to the colonizing pathogen *P. triticina* cells where they triggered RNA silencing of the corresponding genes, resulting in disease suppression. Since siRNAs are generated in the host, this approach is also termed host-induced gene silencing (HIGS) but how the small RNA molecules traffic from the plant into fungal cells is not fully understood (Nunes and Dean 2012).

The same result could be obtained using a transient RNAi approach based on *Agrobacterium tumefaciens*-mediated infiltration (agroinfiltration) of fungal genes capable of forming hairpin-like RNA in wheat plants and trigger RNAi (Panwar et al. 2013b). However a major drawback of agroinfiltration was that significant RNAi of the target fungal genes was transient and observed only within the agroinfiltrated wheat leaf areas (Panwar et al. 2013b), while the BSMV-mediated VIGS of P. *triticina* genes resulted in systemic spread of silencing in wheat plants consistent with virus replication and movement (Panwar et al. 2013a). Compared to agroinfiltration, a systemic viral infection results also in a more-persistent silencing effect that could prove particularly useful for determining the role of fungal genes that are expressed late in infection (Panwar et al. 2013a).

An alternative way to achieve the same objectives is to transform host plants with fragments of the target fungal genes. Highly structured dsRNAs of these fragments are incorporated in plant genome to trigger RNAi and produce siRNAs that would traffic from the plant into the fungal cells. The approach has been exploited successfully with *Fusarium verticillioides* in tobacco (Tinoco et al. 2010), *Blumeria graminis* in barley (Nowara et al. 2010), *Bremia lactucae* in *Lactuca sativa* (Govindarajulu et al. 2014), *F. oxysporum*

f.sp. cubense in banana (Chag et al. 2014), and *P. infestans* in potato (Jahan et al. 2015). Besides the complexity of the transformation and handling of transgenic plants, the risk also exists that HIGS constructs could target and negatively affect the host plant.

Finally, Mascia et al. (2014) have shown that RNAi can be expressed in phytopathogenic fungi by direct transfection with a plant virus-based vector, i.e., without either plant or *Agrobacterium* intermediates. For the purpose, the isolate C71 of *C. acutatum* was transformed with a binary plasmid vector to express constitutively GFP under the regulation of the constitutive translation elongation factor (TEF) promoter from *Aureobasidium pullulans* and the glucoamylase terminator from *Aspergillus awamori*. The transgenic expression of GFP in hyphae and conidia of the resulting transformant CATEF10 was demonstrated by observation under the epifluorescence microscope, while the mitotic stability of the integrated transgene and of its expression was confirmed by more than 20 subcultures in liquid and solid media. Such GFP expression was downregulated easily in CATEF10 cultures following addition of the TMV-GFP vector to culture medium either as sap extracted from infected plants of *Nicotiana occidentalis* or as purified preparation. Similarly to VIGS in plants, the C71 RNAi system generated siRNAs from both the viral RNA and the expressed GFP gene sequence resulting in the almost complete downregulation of the transgenic GFP sequence present in CATEF10, as estimated by quantitative PCR and epifluorescence microscope observations (Mascia et al. 2014). Interestingly, also the TMV sequences of the recombinant vector were targeted by fungal RNAi as demonstrated by the detection of the virus-specific siRNAs confirming that, similarly to true mycoviruses, also plant viruses are recognized as invasive agents and processed to degradation by the fungal RNAi machinery.

Thus, compared to HIGS, agroinfiltration, or other methods to trigger fungal RNAi for functional studies, VIGS approach using a recombinant plant virus vector seems more easy to do, direct, and feasible.

3.4 Current Issues and Future Perspectives in the Use of Plant Virus Infection in Fungi

There are both basic and practical aspects in exploiting plant virus infection in fungi. Basic research requires information on how plant viruses enter fungal mycelia; how they replicate, express, and move; and how long they persist therein. Practical issues include effects of virus infection on fungal growth, metabolism, pathogenicity and persistence, and transmission of the silenced phenotype to fungal progeny.

Hypotheses on how TMV enters mycelia have been formulated already. Once inside, the virus interacts with fungal cells, as demonstrated by the abundant proliferation of the endoplasmic reticulum (ER) and dictyosomal vesicles that may serve as scaffold for viral replication and/or movement like in plants (Liu and Nelson 2013). Preliminary IGL observations with an antiserum raised against TMV replicase indicated that the enzyme accumulated in electron-dense bodies that are part of the vesiculation (Mascia et al. unpublished information). Interestingly, no TMV particles were found in older hyphae, but these retained the extensive membrane proliferation suggesting either that virus replication was limited to the growing tip of the hyphae or that the virus was eliminated in old hyphae that also undergo autophagy (Voigt and Pöggeler 2013). As for the role of other genes coded by the TMV genome, probably the possession of a sequence coding for a movement protein would be irrelevant within a fungal host, as anticipated by Pearson et al (2009) in formulating hypotheses on the origin of mycoviruses. TMV particles should be able to spread throughout the entire mycelium, since the septa separating hyphal cells have pores large enough that allow free flow of organelles and mycovirus particles and thus should not represent a barrier to those of a plant virus. However, like in plants (Liu and Nelson 2013), the formation of an ER-MP-viral RNA complex traveling through mycelia cannot be excluded. Ongoing experiments in our lab with IGL and TMV mutants defective in replicase

(Csorba et al. 2007) or MP (Peiró et al. 2014) would clarify these points on TMV life cycle in fungi.

Practical issues focus on the use of VIGS for functional genomics and its collateral effects on pathogen's characteristics and the persistence of the silenced phenotype in living tissues. Mascia et al. (2014) demonstrated that TMV-GFP replicated in cells of *C. acutatum* with efficiency comparable to that observed in plant cells but unlike in plants, the viral vector did not induce evident detrimental effects to the fungal morphology, growth, and pathogenicity. This is a key point in conducting VIGS studies, as effects on host phenotype and other deleterious characteristics can mask the effect on the gene to be silenced (Ratcliff et al. 2001). On the other hand, attention must be paid for adverse effects of the construct itself on the host. For example, while TMV infection did not alter morpho-functional parameters of *C. acutatum,* both transient and transgenic expression of the reporter gene GFP induced approx.1.5-fold reduction in the growth rate and in the endopolygalacturonase activity, which is an enzyme involved in pathogenicity. When viewed under TEM, cells expressing GFP either transgenically or ectopically showed electron-dense protein aggregates, which were recognized by an antiserum raised against GFP in IGL (Mascia et al. 2014 and unpublished information). These electron-dense bodies were not observed in CATEF10 cells with GFP-silenced phenotype.

As for the persistence of the silenced phenotype, RNAi has been shown to be a potent and elegant system to silence posttranscriptionally selected genes in fungi that have the components of the silencing machinery. However, a striking disadvantage of the method could be the instability of the silencing construct, which may cause reversion of the silenced phenotype to wild type after prolonged cultivation on solid substrates and often after the first mitotic event (Meyer 2008). In the VIGS approach discussed in this chapter, the transgenic expression of GFP in CATEF10 was totally abolished and remained silenced stably up to six subcultures on solid medium, i.e., approximately 65 dpi with TMV-GFP. Fluorescence was monitored at each subculture showing no reversed silencing effect that was observed only at the seventh passage. Therefore, as in plants, the systemic RNAi signal followed fungal growth, and in fact no infectivity was associated with TMV-GFP back inoculated to plants with crushed mycelia of the sixth subculture, providing also direct evidence that the silencing signal targeted both the GFP transcript and the TMV-GFP RNA. This, in turn, poses the question whether the p122 protein suppressor of RNA silencing encoded by TMV would be ineffective in counteracting RNAi in fungal cells. An efficient viral suppressor of RNAi (Csorba et al. 2015) would affect the silenced phenotype and cause reversion to wild type as in plants with the regression of disease symptoms during recovery (Ghoshal and Sanfaçon 2015).

For functional studies in plant–pathogen interactions, it is imperative that the fungal silenced phenotype is maintained also during the infection in host plant. To test this, CATEF10 with silenced GFP was inoculated in wounded apple fruits and leaves of olive seedlings. After 3 weeks, sites of infection necrotized and could not provide any information on the maintenance of GFP silencing, due to fluorescence emitted from the necrotized infected tissues. However, the isolation of the fungus from such fruit or leaves of olive seedlings and propagation on agar plates showed that the GFP in CATEF10 was still silenced, indicating that VIGS silencing of the transgene was maintained also in living tissues.

3.5 Concluding Remarks

Compared with conventional gene knockout strategies in fungi, RNAi has several advantages and fewer drawbacks. One major advantage is that RNAi can be induced transiently, overcoming the need of permanent deletion of specific genes, which may be lethal for the organism and independently of the fungus asexual reproduction pathway.

Its use will need some refined insights and adaptation, for example, to biotrophic fungi, but it is out of doubt that because of its simplicity, it

offers new opportunities in the studies of human, animal, and plant mycology.

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Diverse Roles of Plant and Viral Helicases: Current Status and Future Perspective

4

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Abstract

The ubiquitous helicases form a large family of proteins which are required for all the aspects of nucleic acid metabolism and have been classified into six evolutionarily conserved superfamilies (SF1-SF6). The members of different helicase families are related by elements that unify them at the structural level, despite dissimilarities in their organization and mechanism they employ. The cellular nucleic acids require a transient and local unzipping in order to participate and accomplish various cellular functions such as replication, transcription, and repair, for which the unzipper protein (helicase) is indispensable. In addition to maintain proper growth and development, plants employ various helicases to assist in genome stability. In fact, viruses with smaller genome and limited coding potential, rely largely on host machineries for their infection and thus, are found to code for helicase proteins. This chapter will briefly introduce an updated knowledge on the structural and functional diversity that exists among members of the helicases. Further, the diverse roles of the helicases encoded by plants and plant infecting ssDNA viruses have been discussed.

Keywords

Plant virus • Helicase • Superfamily • Replication • Pathogenesis

4.1 Introduction

Helicases are translocases (motor proteins) that are capable of altering DNA structure by facilitating separation of two strands of a thermodynamically stable dsDNA molecule into unfavored and unstable ssDNA (Matson et al. 1994). This energy-dependent function acts as an essential

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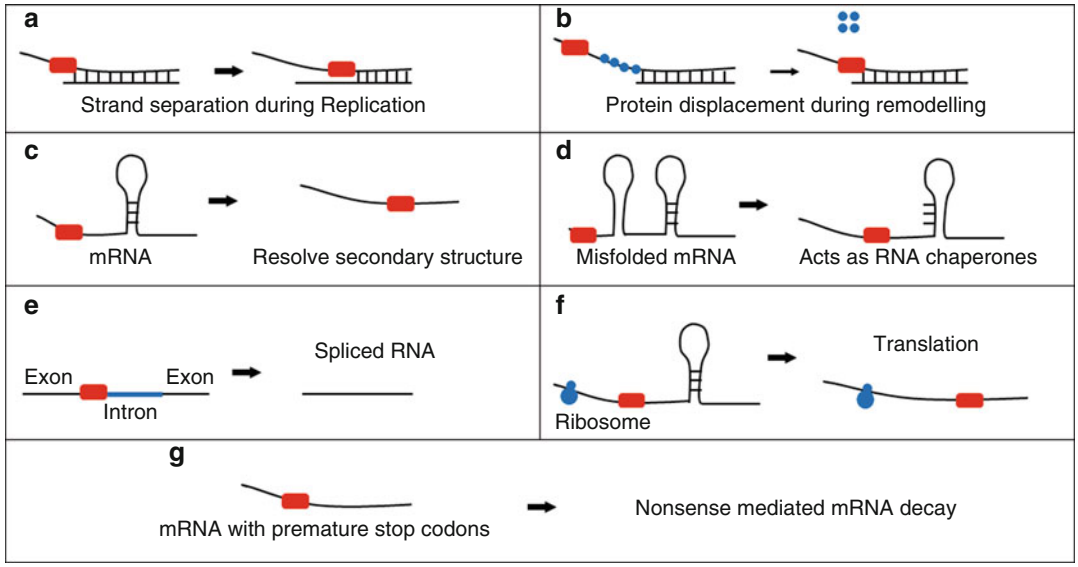

Fig. 4.1 Diverse roles performed by cellular helicases. DNA helicases are employed during replication (**a**) and nucleic acid remodeling process (**b**). RNA helicases help in resolving the secondary structures formed in the mRNA and making it available for various processes (**c**), act as RNA chaperones, and bring about correct folding of the mRNA (**d**). In addition, they also assist in RNA splicing (**e**), in initiation of translation (**f**), and in nonsense-mediated mRNA decay (**g**). The *red-colored boxes* represent the helicase proteins associated with either DNA or RNA

and integral component of diverse protein complexes which participate in various steps involving both DNA and RNA. These helicases are involved in multitude of cellular processes and are evolutionarily conserved (Fig. 4.1). In fact, DNA helicases facilitate metabolic processes such as DNA repair, replication, and recombination, while RNA helicases assist in transcription, RNA splicing, RNA editing, RNA transport, RNA degradation, ribosome biogenesis, and translation machinery (Tanner and Linder 2001). Either absence or defect of helicases in humans causes various pathological disorders which in turn underscores the essentiality of this enzyme in diverse cellular processes (Abdel-Monem et al. 1976).

4.2 Classification

Gorbanlenya and Koonin (1993) proposed a classification of helicases based on the short conserved amino acid sequences, and they have been classified into six superfamilies as SF1, SF2, SF3, SF4, SF5, and SF6. The general classification reveals differences in number of distinct conserved motifs as well as minor differences in the consensus sequences within the conserved motifs among different groups. All these helicases share certain common features such as the presence of ATP-binding motifs and hydrolysis of ATP (ATPase). This ATPase motif shares similar structural elements and topology in relation to RecA, a protein involved in recombination by catalyzing strand exchange within homologous strands of ssDNA and dsDNA in an ATP-dependent manner (Clark and Margulies 1965). These helicases also possess three-dimensional folds which are reminiscent of RecA (RecA-like folds) and forms a functional part of the minimal structural core domain. Furthermore, ATP-binding motifs include a conserved walker A (phosphate-binding loop) and walker B motif (Walker et al. 1982). With the expanding database of the structure of helicases, it became more evident that the tertiary structures of these proteins shared similar spatial arrangements of the conserved helicase motifs.

4.2.1 Non-hexameric Helicases

The DNA helicase from *Bacillus stearothermophilus* (PcrA protein) is the first member of SF1 superfamily for which the crystal structure was solved (Subramanya et al. 1996). A striking similarity between the ATP-binding domain of PcrA protein and the RecA protein, in terms of the topological folding and structure, was also revealed. The characteristic feature of RecA-like fold indicates that it consists of a number of central sheets sandwiched between helices, referred as α/β core domain (Story et al. 1992). RecA-like domain forms the basic catalytic and functional unit of NTPase (Ye et al. 2004). The nucleotide-binding pocket is a cleft that is formed by two adjacent RecA-like domains, wherein nucleotide is bound to one RecA-like domain. However, for nucleotide hydrolysis, the contribution of residues surrounding RecA-like domain is also reported to be necessary. ATP hydrolysis brings motion in the protein domains relative to each other which in turn is transduced into the strand separation of duplex nucleic acids (Fig. 4.2).

Superfamilies 1 and 2 (SF1 and SF2) form the largest and most studied groups of helicases. They were initially identified to comprise of seven motifs, but later on other motifs such as Q motif and TxGx motif were also identified (Gorbalenya et al. 1989). SF1 and SF2 helicases contain two RecA-like domains in a single polypeptide (Fig. 4.2). In motif I resides the walker A box (phosphate-binding P-loop) that contains a highly conserved lysine residue (AxxGxGKT, where x is any amino acid) positioned at the tip of the β1 strand. The amino group of lysine side chain in motif I make contact with the phosphates of the NTP, while the hydroxyl group of threonine forms coordinate bond with the Mg^{2+} ion. The walker B motif (DExx, where x is any amino acid residue) is found within the β3 strand. The negatively charged aspartate residue coordinates with Mg^{2+} ion, whereas glutamate acts as a catalytic base that activates the attacking water molecule during ATP hydrolysis (Story and Steitz 1992). Mutational studies in a protein involved in DNA damage and repair pathway (UvrD protein) suggest that the motif I is not critical in ATP binding; however, it affects ATP hydrolysis, thereby altering its unwinding activity (George et al. 1994). Furthermore, the importance of the residues in motif II of UvrD protein in NTPase activity and the rate of unwinding without any effect on NTP binding has been demonstrated (Brosh

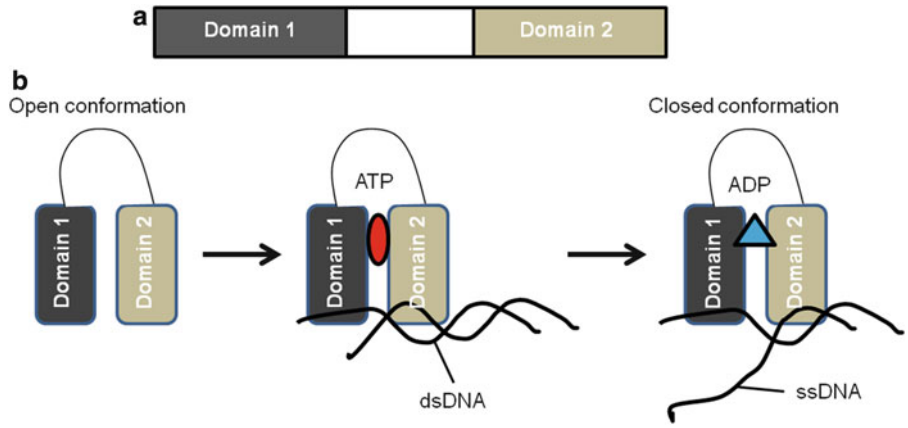

Fig. 4.2 Mechanism of unwinding by SF1 helicase. (**a**) A simplified schematic diagram showing a monomeric helicase protein (SF1) that comprises of two RecA domains (N-terminal and C-terminal referred as domain 1 and domain 2). ATP-binding cleft consists of residues contributed from both domain 1 and domain 2. Domain 1 contributes Walker A and Walker B motif which is required for ATP hydrolysis, while domain 2 possesses the arginine finger. (**b**) Mechanism of strand separation reaction performed by helicase wherein ATP binding and ATP hydrolysis result in relative domain motion which in turn coupled to its translocation along ssDNA. The *red* and *blue* colors depict ATP and ADP, respectively

and Matson 1995). Thus it is established that the highly conserved residues of Walker A and Walker B involved in NTP hydrolysis is not necessarily needed for NTP binding. From the crystal structure of *Escherichia coli* Rep, the residues of motif Ia (TxxAA) and motif V are known to interact with the sugar-phosphate backbone of ssDNA (Korolev et al. 1997). Apart from this, motif III (GDxxQLPP) and motif IV make multiple interactions with ssDNA via hydrogen bonding and stacking. The consensus sequence for motif VI is established as VA(L/Y)TRA(K/R) (Fairman-Williams et al. 2010). In spite of its close proximity to both NTP- and oligonucleotide-binding sites, motif VI makes no direct contact with either NTP or ssDNA. However, it was found to make contact with both NTP-binding motif IV and oligonucleotide interacting motif III, which suggests a role in the coupling of NTP-induced conformational changes to DNA binding. One of the motif III mutants in UL5 also reported to lack unwinding activity but retaining ATPase activity (Graves-Woodward et al. 1997). Similar results were obtained from a point mutation in Motif III of UvrD protein that showed uncoupling of ATPase and unwinding activities (Brosh and Matson 1997). The crystal structure of PcrA protein in complex with DNA and ATP analog (ADPNP) also supported their role in transducing conformational change to DNA-binding site (Velankar et al. 1999).

The first crystal structure from SF2 superfamily was that of hepatitis C virus-encoded nonstructural (NS3) helicase (Yao et al. 1997). Despite the difference in the architecture, this protein shares an overall topology in resemblance to RecA core domain. SF2 superfamily is the largest superfamily which is further subdivided into ten families. DEA(D/H)-box family of RNA helicases is one of the extensively studied group of helicases. They possess a characteristic Q motif which is hypothesized to be involved in adenine recognition (Tanner 2003). All the conserved motifs in SF2 superfamily had been reported to be required for the helicase activity. Although minor sequence differences exists, the majority of the dissimilarity between SF2 and SF1 enzymes are found to be in the motifs III and IV (Korolev et al. 1998). This suggests that the mode of interaction between NTP-binding site and oligonucleotide-binding site may vary between SF1 and SF2. The motifs Ia and III in UvrD/Rep and PiF1-like SF1 families have been proposed to have a role in providing translocation polarity (Saikrishnan et al. 2009).

4.2.2 Hexameric Helicases

Various helicases are reported to assemble as either hexameric or higher-order oligomeric forms. Hexameric helicases possess ring-shaped structures exhibiting two-tiered arrangement. They have either RecA or an AAA+ (ATPases associated with various cellular activities)-like core domain with a NTP-binding site at the interface between the two monomers. The NTP interacts with conserved motifs that line the binding pocket as well as with an arginine residue that is contributed by the adjacent monomer. This arginine residue is analogous to the catalytic arginine residue present in the "arginine finger," which was originally identified in GTPase-activating protein (Ahmadian et al. 1997). In the monomeric helicases, as a consequence of ATP hydrolysis, there is a relative movement between the two RecA-like domains of a single subunit. However, in case of hexameric helicases, this relative motion is between the RecA domains of the two adjacent subunits. This ATP-induced conformational change is sensed by a conserved arginine residue surrounding the nucleotide-binding domain.

The core nucleotide-binding folds of RecA-like and AAA+ proteins are related, but differ in their topology, conserved ATP-binding residues and the orientation of individual motor domains in higher-order quaternary states. Despite these differences, the basic mechanism is similar, whereby ATP hydrolysis brings motion in the protein domains relative to each other which in turn results in strand separation of the duplex nucleic acids.

SF3 family of helicases was originally identified in the genomes of smaller DNA and RNA viruses (Gorbalenya et al. 1989). The members of this family comprise of three motifs, motif A, B, and C, that spans ~100 amino acids long. Similar

to other superfamily helicases, motif A and B of SF3 helicases consist of Walker A and Walker B. In addition, members of this superfamily also possess a SF3 family-specific motif, termed as motif C. Further, B' motif is reported to be sandwiched between Walker B and C motifs. These helicases share more structural resemblance to AAA+ proteins than the RecA (Hickman and Dyda 2005). The salient feature of AAA+ fold of SF3 helicase is that it comprises of the loops inserted into the core domain which interact with DNA or different proteins. The crystal structure of *Papillomavirus* E1 helicase in a complex with ADP and 13-mer DNA has provided insights into the structure and mechanism of DNA unwinding (Enemark and Joshua-Tor 2006). The hexameric E1 helicase has a central channel which is surrounded by protruding loops from the B' motif. ssDNA passes through this channel and interacts via its phosphodiester backbone with the loops from B' motif lining the channel. These DNA-binding loops form a spiral staircase, and the relative position of these DNA-binding loops is found to be correlated with the NTP ligation state (i.e., ATP- or ADP-associated state) of the subunit of helicase (Lee et al. 2008).

SF4 helicases comprise of five conserved sequence motifs, namely, H1, H1a, H2, H3, and H4 (Ilyina et al. 1992). Initially, SF4 members identified from bacteriophages and bacteria were found to be replicative helicases. Motifs, H1 and H2 in these helicases are regarded as Walker A and Walker B motifs which are required for NTP hydrolysis. Motif H3 contains a conserved glutamine residue and is found to be functionally analogous to motif III of the members of SF1 superfamily. The loops from motif H4 protrudes into the central channel of hexameric protein and interacts with ssDNA (Washington et al. 1996). Unlike SF3 members, they show deviations from the sixfold symmetry. As a result, DNA-binding loops, arginine fingers, and subunit interfaces are not found in similar positions. The gene 4 protein (gp4) from T7 bacteriophage is one of the most extensively studied SF4 helicases (Toth et al. 2003). The enzyme comprises primase and helicase domains connected by a flexible linker.

The details on the function of helicases belonging to other superfamilies (SF5 and SF6) are limited. Rho helicase, a member of SF5, is essential for transcription termination. It binds to specific sequences on the nascent RNA and in an ATP-dependent manner unwinds the DNA-RNA hybrid resulting in the release of RNA from the elongation complex. In addition to Walker A, Walker B, and an arginine finger, the members possess a family-specific motif 1a (Gogol et al. 1991; Skordalakes and Berger 2003). Members of the SF6 family contain AAA+ fold (Erzberger and Berger 2006), and one of the best characterized members of this superfamily is MCM complex that is required for eukaryotic replication. It consists of six different subunits and forms heterohexamer (Labib et al. 2000). Not all the subunits possess the helicase activity; only MCM 4, 6, and 7 possess helicase activity (Kaplan and O'Donnell 2004). It also contains two family-specific motifs, namely, S1 and S2.

Further, the expanding structural informations available suggest that these conserved helicase motifs have further evolved to participate in diverse cellular processes (Singleton et al. 2007).

4.3 Functional Roles in Plant Cellular Pathways

Protein annotations have indicated that approximately 1 % of the eukaryotic genome codes for helicases. In *A. thaliana*, >100 genes are predicted to encode either DNA or RNA helicases. Many of these proteins are found to be expressed in all organs and are required for various cellular pathways (Table 4.1). Various DNA helicases are identified to play crucial role in maintaining genome stability (Knoll and Puchta 2011; Mingam et al. 2004). The genes encoding RecQ helicases are reported in almost all organisms; in many species, more than one copy of RecQ helicases are identified (Rossi et al. 2010; Ashton and Hickson 2010). For instance, there are at least seven RecQ helicases reported in plants (Hartung and Puchta 2006). Mostly, the helicase proteins in plants and their interacting partners are identified through homology studies of functionally characterized helicases documented from yeast, from humans, or from other systems. In *A. thaliana*, a functional homologue of human

Table 4.1 List of DNA and RNA helicases involved in various biological processes in the cell

Functions	Helicases	References
Replication	DnaB, HSV UL5, HSV UL9, Rep, PriA, AAV Rep68, Rep78, Dna2, BPV E1, T7gp4A and 4B, T4gp41, SV40 TAG, Polyoma TAG, MCM4/6/7	Korolev et al. (1998), Labib et al. (2000), Matson et al. (1994), Singleton et al. (2007), Yao et al. (1997), Fairman-Williams et al. (2010)
Transcription	TFIIH, SNF2, SWI2, TRCF, Rho, RecQL5	Gogol et al. (1991), Skordalakes and Berger (2003)
Translation	eIF4A, RHA, Ded1	Ray et al. (1985), Singleton et al. (2007)
Recombination	RecB, RecQ, RuvAB, Rho, BLM, UvrD, RecG, Srs2, PDH65, Dda, UvsW	Knoll and Puchta (2011), George et al. (1994), Tanner and Linder (2001), Wu and Hickson (2002), Gorbalenya et al. (1989)
Repair	UvrD, UvrAB, PcrA, BACH1, Rad3, Dna2, XPD, XPB, RecQ, WRN, BLM, RuvB, HDH II, RecD2, Rad51, Rad54	Clever et al. (1997), Klutstein et al. (2008), Velankar et al. (1999), Wu et al. (2002), Gorbalenya et al. (1989)
RNA splicing	UAP56, Brr2, Prp16, Prp22, Prp43	Shen (2009), Zhang et al. (2015), Hotz and Schwer (1998)
RNA chaperone	Dbp5, DDX3	Jarmoskaite and Russell (2014)
mRNA stability	Rh1B, Ski2, Dob1, Dhh1	Liou et al. (2002), Halbach et al. (2012), Pedro-Segura et al. (2008)

BLM protein that belongs to RecQ family, RecQ4A was identified. RecQ4A interacts with AtRMI1 and AtTOP3a and involved in meiotic recombination in plants (Gangloff et al. 1994; Wu et al. 2000; Wu and Hickson 2002). Other members of DNA helicases in *A. thaliana*, RecQ2, and RecQ3 helicases play a vital role in DNA repair and recombination by unwinding the partial duplex DNA.

Yeast homologue of RAD54 in *A. thaliana* has been identified along with RAD51, and their role in DNA repair mechanism has been documented. Both of these are the members of Swi/Snf2 family and are known to interact and enhance each other's activity (Clever et al. 1997). In addition, the heterologous interaction of scRAD51 with AtRAD54 and scRAD54 with AtRAD51 through yeast-two-hybrid experiments have been demonstrated (Klutstein et al. 2008). In *A. thaliana*, a gene encoding for a DEAH-box RNA helicase (LOS4) has been established to positively regulate the transcriptional of CBF proteins (Gong et al. 2002; Gong et al. 2005). LOS4 has been reported to resolve/unwind cold-stabilized secondary structure in the 5′ untranslated region of RNA.

Various RNA helicases have been reported to be involved in the processes such as transcription, pre-mRNA splicing, ribosome biogenesis, mRNA export, RNA degradation, and silencing. For example, eIF4A (eukaryotic initiation factor 4A), a well-characterized RNA helicase, participates in the initiation of translation by resolving the secondary structure in the 5′ untranslated region of the mRNA (Ray et al. 1985). Another RNA helicase, ethylene-responsive 68 (ER68), plays a role in the ethylene response pathway in tomato (Zegzouti et al. 1999). The ER68 is a DEAD-box RNA helicase and is anticipated to be involved in ethylene-regulated gene expression. The requirement of a DAVH box containing RNA helicase, increased size exclusion limit 2 (ISE2), during the embryogenesis of *A. thaliana* is required for maintaining proper plasmodesmata function (Kobayashi et al. 2007).

Post-transcriptional gene silencing (PTGS) plays a critical role in various plant developmental and stress-related pathways (Brodersen and

Voinnet 2006; Shukla et al. 2008). Systemic PTGS is characterized by the presence of 21-nt siRNAs (small interfering RNAs). In *Arabidopsis*, a DExH helicase (SDE3) in concert with SDE1 functions as RNA-dependent RNA polymerase (RDR) and amplifies the 21 nt siRNAs for long-distance transmission (Himber et al. 2003; Dalmay et al. 2001). Like in animals, plant Dicer-like proteins (DCLs) act as a key regulator in the gene silencing machinery. In addition to the presence of PAZ-, RNase III-, and dsRNA-binding domains, all the four Dicer-like proteins (DCL1-4) identified in *A. thaliana* also possess a DExH-RNA helicase domain (Henderson et al. 2006). ESP-3, a RNA helicase, has been reported to be involved in RNA processing and influences both the embryonic development and gene silencing pathways (Herr et al. 2006).

Nonsense-mediated decay (NMD) involves degradation of aberrant mRNA containing premature termination codon and thus prevents the accumulation of truncated proteins. A nuclear localized SF1 RNA helicase, UPF, is found to be indispensable for NMD in *Arabidopsis* plants (Arciga-Reyes et al. 2006). Further, *upf* mutant exhibits various morphological defects with delayed onset of flowering and seedling lethality (Yoine et al. 2006). Moreover, a RING-between-RING (RBR) family of ubiquitin ligase has both RNA binding and DEAH RNA helicase motifs which is necessary for degradation of aberrant polypeptides and the splicing of defective mRNAs (Lucas et al. 2006; Qiu and Fay 2006; Marin et al. 2004).

The role of several helicases in combating various stress responses is evidenced by their overaccumulation during stress conditions (Vashisht and Tuteja 2006). Upregulation of DEAH-box helicases in response to abiotic stresses has also been reported. Pea DNA 45 (PDH45) helicase has been reported to interact with and stimulates topoisomerase I activity (Pham et al. 2000; Nasirudin et al. 2005). PDH45 is a salt stress-induced ATP-dependent DNA and RNA helicase and is expected to participate in the initiation of translation similar to its homologue eIF4A (eukaryotic translation initiation factor 4A). Interestingly, transgenic plants overexpressing PDH45 gene conferred increased salinity tolerance in tobacco (Sanan-Mishra et al. 2005). Similarly another homologue of eIF4A, PDH47, has been found to be expressed in response to salt and cold stress conditions (Vashisht and Tuteja 2005; Vashisht et al. 2005). Recently, a helicase from *O. sativa*, OsSuv3, binds to both DNA and RNA and possesses DNA- and RNA-dependent ATPase activity (Tuteja et al. 2013; Tuteja et al. 2014). Its role has been implicated in improving antioxidant machinery and thereby providing salt-stress tolerance. In yeast homologue of Suv3, K245A and V272L mutations in helicase motif I and motif Ia have been reported to hinder its ATPase activity which in turn affects its helicase activity. Further, in these mutants, misregulation of RNA turnover and the improper mitochondrial DNA maintenance was noticed (Guo et al. 2011).

4.4 Plant Viral DNA Helicases

ssDNA genome containing plant viruses comprises of members of the family *Geminiviridae* and *Nanoviridae*. Geminivirus genome may contain one or two ssDNA molecules, while nanoviruses contain at least six molecules. Due to limited coding capacity, these viruses rely heavily on the host factors for their infection and spread.

4.4.1 Replication Initiator Protein of Geminiviruses

The family, *Geminiviridae*, comprises a large group of ssDNA containing viruses. The smaller genome size (~2.7 kb) of these viruses makes them a preferred choice for use as vectors for expression of foreign genes in plants. Moreover, these viruses depend entirely on the host biosynthetic machinery due to which they make an ideal model system to study plant DNA replication and gene expression (Hanley-Bowdoin et al. 2000). On the basis of their genome organization and insect vectors, the *International Committee on Taxonomy of Viruses* (ICTV) subdivided the *Geminiviridae* family into seven genera as:

Becurtovirus, *Begomovirus*, *Curtovirus*, *Eragrovirus*, *Mastrevirus*, *Topocuvirus* and *Turncurtovirus* (Varsani et al. 2014). Geminiviruses encode 6–8 overlapping ORFs and have adopted a strategy of bidirectional transcription to maximize the gene expression. The genome is arranged with divergent transcription units extending in opposite directions from a highly conserved ~180 nt region (the common region, CR) which contains the replication origin and the promoter for the leftward ORFs. In addition, CR also possess a stretch of 31 nt that includes the characteristic invariant nonamer, 5′-TAATATT↓AC-3′, in which T7-A8 site is required for cleaving as well as for joining of viral DNA during replication (Laufs et al. 1995). This region has a characteristic secondary structure containing a GC-rich stem and an AT-rich loop.

AC1 encodes for replication initiator protein (Rep) which is indispensable for viral DNA replication. Geminiviruses follow rolling circle and recombination-dependent mode of replication (Stenger et al. 1991). Established studies indicate that the geminivirus plus strand DNA synthesis initiates through a DNA cleavage event at the specific site, thereby fulfilling a key requirement for RCR. The 41 kDa Rep protein is the only viral protein that is absolutely required for viral DNA replication (Elmer et al. 1988; Schalk et al. 1989). Rep bears no similarity with known polymerases but instead shares remarkable conservation with replication initiator proteins of bacterial plasmids involved in initiation and termination of rolling circle replication by functioning as site- and strand-specific endonuclease (Koonin and Ilyina 1992).

In addition to its role in replication, Rep protein also performs other functions such as transcriptional regulation (Eagle et al. 1994), autoregulation (Sunter et al. 1993), and suppression of gene silencing (Rodriguez-Negrete et al. 2013). The N-terminal domain contains three motifs (motifs I, II, III) that are characteristic of many rolling circle initiators (Ilyina and Koonin 1992). Motif I (FLTY) is required for sequence-specific dsDNA binding, while motif II (HLH) is a metal-binding site that may be involved in protein conformation and DNA cleavage (Orozco and Hanley-Bowdoin 1998). Motif III (YxxKD/E) is the catalytic site for DNA cleavage, with the hydroxyl group of the tyrosine residue forming a covalent bond with the 5′ phosphoryl group of the cleaved DNA strand. Recently, another conserved region has been identified between motifs II and III which is designated as the GRS (geminivirus Rep sequence) (Nash et al. 2011). Noninfectious nature, inability of supporting viral genome replication, and its incompetency for ssDNA cleavage in GRS mutants' support that these GRS sequence is required for the initiation of rolling circle replication during geminivirus infection. The N-terminal domain along with the oligomerization domain is required for DNA binding, whereas DNA cleavage activity relies in the N-terminal region alone. Moreover, the removal of the first 29 amino acid residues abolished both DNA-binding and DNA-cleaving activity demonstrating that an intact N terminus is required for both the activities. As oligomerization is required prior to DNA binding, it suggests that homo-oligomerization of Rep protein is required for DNA binding, but not for DNA cleavage.

Rep also possesses ATPase activity domain which is residing within the 181–330 amino acid region (Desbiez et al. 1995). The ATPase domain of Rep is characterized by three conserved motifs: Walker A in the P-loop, Walker B, and motif C. Moreover, the presence of these motifs classified Rep as AAA+ (ATPases associated with various cellular activities) in superfamily 3 (SF3) helicases (Clerot and Bernardi 2006). This leads to the hypothesis that Rep may be a putative helicase which was then later confirmed (Choudhury et al. 2006). The helicase activity of the Rep protein has been shown to be depending on the amino acid residues, 121–359, including the C-terminal domain. The N-terminal region is independent of the helicase activity, however, *in vivo*, this N-terminal region helps in recruiting the Rep protein to the correct initiation site for replication from where the neighboring region helicase activity would proceed. For proper helicase activity, oligomerization domain is also needed in consistent with the fact that most helicases are hexamers. Also

mutation of amino acids critical for ATPase activity (K227A) and the region that affects helicase property (i.e., deletion of oligomerization domain) reduces or abolishes the viral DNA replication. Therefore, both the ATPase and helicase activity are essential for the viral DNA replication. Recently, another motif B' which is required for DNA binding during the unwinding process has been identified. Site-directed mutagenesis had also supported the role of two of the critical amino acid, K272 and K289, in coupling of ATP hydrolysis to DNA translocation and DNA binding, respectively (George et al. 2014).

4.4.2 Master Replication Initiator Protein of Nanoviruses

Nanovirus (previously referred as plant *Circovirus*) contains multiple number of circular ssDNA of about 1 kb in size (Pringle 1998). Nanovirus encodes for only one helicase protein, named as master Rep (M-Rep), an essential viral factor for its replication (Timchenko et al. 1999). It has been established that all the Rep proteins are functional and are capable of autonomous replication of their DNA molecule. The available literature suggests that only M-Rep can carry out replication *in trans* of all other DNA genome; however, detailed mechanism of M-Rep-mediated replication is not known yet. Apart from this, it also encodes for another protein named Clink (cell cycle link) to stimulate the DNA replication, not only of nanovirus but also of geminivirus genome (Aronson et al. 2000). This Clink protein of FBNYV is known to interact with plant RBR (retinoblastoma-related) protein through its LxCxE motif; however, this interaction is found absolutely nonessential for the replication (Lageix et al. 2007). Rep1 and Rep2 proteins of FBNYV possess origin-specific endonuclease activity as well as nucleotidyl transferase activity similar to that of geminivirus-encoded Rep protein. Nanovirus-encoded Rep protein also exhibits ATPase activity, and the mutation within the P-loop results in loss of ATP hydrolysis and affects the replication (Timchenko et al. 2006). Not all the proteins of nanovirus are characterized functionally, and hence, the details of nanovirus replication and interaction of the M-Rep with host proteins is still awaited.

4.5 Conclusion and Future Perspectives

Many metabolic pathways in the cell such as replication, transcription, translation, and repair require helicases. They share similarities in basic structural elements, but even members of the same family cannot complement others deficiency. This emphasizes that each one of the helicases are individually essential for the proper functioning of the cell. Like in animals, plants also encode a wide range of helicases that are required for their normal development and maintenance of genomic stability. Few helicases are also found to be overexpressed in adverse conditions. Recent years have witnessed greater advancement in the field of helicase biology which is mainly focused on helicases from the animal system. Nevertheless, homology studies in plant proteins have enabled to identify and characterize a number of helicases involved in various processes; however, its mechanistic role in these biological processes is still lacking.

Being obligate parasite, viruses too naturally exhibit and adopt similar metabolic strategy as their host, and thus, they can obviously modulate the cellular environment. Virus-encoded proteins either resemble with structural elements of host proteins and exhibit favored/preferred interaction or have evolved with the ability to co-opt with the components of cellular machinery. To complete their life cycle, all the viruses require helicase(s) which can be either self-encoded or usurp from the host. On the basis of the available knowledge about the structural information of various helicases across different organisms, drug-designing strategies can prove helpful and successfully generate antiviral inhibitors. Drug designing can be used to screen and identify small-molecule helicase inhibitors that have the following effects on: NTP binding, NTP hydrolysis (via stabilizing ADP-bound state), nucleic acid binding, coupling NTP hydrolysis to translocation (via restricting or

blocking the domain movement or altering the interface cleft), or unwinding of nucleic acid. Alternate approach is by sterically blocking the helicase translocation, or changing the conformation of the helicase protein can also be attempted. Oligomerization has been found to have greater implication on the unwinding activity of hexameric helicases; therefore, small molecules capable of disrupting the oligomerization property of helicase or by inhibiting the crucial interaction with other host protein in larger complex can also facilitate an antiviral state.

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5 Cutting-Edge Technologies for Detection of Plant Viruses in Vegetatively Propagated Crop Plants

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Abstract

Plant virus diseases cause enormous loss which is estimated to be US$60 billion in crop yields worldwide each year. Seed is a propagating material in most of the crop plants, whereas most of the horticultural crops are vegetatively propagated. Horticultural crops like banana, bamboo, citrus, and grapes; commercial crops like sugarcane, black pepper, cardamom, orchids, and bulbous ornamentals; and tuber crops like potato, cassava, yam, etc., have been known to be infected by a range of viruses that belong to different genera and families. In these crops, the primary mode of transmission of viruses is through the use of infected plant propagule like corms, tubers, cutting, grafts, etc. An effective virus management strategy requires an accurate, rapid, and sensitive diagnosis for which understanding the disease cycle of etiological agents and its molecular nature, genome sequence and structure, coat protein information and sequences, etc. should be known in advance to design a detection strategy. Enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR) has been widely used for detection of plant viruses. These methods are time-consuming and laborious and require special skills such as in prior information on taxonomy to detect the pathogen responsible for the disease. On-site or point-of-care methods of detection are not new but limited to clinical use for human diseases. But recently this lateral flow devices (LFDs) are being made available for a number of viruses infecting plants. However, the widespread usage of this technology is delayed probably due to its limitation on robustness and lack of high-throughput nature. In this chapter, we have reviewed the recent developments on the early diagnosis

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using cutting-edge technologies like on-the-spot diagnostic tool lateral flow immunoassay (LFIA), loop-mediated isothermal amplification (LAMP), and multiplex technologies like microarray, microsphere immunoassay, etc. LAMP method is highly specific and requires less time to complete the indexing; however, this technique is yet to replace ELISA and PCR completely in agriculture possibly due to its specificity and viral variants that might escape from detection, and other possible reasons may be that the researchers and policy makers have not yet been convinced. Recent technologies such as rolling circle amplification which is dependent on a circular DNA genome and random hexamers do not require sequence data of the target, and similarly the next-generation sequencing also does not require a priori knowledge on the sequence of the causal agents. Principles and application of these cutting-edge technologies are reviewed in this chapter. The objective of the present chapter is not to cover all the details of diagnostics but to highlight the current status of various cutting-edge diagnostic techniques that can be applied for the biosecurity, by the quarantine departments, international exchange of germplasm, and on-site field detection by farmers, and use in the certification programs.

Keywords
Virus • Diagnostics • Vegetatively propagated crops • LFIA • Multiplex-ELISA RCA • LAMP • NGS

5.1 Introduction

Most of the crop plants are raised for agricultural purposes through either sowing the true seed or using vegetative propagated. The list of important plant viral diseases and their economic yield loss was furnished in Table 5.1. Those which are seed propagated have virus infection but are not easily manageable as they are spread by whitefly and plant hoppers which are very difficult to control using insecticides, as they spread the virus faster. Vegetatively propagated crops like banana, grapes, potato, cassava, sugarcane, and orchids are known to have infected by a large number of viruses, and all of these viruses are primarily transmitted by the use of infected planting materials, and the secondary mode of spread is through aphids and mealy bugs which transmit at a very slower rate; hence, control of vector is comparatively easier than controlling the movement of planting materials. An effective and applicable virus management strategy requires an accurate diagnosis and understanding of the life and disease cycle of etiological agents. Recent developments in molecular techniques have revolutionized the field of diagnostics in agriculture (Sastry 2013). High level of detection, specificity, sensitivity, and accuracy supported with simplicity, amenable to automation, and low cost are the main characteristics of an ideal diagnostic strategy.

Initially plant viruses were diagnosed based on the symptoms that occur on the plants, and later the titer of the viruses was determined using indicator hosts which show hypersensitive reaction with chlorotic and necrotic spots upon inoculation. However, for viruses which are not known to be transmitted by mechanical sap inoculation, indicator host could not be used. In the 1970s the serological techniques became widely used wherein the polyclonal antiserum produced in warm-blooded animals against the viral antigen has been used to detect viruses using gel-based techniques, and later

Table 5.1 Important viral diseases of vegetatively propagated crops and the yield loss

Crop	Disease	Yield loss (%)	Virus	Virus genus	Reference
Banana	Bunchy top	100	Banana bunchy top virus	*Babuvirus*	Dale (1987)
Cassava	Mosaic	24–75	Cassava mosaic virus	*Begomovirus*	Seif (1982)
Cassava	Brown streak disease	100	*Cassava brown streak virus* (CBSV)	*Ipomovirus*	Kaweesi et al. (2014)
			Ugandan cassava brown streak virus (UCBSV)		
Potato	Mosaic	10–100	Potato virus Y	*Potyvirus*	Warren et al. (2005)
Citrus	Mosaic	35.18	Citrus yellow mosaic	*Badnavirus*	Ahlawat et al. (1996)
Citrus		100	Citrus leprosis virus C	*Cilevirus*	Rodrigues (2000)
Sweet potato	Sweet potato viral disease complex	80–90	Sweet potato feathery mottle virus	*Potyvirus* and *Crinivirus*	Mukasa et al. (2006)
			Sweet potato chlorotic stunt virus		
Grapevine	Grapevine leafroll disease	15–20	GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4, GLRaV-7, GLRaV-9, GFLV, GRSPaV, GVA, and GVB	*Closterovirus*	Martelli and Boudon-Padieu (2006)
				Ampelovirus	
				Vitivirus	

different formats of ELISA technique were used for accurate quantification of the antigen (viruses). Sensitivity, specificity, and high throughput, i.e., testing large volume of samples in plate formats and on-site detection instead of lab-based test issues, arose as a problem in the existing technologies. PCR came into use in the late 1980s for detection of plant viruses, and later an improved format of PCR that came into use was the real-time PCR, as the name suggests that the virus could be quantified on real-time basis, i.e., while polymerization of its genomic fragment targeted. These techniques were more accurate and highly sensitive, but they were very costly especially for plant virus detection as the end user is resource poor farmers. Recently, novel techniques such as LAMP, RCA, NGS, microarrays, and microsphere-based magnetic ELISA are being applied as sensitive assays. On-site detection of plant viruses using dipstick or lateral flow immuno assay (LFIA) technologies can be used even by the illiterate farmers or nontechnical persons in the field itself. These assays are now being used in certification purposes and also for ensuring biosecurity. The objective of the present chapter is not to cover all the details of diagnostics but to highlight the current status of various cutting-edge diagnostic techniques that can be applied in the biosecurity, quarantine departments, international exchange of germplasm, on-site field detection by farmers, and certification programs limited to crops that vegetatively propagated.

5.2 Serological Based

5.2.1 Lateral Flow Immunoassay (LFIA) Detection System

Lateral flow immunoassay (LFIA) is used for qualitative or semiquantitative detection and monitoring of viral pathogens in non-laboratory environments. LFIA is simple, efficient, and rapid which makes it possible to identify and evaluate the content of various biologically active substances in a sample in a few minutes without any special skills and equipment and could be used even under field conditions. The basic principle of LFIA is as that of chromatographic principle coupled with immunological recognition system. LFIA basically relies on the interaction between the target virus and immune reagents (antibodies and their conjugates with colloidal particles or nanoparticles) applied on the membrane carriers, the test strips. When the test strip is dipped into the sap of sample being analyzed, the sample liquid flows through membranes and triggers immunochemical interactions resulting in visible coloration in test and reference lines (Von Lode 2005; Price and Kricka 2007). A typical LFIA format consists of a surface layer to carry the sample from the sample application pad via the conjugate release pad along the strip encountering the detection zone up to the absorbent pad (Byzova et al. 2009). The membrane is often thin and fragile, so it is pasted to a plastic layer to allow easy cutting into strips and handling. In addition, robustness is achieved by housing the strips in a plastic holder, where only the sample application window and a reading window are exposed. Current membrane strips are produced from nitrocellulose, nylon, polyethersulfone, polyethylene, or fused silica. At one end of the strip, a sample application pad is provided. The sample application pad is usually made of cellulose or cross-linked silica. Next to the sample application pad is the conjugate release pad, made of cross-linked silica, and lengthwise it is lesser than half the length of sample application pad. Labeled analyte or recognition element(s) is applied and dried on this pad, and after the addition of the sample, this material will interact with the fluid flow; specific interactions will be initiated here and will continue during the chromatographic process. Now, this technology is widely used to detect various plant pathogens and detection of plant viruses made easy using this technology. Many commercial firms are producing these LFIA strips for detection of various pathogens including plant viruses.

The LFIA strip technique has been reported for the detection of viruses (Danks and Barker 2000; Salomone and Roggero 2002; Salomone et al. 2002, 2004; Kusano et al. 2007; Drygin et al. 2009). The use of nanoparticles as labels has led to the improvements in sensitivity and multiplexing capabilities (Jain 2005; Rosi and Mirkin 2005). Metallic nanoparticles composed of gold or silver have many optical and electronic properties, based on their size and composition (Nath et al. 2008). When coupled to affinity ligands, these nanoparticle materials have found important applications as chemical sensor. For example, gold nanoparticles conjugated with specific oligonucleotides can sense complementary DNA strands, detectable by color changes (Mirkin et al. 1996). Other nanoparticles including fluorescent quantum dots and carbon nanotubes have been used in various applications including DNA detection and the development of immunoassays for the detection of pathogens (Bruchez et al. 1998; Edgar et al. 2006; Baptista et al. 2006; Alivisatos et al. 2005).

Express immunochromatographic test strip assays were developed for detection of five plant viruses varying in shape and size of virions, viz., spherical carnation mottle virus, bean mild mosaic virus, rod-shaped tobacco mosaic virus, and filamentous potato viruses X and Y (Byzova et al. 2009). Multi-membrane composites (test strips) with immobilized polyclonal antibodies against viruses and colloidal gold-conjugated antibodies were used for the analysis. These immunochromatographic test strips were shown to enable the detection of viruses both in purified preparations and in leaf extracts of infected plants with sensitivity from 0.08 to 0.5 μg/ml for

10 min. This technique can be adopted for on-site detection of these viruses under field conditions (Byzova et al. 2009); Drygin et al. (2012) have developed immunochromatographic assay for rapid detection of potato virus X (PVX). In this assay time does not exceed 15 min, and the lower limit of the PVX detection in non-clarified leaf extract was 2 ng/ml, and a single measurement required 0.1–0.2 ml of tested solution extracted from 10 to 20 mg of potato. Plum pox virus (PPV) with specific colloidal gold-labeled antibodies using immunochromatographic assay has been developed with a detection limit of 3 ng/ml, and the test duration was just 10 min (Byzova et al. 2010); Yoon et al. (2014) have developed rapid immune-gold strip (RIGS) kit in a novel single strip format to detect on-site detection of *tomato spotted wilt virus* (TSWV), and results could be obtained in 2–5 min.

Safenkova et al. (2012) studied the impact of key factors influencing the analyte detection limit of the sandwich immunochromatographic assay (ICA), namely, the size of gold nanoparticles, the antibody concentration, the conjugation pH, and the characteristics of membranes for the detection of PVX. The antibody-colloidal gold conjugates synthesized at pH 9.0–9.5 at an antibody concentration of 15 μg/mL showed maximum binding with the analyte. The detection limit improved from 80 to 3 ng/mL for a series of nanoparticles with a diameter from 6.4 to 33.4 nm. In the case of larger particles (52 nm in diameter), the detection limit increased and reached 9 ng/mL. A 10 mM phosphate buffer, pH 8, and a 50 mM phosphate buffer, pH 7, were the conditions of choice for the deposition of reactants. The maximum detection limit was 2–3 ng/mL by the standardized method. Recently LFIA have been developed for the on-farm detection of six different plant viruses utilizing specific monoclonal and polyclonal antibodies against cucumber mosaic virus (CMV), groundnut bud necrosis virus (GBNV), large cardamom chirke virus, papaya ring spot virus, peanut mottle virus (PeMoV), and potato virus Y (Bikash Mandal, Personal Communication).

5.2.2 Microsphere Immunoassay

Among the immunoassays, ELISA has been exploited widely in life sciences for the detection of viral pathogens or antibodies to viral proteins or biomarkers for cancer detection. Recently, a breakthrough has been the use of microspheres in the immunoassay (xMAP technology) which has emerged as an alternative for microbial detection (Charlermroj et al. 2013). The basic principle of the technology is that it employs different sets of fluorescence-coded microspheres; each bead set is filled with a combination of dyes which are conjugated with capture antibodies specific to target pathogens, and the detecting antibodies are linked with another fluorophore. There have been several reports using this microsphere technology to detect multiple analytes or biomarkers across a number of fields including human diagnostics (Kellar and Douglass 2003), food microbiology (Dunbar et al. 2003), and plant pathogen detection (Bergervoet et al. 2008). For simultaneous detection of potato-infecting viruses, this bead-based technology has been used (Bergervoet et al. 2008). The use of paramagnetic beads in place of conventional beads in the MIA procedure allows efficient removal of excess sample compounds and reagents which has resulted in lower background values and a higher specificity than a non-wash MIA procedure. They have used MIA technology to detect PVY, PVX, and PLRV in potato leaf extracts which were detected with equal sensitivity and specificity. In MIA, procedure takes longer time due to the fact that the results are not visible by the eye, and therefore all samples have to be analyzed resulting in longer measuring times (Bergervoet et al. 2008); Charlermroj et al. (2013) have developed microsphere immunoassays to simultaneously detect four important plant pathogens: a fruit blotch bacterium *Acidovorax avenae* subsp. *citrulli* (Aac), chili vein-banding mottle virus (CVbMV, potyvirus), watermelon silver mottle virus (WSMoV, tospovirus serogroup IV), and melon yellow spot virus (MYSV, tospovirus). This assay was able to detect all four plant pathogens precisely and accurately with substantially higher sensitivity

than ELISA, and if the same antibody sets were used, its assay time is also shorter.

5.3 Nucleic Acid-Based Detection Assay

5.3.1 Microarray

Microarray- or chip-based technique has become a common tool in molecular biology and biotechnology especially for genotyping by SNPs, functional genomics, genetic mapping, and proteomics. Now this technique is also being applied for detection of plant pathogens including plant viruses. Lee and co-workers were the first to develop cDNA chip for a plant virus using viral cDNA clones and microarray technology (Lee et al. 2003). The cDNA chip was designed for detection and differentiation of the four species of selected cucurbit-infecting tobamoviruses, viz., cucumber green mottle mosaic virus (CGMMV), cucumber fruit mottle mosaic virus (CFMMV), kyuri green mottle mosaic virus (KGMMV), and zucchini green mottle mosaic virus (ZGMMV). Deyong et al. (2005) developed microarray to detect and differentiate 14 different isolates of CMV belonging to different serogroups and subgroups. Microarrays have been proposed as a "multi-target" system capable of testing a full range of organisms in a generic format. This approach would streamline current diagnostic testing methods (Boonham et al. 2003) and improve detection of unknown or unexpected variants. Positively charged membranes can also be used to immobilize cDNA or oligonucleotide probes and RNA extract from infected tissue labeled and hybridized to the membrane to detect infecting viruses. An array of many virus-specific probes can be developed which allows parallel detection. The development of macro- and microarrays with several hundreds or thousands of probes allows for the hybridization and subsequent identification of viruses with only marginal homology to known taxa (Agindotan and Perry 2007; Thompson et al. 2014; Wang et al. 2002). List of microarray developed for plant virus detection is provided in Table 5.2.

In the detection of different isolates of cucumber mosaic virus (CMV) or plum pox virus (PPV), four different cucurbit-infecting viruses belong to *Tobamovirus* genus, and up to 11 viruses infecting cucumber or potato have been reported (Agindotan and Perry 2008; Boonham et al. 2003; Bystricka et al. 2005; Deyong et al. 2005; Lee et al. 2003; Pasquini et al. 2008). Recently, the use of low-density arrays, a technique based on real-time RT-PCR (TaqMan), and antibody microarrays was reported for multiplex detection of up to 13 grapevine viruses (Abdullahi et al. 2005; Osman et al. 2008); Engel et al. (2010) reported an array which contains 570 unique probes designed against highly conserved and species-specific regions of 44 plant viral genomes. This microarray-based detection of plant viruses will have potential value in quarantine departments for the purpose of biosecurity of important diseases not reported in each country.

5.3.2 Loop-Mediated Isothermal Amplification (LAMP)

Isothermal amplification methods are novel detection techniques that have the potential to overcome the cost barriers limiting uptake of PCR-based testing while exceeding the sensitivity and/or specificity of ELISA-based methods. Loop-mediated isothermal amplification (LAMP) is a DNA amplification technique that amplifies DNA with high specificity, efficiency, and rapidity under isothermal conditions (Notomi et al. 2000). LAMP is based on the principle of autocycling with strand displacement and DNA synthesis performed by the *Bst* DNA polymerase (derived from *Bacillus stearothermophilus*), for the detection of any specific DNA sequence (Notomi et al. 2000). This technique uses four to six primers that recognize six to eight regions of the target DNA and provides very high specificity (Notomi et al. 2000; Nagamine et al. 2002). It can be carried out under isothermal condition ranging between 60°C and 65°C and produces large amounts of DNA in a short time (Notomi et al. 2000). The reaction shows high tolerance to biological contaminants (Kaneko et al. 2007),

Table 5.2 Microarray-based detection reported for plant virus species

Species diagnosed	Reference
APLV, APMV, PBRSV, PVA, PVS, PVT, PVV, PVX, PVY, PYVV, TSV	Abdullahi et al. (2005)
PVA, PVS^{A}, PVS^{O}, PVX, PVY	Boonham et al. (2003)
PLRV, PMTV, PVA, PVS, PVX, PVY	Bystricka et al. (2003)
PLRV, PVA, PVM^{O}, PVM^{I}, PVS^{A}, PVS^{O}, PVX, PVY^{O}, PVY^{NTN}	Bystricka et al. (2005), and Sip et al. (2010)
CMV	Deyong et al. (2005)
CFMMV, CGMMV, CMV, KGMMV, PVX, TMV, PMMoV, ZGMMV, ZYMV	Lee et al. (2003)
Six strains of PPV	Pasquini et al. (2008)
CMV, LSV, LMoV, PlAMV	Sugiyama et al. (2008)
PLRV, PVA, PVX, PVY, CMV, PotLV, PVS, TRV, PMTV, PVM, AMV	Agindotan and Perry (2008)
DsMV, LYSV, PVY, ZYMV	Wei et al. (2009)
Grapevine viruses	Engel et al. (2010)
CMV, TICV, ToCV, TSWV, PepMV, TYLCV, TYLCSV, PVY, TMV, ToMV	Tiberini et al. (2010)
ArMV, PNRSV, PPV, PVX, TMV, ToRSV, TRSV, TSWV	Zhang et al. (2010)
Tobamovirus, *Caulimovirus*, *Potexvirus*, *Marafivirus*, *Alphacryptovirus*, and *Furovirus*	Grover et al. (2010)
ArMV, GFLV	Abdullahi and Rott (2009)
KYMV, TYMV, TVCV, AsAV	Grover et al. (2010)
GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4, GLRaV-7, GLRaV-9, GFLV, GRSPaV, GVA, and GVB	Engel et al. (2010)

ArMV arabis mosaic virus, *APLV* andean potato latent virus, *APMV* andean potato mottle virus, *AsAV* asclepias asymptomatic virus, *CMV* cucumber mosaic virus, *CFMMV* cucumber fruit mottle mosaic virus, *CGMMV* cucumber green mottle mosaic virus, *DsMV* dasheen mosaic virus, *GFLV* grapevine fan leaf virus, *GLRaV* grapevine leafroll-associated virus, *GVA* grapevine virus A, *GVB* grapevine virus B, *GRSPaV* grapevine rupestris stem pitting-associated virus, *KGMMV* kyuri green mottle mosaic virus, *KYMV* kennedya yellow mosaic virus, *LYSV* leek yellow stripe virus, *LSV* lily symptomless virus, *LMoV* lily mottle virus, *PBRSV* potato black ringspot virus, *PLRV* potato leaf roll virus, *PMTV* potato mop-top virus, *PepMV* pepino mosaic virus, *PlAMV* plantago asiatica mosaic virus, *PMMoV* pepper mild mottle virus, *PNRSV* prunus necrotic ringspot virus, *PVA* potato virus A, *PVS* potato virus S, *PVT* potato virus T, *PVV* potato virus V, *PVX* potato virus X, *PVY* potato virus Y, *PYVV* potato yellow vein virus, *PPV* plum pox virus, TSV tobacco streak virus, *TMV* tobacco mosaic virus, *ToRSV* tomato ringspot virus, *TRSV* tobacco ringspot virus, *TICV* tomato infectious chlorosis virus, *ToCV* tomato chlorosis virus, *TSWV* tomato spotted wilt virus, *TYLCV* tomato yellow leaf curl virus, *TYLCSV* tomato yellow leaf curl Sardinia virus, *ToMV* tomato mosaic virus, *TYMV* turnip yellow mosaic virus, *TVCV* turnip vein-clearing virus, *ZGMMV* zucchini green mottle mosaic virus, *ZYMV* zucchini yellow mosaic virus

which can help to avoid false-negative results due to the inactivation of the enzyme, a common problem faced in conventional PCR where Taq DNA polymerase is used. As a result, LAMP assays can be performed on simpler equipment (no need of thermal cycler) and often without the need for multistep sample processing. To date, LAMP and reverse transcription LAMP (RT-LAMP) have been used widely to detect plant viruses (Table 5.3). Although LAMP amplification products can also be detected by gel electrophoresis, this long procedure of gel electrophoresis reduces the suitability for field applications. For this reason, SYBR Green I is used as an intercalating DNA dye which can be viewed by a naked eye. Various colorimetric assays including magnesium sulfate ($MgSO_4$), calcium chloride ($CaCl_2$), SYBR® Premix Ex TaqTM II, hydroxynaphthol blue (HNB), GeneFinderTM, SYBR Green I, manganese chloride ($MnCl_2$) combined with calcine, and ethidium bromide have been used in several investigations (Goto et al. 2009; Almasi et al. 2012, 2013; Bhat et al. 2013).

Table 5.3 Application of loop-mediated isothermal amplification in detection of plant virus and limits of detection (sensitivity)

Virus species	Host	Detection limit	Reference
DNA viruses			
Banana bunchy top virus	Banana	100-fold more sensitive than PCR (1 pg/µl plasmid DNA)	Peng et al. (2012a)
Banana streak virus	Banana	100-fold more sensitive than PCR (1 pg/µl plasmid DNA)	Peng et al. (2012b)
Citrus yellow mosaic badnavirus	Citrus	Not reported	Anthony Johnson et al. (2014)
Squash leaf curl	Squash and melon	10 times more sensitive than PCR	Kuan et al. (2010)
Piper yellow mottle virus	Black pepper	100 times more sensitive than PCR	Bhat et al. (2013)
Curly top virus	Sugar beet	Not reported	Almasi et al. (2013)
RNA viruses			
Potato leafroll virus	Potato	Not reported	Ahmadi et al. (2013)
Potato virus Y	Potato	Tenfold more sensitive than RT-PCR and 100-fold more sensitive than ELISA and LFIA	Almasi and Dehabadi (2013), and Przewodowska et al. (2015)
Apple stem grooving virus	Apple	Not reported	Zhao et al. (2014)
Cucumber mosaic virus	Banana	100-fold more sensitive than PCR (1 pg/µl plasmid DNA)	Peng et al. (2012c)
Banana bract mosaic virus	Cardamom	100 times more sensitive than RT-PCR	Siljo and Bhat (2014)
Citrus tristeza virus	Citrus	100 times more sensitive than RT-PCR	Wang et al. (2013)
Citrus yellow vein clearing virus	Citrus	Tenfold higher than RT-PCR	Liu et al. (2015)
Grapevine leafroll-associated virus type 3	Grapevine	RT-LAMP is as sensitive as nested PCR	Walsha and Pietersen (2013)
Papaya ringspot virus	Papaya and cucurbit	1.15×10^{-6} µg of total RNA per reaction (10 times sensitive than RT-PCR)	Shen et al. (2014)
Papaya leaf distortion mosaic virus	Papaya	1.32×10^{-6} µg of total RNA per reaction (10 times more sensitive than RT-PCR)	Shen et al. (2014)
Pepino mosaic virus	Tomato	Sensitive than RT-PCR	Ling et al. (2013)
Cucurbit chlorotic yellows virus	Melon	10^5 times more sensitive than RT-PCR	Wang et al. (2014)
Cucumber green mottle mosaic virus	Watermelon	100-fold more sensitive than RT-PCR	Li et al. (2013)
Tomato necrotic stunt virus	Tomato	8 pg of total tomato RNA or with 1:20,000 dilution of crude tissue extract	Li and Ling (2014)
Tomato chlorosis virus	Tomato	2.0×10^{-7} ng, which is 100 times more sensitive than RT-PCR	Zhao et al. (2015)
Cassava brown streak virus and Ugandan cassava brown streak virus	Cassava	10^{-2} to 10^{-3}	Tomlinson et al. (2013)
Cucumber mosaic virus	Black pepper	100 times more sensitive than RT-PCR	Bhat et al. (2013)
Cymbidium mosaic virus	Orchid	Not reported	Lee et al. (2011)
Tomato spotted wilt virus	Chrysanthemum	100 times more sensitive than IC/RT-PCR	Fukuta et al. (2005)

The LAMP-based detection can be combined and converted into lateral flow dipstick (LFD) device to detect the positive amplification by simple visual inspection, with potential field application. LFDs to detect labels incorporated into the amplification products, allowing multiple products to be discriminated without gel electrophoresis (Tomlinson et al. 2010). Incorporation of two labels allows the product to be detected in a sandwich format: generally, one label is incorporated into the amplification product using a labeled primer, and the second label is incorporated using a labeled detector probe which hybridizes to the amplification product. The detector probe is intended to ensure specificity of detection, because the amplicons must contain a sequence to which the detector probe will hybridize in order to produce a positive result. Detection of LAMP products using lateral flow devices was demonstrated for simultaneous detection of the amplification products for cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) and a plant internal control gene (cytochrome oxidase) (Tomlinson et al. 2013).

5.3.3 Quantum Dots

Quantum dots (QDs) are few nm in diameter, roughly spherical (some QDs have rodlike structures), fluorescent, crystalline particles of semiconductors whose excitons are confined in all the three spatial dimensions. Their potential application in diverse fields can be attributed to the property of quantum confinement. Diagnostics using colloidal QDs has got tremendous hoist from this milestone finding. QDs are robust and very stable light emitters and can be broadly tuned through size variations. Due to phenomenal advancement in nanotechnology, QDs have emerged as pivotal tool for detection of a particular biological marker with extreme accuracy. QDs being very photostable and optically sensitive can be used as labeling and can be easily traced with ordinary equipment. Early detection of pathogens causing plant diseases using quantum dots would prove to be boon in agriculture. One of the most important nanomaterials is fluorescent semiconductor nanocrystals, also known as quantum dots (QDs) which have been widely used for disease diagnosis (Frasco and Chaniotakis 2009).QDs have a number of unique optical properties that are advantageous in the development of bioanalyses based on fluorescence resonance energy transfer (FRET) (Algar and Krull 2007). These biosensors have been widely used in immunoassay (Goldman et al. 2005). Safarpour et al. (2012) developed a specific and sensitive FRET-based QD-antibody biosensor for rapid, accurate, and cost-effective detection of *Polymyxa betae*, the vector of Beet necrotic yellow vein virus (BNYVV). QD-immunofluorescent labeling method for the in vitro and in situ localization of lettuce infectious yellows virus (LIYV) virions was based on the recognition specificity of streptavidin-conjugated QD605 (S-QD605) for biotin-conjugated anti-LIYV IgG (B-aIgG) (Ng 2013). However, these QD-based methods are yet to be used for on-site detection of plant viruses.

5.3.4 Rolling Circle Amplification (RCA)

Rolling circle amplification (RCA), using *Bacillus subtilis* bacteriophage Phi29 DNA polymerase, is a sequence-independent protocol which has been used for the amplification and characterization of circular DNA molecules, including plasmids (Dean et al. 2001; Reagin et al. 2003), and several groups of DNA viruses infecting humans, animals, and plants (Johne et al. 2009). To date, the application of RCA technology to plant-infecting viruses has been limited to the small, single-stranded DNA genomes of viruses in the families *Geminiviridae*, *Nanoviridae*, and *Caulimoviridae*. The method relies on the strand displacement activity of polymerase which allows amplification to occur on newly synthesized template strands without having to cycle temperatures (isothermal amplification). The disadvantages of this method are that nonspecific amplification products are produced in the reaction which requires further methods to identify infecting viruses to the species or strain level such as RFLP or sequencing. Secondly,

long incubation times are required (18–20 h) for amplification (Haible et al. 2006). Since a lot of variations occur in the species of members of *Geminiviridae*, this sequence-independent technique could be used to detect the viruses having no sequence data.

5.3.5 Nucleic Acid-Based Microsphere Assay

Recently, a new platform named Luminex MagPlex-TAG bead system offers a very high degree of multiplex nucleic acid detection (Lin et al. 2011; Boonham et al. 2014). This system incorporates 6.5 μm carboxylated, superparamagnetic polystyrene microspheres that are internally labeled with a spectrally distinct fluorescent dye and pre-coupled with an anti-MagPlex-TAG oligonucleotide sequence. Different microsphere sets can be distinguished by their spectral addresses, and when combined, up to 150 different nucleic acid sequence targets can be simultaneously detected in a single reaction. Luminex MagPlex-TAG microsphere system involves a generic multiplexed RT-PCR step, followed by a multiplexed asymmetric PCR step termed target-specific primer extension (TSPE). In this step, a primer internal to the multiplexed amplification product will hybridize, and be extended, only when there is a sequence match. Resultant TSPE products are biotinylated and labeled with complementary MagPlex-TAG sequences at their 5′ end. TSPE products are then hybridized to the MagPlex-TAG microsphere mixture, and a fluorescent reporter molecule is used to detect incorporated biotin. The bead-TSPE product complexes are then detected on the Luminex instrument. This technology has proven its value for the multiplexed detection of viruses (Foord et al. 2013); Van Brunschot et al. (2014) have developed multiplex xTAG assay to detect all nine known species or members of pospiviroids occurring in different crops, and they demonstrated its usefulness. Multiplexed array using this technology has been shown to be 100% specific, sensitive, and reproducible and has strong potential for use in routine pospiviroid indexing to improve disease management strategies (Van Brunschot et al. 2014). A co-amplified internal control can be incorporated into the assay for quality assurance purposes, while the assay format makes it suitable for utilizing liquid handling robotics.

5.4 Next-Generation Sequencing Technology: An Ever-Expanding Cutting-Edge Technology

Conventional serological or molecular detection and identification methods of plant viruses or viroids depend on prior knowledge or sequence of the virus or viroid of interest. The NGS technologies have provided a very powerful alternative for detection and identification of these pathogens without a priori knowledge (Prabha et al. 2013; Barba et al. 2014). Next-generation high-throughput sequencing technologies are highly efficient, rapid, and low in cost. NGS technologies were available to the scientists at the onset of the twenty-first century. This method is superior to the standard and traditional Sanger DNA sequencing technologies developed in the late 1970s (Barba et al. 2014). NGS has been used as a powerful tool for studies on pathogenomics, especially to detect, identify, and quantify novel viruses in one step. NGS is also sequence-independent and culture-independent approach, which can be used for simultaneous detection of RNA viruses, DNA viruses, and viroids in a plant sample which contains even very low titer. In the past few years, NGS has been successfully used for the rapid identification of pathogens in clinical and public health settings and now in plants. NGS has proved to be a sensitive method for detecting putative infectious agents associated with human tissues and viral transcripts and can be detected at frequencies lower than one in 1,000,000. NGS utilizes a fundamentally different approach from the classic Sanger chain-termination method. It leverages sequencing by synthesis (SBS) technology and keeps tracking the addition of labeled nucleotides as the DNA chain is copied, and it works in a

massively parallel fashion. It generates masses of DNA sequence data that's richer and more complete than it is imaginable with Sanger sequencing. For example, Illumina sequencing systems can deliver data output ranging from 300 kilobases up to 1 terabase in a single run, depending on instrument type and configuration.

5.4.1 NGS in Detection and Discovery of Plant Virus/Viroid Pathogens

Metagenomics developed by NGS technologies has been proven to be sensitive, accurate, and fast in detection and identification of known and unknown viral and viroid, viromes without any bias. The use of the technology in diagnostics has been recently reported for temperate fruit crops (Hadidi and Barba 2012), citrus (Bar-Joseph and Gera 2012), grapevine, and other crops (Martelli 2012). Data generated by these technologies can be used effectively to improve efficiency and reliability of these programs as well as in programs aimed at virus and viroid elimination from vegetatively propagated material. The list of NGS application in plant virus diagnostics is furnished in Table 5.4. The utilization of NGS in plant virology in the near future will definitely increase its usage in virus research and diagnostics. NGS can be exploited in plant certification and quarantine programs which can effectively improve the efficiency and reliability of these programs and in controlling virus and viroid diseases at both the national and international levels, and it would be highly useful for biosecurity purposes not only for plant pathogens but also for animal and human pathogens.

NGS is performed using different platforms and template preparations (Boonham et al. 2014; Massart et al. 2014) and allows the rapid, simultaneous detection of all known or unknown viral sequences present in a sample. In plant viruses, rolling circle amplification could be used to enrich the circular genomes of virus (Wyant et al. 2012) such as species of geminivirus, babuvirus, badnavirus, etc., before going for library preparation for NGS-based virome discovery. Loop-mediated isothermal amplification could also be used for any plant viruses for enrichment so that the plant total RNA/DNA would not interfere in the diagnostics and discovery and the bioinformatic analysis would also be very simple than whole genome sequencing of other microorganisms. Likewise, total RNA preparations can be enriched in viral sequence by purification of mRNAs (Muerhoff et al. 1997). Such an approach has, for example, been used to enrich NA populations for the sequencing of a novel *Cucumovirus*, the gayfeather mild mottle virus (Adams et al. 2009) which was discovered with NGS method.

In order to find out the sequences of unknown virus, the genome could be obtained from partially or completely purified viral particles by various techniques, viz., immune (Wetzel et al. 1992) or print capture (Olmos et al. 1996), simplified partial purification schemes (Muthukumar et al. 2009), or more complex purification schemes through, for example, cesium chloride gradient prior to DNA or RNA extraction. The isolated viral DNA or RNA is finally sequenced using NGS approach (Thapa et al. 2012). NGS has been shown to be a good option for investigating diseases of unknown etiology for, e.g., grapevine (Alabi et al. 2012).

5.5 Conclusion

Vegetatively propagated horticulture and agricultural crops are more nutritive, and some of them like potato, banana, and cassava are staple food for millions of people living in the tropical and subtropical countries. These crops harbor more than a dozen of virus, viroids, and phytoplasmas, and these plants need to be determined free of pathogens before planting them in the field for which highly sensitive, cost-effective, user-friendly, and specific detection kits are necessary. Development of such detection methods keeps on evolving to meet the above said requirements so that virus-free planting materials are made available to the farming community. In addition, biosecurity became an important activity in every country to safeguard the biodiversity and conserve the plant species. Biosecurity cost does not

Table 5.4 Next-generation sequencing of plant viral siRNA, RNA, or DNA from virus-infected plant sample

Virus detected	Host	Strategy	Sequencing platform	Reference
Sweet potato feathery mottle virus	Sweet potato	siRNAs	Illumina	Kreuze et al. (2009)
Sweet potato chlorotic stunt virus				
Sweet potato feathery mottle virus strain RC				
Sweet potato virus C				
(*Potyvirus*), sweet potato chlorotic stunt virus strain WA (*Crinivirus*), sweet potato				
leaf curl Georgia virus (*Begomovirus*), and sweet potato pakakuy virus strain B				
Complete sequence of the Tanzanian strain of Cassava brown streak virus	Cassava	Total RNA + subtractive	Roche 454	Monger et al. (2010)
		hybridization	GS FLX	
Raspberry latent virus	Raspberry	dsRNA	Illumina	Quito-Avila et al. (2011)
Complete nucleotide sequences of two new viruses pepper yellow leaf curl virus	Pepper, eggplant	Purified virons viral RNA	SOLiD	Dombrovsky et al. (2011)
(*Polerovirus*) and eggplant mild leaf mottle virus (*Ipomovirus*) were determined				
Complete genome sequence of Piper yellow mosaic virus	Black pepper	Viral and plant DNA were isolated from virus-enriched fraction	Roche 454	Hany et al. (2014)
Fragments of two additional novel viruses belonging to			GS FLX	
Caulimoviridae were sequenced, and the viruses were tentatively named Piper DNA			Titanium	
virus 1 and 2				
Citrus tristeza virus (CTV)	Citrus	siRNAs	Illumina	Ruiz-Ruiz et al. (2011)
Citrus yellow vein clearing virus, citrus leprosis virus cytoplasmic type 2, citrus vein enation virus			Solexa-Illumina	Loconsole et al. (2012a) Roy et al. (2013) Vives et al. (2013)

(continued)

Table 5.4 (continued)

Virus detected	Host	Strategy	Sequencing platform	Reference
Citrus chlorotic dwarf-associated virus	Citrus	siRNAs and total DNA	Illumina HiSeq2000	Loconsole et al. (2012b)
ASPV, ACLSVASGV, ASPV, ACLSV, ApLV, ApPCLSV, and PCMV	Apple	siRNAs	Illumina HiSeq2000	Maree et al. (2012), and Yoshikawa et al. (2012)
Variants of GLRaV-3, GVA, and an unknown mycovirus	Grapevine	siRNAs	Illumina	Maree et al. (2012)
(Grapevine Syrah 1 virus) GRSPaV, GRVFV, GLRaV-9, and viroids		Total RNA or dsRNA	Roche 454	Al Rwahnih et al. (2009)
Grapevine virus E, *Penicillium chrysogenum* virus, two other mycoviruses, GLRaV-3, GRSPaV, GVA		dsRNA	Illumina	Coetzee et al. (2010)
Viruses of the genera *Foveavirus*, *Maculavirus*, *Marafivirus*, and *Nepovirus*		siRNAs	Illumina	Pantaleo et al. (2010)
Grapevine vein clearing virus		siRNAs	Illumina Genome Analyzer	Zhang et al. (2011)
GRSPaV, GRVFV, GSy 1V, and viroids		siRNAs	Illumina	Giampetruzzi et al. (2012)
Grapevine virus F		dsRNA	Illumina Genome Analyzer IIx	Al Rwahnih et al. (2012a)
Grapevine red leaf-associated virus, GRSPaV, GFV, and viroids		Total RNA treated with DNase	Illumina Genome Analyzer IIx	Poojari et al. (2013)
Grapevine red blotch-associated virus		dsRNA extracted without DNase treatment	Illumina Genome Analyzer IIx	Al Rwahnih et al. (2012b)
PPV, PNRS	*Prunus*	dsRNA	Roche 454	Candresse et al. (2012)
Fig mosaic virus and Fig latent virus 1	Fig	dsRNAs	Illumina	Chiumenti et al. (2012)
Ampelovirus in the family *Closteroviridae*	Blackberry	dsRNAs	Illumina	Thekke-Veetil et al. (2012)
Little cherry virus 1	Cherry	dsRNAs	Roche 454 Pyrosequencing multiplex approach	Candresse et al. (2013)

matter whereas specificity decides in achieving the goal of detecting the targeted pathogens by quarantine departments. Replacement of old techniques lacking sensitivity, specificity, higher cost, etc., is happening in the research arena, and the need of easy to handle, cheaper detection kits for on-site detection even by the end users like farmers, nurserymen, and plant health clinics is increasing day by day. ELISA and their variants have lesser sensitivity than PCR; therefore, PCR dominated in the laboratories for the detection than ELISA. Because of the cost and more sensitivity compared to ELISA, even now it is considered as a user-friendly high-throughput kit for most of the viruses except babuvirus and geminivirus. Now techniques like LAMP became a user-friendly, quick, cost-effective, and highly specific technique which could be used even without PCR machine, and it is possible to exploit this for on-site field detection. For most of the plant viruses now, LAMP method has been made available for the field and lab use. If we look at the literature, more numbers of papers have been published using LAMP; hence, this technique appears to rule in the diagnostic industry in the near future. Microarray, as expected, did not get popular owing to its limitation on the cost and difficulty in usage. RCA is another technique highly suitable for circular DNA virus detection; even without the use of specific primers, it can detect the virus. Wide adoptability of NGS technology may take time as it is very good for indexing precious germplasm and discovery of new viruses without any a priori knowledge, and at present this technology would be limited to labs only as bioinformatic analysis together with multiple step involvement. Fruit trees like citrus, banana, apple, and grapes have to be certified using NGS as it can simultaneously detect large number of viruses, viroids, and phytoplasmas. Once the pathogen-free mother trees are established, then it would be easier to use LAMP and LFIA kits by farmers themselves for field level detection. Finally, it is concluded that LAMP, RCA, LFIA, and microsphere multiplex ELISA are the cutting-edge detection techniques having more utility in all respect compared to conventional ELISA and PCR techniques.

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6 Interactions Among Host and Plant Pararetroviruses: An Ever Evolving Evolutionary Dogma

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Abstract

Plant pararetroviruses are the double-stranded DNA viruses which replicate via reverse transcription like mammalian retroviruses; however, integration is not an essential step in replication. Those pararetroviruses which integrate into the host genome are known as endogenous pararetroviruses (EPRV) and are the fossils of the viruses that existed in their host genomes. There are a large number of reports available confirming the presence of endogenous *Caulimoviridae* sequences in plant genomes. Endogenous caulimovirid sequences have been identified in 27 plant species from 9 different plant families. Among different EPRV, badnaviruses infecting banana known as banana streak viruses (BSVs) and their endogenous counterparts (eBSV) are the most studied. The integration pattern of eBSV is highly complex and is interspaced by host sequences. Evidences suggest that only the integrated sequences present in *Musa* B genome are capable of activation and excise out as episomal viruses, whereas the eBSVs in the *Musa* A genome are rearranged to such an extent that they are not activable even under stress conditions. The coevolution of pararetroviruses, their endogenous counterparts, and host has been discussed in this chapter with special reference to badnaviruses infecting banana.

Keywords

Plant pararetroviruses • Endogenous pararetroviruses (EPRV) • Banana streak viruses • Integration • Co-evolution

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6.1 Introduction

Domestication of wild plants occurred around 10–15,000 years ago. It was well before the plants were domesticated by humans that plant pathogens were coevolving with wild plants. Human movement and gradual distribution of crop plants to new areas led to the simultaneous spread of pathogens (Lovisolo et al. 2003; Jones 2009). Initially cultivated plants might have been infected by diverse plant viruses which have migrated from their wild ancestors (wild relatives or related plant species) (Jones 2009). The increased human activity in movement of plant species across the continents has created instability on plants, vectors, and viruses which led to the increased virus evolution and evolution of new viral genotypes/strains. When new plant species are introduced to the areas where they were not growing earlier or viruses are introduced on new hosts, the sever epidemics occur as these viruses have not co-evolved there with the wild hosts over time. In this chapter our focus will be on interactions among plant pararetroviruses, whose endogenous counterparts exist in their hosts and their interactions with the respective hosts.

6.2 Plant Pararetroviruses (Family *Caulimoviridae*)

Plant pararetroviruses or *Caulimoviridae* are the plant viruses having double-stranded DNA genome comprising of seven recognized genera, viz., *Badnavirus*, *Caulimovirus*, *Cavemovirus*, *Petuvirus*, *Solendovirus*, *Soymovirus*, and *Tungrovirus* (ICTV website accessed in June 2015). Recently described bacilliform Orendoviruses (Geering et al. 2010) and *Rose yellow vein virus* (RYVV) (Mollov et al. 2013) are the other tentative genera in the family. Pararetroviruses replicate *via* transcription/reverse transcription like mammalian retroviruses. The major difference between retroviruses and pararetroviruses is that pararetroviruses do not integrate into the host genome during replication; however, integration is an essential step in the replication of retroviruses (Pfeiffer and Hohn 1983). The replication cycle of pararetroviruses is thus exclusively episomal. The pararetroviruses like that of retroviruses encode a POL gene comprising of protease, reverse transcriptase, and RNase H coding functions (Toh et al. 1983); however, they lack the integrase gene.

6.3 Replication and Translation Strategies in Plant Pararetroviruses

During the infection of plant pararetroviruses, following the entry into the cell, the virion is targeted to the nucleus by a nuclear localization signal located at the N-terminus of coat protein and is exposed on the virion surface (identified in *Cauliflower mosaic virus*, CaMV). Once the virion reaches the nuclear pore, disassembly occurs and double-stranded DNA (dsDNA) enters the nucleus utilizing the importin α pathway or perhaps other pathways as identified in the case of *Rice tungro bacilliform virus* (RTBV) (Hohn and Rothnie 2013). Inside the host nucleus, the discontinuities in the double-stranded DNA (dsDNA) viral genome are sealed by host repair polymerase and ligase to give supercoiled DNA which is associated with histone proteins to form sealed dsDNA minichromosomes (Hull et al. 2000). The transcription takes place in the host cell nucleus, where from the minichromosomal form of viral DNA, a greater-than-genome length transcript (35S or 34S RNA) is being transcribed asymmetrically by host DNA-dependent RNA polymerases. This transcript has a terminal redundancy of about 35 to 270 nt (dependent upon the species), and it serves as a template (pregenomic RNA, pgRNA) for reverse transcription-mediated replication and also as polycistronic mRNA for the translation of open reading frames (ORFs) (all the three ORFs in case of badnaviruses and at least some ORFs in case of other caulimoviruses) (Pooggin et al. 1999). In case of plant pararetroviruses, all the ORFs (in case of viruses of the genera *Petuvirus*, *Soymovirus*, *Cavemovirus*, and *Badnavirus*) are possibly translated from the polycistronic pgRNA as no subgenomic RNA (sgRNA) has been detected. However, in case of genera *Caulimovirus*

and *Soymovirus*, a specific monocistronic mRNA (19S RNA) is produced for the translation of ORF6 (Hohn and Rothnie 2013).

In contrast to retroviruses, the replication cycle of pararetroviruses is episomal and does not involve an integration phase. For synthesis of minus-strand DNA, host cytosolic $tRNA^{met}$ act as primer for reverse transcription by viral RT (Boeke and Corces 1989). The opposite strand is primed from a purine-rich region and polymerized by virally encoded RT and RNase H (Franck et al. 1980; Hull et al. 1986; Verver et al. 1987). The site-specific discontinuities are formed at the priming sites for both minus- and plus-strand DNA synthesis and are made by the oncoming strand displacing the existing strand for a short distance and not ligating to form a closed circle (King et al. 2011).

6.4 Endogenous Pararetroviruses in Plant Genome: Viruses Exist in Host Genome

Retroelements are the general term for those genetic entities which occur both in RNA and DNA stages through the cycles of reverse transcription and transcription (Geering et al. 2010). There is a large diversity of viral retroelements including retroviruses (family *Retroviridae*), pararetroviruses (families *Caulimoviridae* and *Hepadnaviridae*), and long terminal repeat (LTR) retrotransposons (families *Metaviridae* and *Pseudoviridae*, a proposed family) (Hull 2001; King et al. 2011). All the characterized viral retroelements possess *gag-pol* gene core, and other linked adaptive genes, which in turn enables different retroelements to adapt to different ecological niches. The *gag* gene encodes for the capsid protein, whereas the *pol* gene encodes for the aspartic protease and reverse transcriptase with RNase H function (King et al. 2011). In all the viral retroelements, the RT sequences are conserved, as indicated by the presence of conserved motifs, and therefore used to analyze the phylogenetic relationship (Geering et al. 2010; King et al. 2011). Phylogenetic analysis of conserved core RT sequences has been used for determination of correct taxonomic placement and classification of viral retroelements (King et al. 2011). In the literature these endogenous viral sequences present in the host genome are also referred to as endogenous viral elements (EVEs) (Chabannes and Iskra-Caruana 2013; Geering et al. 2014).

EVEs are essentially the fossils of the viruses that existed in their host genomes (Geering et al. 2014; Iskra-Caruana et al. 2010). The endogenous sequences are referred to as viral retroelements that have infected host germ cell lines at some point of time and are thereafter inherited from parent to offspring in Mendelian fashion (Geering et al. 2010). There is a common feature of these viral retroelements which occur as the endogenous in their host genome is that following integration they tend to evolve like pseudogene, and during this process they accumulate inactivating mutations (premature stop codons, frameshift mutations, deletions, and internal recombinations) (Geering et al. 2010). Representative *Metaviridae*- and *Pseudoviridae*-like sequences have been detected in the plants (King et al. 2011). In addition to this a large number of reports are now available confirming the presence of endogenous *Caulimoviridae* sequences in plant genomes (Gayral et al. 2008; Geering et al. 2010; Iskra-Caruana et al. 2010). In the animal genomes, the endogenous retroviruses are known to present, which integrate to their host genome through the virally encoded integrase enzyme; however, unlike retroviruses, the endogenous members of the *Caulimoviridae* do not encode integrase enzyme and thus might have introduced to their host genome through some other mechanism.

6.5 General Mechanisms of Endogenization of Pararetroviruses

There are different proposed mechanisms of the integration of *Caulimoviridae* sequences into the host genomes. They have possibly become integrated either by the recruitment of viral DNA to repair double-stranded breakages in the host chromosomal DNA or by recombination of viral pregenomic RNA with long terminal repeat

(LTR) retrotransposon RNA to form a chimeric molecule followed by its integration through normal retrotransposon mechanisms (Kunii et al. 2004; Gayral et al. 2010). This integration into the plant genome has also been proposed to have occurred through illegitimate recombination (Jakowitsch et al. 1999; Ndowora et al. 1999).

As discussed in the above sections, pararetroviruses (family *Caulimoviridae*; known as caulimovirids; Geering et al. 2014) like retroviruses replicate via reverse transcription. But unlike the retroviruses, the plant pararetroviruses possess a double-stranded DNA genome, and integration is not an essential step in their replication cycle. Over the past two decades, an increasing number of plant-virus sequences have been reported in the plant genomes (Hull et al. 2000; Iskra-Caruana et al. 2010). Sequences of both single-stranded DNA plant viruses (*Geminiviridae*) and double-stranded DNA plant viruses (*Caulimoviridae*) (Gambley et al. 2008; Gayral et al. 2008; Harper et al. 1999a, 2002; Jakowitsch et al. 1999; Ndowora et al. 1999; Staginnus et al. 2009) have been found as endogenous in the host plant genomes. Those pararetroviruses which integrate into the host genome are known as endogenous pararetroviruses (EPRV) (Mette et al. 2002; Staginnus et al. 2009). Endogenous caulimovirid sequences have been identified in 27 plant species from 9 different plant families (Geering et al. 2014). These EPRV or endogenous caulimovirid sequences have been derived from the viruses of five ICTV-recognized genera of family *Caulimoviridae*: *Caulimovirus*, *Petuvirus*, *Badnavirus*, *Solendovirus*, *Tungrovirus*, and a tentative new genus Orendovirus (Teycheney and Geering 2011).

The exact mechanism of integration of EPRV sequences into the plant genomes is not known; however, almost all the described EPRV (endogenous caulimovirid) sequences are fragmented and rearranged when compared with the cognate viral genome, and it does not appear to be a coordinated process controlled by the virus (Geering et al. 2014). This fact is also supported by the absence of virus-encoded integrase enzyme in case of plant pararetroviruses. The integration or endogenization of EPRV appears to be an ancient event, and most of the characterized EPRV sequences are defective (or partial viral genomes) showing the evidence of sequence decay, thus making them incapable of excising out as episomal viruses and thus considered to be replication defective (Teycheney and Geering 2011). There are some cases particularly the characterized loci in *Musa balbisiana*, *Petunia hybrida*, *Nicotiana edwardsonii*, rice, etc. which contain replication-competent endogenous caulimovirid sequences and have the capacity to produce the infectious episomal virus particles under the stress conditions both abiotic and genome stresses (Gayral et al. 2008; Hull et al. 2000; Iskra-Caruana et al. 2010; Kunii et al. 2004; Teycheney and Geering 2011). In addition to the above well-characterized EPRV in the host genomes, they are also known to occur in other plant species, viz., tobacco and its relatives (Jakowitsch et al. 1999), *Poncirus trifoliata* (Yang et al. 2003), potato and relatives (Hansen and Heslop-Harrison 2004), lucky bamboo (Su et al. 2007), tomato (Staginnus et al. 2007), pineapple (Gambley et al. 2008), grapes and poplar (Bertsch et al. 2009), and fig (reviewed by Chabannes and Iskra-Caruana 2013). Three endogenous viral sequences have been described in the plant-virus associations where the infection has been demonstrated to be originating in the hybrids due to the activation of integrated sequences. In the case of *Petunia hybrida* (result of a wild cross between *P. integrifolia* subsp. *inflata* and *P. axillaris* subsp. *axillaris*), the activation of *Petunia vein clearing virus* (PVCV) (Richert-Pöggeler et al. 2003) and the infection of banana streak viruses in *Musa acuminata* (A genome) and *Musa balbisiana* (B genome) hybrids (Chabannes et al. 2013; Gayral et al. 2008; Lheureux et al. 2003) have been reported. Among the EPRVs, the first conclusive evidence for them being infectious came in case of endogenous PVCV (ePVCV) (Richert-Pöggeler et al. 2003). They demonstrated that ePVCV release out as infectious complete virion thus leading to virus infection. These reports have raised the concerns regarding the presence of EPRVs in the host genome which like their exogenous counterparts can lead to epidemics and are of considerable economic importance.

Bertsch et al. (2009) described caulimovirid-like sequences in the genomes of grapevine (*Vitis vinifera*) and *Populus trichocarpa*. Recently these sequences are tentatively classified as members of a new proposed genus *Florendovirus* in the family *Caulimoviridae* (Geering et al. 2014). An extensive colonization of these endogenous caulimovirid sequences was found in the genomes of cultivated and wild plants including *Jatropha curcas*, grapevine, *Ricinus communis*, citrus, *Cucumis sativus*, *Fragaria vesca*, *Glycine max*, rice, potato, tomato, and many others. As these endogenous virus elements (EVEs) were found in flowering plants, they were named as *Florendovirus* (derived from *Flora endogenous virus*) (Geering et al. 2014). In *Vitis vinifera*, 9 % of the endogenous florendovirus loci were located within the introns, thus suggesting their ability to influence host gene expression.

The most studied endogenous sequences are those which are found in the genome of banana (*Musa* spp.) known as endogenous BSV (eBSV). The eBSV and their interactions with banana have been discussed below.

6.6 Endogenous Banana Streak Viruses in *Musa* Genome

The epidemics of banana streak disease are mostly restricted to the East African countries and Nigeria, but BSV has become one of the most widely distributed viruses of banana in the last two decades (Fargette et al. 2006; Iskra-Caruana et al. 2014b; Lockhart and Jones 2000). Banana streak virus (BSV) was not considered a serious threat to banana production until the numerous spontaneous outbreaks of disease were observed in a significant proportion of progenies from interspecific *Musa* hybrids and from tissue culture-derived plants of *Musa* (banana and plantains) in many banana-producing areas with no external source of virus infection (Hull et al. 2000; Iskra-Caruana et al. 2010). The modern cultivated bananas contain various ploidies and combinations of *Musa acuminata* (A genome) and *Musa balbisiana* (B genome), and most widely cultivated banana varieties are sterile triploids. The progenies from different breeding and tissue culture programs found infected with BSV were derived from the parents or mother plants showing no sign of infection. The direct PCR using the specific primers showed the widespread presence of viral sequences in *Musa* plant including from asymptomatic plants (mother as well as progeny) (LaFleur et al. 1996); however, the disease symptoms or virus particles were not observed in all the PCR-positive samples. In contrast to this, based on the viral symptoms and serology, very few of the samples were tested positive (Harper et al. 1999a, b). Researchers have then determined that origin of these field outbreaks could be because of the presence of infectious eBSV sequences present in the *Musa balbisiana* genome (Cote et al. 2010; Dallot et al. 2001; Harper et al. 1999a; Lheureux et al. 2003; Ndowora et al. 1999). The first confirmatory results for the presence of integrated BSV sequences (also known as endogenous BSV) in the *Musa* B genome were presented by Ndowora et al. (1999) and Harper et al. (1999b). They observed that despite the absence of virus particles in the parent lines used in the breeding programs, hybrid progeny of these crosses was frequently infected with *Banana streak OL virus* (BSOLV) (Dahal et al. 1999; Ndowora et al. 1999). An eBSV sequence which had 99 % identity to BSOLV was found integrated in one parent line cv. Obino I'Ewai (*Musa* AAB group), as indicated by hybridization of viral DNA to the genomic DNA of banana and fluorescent in situ hybridization (FISH) studies (Harper et al. 1999b; Ndowora et al. 1999). The integrant eBSOLV in the genome of cv. Obino I'Ewai consist of two viral sequence segments separated by a 6 kb scrambled region containing non-contiguous and inverted viral sequences. These two segments of integrant viral sequences comprise the full activated viral genome (Harper et al. 1999b; Ndowora et al. 1999). Even though the eBSV and other EPRVs in the plant genomes have been discovered, it was earlier thought that these sequences are perhaps non-activable. Ndowora et al. (1999) provided the evidence for the first time that eBSV can activate to cause the episomal virus infection, which was later

confirmed by Cote et al. (2010); Dallot et al. (2001). They found that the virus isolates obtained from the tissue culture-derived micropropagated plants are genetically uniform as compared to the isolates arising from field infection which are generally genetically variable (as indicated by RFLP studies).

Geering et al. (2005a) studied the diversity of integrated *Badnavirus* sequences in the banana genome. A diverse array of eBSV sequences were detected in *M. acuminata*, *M. balbisiana*, and *M. schizocarpa*, as well as two landraces, cvs. "Klue Tiparot" (*Musa* BAB group) and "Obino I'Ewai" (*Musa* AAB group). The 103 PCR clones derived from an array of these *Musa* germplasms showed remarkable variations with 36 distinct sequences sharing less than 85 % identity at nucleotide levels and were divided to three major phylogenetic clades. Nearly half of the sequenced clones had nonfunctional ORFs due to substitutions, additions, or deletions. Authors did not find any commonality in the integrated sequences amplified from *M. acuminata* and *M. balbisiana*, which suggested that integration from the active episomal viruses might have occurred following the speciation of *M. acuminata* and *M. balbisiana* and each integrated sequence resulted from an independent integration event. Southern blotting using the total plant DNA preparation has demonstrated that the endogenous *Banana streak MY virus* (BSMYV) and BSOLV were linked to the B genome and banana endogenous virus (BEV1) to the A genome of banana (Geering et al. 2001, 2005b). Endogenous BSV present in the B genome showed greater diversity, which indicated the existence of greater diversity of episomal badnaviruses in the region where *M. balbisiana* originated (Geering et al. 2005a). The region where *M. balbisiana* had originated includes the Philippines and areas extending from southern China and northern Indochina to northern India (Jones 1999). Most of the BEV derived from *M. acuminata* were in subclades 1A and 1B; however, the BEV sequences amplified from a subspecies occurring in Myanmar near India (*M. acuminata* subsp. *burmannicoides*) clustered in subclusters 1F, 5F, and 5G.

Geering et al. (2001) analyzed the structure of integrated BSV sequences in a range of cultivars and demonstrated using Southern blot hybridization analyses that BSV-OL sequences are associated with the B genome of *Musa*. All the tested cultivars with genetic constitution of BB and AAB were harboring Musa6+8 integrant, a characterized BSOLV integrant by Harper et al. (1999b) and Ndowora et al. (1999); however, such integrant was not present in the *Musa* cultivars with genetic constitution AAA. FISH and DNA fiber stretch hybridization (FSH) analysis showed that the tandemly arrayed repeats of eBSOLV are flanked by a repetitive *Musa* sequence. Geering et al. (2001) have also identified the integrated *Badnavirus*-like sequence from healthy banana cv. Williams, which was integrated to the A genome. Interestingly the hybridization patterns were complex in the AAA and AAB group of cultivars compared to BB group cultivars, suggesting the multiple site of integration in the genome. Although integrated eBSV sequences have been detected both in *Musa acuminata* (A genome) and *Musa balbisiana* (B genome), the evidences till date suggest that only the integrated sequences present in B genome are capable of activation and excise out as episomal viruses, whereas the eBSVs in the A genome are rearranged to such an extent that they are not activable and thus referred to as dead sequences (Gayral et al. 2008; Geering et al. 2010; Iskra-Caruana et al. 2014a, b). Therefore, two types of eBSV have been described in the banana. The first type of integrated sequences is defined by noninfectious sequences with nonfunctional viral open reading frames (ORFs) containing premature stop codons, frameshift mutations, and/or incomplete viral genomes. Such eBSVs are comprised of all the described integrated sequences in *Musa* A genome and many in the *Musa* B genome (Geering et al. 2005a). The second type of eBSV sequences is known as infectious type that contains the complete functional viral genome and thus has the ability to lead to episomal virus infection. These infectious eBSVs have been described to be present in the *Musa* B genome (Geering et al. 2001). Now the BSV species having the infectious eBSV counterpart in the *Musa*

B genome capable of inducing spontaneous infection are widely distributed in all the growing regions of world.

Four naturally widespread BSV species have been identified to be present in integrated forms, viz., BSOLV, *Banana streak IM virus* (BSIMV), BSMYV, and *Banana streak GF virus* (BSGFV) (Chabannes et al. 2013; Gayral et al. 2008; Geering et al. 2001). The characterization of the integration patterns of BSOLV, BSGFV, and BSIMV in the wild seedy banana diploid Pisang Klutuk Wulung (PKW) was performed by Gayral et al. (2008) and Chabannes et al. (2013). The BSGFV sequences were found integrated only into the B genome of cv. PKW and were absent in *Musa acuminata* (Gayral et al. 2008). PKW cv. contained two similar endogenous sequences of BSGFV, with allelic insertion. Although the endogenous viral genome has undergone extensive rearrangement (fragmented, inverted, and partially repeated), both the eBSVs contain the full-length viral genome. The eBSV in PKW corresponds to two alleles of same locus with similar genetic structure and common genetic environment surrounding the eBSV (Gayral et al. 2008). This integration was proposed to be the result of single integration event which was probably recent. In contrast to integrated BSOLV sequences, which reaches to high copy number through a dynamic process of accumulation and elimination (Harper et al. 1999b), the integrated BSGFV in cv. PKW had not undergone any increase in copy number of integrated sequences following single integration event (Gayral et al. 2008). Out of the two alleles harboring the endogenous BSGFV, only one was found to be capable of releasing the activated virus particles through homologous recombination process in PKW (Gayral et al. 2008; Hull et al. 2000).

In the same cv. PKW, the molecular structure, genomic organization, genomic landscape, and infectious capacity of the three endogenous BSV species (BSGFV, BSOLV, and BSIMV) were described by Chabannes et al. (2013). These eBSVs exhibited extensive viral genome duplications and rearrangements in the *Musa* B genome. FISH and segregation analysis in the F1 population indicated that three endogenous BSVs, viz., eBSIMV, eBSOLV, and eBSGFV, were each present at a single locus in the B genome (Chabannes et al. 2013). The eBSOLV and eBSGFV (similar to the findings of Gayral et al. 2008) contained two distinct alleles, compared to two structurally identical alleles in case of eBSIMV. The genotyping of eBSV in both the alleles and expression of infectious episomal virus particles in the progeny indicated that only one allele is infectious and involved in activated BSV infection. Further based on the structure and evolution of eBSVs, authors suggested sequential integration into the plant genome, and the three loci corresponding to these eBSVs were under differential evolution (Chabannes et al. 2013).

Based on the PCR, dCAPS markers, and Southern blot analysis, eBSVs of the three species (BSIMV, BSGFV, and BSOLV) were genotyped in the 77 *Musa* accessions (Gayral et al. 2010; Iskra-Caruana et al. 2014b). The *Musa* samples including all available diploid seedy *M. balbisiana* and its interspecific hybrids with *M. acuminata* were analyzed. All the diploid *M. balbisiana* harbor eBSV for the three BSV species, and interspecific hybrids (*Musa* A x B) had at least one BSV species as endogenous. A massive BSV integration of three BSV species was observed in the majority of B genomes (Gayral et al. 2010). A phylogenetic analysis of *Musa* species/subspecies and integrated sequences indicated the parallel co-evolutionary history of BSV and banana (Gayral et al. 2010; Iskra-Caruana et al. 2014a, b). Although it was earlier suggested by Gayral et al. (2008) that BSV integration to *Musa* genome is a recent event and it has occurred after the *Musa acuminata* and *M. balbisiana* speciation, a conserved locus of each BSV species in the *Musa* B genome further indicated that this integration has happened before the diversification of *M. balbisiana* (Gayral et al. 2010). Integration occurred after *M. acuminata* and *M. balbisiana* speciation around 4.5 million years ago. The eBSGFV was localized in an LTR retrotransposon from the Ty3/gypsy *Metaviridae* family, which enabled the calculation of the substitution rate at the locus, indicating that the origin of this integration was around 0.63 million years ago.

Based on the partial RT/RNase H sequences of BSGFV and BSIMV from Uganda and Colombia and eBSV sequences of the twenty *M. balbisiana* diploid bananas, the phylogenetic relationship between BSV and eBSV was studied (Gayral et al. 2010). The interspecific *Musa* hybrids used in the study were from two centers of origin, viz., India and East Asia (extending from the Philippines to New Guinea) (Perrier et al. 2009). They observed that BSIMV and eBSIMV clustered together, but BSGFV formed three district groups, exhibiting high polymorphism. The comparison of substitution/site suggested a slow evolution rate of eBSGFV compared to that of the episomal counterpart. They hypothesized that these viruses originated from integrants, and all the BSGFV isolates from Uganda clustered independently from eBSGFV. A high identity at nucleotide sequence level (99 %) was observed between eBSV and BSV of three BSV species (BSIMV, BSGFV, and BSOLV) by Chabannes et al. (2013). The low divergence observed among the eBSV and BSV sequences was proposed to reflect a contribution of eBSV to the current viral populations rather than a recent integration into the B genomes (Chabannes et al. 2013). Based on in silico analysis, Gayral and Iskra-Caruana (2009) identified 17 independent integration events in the genome of *M. acuminata*, *M. balbisiana*, and *M. schizocarpa*. They concluded that most of the integration events have occurred following divergence of these banana species and therefore are recent events. Majority of eBSV integrants in banana genome are defective due to pseudogenization driven by evolution of the host genome (Gayral and Iskra-Caruana 2009).

6.7 Role of EPRV

The role of EPRV in plant-virus interaction and the reason behind retaining these sequences by the host genome are not fully understood. The endogenous sequences in the plant genome appear to be the relics of very ancient infection events and are not necessarily associated with the infection or pathogenicity (Geering et al. 2005a, b; Mette et al. 2002). Further, they are neutral components of the plant genomes and sometimes may potentially contribute to the either pathogenicity or virus resistance in the host (Iskra-Caruana et al. 2010). Mette et al. (2002) and Noreen et al. (2007) independently provided the evidence that EPRV sequences may contribute to virus resistance in their respective hosts through transcriptional or posttranscriptional gene silencing of homologous sequences (Hull et al. 2000). When a reporter gene was linked to a tobacco endogenous pararetrovirus enhancer-promoter sequence, it was expressed in stably transformed tobacco *Arabidopsis* plants but was silenced in tobacco plants containing EPRV (Mette et al. 2002). The hypothesis that eBSV sequences could confer virus resistance to the host plant may probably explain the observation that eBSVs are maintained in the *Musa* genome despite the fact that some of them become harmful when activated (Iskra-Caruana et al. 2010). The small interfering RNA (siRNA)-based silencing mechanism probably explains the reason behind wild diploid *Musa balbisiana* cv. PKW, which is resistant to both activation of infectious eBSV and episomal BSV infection (Iskra-Caruana et al. 2010; Lheureux et al. 2003).

6.8 Activation of eBSV Sequences

6.8.1 Factors Influencing Expression of eBSV in *Musa* Cultivars

The BSV integration into the *Musa* genome has possibly originated by illegitimate recombination during the minichromosomal phase (Staginnus et al. 2007). The eBSVs ranging from small incomplete fragment to larger sequences have become integrated into the plant genome via integration in the germinal cell followed by fixation in the plant population by evolutionary forces like natural selection and genetic drift (Gayral et al. 2008). The ability of eBSV sequences in the B genome of *Musa* to form the infectious virus particles de novo triggered by stresses, wounding,

or tissue culture environment has been studied in details during the last decade. Some EPRVs in general and eBSVs in particular are considered to be infectious because of their potential to reconstitute the functional episomal viral genome following the activating stress contributing virus infection (Cote et al. 2010). The infectious eBSV has been known to occur in the *M. balbisiana* genome (Chabannes et al. 2013). There are no reports available that infectious eBSV becomes activated in the diploid seedy *M. balbisiana* genome. However, spontaneous infection has been reported in the interspecific hybrids of *M. acuminata* and *M. balbisiana* (A x B). Under different stress conditions, the eBSV becomes activated to cause episomal BSV infection. These stresses include abiotic stresses such as micropropagation by in vitro culture processes (Dallot et al. 2001), temperature differences (Dahal et al. 1998a, b; 2000) or water stress, and genetic hybridization (Lheureux et al. 2003).

In 2001 Dallot et al. reported that tissue culture systematically triggers the activation of infectious eBSV present in newly created interspecific hybrids. Dallot et al. (2001) selected virus-free FHIA 21 (tetraploid hybrid AAAB) mother plants and found that micropropagation process triggers the expression of infectious eBSOLV. They found that during proliferation stage characterized by the intensive production of neoformed buds, the eBSOLV become activated, whereas rooting and acclimatization stages had little or no effect on activation. The micropropagation process (duration in culture and the way of subdividing the clumps of proliferation) affects the activation of eBSOLV, and 58 % of micropropagated lines were found infected after six in vitro subcultures (Dallot et al. 2001). Regardless of the nature of cultivar (whether synthetic, AAAB and AAB, or natural, such as plantains, AAB), proliferation stage of micropropagation process is the major determinant of activation of endogenous BSV (Cote et al. 2010). They observed large increase in the percentage of infected plants during the first subcultures. A study on the correlation between the duration of tissue culture and the level of activation of infectious eBSOLV showed that after 9–12 proliferation cycles, there was a decrease in percentage of activated BSOLV-positive plantlets for high total produced shoot (TPS) values. They proposed that this decrease was probably due to the concomitant activation of infectious eBSOLV and a decrease in the virus titer in neoformed plantlets, resulting from cell multiplication outcompeting virus replication. The activated episomal BSOLV was detected following tissue culture in a tetraploid *Musa* hybrid FHIA-21 (AAAB), and the activated virions were successfully transmitted to healthy cv. Williams plants by different mealybug species (*Dysmicoccus brevipes*, *Planococcus citri*, and *P. ficus*) (Meyer et al. 2008).

Genetic hybridization was also shown to trigger the activation of infectious endogenous BSV (Lheureux et al. 2003). A high incidence of BSV infection was observed in F1 triploid (AAB) population derived from the cross between episomal virus-free diploid *M. balbisiana* (BB) and tetraploid *M. acuminata* (AAAA) parents. The genetic mechanism behind the release of these episomal BSVs was characterized as a monogenic allelic system depending on a genetic factor called BEL (BSV expressed locus) (Lheureux et al. 2003) which suggested that carrier of this eBSV exists on the *M. balbisiana* diploid parent. In addition ploidy of B genome in *Musa* genotypes also has direct effect on the expression of eBSV (Chabannes and Iskra-Caruana 2013). The diploid *M. balbisiana* cv. such as Pisang Klutuk Wulung (PKW) and Pisang Batu harbor infectious eBSV but are always resistant to BSV (both episomal and integrated forms) (Lheureux et al. 2003). Interestingly, although it has been noted that interspecific hybrids harboring the haploid B genome (AB, AAB, AAAB) have the risk of activation of infectious eBSV, the hybrids with two copies of B genome (ABB and AABB) never show the activation of eBSV even under activating stress conditions (Chabannes and Iskra-Caruana 2013). This activation is thus suggested to be regulated through epigenetic regulation process, which is dosage dependent determined by ploidy state.

6.8.2 Mechanisms of eBSV Activation

As discussed in the above sections, the structure of eBSV in the *Musa* B genome is highly complex and rearranged. The viral genomic fragments appear to be fragmented, inverted, and partially repeated (Chabannes et al. 2013). The full-length infectious viral genome is reconstructed from different segments within the same locus in plant genome (Chabannes and Iskra-Caruana 2013). Iskra-Caruana et al. (2010) proposed a two-step intra-strand homologous recombination (HR)-based model to explain the release of episomal BSGFV from eBSGFV. This model was based on the minimum number of HR steps to reconstitute the full-length infectious circular genome of BSGFV from the characterized eBSGFV-7 integrant. The first HR, called Rec1, occurs between the two inverted repeats of 642 bp separated by 6942 bp. This intramolecular recombination results in turnaround (inversion) of an internal sequence and leads to juxtaposition of two fragments forming a linear eBSGFV genome containing the three ORFs in same orientation. This linear viral genome is flanked by two direct repeats which are involved in second HR step (Rec2). Rec2 produces an eBSV called as "modified eBSGFV-7 locus" carrying a deletion of 7324 bp and results in excision (pop-out) of circular episomal BSGFV molecule (Iskra-Caruana et al. 2010). Based on the characterized eBSOLV-1 allele (Chabannes et al. 2013), a similar two-step HR-based release mechanism has been proposed for BSOLV recently by Chabannes and Iskra-Caruana (2013). In simple terms, the current model of activation of eBSV involves two homologous intra-strand recombination events leading to excision of the "scrambled" region and circularization of the DNA to produce a transcriptionally active form of the virus.

6.8.3 eBSV as Reservoir of BSV

Due to the ability of eBSV to become activated and cause episomal infection under certain conditions, the eBSV-based vertical spread of BSV has been now recognized a serious threat to the banana production and genetic improvement worldwide. The eBSV has a considerable impact on the outbreaks of banana streak disease recorded in countries which were previously free of BSV (Dahal et al. 1998a, b; Harper et al. 2004, 2005; Iskra-Caruana et al. 2010). The plants harboring the eBSV are reservoir as well as sources of viruses and contribute to the global biodiversity of BSV population. The wide molecular diversity of BSV has resulted mainly from the mixture of episomal and endogenous viral sequences (Iskra-Caruana et al. 2010, 2014a). A few molecular differences in the eBSV and their cognate episomal counterparts have indicated that endogenous BSVs have certainly played a significant role in banana-BSV evolution (Gayral and Iskra-Caruana 2009; Gayral et al. 2008). Thus, eBSV can be regarded as a reservoir of BSV biodiversity in that they contribute to the BSV gene pool once activated (Iskra-Caruana et al. 2010). The eBSVs have profound implications for the international germplasm exchange, genetic improvement efforts, mass propagation, and management of banana badnaviruses. Banana streak viruses are quite prevalent in India (Sharma et al. 2014, 2015), and the shortest known BSVs are reported to exist in India which might have evolved during release of eBSV to active episomal form (Baranwal et al., 2014).

6.9 Coevolution of Banana Streak Viruses and Banana

The full genome sequence of DH Pahang, a seedy *M. acuminata* diploid banana (D'Hont et al. 2012), and a wild diploid *M. balbisiana* cv. Pisang Klutuk Wulung (PKW) (Davey et al. 2013) has been completed, which has helped to assess the integrant BSV sequences and their distribution among the 11 chromosomes. Viral integrations were detected at 24 loci covering 10 chromosomes. Similar to the findings of Gayral et al. (2008) and Chabannes et al. (2013), all the integrations were determined to be fragmented and highly reorganized with a size ranging from 100 bp to over 18 kb (D'Hont et al. 2012). These integrant sequences were predicted to be noninfectious. None of these sequences were

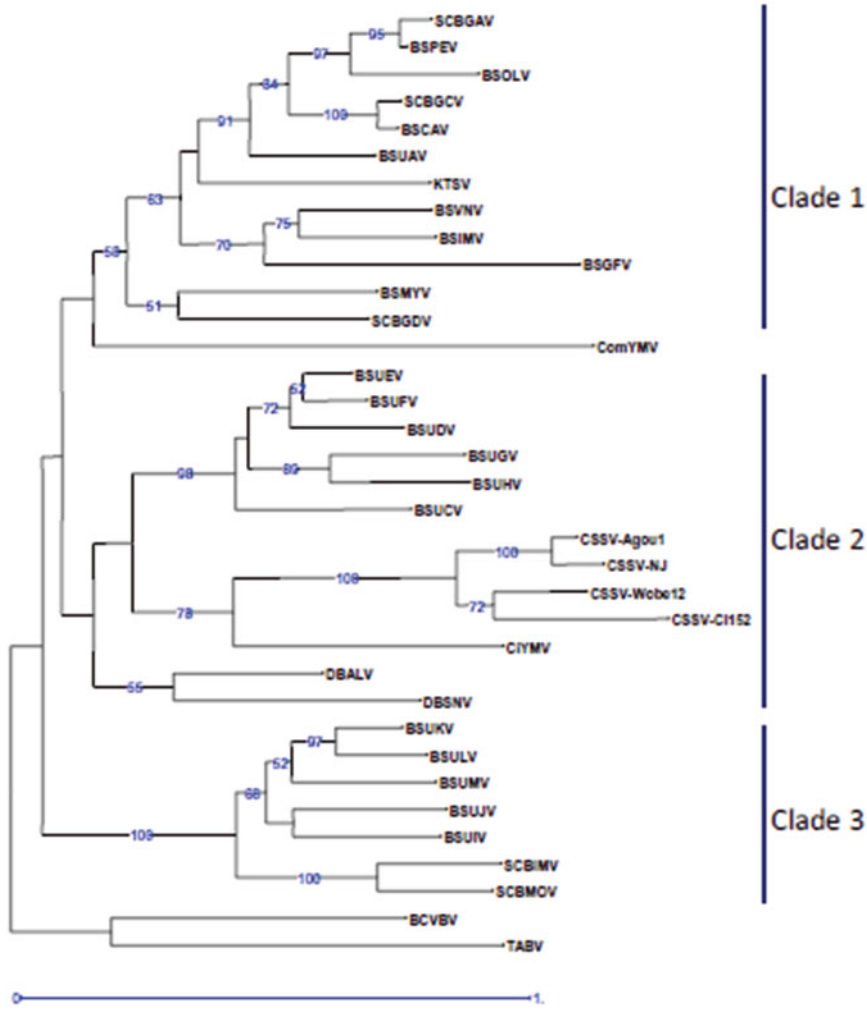

Fig. 6.1 Maximum likelihood (ML) phylogenetic relationship based on the partial conserved 540 bp RT/RNase H sequences. *Taro bacilliform virus* (TaBV) and *Bougainvillea chlorotic vein banding virus* (BCVBV) are out-groups. The GenBank accession numbers are AJ002234 (BSOLV), AY805074 (BSMYV), AY750155 (BSVNV), AY493509 (BSGFV), HQ659760 (BSIMV), HQ593111 (BSCAV), HQ593107 (BSUAV), HQ593108 (BSUIV), AJ968501 (BSUJV), AJ968504 (BSUKV), HQ593109 (BSULV), HQ593110 (BSUMV), J968464 (BSUCV), AJ968465 (BSUDV), AJ968467 (BSUEV), AJ968469 (BSUFV), AJ968470 (BSUGV), AJ968472 (BSUHV), EU034539 (BCVBV), L14546 (*Cacao swollen-shoot virus*, CSSV-Agou1), AJ608931 (CSSV-New Juaben), JN606110 (CSSV-CI152), AJ781003 (CSSV-Wobe12), X52938 (*Commelina yellow mottle virus*, ComYMV), AF347695 (*Citrus yellow mosaic virus*, CYMV), X94576 (*Dioscorea bacilliform AL virus*, DBALV), DQ822073 (*Dioscorea bacilliform SN virus*, DBSNV), AY180137 (*Kalanchoe top-spotting virus*, KTSV), M89923 (*Sugarcane bacilliform MO virus*, SCBMOV), AJ277091 (*Sugarcane bacilliform IM virus*, SCBIMV), FJ824813 (*Sugarcane bacilliform Guadeloupe A virus*, SCBGAV), FJ439796 (*Sugarcane bacilliform Guadeloupe C virus*, SCBGCV), FJ439817 (*Sugarcane bacilliform Guadeloupe D virus*, SCBGDV), AF357836 (TaBV), BEV Banana endogenous virus (Geering et al. 2005a, b). The figure is redrawn from Iskra-Caruana et al. (2014a)

related to BSVs of clade 1 (Iskra-Caruana et al. 2014a) but clustered in clade 2 distributed to four groups (Fig. 6.1). Three of them belong to BSUCV (BEV9), BSUDV (BEV5), and BSUHV (Harper et al. 2005; Geering et al. 2005a) and fourth related to BEV2 (Geering et al. 2005a). These viral sequences belong to at least four distinct *Badnavirus* species (Iskra-Caruana et al. 2014a). It is proposed that the *Badnavirus* species of clade 2 became integrated to banana

genome by an endogenization process following fixation of the ancestor badnaviruses (Iskra-Caruana et al. 2014a, b).

Based on the three clades of BSV described by Iskra-Caruana et al. (2014a) and Iskra-Caruana et al. (2014b), they proposed a model of banana and BSV co-evolution taking in account the different host-virus interactions at different time points during their evolution. At ca. 4.6 MYA a time before the speciation of *M. acuminata* and *M. balbisiana* (Lescot et al. 2008), a *Badnavirus* (ancestor of present-day banana streak viruses) might have become integrated into the banana genome (Iskra-Caruana et al. 2014a). The integration of BSV into the banana genome seems to be opportunistic and a frequent phenomenon (Gayral et al. 2010; Geering et al. 2005a). Under favorable environment, initially viral integration led to better fitness advantage to the infected plants by providing the resistance or tolerance under high viral load in the fields. This situation then led to the fixation of the endogenous *Badnavirus* sequences in the seedy banana population. During the course of evolution, these seedy banana plants harboring the integrants developed strategies to deactivate the activities of viral integrants to escape the potential lethal effects while maintaining the resistance/tolerance characters. This led to the duplication of initially integrated full-length viral genomes and their inversion by recombination, thus forming a different structure of endogenous sequences in banana genome. This structural evolution of the initially integrant sequences was host driven so as to escape the large-scale production of viral genome, which was a threat to host population. Due to this most of banana population might have become resistant to episomal viral population, which in turn has no choice other than to evolve or disappear (Iskra-Caruana et al. 2014a). The second step in the viral evolution, which was virus driven, was representative of an arms race between host and virus. Iskra-Caruana et al. (2014a) proposed that the rearranged duplicated sequences due to the pseudogenization from a BSV ancestor species, which were integrated to banana genome long ago, are actually present-day cluster 2 endogenous sequences, for which the episomal counterparts are not known. Due to the development of resistance/tolerance in the banana ancestors, the original entity of these viruses might have disappeared. As the ancestors of BSV evolved to the present-day episomal BSVs, these banana plants, which although contain the endogenous *Badnavirus* sequences, are susceptible to the evolved BSVs present today (BSVs of clades 1 and 3). Coming to the endogenous counterparts of BSV from clade 1 (i.e., eBSGFV, eBSIMV, and eBSOLV), they became integrated to the *Musa balbisiana* genome following speciation of *M. acuminata* and *M. balbisiana* but before diversification of *Musa balbisiana* (Chabannes et al. 2013). This integration has been driven by the epidemic context in the areas where *M. balbisiana* has originated and diversified. In most of the cases, the seedy diploid *M. balbisiana* are resistant to their episomal BSV counterparts both of endogenous or exogenous origin (Chabannes et al. 2013; Iskra-Caruana et al. 2010; Lheureux 2002). The studies on the integration patterns of the three endogenous BSVs from clade 1 have suggested that the organization of BSOLV and BSGFV is more complex compared to that of BSIMV in the *Musa* B genome (Chabannes et al. 2013). This suggested that integration of these three BSV species was sequential with BSOLV and BSGFV integrated first while the BSIMV more recently. This integration to *Musa* B genome might have occurred somewhere around 0.64 MYA (Iskra-Caruana et al. 2014a). These BSV species are still active in the *Musa* B genome; however, no epidemic has been reported out of this. The transcriptional and post-transcriptional gene silencing of the homologous sequences might have been the mechanisms which escaped the release of these BSVs in *Musa* B genome (Hull et al. 2000). The domestication of banana started around 7000 years ago, and interspecific hybrids were selected for during domestication. This has broken down the gene silencing-based regulation of the viruses from clade 1 and thus maintained the active episomal viruses, which would have been excluded otherwise (as in case of eBSV of clade 2) under natural selection (Iskra-Caruana et al. 2014a). Thus, human interventions have affected

the evolutionary ways of these viruses. The BSVs of clade 3 have evolved purely through the evolution of *Badnavirus* ancestor and are associated with the recent epidemics in East Africa due to several independent introductions. These viruses are not yet fixed to the East African banana genotypes because they are not responsible for better fitness to banana plants. Based on these studies Iskra-Caruana et al. (2014a) and Iskra-Caruana et al. (2014b) hypothesized the common evolutionary pathways of banana and BSVs.

6.10 Conclusion

EPRVs are the counterparts of plant viruses which exist in the genomes of plant from different families. These sequences have significant homology with the exogenous active episomal viruses indicating that endogenization has occurred during some point of evolution. In *Badnavirus-Musa* system where pattern of integration has been studied in details at least for some of banana streak viruses, it indicated that integration has occurred at ancient times during *Musa* evolution. The large array of badnaviruses' diversity infecting banana as affected by human interventions (particularly introduction and spread) has further affected the co-evolution of *Badnavirus-Musa* system. The role of eBSV and purpose of retaining these sequences remain to be investigated in the future, and this may pave the ways in management of pararetroviruses.

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Viral Diseases on Medicinal Plants of North-Eastern Uttar Pradesh

7

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Abstract

Medicinal plant provides perpetual resource base for growing needs of the pharmaceutical industry. Viral diseases have also important position due to huge loss caused by them in plants. Viral diseases are characterised by various symptoms like stunting, chlorosis, colour breaking, reduced leaves, flower distortion, etc. In the present contribution, we have studied medicinal plants of nine (ten) different families which are found to be infected with viral diseases present in North-Eastern Uttar Pradesh, India. These plants are *Acalypha indica, Ageratum conyzoides, Calotropis procera, Cassia tora, Catharanthus roseus, Centella asiatica, Chrysanthemum indicum, Croton bonplandianum*, *Datura metel, Datura stramonium, Helianthus annuus, Hibiscus rosa-sinensis, Jatropha curcas, Ocimum sanctum, Parthenium hysterophorus, Phyllanthus niruri, Pouzolzia indica, Rauvolfia serpentina, Ricinus communis, Solanum nigrum, Tagetes erecta, Withania somnifera* and *Zinnia elegans*. This chapter provides inclusive information about the family, distribution, morphology, pharmacological profile, active constituents and symptoms of viral infections with photographs of infected and non-infected leaves' sample of medicinal plants and viruses reported in North-Eastern Uttar Pradesh, India. The study of the viral diseases will allow developing effective methods of protecting medicinal plants and preserving the quality of raw materials for pharmaceuticals.

Keywords

Medicinal plants • Viral diseases • North-Eastern Uttar Pradesh • Pharmaceuticals

Medicinal plants have always occupied a vital position in human health care. Herbal drugs are preferred over allopathic drugs on account of their efficiency and easy availability and are also

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said to be free from side effect. It is revealing to that about 80 % modern drugs are derived from plants. There is currently a large and ever expanding global population base that prefers the use of natural products in treating and preventing medical problems. This has influenced many pharmaceutical companies to produce new antimicrobial formulations extracted from plants or herbs. At present, plants and herb resources are limited, as far as the search for useful phytochemical is concerned, but these resources are dwindling fast, due to onward march of civilisation. There is a rapid depletion of medicinal plant due to overexploitation and damage to the ecosystem and due to certain diseases caused by bacteria, fungi and viruses. Quite often, plant collectors ignore virus infections of a good number of medicinal plants. This causes loss of efficacy of plant-based medicine. Medicinal plants have a variety of chemical constituents, which have the ability to inhibit the replication cycle of various types of DNA or RNA viruses. A wide variety of active phytochemicals including flavonoids, terpenoids, lignans, sulphides polyphenolics, coumarins, saponins, furylcompounds, alkaloids, polyines, thiophenes, proteins and peptides have been identified in medicinal plants. Due to the presence of these compounds, viral infection in medicinal plants is rare. However, when it occurs, it affects the plant morphologically, physiologically and nutritionally as well as at cellular level. It may also affect the quality of pharmaceutical interest compound found in plants in its healthy stage. Due to the great economic significance of medicinal plants and incomplete characterisation of virus diseases occurring in it, attempts are made to identify and characterise the viruses infecting some medicinal plants.

7.1 Description and Symptomatology of Virus-Infected Medicinal Plants in the North-Eastern Uttar Pradesh

When it is remembered that there are more than 300 different viruses, not counting strains, which affect plants, it is not surprising that this multiplicity is reflected in an equally varied response by the host. Viral diseases have an important status because they not only cause direct damage to the host but also predispose the plant to secondary invaders (Beute 1970). The majority of viral disease leads to overall stunting, reduced leaves, chlorosis, colour breaking, flower distortion and reduced flower (Magie and Poe 1972; Mandahar 1987). In the present study, only those plants which are valued for their medicinal and aromatic qualities are considered. These plants are *Acalypha indica, Ageratum conyzoides, Calotropis* procera*, Cassia tora, Catharanthus roseus, Centella asiatica, Chrysanthemum indicum, Croton bonplandianum Datura metel, Datura stramonium, Helianthus annuus, Hibiscus rosa-sinensis, Jatropha curcas, Ocimum sanctum, Parthenium hysterophorus, Pouzolzia indica, Phyllanthus niruri, Rauwolfia serpentina, Ricinus communis, Solanum nigrum, Tagetes erecta, Withania somnifera* and *Zinnia elegans*. This contribution provides a comprehensive review of the description of plants, family, active constituents, pharmacological profile as a medicinal plant and viruses reported in the survey area with photographs of infected and non-infected leaves' sample of medicinal plant. The literature survey reveals the presence of viruses in these plants worldwide (Table 7.1).

7.2 Description of Virus-Infected Medicinal Plants in North-Eastern Uttar Pradesh

7.2.1 *Acalypha indica* Linn.

Family Euphorbiaceae

Distribution *Acalypha indica* is a weed widely distributed throughout the plains of India. It is deciduous and found in mixed-monsoon forests throughout greater parts of India.

Morphology It is an annual, erect and herbaceous plant of 30–60 cm in height. The leaves are simple, long petiolate and stipulate with rhom-

Table 7.1 Worldwide distribution of virus reported in medicinal plants found in the survey area

S. no.	Medicinal plants	Virus reported	Reference
1.	*Acalypha indica*	Tomato leaf curl virus, Hibiscus chlorotic, Acalypha yellow mosaic virus, ringspot virus, yellow vein mosaic virus, bitter gourd yellow mosaic virus, mung bean yellow mosaic virus	Dafalla and Sidig (1997), Raj et al. (1996), Doan et al. (2003), Muniyappa et al. (2003), Rajinimala et al. (2009), and Green and Kim (1992)
2.	*Ageratum conyzoides*	*Malvastrum* yellow vein virus, *Ageratum* yellow vein virus, *Ageratum* enation virus, Tomato spotted wilt virus, tobacco leaf curl virus, hibiscus leaf curl virus	Jiang and Zhou (2004), Tan and Wong (1993), Parrella et al. (2003), Rajeshwari et al. (2005), and Pandey et al. (2011)
3.	*Calotropis procera*	Tomato spotted wilt virus	Parrella et al. (2003)
4.	*Cassia tora*	Tomato spotted wilt virus	Parrella et al. (2003)
5.	*Catharanthus roseus*	Foxtail mosaic virus; apple mosaic virus, chlorotic stunt virus, periwinkle mosaic, prunus necrotic ring spot virus, tomato spotted wilt virus, Tulare apple mosaic virus, cucumber mosaic virus, *Begomovirus*; *Potyvirus*, CMV	Paulsen and Niblett (1977), Zaidi et al. (1978), Nariani et al. (1978), Chatzivassiliou et al. (2000), Parrella et al. (2003), Brunt et al. (1996), Vemana and Jain (2010), Choi et al. (2014), Ilyas et al. (2013), Maciel et al. (2011), Marwal et al. (2013b), Mazidah et al. (2012), and Srivastava et al.(2012)
6.	*Centella asiatica*	Cucumber mosaic virus,	Cardin and Moury (2010)
7.	*Chrysanthemum indicum*	Tomato aspermy virus, tobacco streak virus, Clerodendron yellow mosaic virus	Procter (1975), Vemana and Jain (2010), Marwal et al. (2013a), and Hollings (1955)
8.	*Croton bonplandianum*	Hibiscus leaf curl virus, Croton yellow vein mosaic virus, geminivirus	Rajeshwari et al. (2005), Verma (1963), Brunt et al. (1996), and Raj et al.(1996)
9.	*Datura metel*	Pepper veinal mottle virus, Datura mosaic virus, pepper mild mosaic virus, Peru tomato virus, cucumber mosaic cucumovirus	Moury et al. (2005), Qureshi and Mahmood (1978), Green and Kim (1991), Fernandez-Northcote and Fulton (1980), and Ali et al. 2012
10.	*Datura stramonium*	Abelia latent tymovirus, pepper veinal mottle virus, tobacco etch virus, tomato ringspot virus, tomato mosaic virus, Serrano golden mosaic virus, tomato spotted wilt virus, cucumber mosaic cucumovirus	Waterworth et al. (1975), Moury et al. (2005), Edwardson (1966), Edwardson et al. (1968), Samuitiene et al. (2003), Green and Kim (1991), Brown and Paulos (1990), Dikova (2011), Ali et al. 2012, and Roggero et al. (2000)
11.	*Helianthus annuus*	Tobacco streak virus, sunflower chlorotic mottle virus, tobacco streak virus, sunflower mosaic virus	Dijkstra (1983), Bejerman et al. (2010), Vemana and Jain (2010), Nagaraju et al. (1997), and Gulya (2002)
12.	*Hibiscus rosa-sinensis*	Hibiscus leaf curl virus, leaf curl mosaic virus	Rajeshwari et al. (2005) and Kumar and Singh (2015)

(continued)

Table 7.1 (continued)

S. no.	Medicinal plants	Virus reported	Reference
13.	*Jatropha curcas*	Jatropha mosaic virus, leaf curl mosaic virus	Aswatha Narayan et al. (2006) and Kumar and Singh (2015)
14.	*Ocimum sanctum*	Alfalfa mosaic virus, tomato spotted wilt virus	Chatzivassiliou et al. (2000), Parrella et al. (2003), and Dikova (2011)
15.	*Parthenium hysterophorus*	Hibiscus leaf curl virus, tobacco streak virus	Rajeshwari et al. (2005) and Vemana and Jain (2010)
16.	*Phyllanthus niruri*	*Ageratum* yellow vein virus	Liu et al. (2008)
17.	*Pouzolzia indica*	No reports available	No reports available
18.	*Rauwolfia serpentina*	Ring spot virus, cucumber mosaic virus	Sastry (1973) and Raj et al. (2007)
19.	*Ricinus communis*	Sunflower ringspot mosaic virus, cassava common mosaic virus, tobacco necrosis virus, beet curly top hybrigeminivirus, tobacco ringspot nepovirus, tobacco rattle tobravirus, tobacco streak virus	Wang et al. (1983), Costa (1940), Brunt et al. (1996), Teakle (1962), Brunt et al. 1996, Stace-Smith (1970), Harrison (1970), and Vemana and Jain (2010)
20.	*Solanum nigrum*	Pepper veinal mottle potyvirus, tomato aspermy virus, cucumber mosaic cucumovirus	Singh (1993), Procter (1975), and Ali et al.(2012)
21.	*Tagetes erecta*	Tomato spotted wilt virus, marigold mottle potyvirus, sunflower mosaic virus, tobacco streak virus	Chatzivassiliou et al. (2000), Naqvi et al. (1981), Gulya et al. (2002), and Vemana and Jain (2010)
22.	*Withania somnifera*	Eggplant mottled dwarf virus, Tobacco leaf curl virus	Al-Musa and Lockhart (1990)
23.	*Zinnia elegans*	Tobacco leaf curl virus, *Zinnia* mild mottle virus, *Bidens* mottle virus, *Zinnia* leaf curl virus, *Ageratum* enation virus, *Zinnia* potyvirus, *Alternanthera* yellow vein virus, Foxtail mosaic virus	Storey (1931), Huertos (1953), Padma et al. (1974), Logan et al. (1984), Haider et al. (2005), Pandey and Tiwari (2012), Kumar et al. (2010), Maritan et al.(2004), Ha et al. (2008), Pandey and Tiwari (2012), Paulsen and. Niblett (1977), and Wislerl (1984)

boid ovate lamina of 5.00 × 3.4 cm in size bearing multicostate reticulate venation. Flowers are minute and arranged in axillary cyme where male flowers are present towards apex of spike and female flowers towards lower parts of spike in clusters of 3–5, subtended by a leafy bract.

Pharmacological Profile as a Medicinal Plant It has been reported to be useful in treating pneumonia, asthma, rheumatism and several other ailments (Chopra et al. (1956)). It has been traditionally used as anthelmintic, cathartic, insanity, purgative and laxative and also used in earache, snakebite and scabies as well as in pyorrhoea, in eczema, in malarial fever and also in mental disorder (Krishnaiah et al. 2009). *Acalypha indica* has been extensively used in Ayurvedic system of medicine for various ailments. It has been reported to possess hepatoprotective; anti-inflammatory, antitussive, antifungal and antibacterial activities. It is also used for healing wounds (Gupta 2010). Powder of dry leaves is used in bed sores. It has also been reported for antifungal and antibacterial activities (Jagatheeswari et al. 2013).

Active Constituents Alkaloids 'acalypus' and 'acalyphine' are cyanogenetic glucoside and triacetonamine, are extremely poisonous to rabbits and cause discoloration of blood and gastrointestinal irritations (Jagatheeswari et al. 2013).

Symptoms of Virus Infection A variety of symptoms were recorded on *Acalypha indica* plants. The infected plants showed yellow-green mosaic symptoms followed by yellow patches intermingled with normal green. There was slight reduction of leaf lamina (Fig. 7.1).

Virus Identification Tomato yellow leaf curl virus has been identified in *Acalypha indica* in North-Eastern Uttar Pradesh (Mall et al. 2014). Suspected infected plants were examined by electron microscopy and nested polymerase chain reaction (PCR) by using CRv301 and CRc1152 primer pairs. The electron micrographs prepared with a leaf-dip method revealed the presence of geminate particles of size 20 nm in diameter. PCR products of the expected size ~ 870 bp were obtained from *Acalypha* samples. The results of the PCR investigation revealed that the *Acalypha* plant showing yellow mosaic was positive for Tomato yellow leaf curl virus (ToLCV). The virus isolate was identified as ToLCV of genus *Begomovirus* and family Geminiviridae.

7.2.2 *Ageratum conyzoides* Linn.

Family Asteraceae

Distribution *Ageratum conyzoides* is an annually occurring global weed. The centre of origin is considered to be Central America and the Caribbean from where it has been distributed to other parts of the world. *A. conyzoides* is a widely and frequently occurring noxious weed in South Asia and is often difficult to control (Swarbrick 1997). *A. conyzoides* can grow from sea level to a very high altitude of up to 2400 m (Dogra et al. 2009). It thrives best in rich, moist, mineral soils with high humidity and tolerates shade. It is a very common weed on wastelands, railway tracks and roadsides (Swarbrick 1997).

Botanical Description It is an erect, annual, branched, slender, hairy and aromatic herb, which grows to approximately 1 m in height. The stems and leaves are covered with fine white hairs, and the leaves are stalked, ovate, 4–10 cm long and 1–5 cm wide, with tip and base somewhat pointed and with round-toothed margins long. The flowers are purple to white, less than 6 mm across and arranged in close terminal inflorescences. The fruit is black and is easily dispersed, while the seeds are photoblastic and often

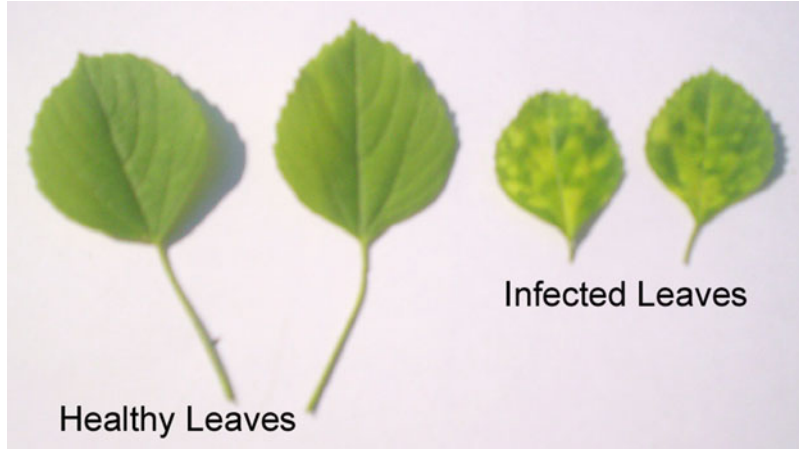

Fig. 7.1 Healthy and infected leaves of *Acalypha indica*

lost within 12 months. The plant grows commonly in waste and on ruined sites. It has a peculiar odour likened in Australia to that of a male goat and hence its name 'goat weed' or 'billy goat weed'(Kamboj and Saluja 2008).

Pharmacological Profile as a Medicinal Plant In Central Africa it is used to treat pneumonia, but the most common use is to cure wounds and burns (Okunade 2002). *A. conyzoides* is also utilised to treat fever, rheumatism, headache and colic (Okunade 2002). In Cameroon aqueous extracts of leaves or the whole plant are used as an antidiabetic. Traditional communities in India use this plant as a bactericide, antidysenteric and antilithic (Okunade 2002), and in Asia, South America and Africa, aqueous extract of this plant is used as a bactericide (Okunade 2002). The leaves are used on cuts, sores; as anti-inflammatory agent, haemostatic, as an insecticide, in headache, in boils, skin diseases, ringworm infection, in typhoid, as an antidote to snake poison, in malarial fever, as antitetanus; for uterine problems, prolapse of anus, for swollen piles, in throat infection, painful gums; on abscess for early suppuration; in wound healing, leucorrhoea and diabetes (Nyemb et al. 2009). The root is used as an antilithic and in infant diarrhoea. It has been reported to have nematocidal activity and have potential use in controlling pests. The plant possesses wide medicinal value and is employed to treat colic, cold and fever, diarrhoea, spasms, pneumonia, headache and rheumatism, but it is commonly used for burns and wounds across the world (Okunade 2002).

Active Constituent The chemical composition shows that *A. conyzoides* contain many bioactive compounds including flavonoids, alkaloids, coumarins, essential oils, chromenes, benzofurans, terpenoids and tannins (Kamboj and Saluja 2008). Leaves and flowers yield 0.02 % essential oil with powerful nauseating odour essential oils containing phenols (eugenol) 5 %, consisting almost entirely of a phenolic ester similar to ethyl eugenol (Chopra et al. 1956).

Symptoms of Virus Infection The leaves showed vein-clearing symptom, i.e. the vein becomes light in colour. The infected plants were weak and set reduced flower (Fig. 7.2).

Virus Identification *Ageratum conyzoides* has been reported as host for many crop diseases (Ekeleme et al. 2005). It has been described as natural host of begomoviruses, viz., *Ageratum* yellow vein virus from China, Japan, Indonesia, Taiwan, Pakistan and Singapore (Tan and Wong 1993; Tan et al. 1995; Sukamto et al. 2005; Haider 1996; Stanley et al. 1997; Saunders et al. 2000; Mansoor et al. 2003; Liu et al. 2008),

Fig. 7.2 Healthy and infected leaves of *Ageratum conyzoides*

Ageratum yellow vein Taiwan virus from Taiwan and China (Wu et al. 2008), Tomato yellow leaf curl Tanzania virus from Tanzania (Kashina et al. 2003), Pepper yellow leaf curl Indonesia virus from Indonesia (Shibuya et al. 2007; Sakata et al. 2008), *Ageratum* yellow vein China virus from China and the Philippines (Xiong et al. 2007), *Ageratum* yellow vein Java virus from Indonesia (Kon et al. 2007), *Ageratum* yellow vein Singapore virus from Singapore (Wong et al. 1993), *Ageratum* enation virus from Pakistan (Tahir et al. 2015; Briddon et al. 2002) and *Ageratum* leaf curl virus from China (Huang and Zhou 2006). *Ageratum* enation virus (AEV) has been reported from North-Eastern Uttar Pradesh. It is a *Begomovirus* on *A. conyzoides* (Kumar et al. 2011; Pandey et al. 2011).

7.2.3 *Calotropis procera* Linn., R. Br.

Family Asclepiadaceae

Distribution *C. Procera* is drought-resistant and salt-tolerant to a relatively high degree, and it disperses seeds through wind and animals. It quickly becomes established as a weed along degraded roadsides and lagoon edges and in overgrazed native pastures. It has a preference for and is often dominant in areas of abandoned cultivation especially sandy soils in areas of low rainfall, assumed to be an indicator of overcultivation. *C. Procera* is native to India, Pakistan, Nepal, Afghanistan, Algeria, Iran, Iraq, Israel, Kenya, Kuwait, Niger, Nigeria, Oman, Saudi Arabia, United Arab Emirates, Vietnam, Yemen and Zimbabwe.

Botanical Descriptions *Calotropis procera* occurs as a single or many stemmed soft-wooded shrub and occasionally a tree reaching 6 m. All parts of the plant exude white milky latex when cut. The bark is thick, rough and corky and yellow-brown in colour; twigs are green and fleshy and may have a covering of tomentum (white fur-like hairs). Leaves are opposite-decussate, simple, ovate to obovate with 4–6 pairs of subopposite nerves prominent on the abaxial surface, an acute apex, sessile (almost decurrent) base, a pale green colour and quite large which is about 30 × 25 cm. Inflorescences arise from the base of the leaves in pedunculate (c.7 cm) cymes of 3–20. Flowers consist of five small triangular dirty white sepals, five thick ovate petals (c1cm x 1 cm) which are white at the base and purple at the tips and five purple tipped stamens, which surround a white five-lobed stigma. Fruits consist of green, spongy ovoid fruits (follicles), up to 15 cm long by 10 cm wide. They split open to release plumed, papery light brown seeds with a pappus of white filaments up to 6 cm long on one side. The main flowering period would be from March to October (Nandkarni 2000).

Pharmacological Profile as a Medicinal Plant Compounds derived from the plant have been found to have emeto-cathartic and digitalic properties (Chopra et al. 1956). The latex contains a proteolytic enzyme called caloptropaine (Kishore and Chopra 1997). An infusion of bark powder is used in the treatment and cure of leprosy and elephantiasis. The root bark is an emetic and the flower a digestive, and a tonic is used for asthma and catarrh. Roots (extremely poisonous) are applied for snakebite. The milky sap is used as a rubefacient and is also strongly purgative and caustic. The latex is used for treating ringworm, guinea worm blisters, scorpion stings, venereal sores and ophthalmic disorders and is also used as a laxative (Basu and Chaudhuri 1997).

Active Constituent Phytochemical studies on *Calotropis procera* have afforded several types of compounds such as cardenolide, triterpenoids, alkaloids, resins, anthocyanins and proteolytic enzymes in latex, flavonoids, tannins, sterol, saponins and cardiac glycosides. Flowers contain terpenes, multiflorenol and cyclisadol (Chopra et al. 1956). The leaves contain mainly the amyrin, amyrin acetate, ß-sitosterol, ursolic acid, cardenolides, calotropin and calotropagenin. The latex contains caoutchouc, calotropin, calotoxin 0.15 %, calactin 0.15 %, uscharin 0.45 %, trypsin, voruscharin, uzarigenin, syriogenin and proceroside (Sharma et al. 2011).

Symptoms of Virus Infections Infected plants are found to show mosaic-like symptoms having yellow-green intermingled rings. There was a slight reduction of leaf lamina (Fig. 7.3).

Virus Identification Groundnut bud necrosis virus has been identified in *Calotropis gigantea* (Bhaskara et al. 2011).

7.2.4 *Cassia tora* Linn.

Family Fabaceae; subfamily, Caesalpiniaceae

Distribution *Cassia tora* (subfamily, Caesalpinioideae; family, Leguminosae/Fabaceae) is a small shrub which grows up in warm moist soil throughout the tropical parts of Asian and African countries. It is known by different names in different places like wise Foetid *Cassia tora*, Sickle Senna, Wild Senna, sicklepod, coffee Pod, Tovara, Chakvad and ringworm plant.

Botanical Descriptions *C. tora* has 10 cm long pinnate leaves, and each leaf has three pairs of leaflets that are opposite, ovate, oblong and oblique at the base. The yellow-coloured flowers are bearded in the axil of the leaves. The flowers consist of half inch diameter five petals. The seeds of *C. tora* are rhombohedral and brown in colour. The *C. tora* gets flowers in the rainy season and the fruits in the winter season. *C. tora* leaves, seeds and roots are utilised as food ingredients since long (Ingle et al. 2012).

Pharmacological Profile as a Medicinal Plant Many medicinal properties such as antimicrobial, antihepatotoxic and antimutagenic activities have been attributed to this plant. It has been used to treat constipation, oedema, glaucoma, nyctalopia, conjunctivitis, hypertension, hypercholesterolaemia and liver damage and is sometimes eaten as vegetable. It is very useful in treating skin diseases like ringworm and itching or body scratch and psoriasis. The alcoholic or vinegar maceration of pounded fresh leaves is used externally to treat eczema and dermatomycosis. Decoction of the fruit of *Cassia tora* is used in the treatment of fever. Since the herb acts as a kapha and vata dosha suppressant, it acts as a nerve tonic (Shukla and Kumar 2013). It is consumed in worm infestation and cures the infection occurring in the body. *Cassia tora* acts as a liver stimulant, mild laxative and heart tonic. The herb helps the body in maintaining the normal level of cholesterol. Its paste is used for treating skin ailments and also for getting rid of chronic diseases. *Cassia tora* proves worthwhile in treating piles and haemorrhoids as well as relieving the pain caused on excretion. Its pow-

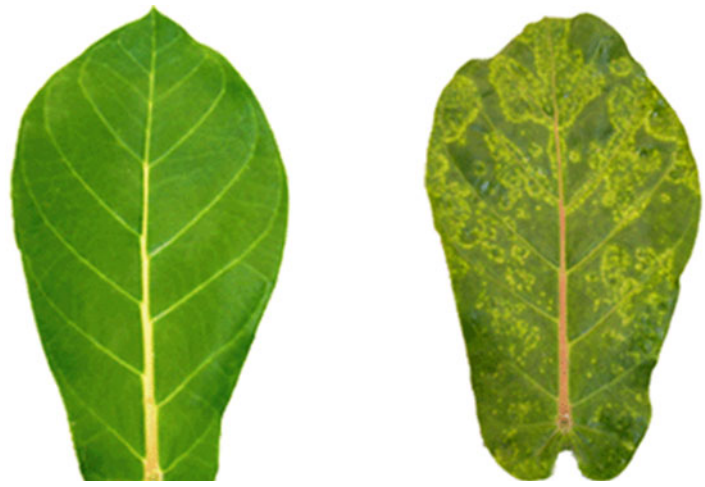

Fig. 7.3 Healthy and infected leaves of *Calotropis procera*

der proves useful in combating indigestion, toning up heart muscles and purifying blood. The juice extracted from its leaves is used in case of skin ailments, rashes and allergies. It is also used as an antidote in case of various poisonings. The leaves and seeds of *Cassia tora* are useful in leprosy, flatulence, colic, dyspepsia, constipation, cough, bronchitis and cardiac disorders (Shakywar et al. 2011). Many medicinal properties such as antimicrobial, antihepatotoxic and antimutagenic activities have been attributed to this plant. It has been used to treat constipation, oedema, glaucoma, nyctalopia, conjunctivitis, hypertension, hypercholesterolaemia and liver damage and is sometimes eaten as vegetable (Ingle et al. 2012).

Active Constituent The bioactive constituents of *C. tora* are anthraquinones, including 1-desmethylaurantion-obtusin, 1-desmethylchryso-obtusin, aurantio-obtusin, chrysoobtusin and obtusin (Meena et al. 2010). The seeds of *C. tora* contain a variety of bioactive anthraquinones, including chrysophanol, emodin and rhein, which are mainly responsible for their pharmacological actions. From the seeds of *C. tora*, an anthraquinone glucoside was also isolated and characterised as alaternin 2-O-β-D-glucopyranoside. From the roasted seeds of *C. tora*, a new naphthopyrone glycoside was isolated and characterised as 10-[(ß-D-glucopyranosyl-(1>6)-O-ß-D-glucopyranosyl) oxy]-5-hydroxy-8-methoxy-2-methyl4H-l-naphtho [1,2-b] pyran-4-one (isorubrofusarin gentiobioside). Along with isorubrofusarin, gentiobioside, alaternin and adenosine were also isolated and identified. From the leaves of *C. tora*, ononitol monohydrate, which is structurally similar to glycoside, was isolated (Meena et al. 2010).

Symptoms of Virus Infections The plants were weak in comparison to the healthy ones. The infected leaves showed vein banding, i.e. all the other parts of leaf lamina were light in colour, while the veins of the leaves were dark in colour and became thick (Fig. 7.4).

Virus Identification Cassia mosaic virus was first reported in *Cassia tora* by Van Velsen (1961). Infection of *Cassia tora* by tobacco etch virus in the field is reported from Florida (Anderson 1954). In inoculation tests, using carborundum, at the Florida Agricultural Experiment Station, Gainesville, young C. *tora* plants developed systemic infection by the *Cassia* strain and mild and severe strains of tobacco etch (Anderson 1954).

Fig. 7.4 Healthy and infected leaves of *Cassia tora*

7.2.5 *Catharanthus roseus* L(G). Don

Family Apocynaceae

Distribution The plant is a native of Madagascar and hence the name Madagascar periwinkle. It is distributed in West Indies, Mozambique, South Vietnam, Sri Lanka, the Philippines and Australia. It is well adapted to diverse agroclimatic situations prevalent in India and is commercially cultivated in the states of Tamil Nadu, Karnataka, Gujarat, Madhya Pradesh and Assam. The USA, Hungary, West Germany, Italy, Netherlands and the UK are the major consumers.

Botanical Descriptions Leaves are oblong or ovate, opposite, short-petioled and smooth with entire margin. Flowers are borne on axils in pairs. There are three flower colour types, pink, pink-eyed and white. Calyx: with five sepal, green, linear, subulate. Corolla tube is cylindrical with five petals, rose-purple or white with rose-purple spot in the centre; throat of corolla tube hairy, forming a corona-like structure. The anthers are epipetalous borne on short filaments inside the bulging distal end of corolla tube converging conically above the stigma. Two characteristic secretary systems, namely, a column-like nectarium on both sides of pistil and a secretory cringulam circling the papillate stigma with a presumed role in pollination-fecundation process, are present. Ovary bicarpellary, basally distinct with fused common style and stigma. The dehiscent fruit consists of a pair of follicles each measuring about 25 mm in length and 2.3 mm in diameter, containing up to thirty linearly arranged seeds with a thin black tegument. On maturity, the follicles split along the length dehiscing the seeds.

Pharmacological Profile as a Medicinal Plant *Catharanthus roseus* (periwinkle or *Vinca*) is an erect herbaceous perennial plant which is a chief source of patented cancer and hypotensive drugs. It is one of the very few medicinal plants which have a long history of uses as diuretic, antidysenteric, haemorrhagic and antiseptic. It is known for use in the treatment of diabetes in Jamaica and India (Nammi et al. 2003). The alkaloids vinblastine and vincristine present in the leaves are recognised as anticancerous drugs. Vinblastine in the form of vinblastin sulphate is available in market under the trade name 'VELBE' and vincristine sulphate as 'NCOVIN' (Eli Lilly). Vinblastine is used in combination with other anticancer agents for the treatment of lymphocytic lymphoma, Hodgkin's disease, testicular carcinoma and choriocarcinoma. Vincristine is used in acute leukaemia, lymphosarcoma and Wilm's tumour. Its roots are a major source of the alkaloids raubasine (ajmalicine), reserpine and serpentine used in the preparation of antifibrillic and hypertension-relieving drugs. It is useful in the treatment of choriocarcinoma and Hodgkin's disease – a cancer affecting the lymph glands, spleen and liver (Nammi et al. 2003). Its leaves are used for curing diabetes, menorrhagia and wasp stings. Root is tonic, stomachic, hypotensive, sedative and tranquilliser (Chopra et al. 1956).

Active Constituent More than 130 alkaloids and related compounds have so far been isolated and characterised from the plant (Punia et al. 2014; Nejat et al. 2015). These alkaloids include monomeric indole alkaloids, 2-acyl indoles, oxindole, α-methylene indolines, dihydroindoles, bisindole and others. Dry leaves contain vinblastine (vincaleucoblastine or VLB) 0.00013–0.00063 % and vincristine (leurocristine or LC) 0.0000003–0.0000153 % which have anticancer activity (Virmani et al. 1978). Other alkaloids reported are vincoside, isovincoside (strictosidine), catharanthine, vindolinine, lochrovicine, vincolidine, ajmalicine (raubasine), reserpine, serpentine, leurosine, lochnerine, tetrahydroalstonine, vindoline, pericalline, perivine, periformyline, perividine, carosine, leurosivine, leurosidine and rovidine (Van De Heijden et al. 2004). The different alkaloids possessed anticancerous, antidiabetic, diuretic, antihypertensive, antimicrobial, antidysenteric, haemorrhagic, antifibrillic, tonic, stomachic, sedative and tranquillising activities.

Lochnericine" from the dried root bark; alstonine and serpentine are also isolated from roots (Gorman et al. 1959).

Symptoms of Virus Infections The leaves of the plant show yellow-green mosaic-like symptoms, i.e. an irregular intermingling of normal green and yellow area of leaves (Fig. 7.5).

Virus Identification Espinha and Gaspar (1997) reported cucumber mosaic virus (CMV) infection in *C. roseus*, showing mild mosaic, chlorosis and plant distortion. Meanwhile, Tomato spotted wilt virus (TSWV) has also been reported in *C. roseus* with black spots, systemic mosaic, leaf deformation and browning of larger leafs at the bottom part of the plant (Chatzivassiliou et al. 2000). Samad et al. (2008) reported the natural infection of *C. roseus* with an isolate of CMV in India. *Zantedeschia* mild mosaic virus (ZaMMV, Huang and Chang 2005), Carnation mottle virus (Singh et al. 2005), Potato yellow vein virus (PYVV, Salazar et al. 2000) and Tomato spotted wilt virus (TSWV, Chatzivassiliou et al. 2000) have been reported in this plant. A *Potyvirus* has been identified in Gorakhpur in *Catharanthus roseus* (Srivastava et al. 2012). In the purified preparations, long flexuous filamentous particle was observed with model length of 760 nm and 14 nm width.

7.2.6 *Centella asiatica* Linn.

Family Umbelliferae (Apiaceae)

Distribution *Centella asiatica* is found throughout tropical and subtropical regions of India up to an altitude of 600 m. The plant has been reported to occur also at high altitudes of 1550 m in Sikkim and 1200 m in Mount Abu (Rajasthan). The plant is indigenous to South-East Asia, India and Sri Lanka, parts of China, the Western South Sea Islands, Madagascar, South Africa, South-Eastern USA, Mexico, Venezuela, Columbia and Eastern South America.

Botanical Descriptions *Centella asiatica* is a slender trailing herb, rooting at the nodes. It has long, reddish, prostate stem emerging from the leaf axils of a vertical root stock. Leaves are orbicular, reniform, entire, crenate, glabrous, 1.3–7 cm in diameter. Flowers are sessile, white or reddish and covered by bracts, and 3–6 flowers are arranged in an umbel. Fruits are small, com-

Fig. 7.5 Infected and healthy plants of *Catharanthus roseus*

pressed and 8 mm long; mericarps are curved, rounded at the top, broad and 7–9 ridged. Seeds are compressed laterally. This has a characteristic odour, greyish green colour and bittersweet taste.

Pharmacological Profile as a Medicinal Plant In Ayurveda, it is a popular rasayana drug and is used as medhya rasayana in the CNS disorders like epilepsy, schizophrenia and cognitive dysfunction. It also finds use in renal stones, leprosy and skin diseases, anorexia and asthma. In other traditional systems, it has been additionally used in the management of diarrhoea, cholera, measles, jaundice, leucorrhoea, haematemesis, hepatitis, urethritis, toothache, syphilis, smallpox, neuralgia, rheumatism, toothache and varices and as an antipyretic, analgesic and anti-inflammatory (Singh et al. 2010). It is also recomended in diseases of skin, nerves and blood. Leaves are taken as tonic and improve memory and are useful in syphilitic skin diseases both internally and externally. It has antimicrobial, cytotoxic and antioxidant activity (Ullah et al. 2009).

Active Constituents The major principles in the plant are the triterpenes, asiatic acid and madecassic acid and their derived triterpene ester glycosides, asiaticoside and madecassoside. The whole plant on extraction with diethyl ether has yielded β-pinene, α-terpinene, bornyl acetate, α-copaene, β-elemene, β-caryophyllene, trans-β-farnesene, germacrene-D and bicycloelemene through GC-MS analysis (Singh et al. 2010). The roots are rich in amino acids, specially aspartic, glutamic, serine, threonine, alanine, lysine and histidine (Chopra et al. 1956).

Symptoms of Viral Infection The plants show chlorosis (weakens green colour); initially the young growing leaves are infected. Chlorotic tissues contain less chlorophyll and appear pale green or yellowish in colour. In extreme case of chlorosis, leaves show bleaching in which chlorophyll completely disappears (Fig. 7.6).

Viruses Reported Cardin and Moury (2010) reported cucumber mosaic virus in *Centella asiatica* in Madagascar.

7.2.7 *Chrysanthemum indicum* Linn.

Family Asteraceae

Distribution Widely cultivated for ornamental and medicinal purposes

Fig. 7.6 Healthy and infected plants of *Centella asiatica*

Botanical Descriptions It is an erect or ascending, somewhat whitish, hairy herb, perennial, aromatic, 30–60 cm high. Leaves: thin, pinnately lobed, ovate to oblong-ovate, and 4–6 cm long; lobes 2–3 on each side, ovate or oblong-ovate and sharply toothed; upper surface of the leaves deep green while the under surface grey-green. Flowers: in heads; heads yellow, peduncled, corymbosely panicled and 1.5–2.5 cm in diameter; involucre bracts oblong or elliptic and as large as the achenes. Receptacle smooth or pitted, not paleaceous. Ray flowers 1-seriate, female, ligule spreading, disc flowers numerous, perfect, limb 4- to 5-fid; fruits: achenes, very small, cuneate-oblong, somewhat compressed and grooved.

Pharmacological Profile as a Medicinal Plant *Chrysanthemum indicum* has been used as a herbal medicine, which is prescribed for anti-inflammatory, analgesic, antipyretic purposes and the treatment of eye disease in Chinese traditional preparations. It showed inhibitory activity against rat lens aldose reductase and against nitric oxide (NO) production in polysaccharide-activated macrophages (Yoshikawa et al. 1999, 2000). It was also proved to be effective to inhibit the agglutination of blood platelet and promote the myocardial blood circulation and white cell phagocytosis, and therefore it was used to treat many diseases such as furuncle (Zhang 1997). It is a perennial, aromatic, medicinal plant used in the traditional folk medicine in Asia and Europe, against nephritis and women's diseases and in the treatment of neurological problems and headache (Chang et al. 2010). It was also found to have antimicrobial, antioxidant and anti-inflammatory properties (Park et al. 2012). Extracts of chrysanthemum plants (stem and flower) have been shown to have a wide variety of potential medicinal properties, antibacterial (Sassi et al. 2008) and antiarthritis (Xie et al. 2008). It has been taken orally or used externally to treat various clinically infectious diseases and dermatitis, such as influenza, tonsillitis, stomatitis, bronchitis, pneumonia, appendicitis, dermatitis and furuncle (Park et al. 2012). It possesses antihyperkinesia, antiasthma, antimicrobial, antiviral and parasitical activities (Chang et al. 2010).

Active Constituents Sixty-three volatiles were detected and the abundant volatiles included 2,6,6trimethyl-bicyclo[3.1.1]hept-2-en-4-ol, 2-(2,4-hexadiynylidene)-1,6-dioxaspiro[4.4]non-3-ene, germacrene-D, α-neoclovene, eucalyptol, α-pinene. Ten flavonoids were identified. Quercitrin, myricetin and luteolin7-glucoside were abundant flavonoids. It is considered that *Chrysanthemum indicum* flower is a good source of natural quercitrin and myricetin, which is significant for the development of potential pharmaceuticals (Wu et al. 2010).

Symptoms of Virus Infection The infected plant is characterised by vein-clearing and mosaic symptoms in their leaves (Fig. 7.7).

Virus Identification Holling (1955) first investigated virus on chrysanthemum. An expected PCR product of size ~500 bp was amplified from total DNA extracts of symptomatic leaf samples with universal primers on the gene of coat protein region of *Begomovirus* DNA-A component. The presence of begomoviruses was also confirmed by Southern blot analysis using control cloned DNA-A probe of Cotton leaf curl virus. Sequence analysis of the virus infecting *Chrysanthemum indicum* showed 99 % nucleotide sequence identity with Clerodendron yellow mosaic virus (EF408037). Sequence analysis was carried out using BLAST, which revealed that the isolated *Begomovirus* (JN998441) infecting *Chrysanthemum indicum* showed 99 % sequence identity with Clerodendron yellow mosaic virus (EF408037) and Clerodendron golden mosaic China virus (FN645907) (Marwal et al. 2013a).

7.2.8 *Croton bonplandianum* Baill.

Family Euphorbiaceae

Distribution *Croton bonplandianum* Baill. (Euphorbiaceae), commonly known as 'Ban Tulsi', is a perennial herb found in wastelands and roadside areas in India.

Fig. 7.7 *Chrysanthemum indicum* healthy and infected leaves

Botanical Descriptions A much-branched woody herb, 20–50 cm tall, branches moderately stellate-hairy to subglabrous. Leaves alternate or subopposite, petiolate, petioles 2–6 mm long, slender, sparsely to densely stellate-hairy, leaf blade narrowly ovate-lanceolate. Inflorescence: terminal, 5–9 cm long, sparsely stellate-hairy to subglabrous. Flowers: laxly distributed. Male flowers: pedicellate, slender, glabrous, petals smaller than sepals, white, hairy at the base, stamens 12. Female flowers: present at the base of the inflorescence, stout, densely stellate-hairy, sepals lanceolate; ovary 1 mm in diameter, broadly ellipsoid, densely hairy. Fruit: a capsule, pale brown, sparsely stellate-hairy; seeds are ellipsoid, grey and minutely foveolate.

Pharmacological Profile as a Medicinal Plant Traditionally, this plant is used to treat liver and skin disease including ringworm infection and also to cure the swelling of the body. Bark and roots of *C. bonplandianum* are alterative and cholagogue. Leaves of this plant are medicinally used for the treatment of cuts and wounds, venereal sores and cholera. The seeds are used for the treatment of jaundice, acute constipation, abdominal dropsy and internal abscesses. The genus *Croton* is rich in secondary metabolites including alkaloids and terpenoids and also possesses toxic components, phorbol esters. Phytochemically, the plant *C. bonplandianum* has been reported to contain rutin (C18H36O19) as main constituent, crotsearinine and crotasparine and its methyl derivatives aphorbol (Singh et al. 2014).

Active Constituents Plant and leaves contain alkaloids sparsiflorine, crotoflorine, crotsparine, crotsparinine, proaporphine, isoquinoline dienone, N-methylcrotsparine and N-methylcrotsparinine. Leaves and stem contain ß-sitosterol and taraxerol, vomifoliol, ursolic acid and tetrahydroglazievine. Leaves also contain rutin. Seeds contain phorbol diesters, phorbol trimesters, cocarcinogen, alkaloid, 3-OMe-4, 6-di-OH-morphinan-dien-7-one and norsinoacutine The roots in addition to ß-sitosterol contain phenolic quinonoid alkaloid norsinoacutine and 3-methoxy-4,6-dihydroxy morphinan-dien-7-one (Asolkar et al. 1992).

Symptoms of Viral Infection The plants were weak in comparison to the healthy ones. The infected leaves showed chlorosis symptoms. The newly born leaves become light in colour (Fig. 7.8).

Fig. 7.8 Healthy and infected plants of *Croton bonplandianum*

Virus Identification Croton yellow vein mosaic virus (CYVMV) is a widely occurring *Begomovirus* in *Croton bonplandianum.* CYVMV produced bright yellow vein symptoms in croton. The complete genome sequences of four isolates originating from northern, eastern and southern India revealed that a single species of DNA-A and a croton yellow vein mosaic betasatellite (CroYVMB) were associated with the yellow vein mosaic disease of croton (Hussain et al. 2011). The sequence identity among the isolates of CYVMV DNA-A and CroYVMB occurring in diverse plant species was 91.8–97.9 % and 83.3–100 %, respectively (Snehi et al. 2011; Pramesh et al. 2013).

7.2.9 *Datura metel* Linn.

Family Solanaceae

Distribution Datura is distributed throughout the world, particularly the warmer regions. They are found commonly in wastelands, gardens and roadsides. They are distributed in rich localities under semiarid and arid regions of Punjab, Haryana, Rajastan and Gujarat and the Central Plateau of Andhra Pradesh and Maharashtra and the southern peninsular region of Tamil Nadu.

Botanical Descriptions The genus *Datura*, belonging to the family Solanaceae, consists of annual and perennial herbs, shrubs and trees. Three species, viz. *Datura metel* Linn., *D. stramonium* Linn. and *D. innoxia* Mill. are medicinally important. *D. innoxia* mill. and *D. metel* Linn. (var. alba and var. fastuosa) are the choice drug plants, rich in hyoscine. *D. metel* Linn. is the most common in India. The names, *D. metel* Linn, *D. fastuosa* Linn, *D. alba* Nees., *D. fastuosa* Linn. var. alba (Nees) C.B. Clarke and D. metel Linn. var. fastuosa (Linn.) Safford are synonymously used by many workers. Two varieties are often noted in *D. metel* Linn., namely, the white flowered var. alba and purple flowered var. fastuosa. *D. metel* Linn. is an erect succulent branched undershrub divaricate often with purplish branches and ovate pubescent leaves which are oblique at the base of lamina. Flowers are large, solitary, short pedicelled, purplish outside and white inside. Fruits are subglobose capsules covered all over with numerous, fleshy prickles, irregularly breaking when mature. Seeds are numerous, smooth and yellowish brown.

Pharmacological Profile as a Medicinal Plant Downy datura or thorn apple is an erect branched undershrub, and its intoxicating and narcotic properties have been made use of by man since ancient times. The plant and fruit are spasmolytic, anticancerous and anthelmintic (Asolkar et al. 1992). Leaves and seeds are inhaled in whooping cough, asthma and other respiratory diseases. Root, leaf and seed are febrifuge, antidiarrhoeal and anticatarrhal and are used in insanity, cerebral complications and skin diseases. Leaf is antitumour, antirheumatic and vermicide. Flower is antiasthmatic and anaesthetic and is employed in swellings and eruptions on face. Fruit juice is used in earache and seed decoction in ophthalmia. For the rheumatic swellings of joints, lumbago, sciatica and neuralgia, warm leaf smeared with oil is used as a bandage or sometimes the leaf is made into a poultice and applied. The root boiled with milk is used to cure insanity. It is also an ingredient in the ayurvedic preparation Kanakasava used in bronchial troubles, and the Unani formulations 'Roghan dhatur' used as massage oil for the paralysed part. The alkaloids of pharmaceutical interest present in the plant are hyoscyamine, hyoscine and meteloidine. Datura is the chief commercial source of hyoscine available from a natural source. Hyoscine, in the form of hyoscine hydrobromide, is used as a preanaesthetic in surgery, child birth and ophthalmology and in the prevention of motion sickness. It is also employed in the relief of withdrawal symptoms in morphine and alcoholic addiction, paralysis agitans and postencephalitic parkinsonism and to allay sexual excitement. Hyoscyamine and its salt hyoscyamine sulphate and hyoscyamine hydrobromide are used in delirium, tremor, mania and parkinsonism (Neeraj et al. 2013).

Active Constituent Tropane alkaloid such as scopolamine, hyoscyamine and atropine. The alkaloids hyoscyamine and hyoscine (scopolamine) and meteloidine are found in all parts of the plant. The total alkaloid content is 0.26–0.42%. Fruits contain daturaolone and daturadiol, while roots contain additionally ditigloyloxy tropane derivatives, tigloidine, apohyoscine, norhyoscine, norhyocyamine, cuscohygrine and tropine. Other alkaloids isolated from the plant are apohyoscyamine, DL-scopolamine, normeteloidine, tigloylputrescine, scopine, nortigloidine, tropine, pseudo valeroidine, fastudine, fastunine, fastusinine, 7-hydroxy-3, 6-ditigloyloxytropane (2) datura nolone and fastusic acid. The physiological effects of hyoscyamine are qualitatively the same as those of its racemic derivative atropine. This is relatively more active in its paralysing affect on nerve endings and less active in its stimulant action on the central nervous system. The sedative and hypnotic action of hyoscyamine is weaker than that of hyoscine. Atropine has a stimulant action on the central nervous system and depresses the nerve endings to the secretary glands and plain muscles. The plant or the different alkaloids have narcotic, anthelmintic, spasmolytic anaesthetic, sedative, ophthalmic, anticancerous, antitumour, antirheumatic, antiasthmatic, antidiarrhoeal and anticatarrhal activities (Neeraj et al. 2013).

Symptoms of Virus Infection on Leaves The infected plant showed mosaic and deformity symptoms (Fig. 7.9).

Virus Identification The causal virus of leaf distortion disease of *D. metel* was identified as a potyvirus (Prasanna et al. 1996). The virus was transmitted non-persistently by *Aphis craccivora* and *Myzus persicae* but not transmitted through seeds. The virus in the *D. metel* leaf sap stored at 25°–30°C retained infectivity for 5 days and was infective up to 10^{-5} dilutions and at 50 °C. Partially purified preparations contained flexuous filamentous virus particles (Prasanna et al. 1996).

7.2.10 *Datura Stramonium* Linn.

Family Solanaceae

Distribution *Datura* is distributed throughout the world, particularly the warmer regions. Out

Fig. 7.9 *Datura metel* infected and healthy leaves

of 15 species reported from different parts of the world, only 10 are known to occur in India. *Datura stramonium* is indigenous to India. They are found commonly in wastelands, gardens and roadsides.

Botanical Descriptions *Datura stramonium* is commonly known as Jimson weed. It is 60–120 cm or more tall, branched and pubescent plant. Leaves are 8–17 × 4–13 cm, ovate, sinuately dentate and minutely puberulose. The flowers are trumpet-shaped, white to creamy or violet and 6–9 cm long. Mostly found in temperate and subtropical region. Humans use different plant for treatment and still are in search of medicinal value plants. *Datura stramonium* has both poisonous and medicinal uses (Sayyed and Shah 2014).

Pharmacological Profile as a Medicinal Plant Leaves are used in asthma treatment. The primary biologically active substances in *Datura stramonium* are the alkaloids atropine and scopolamine. Atropine has been used in treating Parkinson's disease, peptic ulcers, diarrhoea and bronchial asthma. Its leaves mucilage and polyvinylpyrrolidone combination can be used as a matrix forming material for making sustained release matrix tablets. *Datura stramonium* is a natural source of antioxidants and phytochemical having antimicrobial activities. Its extracts show significant antimicrobial activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus niger* and *Fusarium species* (Reddy 2009). The secondary metabolites of *Datura stramonium* are highly effective against different types of diseases such as antidiabetic, antiviral, etc. Water extract shows insecticidal activities. *Datura stramonium* is used in Ayurvedic system for curing various human ailments, including ulcers, wounds, inflammation, rheumatism and gout, sciatica, bruises and swellings, fever, asthma, bronchitis and toothache. Extracts of leaves show better efficacy than the stem and root. Leaves are used in treatment of parkinsonism and haemorrhoids (Sayyed and Shah 2014).

Active Constituent *Datura stramonium* contains sixty-four tropane alkaloids. It contains alkaloids, tannins, carbohydrates and proteins and used in medicine due to its analgesic and antiasthmatic activities. Two new tropane alkaloids, 3phenylacetoxy-6, 7-epoxynortropane and 7-hydroxyapoatropine, were tentatively identified. The alkaloids scopoline, 3-(hydroxyacetoxy) tropane, 3-hydroxy-6-(2-methylbutyryloxy) tropane, 3a-tigloyloxy-6-hydroxytropane, 3,7-dihydroxy-6-tigloyloxytropane, 3-tigloyloxy -6-propionyloxytropane, 3-phenylacetoxy-6,

7-epoxytropane, 3-phenylacetoxy-6-hydroxytropane, aponorscopolamine, 3a,6a-ditigloyloxytropane and 7-hydroxyhyoscyamine are reported for the first time for this species (Berkov et al. 2006). The main components of essential oil were sterols and their derivatives, and the major constituents of *Datura stramonium* essential oil are sterols and their derivatives and 5.alpha.-ergosta-7, 22-dien-3.beta.-ol (16.53 %), 3-hydroxycholestan-5-yl, acetate (14.97 %) and 26, 26-dimethyl-5, 24(28)-ergostadien-3.beta.-ol (10.39 %) (Wang and You 2012).

Symptoms of Virus Infection on Leaves The infected plant showed yellow-green mosaic symptoms and reduction in size (Fig. 7.10). It showed severe mosaic with blistering of the leaves.

Virus Identification TVBMV was isolated from a *Datura stramonium* in China (Roggero et al. 2000). The plant was also infected by Cucumber mosaic virus (CMV). The 3′-end of the viral genome was amplified by reverse transcription-polymerase chain reaction (RT-PCR) using primers derived from the Potyviridae primers and cloned in p Blue Script. The sequence of 1,630 bp (GenBank AF274315) was determined on both DNA strands and found to have approximately 94 % homology with other TVBMV sequences (L 28816 from Tennessee, X77637 from Taiwan and AB020524 from Japan).

7.2.11 *Helianthus annuus* L.

Family Asteraceae

Distribution Native to western North America. Introduced early in Europe and Russia, the species has now spread to both tropical and temperate countries and is commercially grown for oil and found in the gardens in India.

Botanical Descriptions *Helianthus annuus* L. is a stiff, plump and common annual plant 1–3 m high. It has initially tap roots, but when plant becomes mature, then develops large, fibrous and lateral roots. Stems of this plant are 1–6 ft tall, hispid, round and unbranched. Stem length is determined by the number of internodes. Leaves are arranged in such a way that lowers leaves are mostly opposite along the stem and upper leaves alternate along the stem. Leaves are mostly ovate shaped and mostly 4–20 cm long and 3–15 cm wide or more, apex acute to abruptly acuminate and margins are serrate. Inflorescence of this

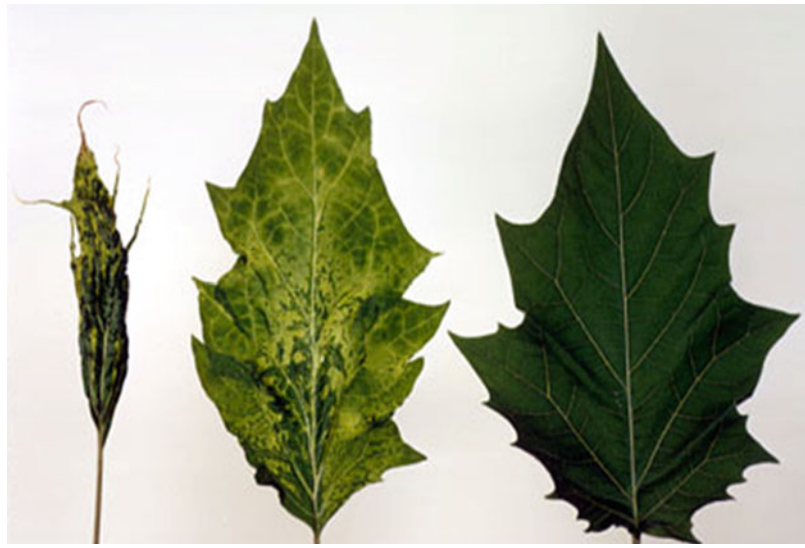

Fig. 7.10 Infected and healthy leaves of *Datura stramonium*

plant is a capitulum composite heads, solitary at terminal of peduncle or terminal on a branch or axillary, ray and disc florets present. Ray flowers are sterile, 0.6–1.6 inch long, and ligules are yellow. Disc flowers are perfect, corolla lobes 5, 0.2–0.3 inch long, tubular and purple brown to yellow. Pappus is two and deciduous and ovary is inferior in disc flower. Fruit of *H. annuus* is an achene. Achenes vary from 7–25 mm in length and 14–13 mm in width. The head of sunflower is made up of 1000–2000 individual flowers which are connected at a common receptacle. It has ray flowers which are ligulated around boundary and are without stamens and pistils, and the other flowers are perfect (Bashir et al. 2015).

Pharmacological Profile as a Medicinal Plant Medicinally, seeds are diuretic and expectorant and used for colds, coughs and throat and lung ailments. Reported to be anodyne, antiseptic, aphrodisiac, bactericidal, deobstruent, diuretic, emollient, expectorant, insecticidal, malaria preventative, sunflower is a folk remedy for aftosa, blindness, bronchiectasis, bronchitis, carbuncles, catarrh, cold, colic, cough, diarrhoea, dysentery, dysuria, epistaxis, the eyes, fever, flu, fractures, inflammations, laryngitis, lungs, malaria, menorrhagia, pleuritis, rheumatism, scorpion stings, snakebite, splenitis, urogenital ailments, whitlow and wounds (Chopra et al. 1956). A tea made from leaves is astringent, diuretic and expectorant; it is used in the treatment of high fevers. The crushed leaves are used as a poultice on sores, swellings snakebites and spider bites. A tea made from the flowers is used in the treatment of malarial and lung ailments. The seed is also considered to be diuretic and expectorant. It has been used with success in the treatment of many pulmonary complaints (Bashir et al. 2015).

Active Constituent The active compounds reported are (2R)-2-hydroxyl-N-[(2S, 3S, 4R, 10E)-1, 3, 4-trihydroxyicos-10-en-2-y1]docosanamide, (2R,3R)-2,3-dihydroxy-N[(2S,3S,4R,10E)-1,3,4-trihydroxyicos-10-en-2-yl] docosanamide, N-(2-phenylethyl) tetracosanamide and (2R)-N-[(2S, 3S,4R,8E)]-1-(β-d-glucopyranosyloxy)-3,4-dihydroxyoctadec-8-en-2-yl]-2-hydroxyhexadecanamide (Bashir et al. 2015).

Symptoms of Virus Infection Infected plants show a wide range of symptoms, ranging from stunting, mottling, leaf distortion and colour break in flowers. The infected plant is characterised by mosaic and vein-clearing symptoms (Fig. 7.11).

Virus Identification Tobacco streak virus (TSV) was isolated from a plant of sunflower (*Helianthus annuus*) showing severe necrosis and chlorosis in the leaves. The virus was identified as TSV by serology (Sharman et al. 2008). Sunflower chlorotic mottle virus (SuCMoV) has been biologically, structurally and serologically characterised as a potyvirus, and the term sunflower chlorotic mottle virus (SuCMoV) has been proposed based on its molecular properties (Bejerman et al. 2010).

7.2.12 *Hibiscus rosa-sinensis* Linn.

Family Malvaceae

Distribution China rose or *Hibiscus rosa-sinensis* is native to Tropical Asia. It is cultivated widely as an ornamental flowering plant in tropical and subtropical regions and is esteemed for its aesthetic value, which has been popular particularly in Indian landscape gardens from ancient times.

Botanical Descriptions Roots are cylindrical of 5–15 cm length and 2 cm in diameter, off-white in colour, light brown transverse lenticies. Roots taste sweet and mucilaginous. Leaves are simple ovate or ovate-lanceolate. Leaves are entire at the base and coarsely toothed at the apex. Taste is

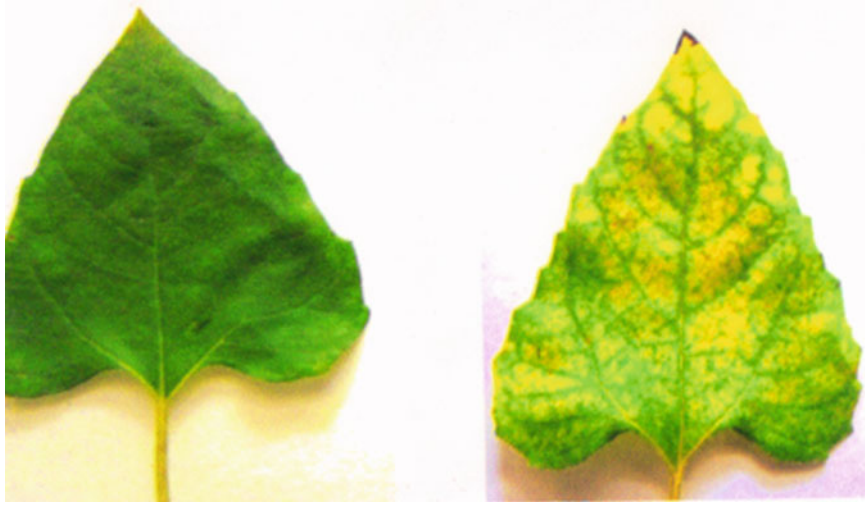

Fig. 7.11 Healthy and infected leaves of *Helianthus annuus*

mucilaginous. Flowers are pedicellate, actinomorphic, pentamerous and complete. Corolla consists of five petals, red in colour and about 3 inches in diameter. The fruit (very rarely formed) is a capsule about 3 cm long.

Pharmacological Profile as a Medicinal Plant Flowers are the economically important part of the plant and are used in decorations. Root is used in cough, a substitute for althaea. Leaves are emollient and aperients. Flowers are emollient; infusion of petals is given as a demulcent and refrigerant drink in fevers. In traditional medicine, the leaves of the plant are used in fatigue and skin disease. Fresh root juice of the plant is given for gonorrhoea and powder root for menorrhagia. Flowers of the plant are used in epilepsy, leprosy, bronchial catarrh and diabetes (Kumar and Singh 2012).

Active Constituent Leaves and stems contain β-sitosterol, stigmasterol, taraxeryl acetate and three cyclopropane compounds and their derivatives. Flowers contain cyanidin diglucoside, flavonoids and vitamins, thiamine, riboflavin, niacin and ascorbic acid (Ghani 2003). Quercetin-3-diglucoside, 3,7-diglucoside, cyanidin3,5-diglucoside and cyanidin-3-sophoroside-5glucoside have been isolated from deep yellow flowers; all above compounds and kaempferol-3xylosylglucoside have been isolated from ovary white flowers (Rastogi and Mehrotra 1993).

Symptoms of Viral Infection The infected leaves are smaller in size and in extreme cases the blister-like appearance is present. The flowers are reduced in number and size (Fig. 7.12).

Virus Identification Five viruses infecting *H. rosa-sinensis* that have been characterised previously are *Hibiscus* chlorotic ringspot virus (HCRSV, genus *Carmovirus*) (Raju 1985; Jones and Behncken 1980), *Hibiscus* latent ringspot virus (HLRSV, genus *Nepovirus*), *Hibiscus* yellow mosaic virus (genus *Tobamovirus*), eggplant mottled dwarf virus (EMDV, genus *Nucleorhabdovirus*) and okra mosaic virus (OkMV, genus Tymovirus). In India, leaf curl disease of *Hibiscus* was first reported in the northern states during the 1950s. The agent of HLCuD was transmitted in a persistent manner by the whitefly, *Bemisia tabaci* (Gennadius) (Aleyrodidae, Hemiptera), which indicated that the disease may be caused by a *Begomovirus*. Further evidence was obtained when *H. rosa-*

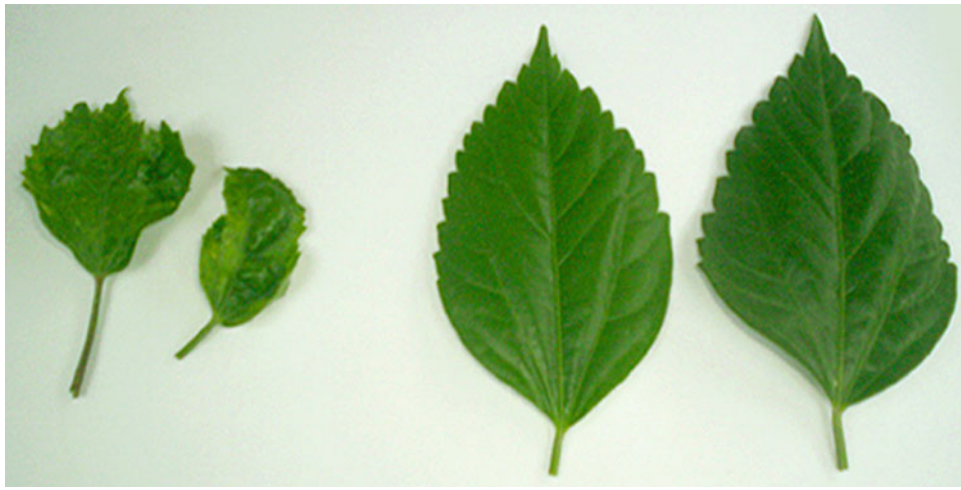

Fig. 7.12 Infected and healthy leaves of *Hibiscus rosa-sinensis*

sinensis was found to be a natural host of a *Begomovirus* (Rajeshwari et al. 2005). Tomato mosaic virus was reported in *Hibiscus rosa-sinensis* (Huang et al. 2004). Vein yellowing of *Hibiscus rosa-sinensis* caused by eggplant mottled dwarf virus was reported in Southern Italy (De et al. 2008; Stradis et al. 2008).

7.2.13 *Jatropha curcas* Linn.

Family Euphorbiaceae

Distribution Native of tropical America, grown in various parts of India as field barrier

Botanical Descriptions *Jatropha curcas* L. is a major commercial biodiesel fuel crop grown on 98 million acres (39.66 million ha) in India. This is a drought-resistant perennial biodiesel plant (Rangaswamy et al. 2005). *Jatropha* (*Jatropha curcas* L.) also known as physic nut is a drought-resistant perennial plant, which is popularly cultivated in the tropics as a living fence. The tree is of significant economic importance for its numerous industrial and medicinal uses. The oil extracted from *Jatropha* seeds is being used as biofuel for diesel engines, thus *Jatropha* has a great potential to contribute to the renewable energy source.

Pharmacological Profile as a Medicinal Plant Root bark is applied externally in rheumatism and is used in sores. Leaves are rubefacient, suppurative and insecticidal and are used in foul ulcers, tumours and scabies and given internally for jaundice. Leaves locally applied to breast to increase secretion of milk. Decoction of leaves is useful in stomach ache, diarrhoea and cough. Twig is used for toothbrushing in swollen gums to the mammal as a lactagogue and rubefacient. Stem bark is used for wounds of animal bites. Fruit and seed are anthelmintic and are useful in chronic dysentery, urinary discharges, abdominal complaints, anaemia and diseases of the heart. Seeds are acro-narcotic and used against wart and cancers, also to promote hair growth. Seed and oil are purgative (Joshi 2006). *Jatropha* has been found to have strong molluscicidal activity and the latex to be strongly inhibitory to watermelon mosaic virus.

Active Constituent Leaves contain apigenin, vitexin and isovitexin, α-amyrin and stigmasterol along with new flavonoid glycosides found in leaves and twigs. Seeds contain fatty oil, phyto sterols, phyto sterolin (glucoside of phytosterol),

curcin, palmitic, oleic and linoleic acid (Joshi 2006) and a large amount of sucrose and a resinous matter having nauseating, purging and griping effect (Chopra et al. 1956)

Symptoms of Virus Infection on Leaves The disease is characterised by marked reduction of leaf size, upward rolling, the margins prominent and puckering of leaf margin and main veins (Fig. 7.13). There is stunted growth and no fruit produced in severely infected plants.

Virus Identification Out of the 22 viruses infecting *Jatropha*, mosaic and leaf curl diseases are the most devastating (Paszkowski et al. 1993). The virus is obligately transmitted by an insect vector, which can be the whitefly *Bemisia tabaci* or can be other whiteflies. This vector allows rapid and efficient propagation of the virus because it is an indiscriminate feeder. Gel diffusion tests were performed with antiserum to *Cucumber mosaic virus* (CMV). Leaf sap of infected plants reacted with PVAS-242a, indicating the presence of CMV. Reverse transcription (RT)-PCR assays with CMV coat protein gene-specific primers (GenBank Accession Nos. AM180922 and AM180923) and total nucleic acid extracted from symptomatic *J. curcas* leaf tissue yielded the expected ~650-bp amplicon, which was cloned and sequenced (GenBank Accession No. EF153739). BLAST analysis indicated 98–99 % nucleotide identity with CMV isolates (GenBank Accession Nos. DQ914877, DQ640743, AF350450, AF281864, X89652, AF198622, DQ152254, DQ141675 and DQ028777). Phylogenetic analysis showed that the *J. curcas* isolate was more closely related to Indian isolates of CMV belonging to subgroup Ib (Raj et al. 2008).

7.2.14 *Ocimum sanctum* Linn.

Family Lamiaceae

Distribution *Ocimum sanctum*, or holy basil, is an aromatic plant that is native to the tropics of Asia and Africa and is widespread as a cultivated plant and weed and is found throughout India, cultivated but doubtfully indigenous.

Botanical Descriptions It is a small shrub with many branches and strongly scented green leaves. The tulsi shrub is an erect plant which grows up to 50–60 cm tall. The leaves are ovate and slightly toothed. The flowers are purplish to white. There are two main types grown in India, green-leaved holy basil (Sri Tulsi) and purple-leaved holy basil (Krishna Tulsi).

Fig. 7.13 Healthy and infected leaves of *Jatropha curcas*

Pharmacological Profile as a Medicinal Plant *Ocimum sanctum* has anti-inflammatory properties as it reduces vata. Hence, its external application on swollen parts helps to diminish swelling and pain. It helps in many skin disorders. It is effective in skin rashes, insect bites and itching. Leaves of this plant are effectively used in ringworm infections and leucoderma. Fresh juice of *Ocimum sanctum* leaves is used in nasya karma. This method helps to relieve headache and diseases of the head and neck. Tulsi leaves act as nervine tonic and help to sharpen memory. Paste and juice of *Ocimum sanctum* leaves help to reduce acne, pimples and scars. According to Ayurveda, preparations of *Ocimum sanctum* are beneficial in indigestion, intestinal parasites and constipation. Crushed leaves of *Ocimum sanctum* are very effective in fever, cough, bronchitis and other diseases of the lungs. It helps in expectoration of excess mucous secretion. *Ocimum sanctum* acts as a cardiac tonic and purifies blood. Seeds of *Ocimum sanctum* are effective in premature ejaculation. Consuming 10–12 leaves of *Ocimum sanctum* per day helps to reduce stress. Juice of leaves is diaphoretic and antiperiodic, stimulates expectorant and is used in catarrhal and bronchitis and dropped into ear as a remedy for earache. Infusion of leaves is used as stomachic and is used in gastric disorders of children and in hepatic affections (Kumar et al. 2013).

Active Constituents In dried leaf powder 49 components were found, and major components were 1-methyl eugenol (89.20 %), 2-eugenol (5.29 %); in methanolic extract, 1-stigmast-5-en-3-ol (17.46 %), 2-stigmast-5, 22-dien-3-ol (13.13 %) and 3-methyl eugenol (6.19 %) were found in majority; in acetonic extract, 1-methyl eugenol (25.31 %) and 2-neophytadiene (7.77 %) were found in majority; and in petroleum ether extract, 1-methyl eugenol (20.97 %), 2-octadecane (17.50 %) and 3-β-caryophylene (8.22 %) were found in majority. The leaf volatile oil contains eugenol (1-hydroxy-2-methoxy-4-allylbenzene), euginal (also called eugenic acid), ursolic acid, carvacrol (5-isopropyl-2-methylphenol), linalool (3,7-dimethylocta-1,6-dien-3-ol), limatrol, caryophyllene, methyl chavicol (also called estragole: 1-allyl-4-methoxybenzene), while the seed volatile oil has fatty acids and sitosterol. The aqueous extract of *O. sanctum* leaves revealed alkaloids, flavonoids, tannins and carbohydrates (Kumar et al. 2013; Chopra et al. 1956).

Symptoms of Viral infection The plant is characterised by puckering of the leaves. The symptoms generally appear first on new growth (Fig. 7.14).

Virus Identification Cucumber mosaic virus on *Ocimum sanctum* is reported (Raj et al. 1997). Tomato spotted wilt virus (TSWV) is reported to infect *Ocimum sanctum* (Parrella et al. 2003).

7.2.15 *Parthenium hysterophorus* Linn.

Family Asteraceae

Distribution *Parthenium hysterophorus* is an invasive exotic weed in India, and it occupies farm- and wastelands, pastures and roadsides. It belongs to the family Compositae and commonly known as carrot weed/congress grass causing pollen allergy to humans and animals.

Botanical Descriptions It grows to 1–1.5 m high, developing many branches in its top half when mature. Pale green leaves, up to 2 mm long, deeply lobed and covered with fine soft hairs. Flowers: small creamy white flowers on stem tips and 4–10 mm in a five-sided shape. Flowers contain four to five black seeds that are wedge-shaped, 2 mm long with two thin, white scales.

Pharmacological Profile as a Medicinal Plant It is used in neurologic disorders, urinary tract infections, dysentery and malaria. Fruits and seeds are said to be useful in the treatment of

Fig. 7.14 Healthy and infected plants of *Ocimum sanctum*

leucoderma. Roots are considered as an abortifacient. It is a promising weed for hepatic amoebiasis. The perusal of available literature shows that it has some chemicals, which can be effective in the treatment of cancer (Patel 2011).

Active Constituents It contains a bitter glycoloid parthenin and a major sesquiterpene lactone. Other phytotoxic compounds are hysterin, ambrosin, flavonoids such as quercetagetin 3,7 dimethyl ether and 6-hydroxykaempferol, anisic acid, p-anisic acid, chlorogenic acids, furfural acid, sitosterol and some unidentified alcohols (Patel 2011).

Symptoms of Viral Infection The infected plant is characterised by mosaic and mottling. The plant becomes weak and in severe cases the complete distortion of plants (Fig. 7.15). Symptoms on *Parthenium* weed included chlorotic spots/ chlorotic ring spots which turn to necrotic followed by mosaic and wilting of leaf.

Virus Identification In DAC-ELISA, field-infected and sap-inoculated *Parthenium* and cowpea samples have shown positive reaction against Groundnut bud necrosis virus (GBNV) using specific polyclonal antisera supplied by ICISAT, India GBNV which belongs to the genus *Tospovirus* and family Bunyaviridae (Vemana et al. 2015). Tobacco streak virus has been also reported in *Parthenium hysterophorus* (Vemana and Jain 2010).

7.2.16 *Phyllanthus niruri* Linn.

Family Euphorbiaceae

Distribution *Phyllanthus niruri* (Bhumi amla) is an annual herbaceous plant which grows to life in the terrain regions of Jharkhand, Chhattisgarh, Bihar and other states of India. It is common to rainy forests of the Bahamas, Southern India and China.

Botanical Descriptions *Phyllanthus niruri* is a small erect annual herb that grows 30–40 cm in height. It is indigenous to the rainforests of the Amazon and other tropical areas throughout the world, including the Bahamas, southern India and China. *P. niruri* is quite prevalent in the Amazon and other wet rain forests, growing and spreading freely (much like a weed).

Pharmacological Profile as a Medicinal Plant *Phyllanthus niruri* is also known as

Fig. 7.15 Infected and healthy leaves of *Parthenium hysterophorus*

Bhumi amla. The plant is gaining immense popularity owing to its hepatoprotective activities in viral hepatitis A and B. Not only against viral infections of the liver, *Phyllanthus niruri* is extremely beneficial against viral infections of all kinds in general. It is used as a diuretic in dropsical affections, gonorrhoea and other troubles of genitor-urinary tract and is an effective treatment for urolithiasis (Boim et al. 2010). It is also reported for its anti-HIV activities and anti-hepatitis B virus (HBV) (Naik and Juvekar 2003). It is used in stomach troubles such as dyspepsia, colic, diarrhoea and dysentery and also for dropsy and diseases of the urinogenital systems. Fresh root is a remedy for jaundice. Infusion of young shoots is given in dysentery (Joshi 2006).

Active Constituents The main active principles of the herb are phyllanthin and hypophyllanthin. Leaves contain phyllanthin and hypophyllanthin. Three lignans niranthin, nirteralin and phyletralin are obtained from leaves. Stem contains saponin (Joshi 2006).

Symptoms of Virus Infection The infected plant is characterised by mosaic and mottling. The plant becomes weak and in severe cases the complete distortion of plants (Fig. 7.16).

Virus Identification Identification of virus is yet to be done.

7.2.17 *Pouzolzia indica* Gaud.

Family Urticaceae

Distribution This is found throughout India.

Botanical Descriptions A perennial herb, very variable in size and habit; stem erect or prostrate, 15–30 cm long. Leaves: 2–3.8 cm long; ovate or ovate-lanceolate; obtuse, acute or acuminate; entire. Flowers minute, in small axillary androgynous clusters.

Pharmacological Profile as a Medicinal Plant Plant is used in syphilis, gonorrhoea and snake poison (Chopra et al. 1956). Leaves are anthelmintic and vulnerary and used as a cicatrisant for gangrenous ulcers, in syphilis and gonor-

Fig. 7.16 Infected and healthy leaves of *Phyllanthus niruri* Linn.

rhoea. Leaf juice is used as galactagogue. Poultice of the herb is applied to sores and boils and to relieve stomach ache. The methanolic extract from the aerial parts of *Pouzolzia indica* shows antiproliferative effect on NB4 and HT93A acute leukemic cell lines with the IC50 values of 28.5 and 49.8 μg/mL, respectively (Sangsuwon et al. 2013).

Active Constituents The compounds isolated using chromatographic technique contained friedelin 1, 28-hydroxy-3-friedelanone 2 and 7-methoxy-coumarin; 6, 7-dimethoxy-coumarin 4, scopoletin 5 and methyl caffeate; and sitosterol glucoside and a supposed glycosphingolipid (Sangsuwon et al. 2013).

Symptoms of Viral Infection The plants showed chlorotic symptoms. The symptoms generally appear first on new growth; the leaves become light green in colour and reduced in size (Fig. 7.17).

Virus Identification Identification of virus is yet to be done.

7.2.18 *Rauvolfia serpentina* Benth. ex Kurz.

Family Apocynaceae

Distribution *Rauvolfia serpentina* is native to India. Several species of *Rauvolfia* are observed growing under varying edaphoclimatic conditions in the humid tropics of India, Nepal, Burma, Thailand, Bangladesh, Indonesia, Cambodia, the Philippines and Sri Lanka. In India, it is cultivated in the states of Uttar Pradesh, Bihar, Tamil Nadu, Orissa, Kerala, Assam, West Bengal and Madhya Pradesh. Thailand is the chief exporter of *Rauvolfia* alkaloids followed by Zaire, Bangladesh, Sri Lanka, Indonesia and Nepal. In India, it has become an endangered species and hence the government has prohibited the exploitation of wild-growing plants in the forest and its export since 1969.

Botanical Descriptions *R. serpentina* is an erect perennial shrub generally 15–45 cm high, but growing up to 90 cm under cultivation. Roots are nearly vertical, tapering up to 15 cm thick at

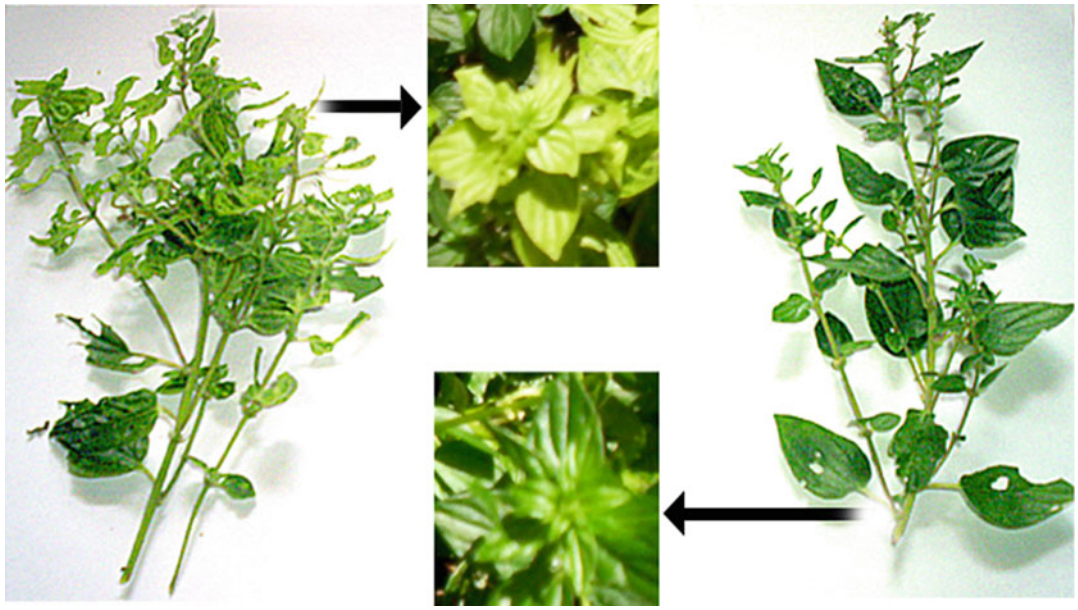

Fig. 7.17 Infected and healthy plants of *Pouzolzia indica*

the crown and long, giving a serpent-like appearance, occasionally branched or tortuous developing small fibrous roots. Roots are greenish-yellow externally and pale yellow inside and have an extremely bitter taste. Leaves are born in whorls of 3–4 elliptic-lanceolate or obovate, pointed. Flowers are numerous, borne on terminal or axillary cymose inflorescence, corolla tubular, 5-lobed, 1–3 cm long, whitish-pink in colour, stamens 5, epipetalous, carpels 2, connate, style filiform with large bifid stigma. Fruit is a drupe, obliquely ovoid and purplish black in colour at maturity with stone containing 1–2 ovoid wrinkled seeds. The plant is cross-pollinated, mainly due to the protogynous flowers.

Pharmacological Profile as a Medicinal Plant Serpentwood is an erect, evergreen, perennial undershrub whose medicinal use has been known for 3000 years. The root is a sedative and is used to control high blood pressure and certain forms of insanity. In Ayurveda it is also used for the treatment of insomnia, epilepsy, asthma, acute stomach ache and painful delivery. It is used in snakebite, insect stings and mental disorders. It is popular as 'Madman's medicine' among tribals. 'Serpumsil' tablet for high blood pressure is prepared from *Rauvolfia* roots. Reserpine is a potent hypotensive and tranquilliser, but its prolonged usage stimulates prolactin release and causes breast cancer. The juice of the leaves is used as a remedy for the removal of opacities of the cornea. Root is hypnotic, sedative, specific for insanity, remedy in painful affections of the bowels and in decoction employed in labours to increase uterine contractions and reduces blood pressure. Juice of leaves is used for removal of opacities of the cornea of the eyes. *Rauvolfia* root is bitter, acrid, laxative, anthelmintic, thermogenic, diuretic and sedative (Pakrashi and Akkhari 1968).

Active Constituents Over 200 alkaloids have been isolated from the plant. The alkaloids are classified into three groups, viz. reserpine, ajmaline and serpentine groups. Reserpine group comprising reserpine, rescinnamine, deserpine, etc. act as hypotensive, sedative and tranquillising agent. Overdose may cause diarrhoea, bradycardia and drowsiness. Ajmaline, ajmalicine, ajmalinine, iso-ajmaline, etc. of the ajmaline group stimulate the central nervous system, respiration and intestinal movement with slight

hypotensive activity. Serpentine group comprising serpentine, serpentinine, alstonine, etc. is mostly antihypertensive. Its dried root is the economical part which contains a number of alkaloids of which reserpine, rescinnamine, deserpidine, ajamalacine, ajmaline, neoajmalin, serpentine, α-yohimbine are pharmacologically important. The root yields 0.8–13 % total alkaloids consisting of ajmaline, ajmalinine, yellow alkaloids, serpentine, serpentinine and amorphous bases; root bark yields 0.1 % iso-ajmaline and 1.0 % neo-ajmaline. Besides the alkaloids root contains oleoresin and a sterol, serposterol; the resin fraction is physiologically active. A resin fraction produces sedative and hypotonic effect in experimental animals. Alkaloid-free oleoresin fraction from the roots produced sedative and hypnotic effects in animals (Chopra et al. 1956).

Symptoms of Viral Infection The leaves show ring spot symptoms in which lesions are formed by concentric rings of dark and light tissue and are termed ring spot. Necrosis accompanies them (Fig. 7.18).

Fig. 7.18 Infected and healthy leaves of *Rauvolfia serpentine*

Virus Identification Raj et al. (2007) reported the natural occurrence of CMV (Cucumber mosaic virus) infection on *Rauvolfia serpentina* in Lucknow, India. The symptom was severe mosaic and stunting of the whole plant. The virus was transmitted by sap inoculation to indicator plants *Nicotiana tabacum* cv. White Burley, *N. rustica* and *N. glutinosa*, which produced necrotic local lesions and systemic mosaic. The virus also reacted positively with antiserum of CMV in gel double diffusion tests, indicating the virus was CMV. Supyani et al. (2014) reported another virus on the basis of transmission assay in *R. serpentina* and the virus was not CMV.

7.2.19 *Ricinus communis*

Family Euphorbiaceae

Distribution It is a native of North-East tropical Africa. It is found throughout India, cultivated and found wild up to 2400 m.

Botanical Descriptions *Ricinus communis* is a monoecious evergreen shrub growing up to 4 m. Leaves are alternate, palmately lobed, Petiolate, Lobes are lanceolate, thinly pubescent below, margin serrate and apex acuminate. Paniculate racemes are terminal with male flowers below and female ones above. Perianth is cupular, splitting into 3–5 lobes, lanceolate, valvate, margin in rolled and acuminate. Filaments of stamen are connate and repeatedly branched with divergent anther cells. Sepals are five, subequal, lanceolate, valvate and acute. Ovary is globose, echinate, 3-locular with three ovules and pendulous. Styles are three, stout, papillose, stigmatiferous. Capsules are 3-lobed and prickly with oblong seeds having smooth testa and marbled, shiny and carunculate. *R. bronze* King and *R. africanus* are two good garden varieties which are known as Italian and East Indian Castors, respectively.

Pharmacological Profile as a Medicinal Plant Castor is a perennial evergreen shrub. The Sanskrit name 'erandah' describes the property of the drug to dispel diseases. It is considered as a reputed remedy for all kinds of rheumatic affections. They are useful in gastropathy such as gulma, amadosa, constipation, inflammations, fever, ascitis, strangury, bronchitis, cough, leprosy, skin diseases, vitiated conditions of vata, colic, coxalgia and lumbago. The leaves are useful in burns, nyctalopia and strangury and for bathing and fermentation and vitiated conditions of vata, especially in rheumatoid arthritis, urodynia and arthralgia. Flowers are useful in urodynia and arthralgia and glandular tumours. Seeds are useful in dyspepsia and for preparing a poultice to treat arthralgia. The oil from seeds is a very effective purgative for all ailments caused by vata and kapha. It is also recommended for scrotocele, ascites, intermittent fever, gulma, colonitis, lumbago, coxalgia and coxitis. Oil is also used for soap making. Fresh leaves are used by nursing mothers in the Canary Island as an external application to increase the flow of milk. Castor oil is an excellent solvent of pure alkaloids, and solutions of atropine, cocaine, etc. are used in ophthalmic surgery. It is also dropped into the eye to remove the after-irritation caused by the removal of foreign bodies (Jena and Gupta 2012). Root, stem and seed are diuretic. The roots are sweet, acrid, astringent, thermogenic, carminative, purgative, galactagogue, sudorific, expectorant and depurative. Leaves are diuretic, anthelmintic and galactagogue. Seeds are acrid, thermogenic, digestive, cathartic and aphrodisiac. Oil is bitter, acrid, sweet, antipyretic, thermogenic and viscous (Jena and Gupta 2012). Castor oil forms a clean, light-coloured soap, which dries and hardens well and is free from smell. The oil varies much in activity. Castor oil is an excellent solvent of pure alkaloids. The oil furnishes sebacic acid and caprylic acid. Root and stem are antiprotozoal and anticancerous (Jena and Gupta 2012).

Active Constituents The bean coat yielded lupeol and 30-norlupan-3β-ol-20-one. Roots, stems and leaves contain several amino acids. Flowers gave apigenin, chlorogenin, rutin, coumarin and hyperoside. Castor oil is constituted by several fatty acids (Chopra et al. 1956). Seed coat contained 1.50–1.62 % lipids and higher amounts of phosphatides and non-saponifiable matter than seed kernel. Fresh leaves protected against liver injury induced by carbon tetrachloride in rats, while cold aqueous extract provided partial protection.

Symptoms of Virus Infections Symptoms consisted of yellow speckled leaves with significant mottled areas; some leaves also contained an arabesque line pattern. The infected plants had leaves showing puckering, and later leaf laminar is considerably reduced and distorted (Fig. 7.19).

Virus Identification A castor bean isolate of *Olive latent virus 2* was reported by Grieco et al. (2002) and Parrella et al. (2007). The virus was identified serologically by particle decoration with an OLV2 antiserum, raised against an Italian OLV2 isolate (Grieco et al. 1992) and by sequencing the movement gene (MP) and the coat protein gene (CP) (Parrella et al. 2007). Fareed et al. (2012) reported a cotton leaf curl Burewala virus infecting *Ricinus communis.*

7.2.20 *Solanum nigrum* Linn.

Family Solanaceae

Distribution It is seen wild throughout India. It is an erect, divaricately branched, unarmed and suffrutescent.

Botanical Descriptions Leaves are ovate or oblong, sinuate-toothed or lobed and glabrous. Flowers are 3–8 in extra-axillary drooping subumbellate cymes. Fruits are purplish black or reddish berries. Seeds are many, discoid, yellow and minutely pitted. The whole plant is useful in vitiated conditions of tridosha, rheumatalgia,

Fig. 7.19 Healthy and infected leaves of *Ricinus communis*

swellings, cough, asthma, bronchitis, wounds, ulcers, flatulence, dyspepsia, strangury, hepatomegaly, otalgia, hiccup, ophthalmopathy, vomiting, cardiomyopathy, leprosy, skin diseases, fever, splenomegaly, haemorrhoids, nephropathy, dropsy and general debility. The plant is bitter, acrid, emollient, antiseptic, anti-inflammatory, expectorant, anodyne, vulnerary, digestive, laxative, diuretic, cardiotonic, depurative, diaphoretic, febrifuge, rejuvenating, sedative, alterant and tonic.

Active Constituents Solanine and related glycoalkaloids

Pharmacological Profile as a Medicinal Plant Bruised fresh leaves are used externally to ease pain and abate inflammation. Their juice is used for ringworm, gout and earache. Chinese experiments confirm that the plant inhibits growth of cervical carcinoma (Chopra et al. 1956).

Symptoms on leaves The infected plant showed mosaic-like symptoms and distortion (Fig. 7.20).

Virus Identification *S. nigrum* is known as a virophilous species, as host for more than 40 viruses (Kazinczi et al. 2004). The study conducted by Kazinczi et al. confirmed the occurrence of Obuda pepper virus (ObPV) Pepino mosaic virus (PepMV) on *S. nigrum* L. (black nightshade) PepMV was identified and isolated recently in from tomato in Hungary (Forray et al. 2004) on *S. nigrum L.* (black nightshade). Mild chlorotic mottling occurs in inoculated leaves, which later abscise. Systemic mottling occurs in subsequent growth (Kazinczi et al. 2006).

7.2.21 *Tagetes erectus* Linn.

Family Asteraceae

Distribution They are grown in gardens in India.

Botanical Descriptions An annual ornamental erect herb, about 60 cm high and strongly aromatic. Leaves pinnately divided segments lanceolate-serrate and strongly aromatic. Flowers are in terminal heads; colour ranges from a light sulphur-yellow to a deep orange.

Fig. 7.20 Healthy and infected plants of *Solanum nigrum*

Pharmacological Profile as a Medicinal Plant The whole herb is anthelmintic, aromatic, digestive, diuretic, emmenagogue, sedative and stomachic. It is used internally in the treatment of indigestion, colic, severe constipation coughs and dysentery. Externally, it is used to treat sores, ulcers, eczema, sore eyes and rheumatism. The leaves are harvested as required for immediate use during the growing season, while the flowering plant can be dried and stored for later use. A paste of the leaves is applied externally to treat boils, carbuncles and earaches. The flowers are carminative, diuretic and vermifuge. A decoction is used to treat colds and mumps. It is applied externally to treat skin diseases, conjunctivitis and sore eyes. The root is laxative (Asolkar et al. 1992).

Active Constituents Flowers contain essential oil, colouring matter, pigments, quercetagetin and phenolics, syringic acid, methyl-3, 5-dihydroxy-4-methoxy benzoate and quercetin. Whole plant yields 0.01 % essential oil that contains d-limonene, ocimene, l-linalyl acetate, l-linalool, tagetone and nonanal. Mono- and diesterified luteins have also been isolated from petals. Dried petals also contain quercetagitrin and a glucoside of quercetagetin. The plant also contains ethylgallate and three contact allergens, butenylbithiophene, α-terthienyl and hydroxytremetone and essential oil and colouring matter; Indian flowers contain the pigment quercetagetins (Chopra et al. 1956). Whole plant yields 0.01 % essential oil; quercetagitrin, a glucoside of quercetagetin, is isolated from the dried petals.

Symptoms of Virus Infection Diseased plants showed mosaic, mottling and stunting. The affected leaves were reduced in size. The diseased plant remains stunted and produces poorly developed flower (Fig. 7.21).

Identification of Virus Marigold mottle *Potyvirus* was first reported in *Tagetes erecta* from India by Naqvi et al. 1981. It is transmitted by a vector, an insect, *Myzus persicae*. Virus transmitted by mechanical inoculation. Virions are filamentous, not enveloped and usually flexuous with a clear modal length of 675 nm. The virus isolated from infected leaf tissue under electron microscope showed the presence of isometric virus particles measuring about 29 nm in diameter, resembling those of cucumoviruses in diseased *Tagetes erecta* obtained from plant growing at Aligarh, India (Singh et al. 1999).

Fig. 7.21 Infected and healthy plant of *Tagetes erectus*

7.2.22 *Withania somnifera* L. Dunal.

Family Solanaceae

Distribution *Withania somnifera* (Ashwagandha) is believed to have oriental origin. It is found wild in the forests of Mandsaur and Bastar in Madhya Pradesh, the foot hills of Punjab, Himachal Pradesh, Uttar Pradesh and western Himalayas in India. It is also found wild in the Mediterranean region in North America. In India it is cultivated in Madhya Pradesh, Rajastan and other drier parts of the country.

Botanical Descriptions *W. somnifera* (Indian ginseng or Winter cherry) is erect, evergreen, tomentose shrub and 30–75 cm in height. Roots are stout, fleshy, cylindrical, 1–2 cm in diameter and whitish brown in colour. Leaves are simple, ovate, glabrous and opposite. Flowers are bisexual, inconspicuous, greenish or dull yellow in colour and born on axillary umbellate cymes, comprising five sepals, petals and stamens each; the two-celled ovary has a single style and a bilobed stigma. The petals are united and tubular. The stamens are attached to the corolla tube and bear erect anthers which form a close column or cone around the style. Pollen production is poor. The fruit is a small berry, globose, orange red when mature and is enclosed in persistent calyx. The seeds are small, flat, yellow and reniform in shape and very light in weight.

Pharmacological Profile as a Medicinal Plant *W. somnifera* is considered to be one of the best rejuvenating agents in Ayurveda. Its roots, leaves and seeds are used in Ayurvedic and Unani medicines to combat diseases ranging from tuberculosis to arthritis. The pharmacological activity of the plant is attributed to the presence of several alkaloids and withaniols. Roots are prescribed in medicines for hiccup, several female disorders, bronchitis, rheumatism, dropsy, stomach and lung inflammations and skin diseases (Pant et al. 2012). Its roots and paste of green leaves are used to relieve joint pains and inflammation. It is also an ingredient of medicaments prescribed for curing disability and sexual weakness in male. Leaves are used in eye diseases. Seeds are diuretic. It is a constituent of the herbal drug ‘Lactare’ which is a galactagogue. Ashwagandha was observed to increase cell-mediated immunity, prevent stress-induced changes in adrenal function and enhance protein synthesis. Milk fortified with it increases total proteins and body weight. It is a well-known rejuvenating agent capable of imparting long life, youthful vigour and intellectual power. It improves physical strength and is prescribed in

all cases of general debility. Ashwagandha powder (6–12 g) twice a day along with honey and ghee is advised for tuberculosis in Sushruta Samhita. It also provides sound sleep (Baghel 2013).

Active Constituents Ashwagandha roots contain alkaloids, starch, reducing sugar, hentriacontane, glycosides, dulcitol, withaniol acid and a neutral compound. Wide variation (0.13–0.31 %) is observed in alkaloid content. At present, 138 withanolides have been reported (Baghel 2013); Majmudar (1955) isolated eight amorphous bases such as withanine, somniferine, somniferinine, somnine, withananine, withananinine, pseudowithanine and withasomnine. Other alkaloids reported are nicotine, tropine, pseudotropine, 3,α-tigloyloxytropane, choline, cuscudohygrine, anaferine, anahygrine and others. Free amino acids in the roots include aspartic acid, glycine, tyrosine, alanine, proline, tryptophan, glutamic acid and cystine. Leaves contain 12 withanolides, alkaloids, glycosides, glucose and free amino acids. Berries contain a milk-coagulating enzyme, two esterases, free amino acids, fatty oil, essential oil and alkaloids. Methods for alkaloid's analysis in Asgandh roots have also been reported (Majmudar 1955). Withania roots are astringent, bitter, acrid, somniferous, thermogenic, stimulant, aphrodisiac, diuretic and tonic. Leaf is antibiotic, antitumourous, antihepatotoxic and anti-inflammatory. Seed is milk coagulating, hypnotic and diuretic.

Symptoms of Virus Infection The leaves of *Withania somnifera* become considerably small in size and become leathery. The plant reduces in size and does not produce healthy flowers (Fig. 7.22). A yellow mosaic disease on *W. somnifera* with a significant disease incidence was observed at Aligarh, Lucknow in Uttar Pradesh.

Virus Identification The *Begomovirus* association with the disease of *W. somnifera* was identified on the basis of virus transmission by whitefly, host range study, electron microscopic observations and positive amplification of expected size (~800 bp, 1.2 Kb) DNA bands by PCR using two pairs of *Begomovirus*-specific primers. The impact of *Begomovirus* infection on growth and biomass yield *W. somnifera* plants was also studied which indicated significant losses to its biomass yield (Baghel et al. 2010).

7.2.23 *Zinnia elegans* Linn

Family Asteraceae

Fig. 7.22 Healthy and infected plant of *Withania somnifera*

Distribution *Zinnia* is a genus of plants of the sunflower tribe within the daisy family. They are native to scrub and dry grassland in an area stretching from the Southwestern USA to South America, with a centre of diversity in Mexico. It is cultivated in the gardens in India.

Botanical Descriptions They typically range in height from 10 to 100 cm tall. Leaves are opposite and usually stalkless (sessile), with a shape ranging from linear to ovate and pale to middle green in colour. The flowers have a range of appearances, from a single row of petals to a dome shape, with the colours white, chartreuse, yellow, orange, red, purple and lilac. The flowers may be single, semi-double or double, in shades of scarlet, rose, orange, yellow or white. Seeds are sown during summer directly or in trays; germination temperature is 25 °C.

Pharmacological Profile as a Medicinal Plant Plant used for nose and throat troubles (Chopra et al. 1956). *Z. elegans* inhibited the growth of fungus *Fusarium moniliforme* (Hafiza et al. 2002). *Zinnia elegans* leaves play an important role in the antioxidant hepatoprotective activity against CCl4 toxicity (Mohamed et al. 2015).

Active Constituents Flavonoids are major compounds in flower and herb of *Zinnia*. Flavonoid, glycosides, tannins, anthocyanins, saponins and phenols are commonly found in *Zinnia* (Yamaguchi et al. 1990).

Symptoms of Virus Infection The infected plant is characterised by leaf curling, foliar deformation and distortion symptoms. The flower shows deformations (Fig. 7.23).

Virus Identification Tobacco leaf curl virus from Tanzania was the first reported virus on *Zinnia* (Storey 1931). Later, *Zinnia* mosaic virus (Huertos 1953), *Zinnia* mild mottle virus from India (Padma et al. 1974), *Bidens* mottle virus (Logan et al. 1984), *Zinnia* leaf curl virus (Haider et al. 2005), *Ageratum* enation virus (Kumar et al. 2010), *Zinnia* leaf curl virus, *Zinnia* potyvirus (Maritan et al. 2004) and *Zinnia* leaf curl Pakistan virus (AM040438) were reported by different workers around the world. Ha et al. (2008)

Fig. 7.23 Healthy and infected plants of *Zinnia elegans*

found *Alternanthera* yellow vein virus affecting *Zinnia* with leaf curling symptoms in Vietnam. *Zinnia* leaf curl virus was isolated from Gorakhpur on the basis of polymerase chain reaction (PCR) performed with the *Begomovirus*-specific primers (TLCV-CP). Amplicon obtained were directly sequenced and submitted in the GenBank (GQ412352), and phylogeny was constructed with the available identical sequences in the GenBank. Based on the highest similarity 97 % at nucleotide and 99 % at amino acid level and closest relationship with isolates of Zinnia leaf curl virus, the isolate was considered an isolate of *Zinnia* leaf curl virus (Pandey and Tiwari 2012).

Medicinal plant enables sustainable resource based on growing needs of the pharmaceutical industry. These plants are attacked and damaged by variety of insect pest and pathogen like fungi, bacteria, viruses, MLOs etc. To prevent transmission of pathogen and disease and to reduce the yield loss, prevention of diseases must be done at all stages, including use of healthy and disease free seeds. However, little information is avalible on the distribution and species diversity of viruses invading medicinal plants in North-Eastern Uttar Pradesh. In the present investigation 23 medicinal plants of ten different families, i.e. Apocynaceae, Apiaceae, Asclepiadaceae, Asteraceae, Caesalpiniaceae, Euphorbiaceae, Lamiaceae Malvaceae, Solanaceae and Urticaceae have been studied. We have considered viral infection of only those plants which have their aromatic and medicinal value; we have not considered here vegetables and fruits which have medicinal value. During diagnosis of plant diseases only visual signs are not always accurate; to identify the causal factors.This fact is evident in several cases where one and the same signs or symptoms may be caused by different factors (Sultana et al. 2014). The study of the etiology of diseases will allow developing effective methods of protecting plants and harvesting and preserving the quality of raw materials for phyto-pharmaceuticals.

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Geminivirus: Indian Scenario

8

Koushlesh Ranjan, Riaz Ahmed Siddique, Mahesh Kumar Bharti, and Jitender Singh

Abstract

Geminiviruses are a diverse group of plant virus which infects several crop species in India. Based on host range, insect vector, and genome organization, geminiviruses are classified into four genera: *Begomovirus*, *Curtovirus*, *Topocuvirus*, and *Mastrevirus. Begomovirus* are isolated from many of the Indian crop plants such as tomato, chilies, cotton, pulses, papaya, cucurbits, okra, etc. Begomoviruses are transmitted by whiteflies. They possess a bipartite genome of approximately 2.6 kb in size. Begomovirus genome may also possess satellite DNA molecules along with its genome. The infections of begomoviruses lead to significant economic loss to Indian crops. Therefore research on begomoviruses in India has been mainly focused on the molecular diagnosis of virus along with phylogenetic analyses, DNA replication, transgenic resistance, etc. Various techniques used for geminiviruses are antibody-based detection methods such as enzyme-linked immunosorbent assay (ELISA), nucleic acid hybridizations, dot blot, Southern blot, and polymerase chain reaction (PCR) along with direct visualization of virus using electron microscopy.

Keywords

Begomovirus • *Topocuvirus* • *Curtovirus* • *Mastrevirus* • Rolling circle amplification • Plant virus

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8.1 Introduction

Geminiviruses are one of the most devastating viruses of several crops in India and other tropical and subtropical countries. Based on insect vector, host range, and genome organization, geminiviruses are broadly categorized in four genera: *Mastrevirus*, *Begomovirus*, *Curtovirus*,

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and *Topocuvirus* (Hull 2002). Geminiviruses are large and diverse group of plant viruses. They have unique paired icosahedral capsids (Lazarowitz 1992). They are characterized by single-stranded DNA (ssDNA), circular genomes. The ssDNA genome replicate via double-stranded DNA (dsDNA) intermediates in infected host cell nucleus (Hanley-Bowdoin et al. 1999; Gutierrez 1999).

8.2 Geminivirus Genome Structure

Geminiviruses are classified into four genera: *Begomovirus*, *Curtovirus*, *Topocuvirus*, and *Mastrevirus*. Out of these four genera, begomoviruses are commonly reported from Indian crop. *Begomovirus* are transmitted by the whitefly (Chakraborty et al. 2008). Begomovirus genomes can be classified into monopartite or bipartite depending on the presence of one or two circular ssDNA components in its genome. The majority of begomoviruses are bipartite having both DNA-A and DNA-B molecules, of 2600–2800 nucleotides (nt) each (Fig. 8.1). However, some Old World tomato viruses like tomato yellow leaf curl virus (TYLCV) and tomato leaf curl virus-Australia (ToLCV-[Au]) require only DNA-A component to infect plants (Dry et al. 1993; Navot et al. 1991). Additionally, some monopartite begomoviruses also possesses a different type of ssDNA component called as DNA1. However, majority of monopartite viruses also contain an additional DNA-β component having unknown function along with a DNA1 component (Briddon et al. 2004). Irrespective of genome organization, all the begomoviruses possess the ability to undergo recombination and pseudorecombination processes resulting in the evolution of new viral strain which may be more virulent and resistant strain.

Mastreviruses have geminate particles, apparently consisting of two incomplete icosahedra joined together. The virion particle consists of 22 pentameric capsomers and 110 identical protein subunits. They have circular, monopartite, ssDNA genome of about 2.6–2.8 kb in size (Fig. 8.2). The viral genome is replicated through dsDNA intermediate. The rolling circle replication mechanism is initiated and terminated by replication (Rep) protein. The DNA replication is completed by

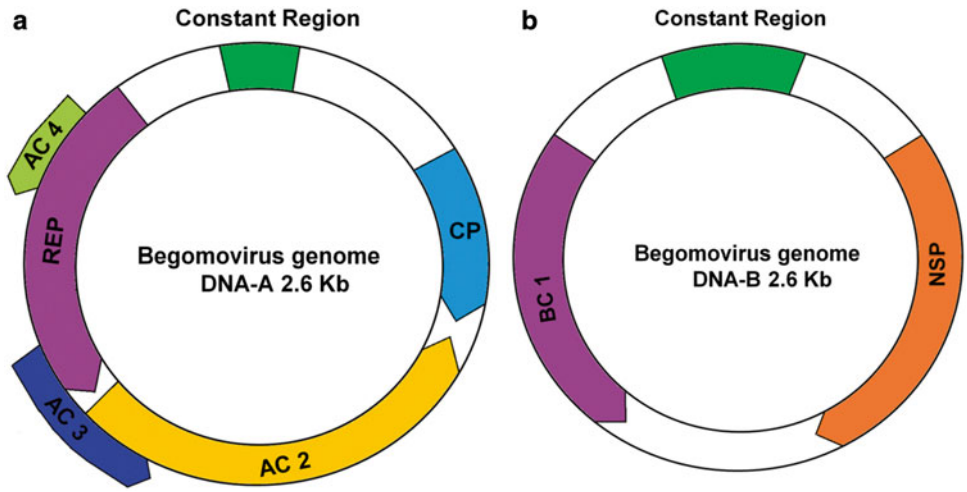

Fig. 8.1 (**a** and **b**) *Begomovirus* genome components showing coat protein (*CP*), replication-associated protein (*Rep*), and nuclear shuttle protein (*NSP*)

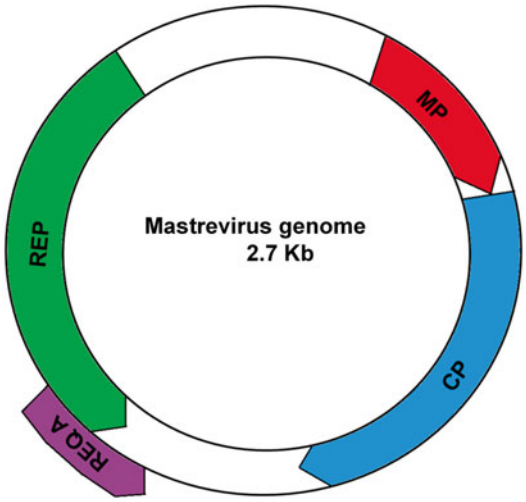

Fig. 8.2 *Mastrevirus* genome component showing coat protein (*CP*), replication-associated protein (*Rep*), and movement protein (*MP*)

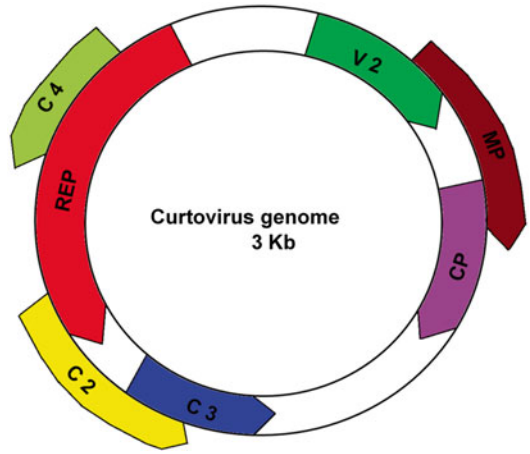

Fig. 8.3 *Curtovirus* genome component showing coat protein (*CP*), replication-associated protein (*Rep*), and movement protein (*MP*)

host DNA polymerase enzyme. The genes are bidirectional transcribed from a control region, the long intergenic region (LIR). It contains a virion-sense-specific origin of replication as well as virion (V) and complementary (C) sense promoters. The short intergenic region (SIR) contains bidirectional polyadenylation signals. It may also act as origin of replication for complementary strand. Mastreviruses possess three specific genes which are involved in infection to plants. These are movement protein, MP (V1 ORF); coat protein, CP (V2 ORF); and replication-associated protein, Rep (C1 and C2 ORFs also called RepA and RepB, respectively). The MP appears to localize to cell walls in infected cells and is presumed to attach to plasmodesmata. The MP gene sequence is the most variable sequence apart from the LIR (Padidam et al. 1995). The CP is single capsid protein. It has role in vector transmission. The CP is relatively conserved among mastreviruses. It is one of the two protein sequences used to show relationships among *Geminiviridae* (Padidam et al. 1995). Rep (or Reps) protein is necessary for viral replication using rolling circle replication mechanism.

Curtovirus have monopartite, circular, ssDNA genome of 3.0 kb in size (Fig. 8.3). The 3' terminus of genome do not have poly (A) region. The coding regions are found in both the virion (positive) and its complementary (negative) strands. The genome is replicated through rolling circle amplification mechanism. The replication (Rep) protein initiates and terminates rolling circle replication. The transcription is bidirectional from the intergenic region. There are seven proteins produced by transcription on V sense, viz., movement protein (MP), CP, and V2 protein, and on C sense, viz., Rep, C2, replication enhancer (REn), and C4.

Topocuvirus genome is monopartite, circular, and ssDNA of 2.86 kb (Fig. 8.4). The 3′ terminus of genome do not possess poly (A) tract. The coding regions are found in both virion (positive) strand and its complementary (negative) strands. The genome is replicated by rolling circle replication which is initiated and terminated by replication (Rep) protein. The host DNA polymerase is used for viral DNA replication. The genome transcription is bidirectional and initiated from the common region (CR). The V sense produces V2 and CP protein and C sense Rep, C2, C3, and C4 proteins.

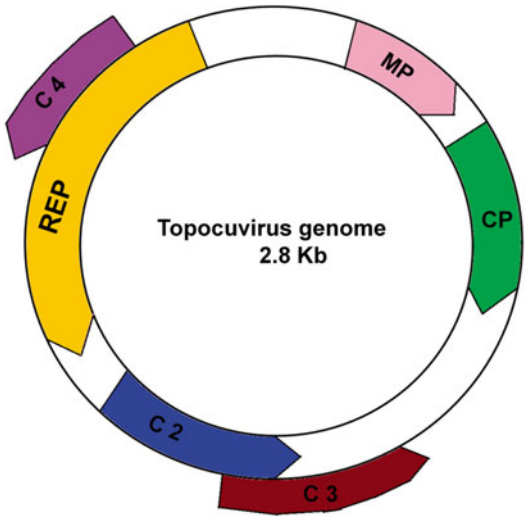

Fig. 8.4 *Topocuvirus* genome component showing coat protein (*CP*), replication-associated protein (*Rep*), and movement protein (*MP*)

8.3 Geminivirus Infection in Indian Scenario

Geminiviruses cause severe economic losses to Indian crop. Several geminiviruses have been isolated from different crop species in India. Several workers have been working extensively on infectivity, sequence and phylogenetic analysis, virus-host interactions, functions of viral proteins, etc. of these viruses. Till date they are reported from various plant species in our country. The given table (Table 8.1) represents the geminivirus diversity in India (Fauquet et al. 2008; Marwal et al. 2012).

8.4 Bitter Gourd

The yellow mosaic disease (YMD) lowers the several nutrients and antioxidant in bitter gourd (*Momordica charantia*) (Raj et al. 2005). The PCR and southern hybridization confirmed the causative agent of disease as begomovirus. The virus was given a specific name as bitter gourd yellow mosaic virus (BGYMV) (Raj et al. 2005). Later on it was demonstrated that BGYMV was transmitted by whitefly (Rajinimala et al. 2005). Later on, Indian cassava mosaic virus (ICMV) was also found in bitter gourd using immunological and PCR analysis (Rajinimala and Rabindran 2007).

8.5 Bhindi

The Indian vegetable bhindi (*Abelmoschus esculentus*) is severely infected by *Begomovirus*. The bhindi yellow vein mosaic virus (BYVMV) was first reported from Bombay in India (Kulkarni 1924). Later on morphological and serological study showed that BYVMV belongs to *Begomovirus* genus (Harrison et al. 1991). Recently, okra isolate (OY131) of BYVMV was isolated from bhindi plant in Palem region of New Delhi showing reduced leaves, vein twisting and yellow vein mosaic, and a bushy appearance. The complete genome sequencing showed that DNA-A and DNA-B component have been comprised of 2746 and 2703 nucleotides, respectively (Venkataravanappa et al. 2012a, b). 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 host. However, the typical symptom of disease is shown when BYVMV is agro-inoculated with DNA-β to bhindi (Jose and Usha 2003).

In the study of V- and C-sense promoters of BYVMV in transgenic *Nicotiana benthamiana*, plant showed that the C-sense promoter is stronger than the V-sense promoter in the absence of AC2 protein. AC2 protein was shown as a weak suppressor of silencing. However, AC4, a strong silencing suppressor, produced abnormal phenotype when expressed in transgenic *Nicotiana benthamiana* plants (Gopal et al. 2007).

8.6 Cotton

Cotton leaf curl disease was observed for the first time in India in northwest region (Rishi and Chauhan 1994). In India, cotton leaf curl disease is caused by several monopartite begomoviruses having a satellite DNA-β fragment (Kirthi et al.

Table 8.1 Geminivirus diversity in India (Fauquet et al. 2008; Marwal et al. 2012)

S.n	Plant species/virus name	Accession number	Abbreviation used for virus species
Begomovirus			
1.	**Bhindi yellow vein mosaic virus**		
	Bhindi yellow vein mosaic virus-India [India: Madurai]	AF241479	BYVMV-IN[IN: Mad]
2.	**Chili leaf curl virus**		
	Chili leaf curl virus-A [India: 2005]	DQ673859	ChiLCV-A[IN: 05]
	Chili leaf curl virus-India [India: Papaya: 2005]	DQ989326	ChiLCV-A[IN: Pap: 05]
	Chili leaf curl virus-India [India: Varanasi: 2006]	EF190217	ChiLCV-A[IN: Var: 06]
3.	**Cotton leaf curl Bangalore virus**		
	Cotton leaf curl Bangalore virus [India: Bangalore: 2004]	AY705380	CLCuBV-[IN: Ban: 04]
4.	**Cotton leaf curl Multan virus**		
	Cotton leaf curl Multan virus-Bhatinda [India: Bhatinda]	DQ191160	CLCuMV-Bha[IN: Bha]
	Cotton leaf curl Multan virus-Hisar [India: Hisar: 1999]	AY765253	CLCuMV-His[IN: His: 99]
	Cotton leaf curl Multan virus-India [India: New Delhi2: 2003]	AY795605	CLCuMV-IN[IN: ND2: 03]
5.	**Indian cassava mosaic virus**		
	Indian cassava mosaic virus- India [India: Maharashtra: 1988]	AJ314739	ICMV-IN[IN: Mah: 88]
	Indian cassava mosaic virus-Kerala [India: Kerala 2: 2002]	AJ575819	ICMV-Ker[IN: Ker2: 02]
	Indian cassava mosaic virus-Kerala [India: Kerala 3: 2002]	AJ575820	ICMV-Ker[IN: Ker3: 02]
6.	**Mung bean yellow mosaic virus**		
	Mung bean yellow mosaic virus [India: Haryana: 2001]	AY271896	MYMV-[IN: Har: 01]
	Mung bean yellow mosaic virus [India: Madurai: Soybean 2]	AJ582267	MYMV-[IN: Mad: Sb2]
	Mung bean yellow mosaic virus [India: Namakkal B1: 2005]	DQ865202	MYMV-[IN: NamB1: 05]
	Mung bean yellow mosaic virus [India: Namakkal B2: 2005]	DQ865203	MYMV-[IN: NamB2: 05]
7.	**Mung bean yellow mosaic India virus**		
	Mung bean yellow mosaic India virus [India: Punjab: 2005]	DQ400847	MYMIV-[IN: Pun: 05]
	Mung bean yellow mosaic India virus [India: New Delhi: Cowpea: 2005]	DQ289153	MYMIV-[IN: ND: Cp: 05]
	Mung bean yellow mosaic India virus [India: Varanasi: Cowpea]	AY618902	MYMIV-[IN: Var: Cp]
8.	**Papaya leaf curl virus**		
	Papaya leaf curl virus-India [India: Lucknow]	Y15934	PaLCuV-IN[IN: Luc]
9.	**Radish leaf curl virus**		
	Radish leaf curl virus [India: Varanasi: 2005]	EF175733	RaLCV-[IN: Var: 05]

(continued)

Table 8.1 (continued)

S.n	Plant species/virus name	Accession number	Abbreviation used for virus species
10.	**Tomato leaf curl Bangalore virus**		
	Tomato leaf curl Bangalore virus-A [India: Bangalore 1]	Z48182	ToLCBV-A[IN: Ban1]
	Tomato leaf curl Bangalore virus-B [India: Bangalore 5]	AF295401	ToLCBV-B[IN: Ban5]
	Tomato leaf curl Bangalore virus-C [India: Bangalore: AVT1]	AY428770	ToLCBV-C[IN: Ban: AVT1]
11.	**Tomato leaf curl Gujarat virus**		
	Tomato leaf curl Gujarat virus [India: Mirzapur: 1999]	AF449999	ToLCGV-[IN: Mir: 99]
	Tomato leaf curl Gujarat virus [India: Vadodara: 1999]	AF413671	ToLCGV-[IN: Vad: 99]
12.	**Tomato leaf curl Kerala virus**		
	Tomato leaf curl Kerala virus [India: Kerala II: 2005]	DQ852633	ToLCKeV-[IN: KerII: 05]
13.	**Tomato leaf curl Karnataka virus**		
	Tomato leaf curl Karnataka virus-Bangalore [India: Bangalore: 1993]	U38239	ToLCKV-Ban[IN: Ban: 93]
	Tomato leaf curl Karnataka virus-Janti [India: Janti: 2005]	AY754812	ToLCKV-Jan[IN: Jan: 05]
14.	**Tomato leaf curl New Delhi virus**		
	Tomato leaf curl New Delhi virus-India [India: Hissar: Cotton: 2005]	EF063145	ToLCNDV-IN[IN: His: Cot: 05]
	Tomato leaf curl New Delhi virus-India [India: New Delhi: Mild: 1992]	U15016	ToLCNDV-IN[IM: ND: Mld: 92]
15.	**Tomato leaf curl Pune virus**		
	Tomato leaf curl Pune virus [India: Pune: 2005]	AY754814	ToLCBV-[IN: Pun: 05]
16.	**Tomato leaf curl Rajasthan virus**		
	Tomato leaf curl Rajasthan virus [India: Rajasthan: 2005]	DQ339117	ToLCBV-[IN: Raj: 05]
Mastrevirus			
1.	**Unassigned isolates in genus**		
	Bajra streak virus		BaSV

2002). Tomato plant may act as alternate host for at least some strains of the virus (Khan and Ahmed 2005). In India at least four begomoviruses are considered as causative agents of this disease, namely, cotton leaf curl Multan virus, tomato leaf curl Bangalore virus (ToLCBV), cotton leaf curl Rajasthan virus (CLCuRV), and cotton leaf curl Kokhran virus (CLCuKV) (Ahuja et al. 2007). The disease is characterized by darkened and swelled vein, leaf curling, and enations on the undersides of leaves that develop into cup-shaped, leaflike structures. Recently, the recombinant strains (CLCuV-SG01 and CLCuV-SG02) of cotton leaf curl virus with other begomoviruses have been reported from Rajasthan (Kumar et al. 2010).

8.7 Legumes

Geminiviruses have been isolated from several legume species in India. Yellow mosaic disease (YMD) in several legumes, viz., mung bean (*Vigna radiata*) and black gram (*Vigna mungo*),

was first reported (Nariani 1960). The YMD is caused by begomoviruses having bipartite genomes (Karthikeyan et al. 2004). YMD of legumes in India is caused by four different species of begomoviruses (Qazi et al. 2007). However, two species, i.e., mung bean yellow mosaic virus (MYMV) and mung bean yellow mosaic India virus (MYMIV) (Fauquet and Stanley 2003), are most prevalent in India. The MYMIV infect several species of legumes such as soybean (*Glycine max*), French bean (*Phaseolus vulgaris*), black gram, cowpea (*V. unguiculata*), and mung bean (Varma et al. 1992). The remaining species, i.e., horse gram yellow mosaic virus and Dolichos yellow mosaic virus (Maruthi et al. 2006), have been reported rarely. Recently, a bipartite begomovirus isolate causing YMD in black gram has been identified in South India. The DNA-A component of this virus is a variant of MYMV and DNA-B, a variant of MYMIV. However, this virus showed distinct characteristic symptom in different legumes hosts (Haq et al. 2011).

8.8 Chili

Begomovirus have also been reported from Indian chili. The causative agent of chili leaf curl disease (ChLCD) was reported as *Begomovirus* in India. The DNA-A sequence analysis revealed that a monopartite begomovirus was shown to be associated with the disease. The causative agent was found as a strain of the chili leaf curl virus (ChiLCV) of Pakistan (ChiLCV-PK [PK: Mul: 98]) origin (Senanayake et al. 2006). The complete gene sequencing of the virus showed that it shares 95 % sequence identity with ChiLCV-PK [PK: Mul: 98]. Moreover, ChiLCV isolated from Punjab was also found to be associated with tomato leaf curl Joydebpur virus, reported from Joydebpur, Bangladesh (Shih et al. 2006). The complete genome of a *Begomovirus* and its cognate DNA-β satellite component was sequenced and analyzed from chili plant infected with chili leaf curl disease (ChLCD), from Varanasi, India (Chattopadhyay et al. 2008). A very high incidence of ChLCV in chili was observed in Meerut district of Western Uttar Pradesh in 2011 with symptoms of crowding and yellowing of leaves, upward leaf curling, and stunting of whole plants. The PCR and phylogenetic analysis showed that tomato leaf curl New Delhi virus (ToLCNDV) and associated betasatellite were responsible for ChLCD in chili (Singh et al. 2013).

8.9 Papaya

The papaya leaf curl disease has serious impact on papaya cultivation in North India. The disease is caused by papaya leaf curl virus (PLCV). The PCR-based diagnosis confirmed the presence of geminivirus in papaya in Uttar Pradesh state (Singh et al. 2007). In one of the other incidences in Uttar Pradesh, PCR and Southern blot hybridization with geminiviral specific DNA probes also prove the causative agent of papaya leaf curl disease as PLCV (Sangeeta et al. 1998). However, the mixed infection of PCLV along with other virus in papaya is also observed. A mixed infection of papaya leaf curl virus (PLCV), a bipartite geminivirus, and papaya ring spot virus (PRSV) used to hamper the production of papaya in many parts of world. Therefore, a duplex PCR for rapid and reliable detection of these viruses has been standardized (Usharani et al. 2013). Recently, the siRNA techniques have been used to counter the resistance against geminiviruses infecting papaya (Saxena et al. 2011).

8.10 Tobacco

Leaf curl disease of tobacco (TbLCD) is endemic in India. The begomovirus having monopartite with betasatellite and an alphasatellite association was reported as a causative agent of disease in tobacco from Pusa, Bihar. The isolate was named as tobacco leaf curl Pusa virus [India: Pusa: 2010]. A betasatellite, associated with TbLCD, was found to be a variant of tomato leaf curl Bangladesh betasatellite [India: Rajasthan: 2003], sharing 90.4 % sequence identity. An alphasatellite, detected in the diseased plants, had 87 % nucleotide sequence identity with tomato

leaf curl alphasatellite. The nucleic acid sequence analysis showed that the begomovirus a product of recombination of multiple begomovirus complexes (Singh et al. 2011). A new strain (FB01) of tobacco curly shoot virus (TbCSV) has been from Varanasi, Uttar Pradesh, which shows curly shoot symptoms reported in common bean plants (Venkataravanappa et al. 2012a, b).

8.11 Tomato

Indian tomato plant is also infected by geminiviruses. Tomato leaf curl disease (ToLCD) was first reported from Nort India (Vasudeva and Sam Raj 1948) and subsequently from central India and Southern India (Sastry and Singh 1973). ToLCD is characterized by leaf curling, vein clearing, and stunting in tomato plant (Saikia and Muniyappa 1989). Several begomoviruses associated with ToLCD have been cloned and sequenced from India. Two isolates from New Delhi, tomato leaf curl New Delhi virus-severe (ToLCNDV-Severe) and tomato leaf curl New Delhi virus-mild (ToLCNDV-Mild), with bipartite genomes, sharing 94 % identity in the DNA-A component (with identical DNA-B components), were reported earlier (Padidam et al. 1995). The nucleic acid sequence analysis of two isolates, ToLCV-Ban-2 and ToLCV-Ban-4, from Bangalore showed 91 % identity with each other. In addition, the DNA sequence-based study identified two more isolates, namely, tomato leaf curl Bangalore virus ToLCBV [ToLCBV-Ban-5] and [ToLCBV-Kolar] from same geographical region (Kirthi et al. 2002). The sequence analysis of CP gene of Indian tomato leaf curl viruses (ToLCVs) showed its highly diverse nature. Indian ToLCVs form five distinct clusters with four of them that represent the known tomato leaf curl viruses, while one cluster showed maximum identity with Croton yellow vein mosaic virus (Reddy et al. 2005). The Indian isolates of ToLCV contain two components, A and B, which are required for systemic movement and symptom development in host plant. In one of the studies, the genome organization of ToLCV-India was found similar to other whitefly-transmitted geminiviruses (WTGs) having bipartite genomes (Padidam et al. 1995).

8.12 Potato

The first-time natural occurrence of a geminivirus from potatoes in India was reported by immunoelectron microscopy. The viral agent was named as potato apical leaf curl begomovirus (Garg et al. 2001). The infected potato plants usually show stunting, crinkling, vein thickening, and leaf distortion (Fig. 8.5). A PCR-based assay using primers specific to the coat protein (AV1) and replicase (AC1) gene regions was standardized for the detection of tomato leaf curl New Delhi virus-potato (ToLCNDV-Potato) which is the causal agent of apical leaf curl disease in potato (Jeevalatha et al. 2013). The double-antibody

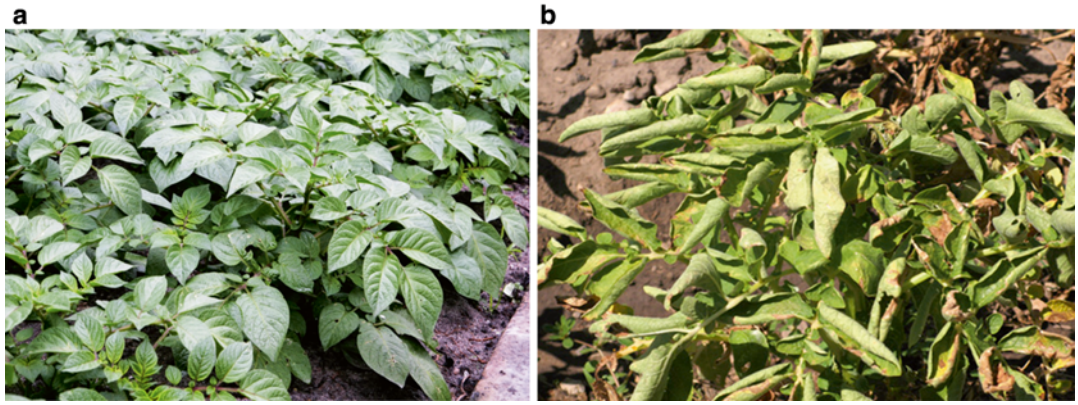

Fig. 8.5 (**a**) Potato plant showing healthy leaves. (**b**) Potato plant showing typical leaf curl disease symptoms

sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and nucleic acid hybridization also revealed the causative agent of apical leaf curl symptoms in potato as begomovirus (Venkatasalam et al. 2005). The causal virus was named as potato apical leaf curl virus (PALCV) based on PCR amplification of DNA-A and DNA-B component (Venkatasalam et al. 2011).

8.13 Mentha

Geminiviruses were also found to be associated with *Mentha* (mint). The nucleic acid sequence analysis from symptomatic plants showed the begomovirus association. The sequence study showed 93 % identity with a tomato leaf curl Pakistan virus (Samad et al. 2008). Subsequently, several begomoviral DNA-A was sequenced completely from Indian mentha plant. They shared 94 % sequence identity with tomato leaf curl Karnataka virus-Bangalore [India: Bangalore: 1993], a betasatellite, cotton leaf curl Multan betasatellite [India: New Delhi 2: 2004], and a new satellite molecule (DNA-II). The virus isolate was named as tomato leaf curl Karnataka virus-Bangalore [India: Ludhiana: Mentha: 2007] (Borah et al. 2010).

8.14 Diagnosis of Geminivirus

Various species of geminiviruses from different crop plants have been isolated from India. The laboratory diagnosis is mainly based on the detection of either viral nucleic acid or virus-specific antigen. The various diagnostic assays used for geminivirus diagnosis are the following.

8.15 Enzyme-Linked Immunosorbent Assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) is a test that uses antigen-antibody reaction along with a color agent to identify a substance. It utilizes the components of the immune system to detect immune responses in the body. The ELISA test involves an enzyme, antibody, and antigen molecule. After the development of microtiter plate-based ELISA reaction, it became a popular method for identification and quantification of plant viruses (Duan 1995). ELISA is simple to perform, sensitive, and easily used in large scale analysis. There are several modifications in ELISA test that have been incorporated. They mostly differ in the quality and specificity of the reagents. In India monoclonal antibodies against tomato leaf curl virus (ToLCV) have been utilized for the detection of begomovirus infections in tomato and other crop species (Devaraja et al. 2003). In North India, potato apical leaf curl virus (PALCV) has been successfully detected and quantified using double-antibody sandwich ELISA (DAS-ELISA) (Venkatasalam et al. 2005). Later on DAS-ELISA and triple-antibody sandwich ELISA (TAS-ELISA) were also validated for the detection and quantification of *tomato leaf curl Bangalore virus* (ToLCBV) (Devaraja et al. 2005).

Although ELISA is a sensitive method for geminivirus detection, it has limitations of low specificity. The virus showed low antigenicity to its specific antibody developed in other species of animals. Moreover, geminiviruses shared a high degree of genome identity which results in many identical shared epitopes on virus surface. This results in high level of cross-reaction between different geminiviruses and causes difficulty in individual virus identification of closely related species. Therefore, new modifications in geminivirus-specific ELISA should be incorporated so as to enhance the sensitivity and specificity.

8.16 Dot Immunobinding Assay (DIBA)

Dot immunobinding assay (DIBA) is a simple, sensitive, and highly reproducible immunoassay. The sample to be checked is incubated on nitrocellulose membrane (NCM) disk. The sample is detected on NCM using suitable antigen or antibody. The presence of antigen-antibody complex is directly demonstrated by enzyme-conjugated antiglobulins and substrate. It can

detect multiple samples within 4–6 h of operational period (Sumi et al. 2009). DIBA is rapid and simple to perform than any other immunoassays such as enzyme-linked immunosorbent assay in the detection of antigen and antibody. DIBA has been used in the detection of tomato yellow leaf curl virus (TYLCV) (Hajimorad 1996).

8.17 Dot-Blot Hybridization (Nucleic Acid Spot Hybridization)

In this method the viral nucleic acid is applied on a nylon membrane and is allowed to hybridize with labeled viral nucleic acid as probe. The crude extract of DNA from infected plant tissue is ground in water or 0.4 M NaOH and applied on nylon membrane as a dot. Then it is hybridized with radioactively labeled viral DNA probe. Dot-blot hybridization is a very sensitive and specific method. It can detect and quantify the nanogram level of DNA (Caciagli et al. 2009). The geminiviruses from Indian ornamental crotons have been detected using this method (Raj 1996).

8.18 Southern Blot

Southern blot is a routine method for the detection of specific DNA segments from a variety of biological sample. The DNA fragments are transferred electrophoretically to a filter membrane and subsequently detected using hybridization probe (Southern 1975). In Southern blot, total DNA of infected plant is isolated and transferred on a membrane and hybridized with virus-specific radiolabeled nucleic acid probe. This method has been used for qualitative and quantitative determination of many geminiviruses, e.g., tomato leaf curl Gujarat virus- Varanasi, chili leaf curl Varanasi virus, squash leaf curl virus, and tomato leaf curl New Delhi virus (Chakraborty et al. 2008 Singh 2009).

8.19 Polymerase Chain Reaction (PCR) Assay

PCR is routinely used for the detection of plant viral genome. It is much more sensitive and specific than nucleic acid hybridization and ELISA assay. Rapid improvement in DNA polymerase enzyme of PCR and sequencing technology make it relatively easy to amplify and sequence full-length geminivirus genome. The specific primer set for different geminiviruses enable PCR to detect specific virus.

8.20 Geminivirus Detection Using Degenerate Primer

Degenerate primers are mixtures of similar, but not identical primers. They are used for amplification of same gene from different organisms. These genes are usually similar but not identical in their nucleotide sequence. Nucleic acid sequence analysis showed that all the begomoviruses share region of high homology in their genomes. Thus PCR using degenerate primer assay is proved as sensitive and effective assay for begomovirus identification (Ieamkhang et al. 2005; Ruhui et al. 2008).

8.21 Multiplex PCR

Multiplex polymerase chain reaction (Multiplex PCR) amplifies several different DNA targets simultaneously in a single PCR reaction. It uses multiple primers for genomic DNA samples and temperature-mediated DNA polymerase in a thermal cycler. All the primer pairs are optimized in such a way that all primer pairs can work at the same annealing temperature during PCR. A multiplex PCR was successfully validated for simultaneous detection of East African cassava mosaic Cameroon virus (EACMCV) and African cassava mosaic virus (ACMV) from cassava plant (Olufemi et al. 2008). Multiplex PCR can detect multiple geminiviruses at a time in a single

reaction. However, it can detect only a limited number of targets effectively simultaneous in simultaneous reaction. As the number of primers increases in PCR reaction, unexpected interactions may take place.

8.22 Real-Time Polymerase Chain Reaction (Real-Time PCR)

The real-time polymerase chain reaction is a PCR-based molecular biology technique which is used to amplify and simultaneously quantify a targeted DNA molecule from biological samples. The diagnosis of plant viruses using real-time PCR studies showed that real-time PCR exhibits greater sensitivity and specificity than conventional PCR and molecular hybridization assay (Korimbocus et al. 2002; Boonham et al. 2004). For its higher specificity and sensitivity, it has also been applied to detect viruses. In TaqMan probe-based detection fluorescence is generated by the cleavage of a TaqMan probe by the 5′ exonuclease activity of Taq DNA polymerase. The TaqMan-based technology (Higuchi et al. 1993; Heid et al. 1996) provides accurate and sensitive methods to quantify nucleic acids. The fluorescence measured is directly related to the amount of DNA by analyzing samples containing known amounts of reference DNA, in parallel reactions.

8.23 Rolling Circle Amplification

Rolling circle replication is a unidirectional nucleic acid replication which can rapidly synthesize multiple copies of circular molecules of nucleic acid. It can be used for diagnosis of geminiviruses. It is better and cheaper than antibody- and nucleic acid-based (PCR) detection method for geminivirus diagnosis. Moreover, Rolling Circle Amplification (RCA) procedure is cheaper and simple in handling and can detect viruses having circular DNA genome without actual sequence information. RCA produces microgram quantities of DNA from few copy of DNA in a few hours (Fig. 8.6). It utilizes bacteriophage φ29 DNA polymerase for exponential amplification of circular DNA templates (Dean et al. 2001). The φ29 DNA polymerase also ensures high-fidelity DNA replication because it has proofreading activity during DNA replication (Johne et al. 2009). Usually the starting material for diagnosis can be viral nucleic acid cloned in plasmid in

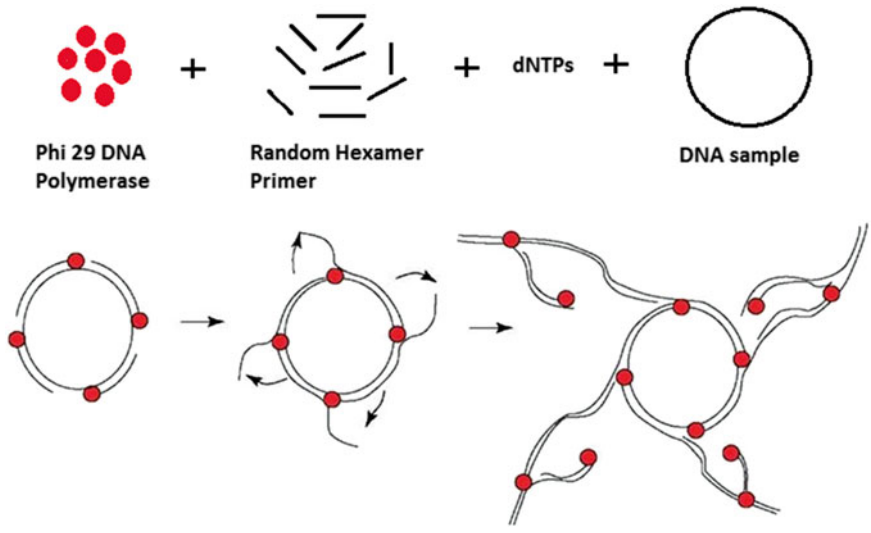

Fig. 8.6 *Rolling circle* amplification of *Geminivirus* genome using *random hexamer primers* and φ29 *DNA polymerase. Random hexamer primers* bind to the circular DNA template and generate multiple replication forks and amplification occurs

bacterial cells, intact M13 phage, etc. RCA can be used as a reliable diagnostic method for the detection of geminiviruses along with other viruses having small, circular, single-stranded DNA genomes.

8.24 Conclusion and Future Prospects of Geminiviruses

Geminiviruses are endemic in Indian crops probably due to warm tropical climate and intensive crop cultivation. Out of four major species of geminiviruses, begomoviruses cause major losses in many of Indian crop plants. However, other viruses, *Mastrevirus*, *Curtovirus*, and *Topocuvirus* are not much reported from India. The Indian begomoviruses have broad overlapping host range and they are reported from different crop species. The broad overlapping host range system may be due to mixed cropping systems and polyphagous nature of the whiteflies vector prevalent in the country. The various bioinformatics tools suggest that begomoviruses are undergoing recombination with other begomoviruses and they are producing highly virulent species such as emergence of cassava begomoviruses in the African continent. Recently the emergence of a large number of betasatellites and alphasatellites associated with begomoviruses in India is also reported. Thus now it became an area of immense importance for investigation and disease control for begomovirus diseases in India. The methods of controlling begomovirus infections in crop plants are expanding. The reports of success in controlling begomoviruses with transgenes are encouraging. Well-characterized resistance genes hold a lot of promise in controlling begomoviruses. However, only a few resistance genes have been known till date which can be used for production of begomovirus resistance crop varieties. Hence, extensive search must be undertaken to search for begomovirus-resistant genes in wild varieties of crop plants. Moreover, begomoviruses are transmitted by whiteflies vector. Therefore, virus-vector interaction is a crucial step in the spread of begomoviruses in the field. Thus, virus-vector interaction also needs to be carefully taken into consideration. These measures need to be urgently deployed to field level to assure crop protection due to begomoviral infections in India.

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Emerging Satellites Associated with Begomoviruses: World Scenario

9

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Abstract

Begomoviruses are abundant and worldwide in occurrence that cause economically important diseases not only in a vast range of crop plants but also in many weed plants which serve as reservoir host plants. Begomoviruses are associated with satellite molecules called as betasatellite and alphasatellite with DNA genomes approximately half the size of begomovirus DNA genomes. These satellites are also emerging very fast and are found abundantly in a number of crop plants associated with begomoviruses. Betasatellites are reported from the Old World, till today no betasatellite is reported from the New World though alphasatellites are now being reported from the New World too. Alphasatellites were earlier reported to be associated with monopartite begomoviruses only, but now they are reported with bipartite begomoviruses as well. This indicates their continuous emergence due to increasing host range. Genes encoded by the betasatellites (βC1) play important roles in the induction of symptoms and in gene silencing as suppressor of transcriptional and posttranscriptional gene silencing. Alphasatellites as such do not have any role in pathogenicity of begomoviruses. Some alphasatellites can attenuate disease symptoms caused by begomovirus-betasatellite complexes in the early stages of infection.

Keywords

Begomovirus • Alphasatellite • Betasatellite • Diversity

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9.1 Introduction

Geminiviruses (family *Geminiviridae*) are a large group of viruses infecting plants and are responsible for causing crop losses worldwide and are transmitted by insect vectors (white flies). The

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geminivirus genome is a single-stranded DNA which is encapsidated by twin incomplete icosahedral particle. There are seven genera in the family *Geminiviridae: Begomovirus*, *Becurtovirus*, *Curtovirus*, *Eragrovirus*, *Mastrevirus*, *Topocuvirus*, and *Turncurtovirus* based on their host range, insect vector, and genome organization (Varsani et al. 2014). The genus *Begomovirus* have emergent pathogens of crops throughout the tropical and subtropical regions of the world. Continuous emergence of begomoviruses is a threat to vegetable production in Southeast Asia (Varma and Malathi 2003; Varma et al. 2011, 2012, 2013). *Begomovirus* is the largest genus of plant viruses with respect to the number of species (288) presently recognized by the International Committee on Taxonomy of Viruses (ICTV) (Brown et al. 2015). Whitefly-transmitted geminiviruses cause tremendous losses in the number of vegetable and cereal crops throughout the American and the Caribbean Basin, the Mediterranean Plain, India, and Southeast Asia. Various crops are affected by begomoviruses such as cassava, cotton, bean, pepper, and tomato (Brown et al. 2015; Varma et al. 2011). In the Old World (OW; Africa, Asia, Australia, and Europe), mostly monopartite, begomoviruses are present with very few having a bipartite genome in comparison to begomoviruses native to the New World (NW; the Americas) which are almost exclusively bipartite, with only a single monopartite virus identified so far. However, a number of monopartite begomoviruses do occur in the NW as well as a result of their introduction from the OW (Sánchez-Campos et al. 2013; Melgarejo et al. 2013). New World and Old World begomoviruses are different genetically, and they fall in a separate group in phylogenetic analyses. The OW viruses show a greater genetic diversity and have an additional, conserved gene (known as V2 for the monopartite and AV2 for the bipartite viruses) which is absent in the NW begomoviruses.

Geminiviruses have monopartite genomes except begomoviruses. The begomovirus genome is bipartite (two DNA components; DNA-A and DNA-B, 2.6 kb) or monopartite (similar to DNA-A, 2.8 kb). With a few exceptions (Albuquerque et al. 2011; Choi et al. 2012), there is always association of betasatellite or alphasatellites or both in monopartite begomoviruses (Briddon et al. 2003). There are some reports available where satellites have also been found to be associated with bipartite begomoviruses (Romay et al. 2010; Jyothsna et al. 2013; Sivalingam and Varma 2012).

Till date two satellite DNA molecules associated with begomoviruses are reported: betasatellite and alphasatellite. Earlier known as DNA-β and now called as betasatellites, these molecules are found associated with monopartite begomoviruses, ~1360 nt in length (half the size of the helper virus genome). These betasatellites do not have any similarity in sequence with the helper viruses and are dependent on them for the vector transmission, movement, and replication. Trans-replication is also reported in New World begomoviruses (Nawaz-ul-Rehman et al. 2012). Betasatellites have a very conserved genome with adenine-rich region (A-rich), which is known as the satellite-conserved region (SCR). This conserved region is also present as single open reading frame (ORF) in the complementary strand in *Tomato leaf curl virus*-sat (ToLCV-sat) and codes for the *β*C1 protein (Briddon et al. 2003, 2008). The satellite-conserved region is similar to the origin of replication of geminiviruses and nanoviruses, and it has a hairpin structure with a loop sequence TAA/GTATTAC (Briddon et al. 2003). Betasatellites help in augmentation of the accumulation of their helper begomoviruses and also help in the enhancement of the symptoms in some host plants (Briddon et al. 2001; Nawaz-ul-Rehman and Fauquet 2009; Patil and Fauquet 2010; Saunders et al. 2000), which is due to *β*C1 protein and its role as suppressor of silencing (Cui et al. 2005). Alphasatellites and betasatellites need helper virus for replication and symptom attenuation (Idris et al. 2011).

Beside betasatellites, some begomoviruses are also associated with an additional single-stranded DNA component, which was previously called as DNA-1, and now it is known as alphasatellite (Briddon et al. 2004). These molecules are mostly

half the size of begomovirus DNA components (~1375 nt) and show a common organization which consist of a single ORF and it codes for a Rep protein which shows resemblance to nanoviruses (Mansoor et al. 1999; Saunders and Stanley 1999). For insect transmission and movement within the plants, these molecules need a helper begomovirus but in host plants they are capable of self-replication (Saunders and Stanley 1999; Saunders et al. 2000). In some cases these alphasatellites have also been shown to reduce the begomovirus-betasatellite symptoms by reducing betasatellite DNA accumulation (Idris et al. 2011). Two different alphasatellites have been found to be associated with New World begomoviruses. In Brazil, they were found to associated with two bipartite begomoviruses which infect weeds (Euphorbia mosaic virus and Cleome leaf crumple virus), and in both the cases, they contain the typical conserved genome features of alphasatellites, including a gene encoding a Rep protein, an A-rich region, and a hairpin structure similar to those of alphasatellites reported from Africa (Paprota et al. 2010). The alphasatellite-like molecule reported from Venezuela was associated with the *Melon chlorotic mosaic virus* which is a bipartite begomovirus, and it had all the genome features of this type of DNA satellite, and its sequence is different from that of Old World alphasatellites (Romay et al. 2010).

Alphasatellites were also isolated from dragonflies which transmit mastreviruses from agricultural fields in Puerto Rico (Rosario et al. 2013). Cotton leaf curl Multan alphasatellite (CLCuMA) and a Guar leaf curl alphasatellite (GLCuA) were detected in different field samples of wheat which were infected with *Wheat Dwarf India Virus* (WDIV) (*Mastrevirus)* (Kumar et al. 2014).

9.2 Betasatellites

Satellite virus and satellite RNA are commonly found associated with RNA viruses. They are defined as virus or nucleic acid that depends on a helper virus for replication, but lacks nucleotide sequence homology to the helper viral genome. In addition, satellite RNA depends on helper virus for transmission by encapsidation within the same coat protein coded by helper virus along with helper virus nucleic acid. A majority of satellites interfere with the replication of helper virus and cause attenuation in symptoms but some contribute to increased severity of symptoms (Murant and Mayo 1982; Collmer and Howell 1992; Dry et al. 1997 and Mansoor et al. 2003). No DNA satellite was found to be associated with any plant DNA viruses till 1997. However, in a prokaryotic system, bacteriophage P4 has been classified as a satellite DNA with the genome size of 11.6 kb containing 13 functional genes; the p4 DNA maintains itself as a multiple copy plasmid in the infected host cells, and it depends on helper phage for lyses (Dry et al. 1997). Unlike RNA satellite the P4 DNA bacterial phage satellite does not depend on a helper for replication and transmission. In 1997, a novel subviral agent, satellite DNA of 682 nucleotides in length, was found associated with a monopartite *Tomato leaf curl virus* originating from Australia (ToLCV-sat). The satellite DNA depends on the helper virus for its replication, encapsidation, and transmission and has no role in disease pathogenesis (Dry et al. 1993).

The first full-length betasatellite (1347 nts in length) was identified in 1999 in an Ageratum yellow vein virus (AYVV)-infected *Ageratum conyzoides* plant showing yellow vein symptoms (Saunders et al. 2000). Since then, many begomovirus-betasatellite disease complexes have been shown to be responsible for economically important diseases in different plant species in Africa and Asia, especially in China and the Indian subcontinent. These virus complexes elicit various disease symptoms, including leaf curling, enations, and yellow veins, and are major threats to crops (Mansoor et al. 2003, 2006). The complexes also cause diseases in a wide range of dicotyledonous host species within at least 37 different genera in 17 families that include vegetable crops, fiber and ornamental plants, and many weeds (Zhou 2013).

The betasatellite molecule can be defined as symptom modulating, circular, single-stranded

DNA that lacks sequence homology with helper virus except the loop sequence of nonanucleotide TAATATT/AC, seen in begomoviruses, and depends on helper virus for replication and transmission (Saunders et al. 2000; Mansoor et al. 2003; Briddon et al. 2003 and Zhou et al. 2003). Most of the characterized begomoviruses associated with betasatellites are monopartite and occur in the Old World. Betasatellites are indispensable for induction of typical virus symptoms in host plants.

9.2.1 Structural Features of Betasatellite

Analysis of nucleotide sequence of betasatellite molecular data suggests that there is no obvious sequence homology with helper begomovirus DNA-A components except for the nonanucleotide sequence, TAATATT/AC. It is contrasting to what is seen in bipartite begomoviruses, wherein approximately 200 nucleotides are common for the DNA-A and DNA-B belonging to the same species. The betasatellite possesses three major regions: satellite-conserved region (SCR), adenine-rich region (a rich region), and an open reading frame βC1 (ORF βC1) (Briddon et al. 2003 and Zhou et al. 2003; Sivalingam et al. 2010). SCR is -200nt in length and consists of stem and loop (S-L) region, and it is conserved for all betasatellite molecules. The most conserved region (93–100 %) within SCR is approximately 115 nucleotides at the 5′ region of SCR (Zhou et al. 2003). The SCR may be having iteron sequence for protein (Rep), binding of which will mediate replication. This specific Rep binding sequence is not yet identified in betasatellite as in the case of bipartite DNA-A and DNA-B. However, in few betasatellite iteron sequences have been identified. In Ageratum yellow vein disease (AYVD), Saunders et al. in 2000 found that DNA-A of Ageratum yellow vein virus AYVV has GGTACTCA as its iteron, the same sequences were not found in betasatellite. But similar sequences GCTACGCA and GGTACAACA were identified in upstream of S-L portion suggesting its function as an iteron-binding sequence for Rep (Saunders et al. 2000), but experimental evidence are lacking mutation experiments, and sequence comparison between ToLCV and ToLCV-Sat in Australia revealed another stem and loop segment called S-L II (Dry et al. 1997). Based on nucleotide sequence analysis, Briddon and group (2003) found that SCR has cryptic Rep binding site for trans-replication of betasatellite by helper begomovirus and identified five-base-pair core sequences, GGN1N2N3, and a variable number of addition of nucleotide which are species specific. These iteron sequences are possibly generic recognition sequences for rep rather than species specific. Multiple alignment of SCR of betasatellite associated with Bhendi yellow vein mosaic disease (BYVMD), Cotton leaf curl disease (CLCuD), AYVD, and ToLCV-sat showed that there are no iteron sequences as presented in their helper virus, but the sequence GCTACGC occurred twice in upstream of S-L; this sequence may be an iteron sequence for rep (Jose and Usha 2003). The Rep binding site for satellite DNAs associated with *Cotton leaf curl Gezira virus* (CLCuGV) originating from the Nile Basin consists of directly repeated sequence (CGGTACTCA) and an inverted repeated sequence (TGATGACCG) occurring in the text of 17 nucleotide motif (Idris et al. 2005).

A-rich region is approximately 160–280 nucleotides in length, located upstream of SCR, from 750 to 1000th nucleotide coordinate (Zhou et al. 2003). This region is maintained in all the betasatellite molecules including ToLCV-sat from Australia. It is hypothesized that A-rich region may arise due to duplication of the sequence that they may act as "stuffer" (a region of arbitrary sequences) required to the size of the betasatellite molecule to get encapsidated inside the coat protein (Saunders et al. 2000). However, Briddon et al. (2003) suggest it to have a function in complementary strand DNA replication.

The betasatellite encodes many ORFs with the predicted protein molecular weight of more than 4kDa, but only one ORF is present in complementary strand (approximately from 550 to 200th nucleotide coordinate), encode−13.5 kDA

to−17.5 kDa protein called βC1, which is known to be functional. ORF βC1 is positionally conserved in all betasatellites characterized so far, from different geographical locations and diverse host species. There is a TATA box upstream of the ORF and polyadenylation signal for transcription to occur (Briddon et al. 2003; Mansoor et al. 2003; Bull et al. 2004 and Cui et al. 2004a, b).

In a large number of infected samples analyzed, defective deletion mutants of betasatellite have been found to occur in nature. The mutant maintains SCR and A-rich region but the deletion was observed in ORF βC1 (Briddon et al. 2003 and Bull et al. 2004).

9.2.2 Betasatellites Associated with Begomovirus

Betasatellite molecules isolated and characterized so far are found to be associated with many diseases caused by monopartite begomoviruses (Briddon et al. 2001, 2003; Mansoor et al. 2003; Saunders et al. 2000; Zhou et al. 2003, Radhakrishnan et al. 2004, Jose and Usha 2003; Bull et al. 2004; Xiong et al. 2005; Singh et al. 2011, 2012) and some are found associated with bipartite begomoviruses (Malathi et al. 2004; Rouhibakhsh and Malathi 2005). As far as identification of betasatellite is concerned; a major breakthrough came while investigating Ageratum yellow vein disease (AYVd), which was known to be caused by monopartite begomovirus having DNA-A alone. Inoculation with DNA-A alone did not produce any typical symptoms in ageratum as seen in the field (Stanley et al. 1997). Rigorous attempts made to isolate another component, DNA-B were unsuccessful. Several recombinant DNA of approximately half the size to helper genome were isolated from an yellow vein disease infected *Ageratum conyzoides* plants and they were characterized.

Some of the recombinant clones had part of DNA-A sequence and sequence of unknown origin. Using primers in PCR approach betasatellite and alphasatellite were isolated and sequenced (Mansoor et al. 1999 and Saunders et al. 2000). Sequenced data revealed that the recombinant/defective components had the sequence of unknown origin and shared no homology with helper DNA-A except for TAATATTAC.

Betasatellites are capable of being *trans*-replicated by different begomoviruses, so a variety of begomovirus-betasatellite complexes can occur. For example, the cotton leaf curl disease (CLCuD) in Pakistan is reportedly caused by association of a single betasatellite, Cotton leaf curl Multan betasatellite (CLCuMuB), with at least six begomovirus species, either as single or multiple infections (Nawaz-ul-Rehman et al. 2012). African CLCuD-associated begomovirus *Cotton leaf curl Gezira virus* (CLCuGV) was identified in cotton from southern Pakistan where CLCuMuB is known to be found (Tahir et al. 2011). It has also been reported that true monopartite begomoviruses, such as *Tomato yellow leaf curl virus* (TYLCV) and *Papaya leaf curl China virus* (PLCCNV), can *trans*-replicate betasatellites (Zhang et al. 2009; Zhang et al. 2010). These observations raise an alarming scenario in which true monopartite begomoviruses may form new disease complexes by acquiring other begomovirus betasatellites in mix-infected plants. Indeed, divergent isolates of Tomato yellow leaf curl disease (TYLCD) from Oman is associated with a betasatellite (Khan et al. 2008), and a severe symptom phenotype in tomato in Mali is caused by a novel begomovirus-betasatellite complex resulting from reassortment (Chen et al. 2009). Cotton leaf curl Gezira betasatellite (CLCuGB), initially identified in the Nile basin, has been identified in West Africa, associated with diseased okra and tomato (Chen et al. 2009; Kon et al. 2009; Shih et al. 2009; Tiendrebeogo et al. 2010; Idris et al. 2014).

9.2.3 Diversity of Betasatellite

Information on the existence and diversity of DNA satellite molecules associated with monopartite begomoviruses has been mainly from Asia (Bull et al. 2004; Nawaz-ul-Rehman and Fauquet 2009; Sivalingam et al. 2010). Identification of betasatellite molecules, which are associated with monopartite begomoviruses widely distributed in the Old World, led to inves-

tigation on the molecular variability in the betasatellite. Considerable variation has been found in nucleotide sequence of full-length betasatellite. A-rich region and amino acid sequence of ORF βC1. However, conservation was also found in the ORF βC1. A major initiative was taken to determine the variability in Yunana province of China (Zhou et al. 2003), Pakistan, India, Egypt, Singapore, UK, (Briddon et al. 2003) and East and South east Asian countries (Bull et al. 2004). The nucleotide sequence of complete betasatellite was compared with betasatellite earlier reported from CLCuD, Bhendi yellow vein mosaic disease (BYVMD) and Agretum yellow vein disease (AYVD) originating from India, Pakistan and Singapore (Saunders et al. 2000; Jose and Usha 2003, Radhakrisnan 2003, Zhou et al. 2003). Analysis of nucleotide sequence of eighteen betasatellite molecules with their corresponding helper DNA-A was done. This analysis include 13 betasatellite associated with *Tomato yellow leaf curl China virus* (TYLCCNV), four betasatellite with *Tobacco curly shoot virus* (TbCSV) and one with *Cotton leaf curl Multan virus* (CLCuMV). Based on their analysis, they proposed a species concept that betasatellite molecules sharing 72–99 % identity belong to one species and betasatellite sharing 36–57 % identity to be considered as different species, with the exception of betasatellite associated with *Malvastrum Yellow Vein Virus* (MYVV-Y47) (62–67 % identity) and betasatellite *with Cotton leaf curl Rajasthan virus* CLCuRaV.

Briddon et al. 2003 showed that nucleotide sequence similarity was 49–99 % between the betasatellite molecules, but for betasatellite molecules originating from different disease and/or geographical locations (called unrelated betasatellite) nucleotide similarity was up to 71 % indicating their association with their helper /disease in a geographical isolation. Amino acid sequence comparison of βC1 showed low level of sequence identity (35–50 %) between βC1 of betasatellite molecules.

Bull et al. 2004 attempted to understand the diversity within betasatellite isolated from East and Southeast Asia. They showed that there was more diversity within the region due to the limited movement of begomovirus-betasatellite complex and suggest that all the betasatellite molecules originated from a common ancestor and further got adapted according to the host.

Betasatellites are reported from various crops and along with different viruses in India. This shows their diversity and emergence (George et al. 2014; Kumar et al. 2014; Srivastava et al. 2013a, b; Singh et al. 2012) . Betasatellites reported from different countries is depicted in the world map in Fig. 9.1. Betasatellites reported from various crops is being tabulated in the Table 9.1.

9.2.4 Recombinant Betasatellite

Evidence for recombinant betasatellite is based on nucleotide sequence analysis. The betasatellite associated with CLCuRV can be divided into two parts (Zhou et al. 2003). Part one is 184 nt located between 1065 and 1269, which has only 62 % identity with betasatellite of CLCuMV, but rest of the region showed 98 % similarity with the same betasatellite. This indicate that betasatellite associated with CLCuRV and CLCuMV evolved by recombination (Zhou et al. 2003). Another example is betasatellite associated with Tomato leaf curl disease (TomLCD) in Pakistan (TomLCDβ01-Pak). This betasatellite molecule evolved by natural recombination between betasatellite associated with TomLCD and CLCuD (CLCD β02-Pak) as the SCR of TomLCDβ02-Pak showed 90 % nucleotide identity with TomLCDβ02-Pak and only 77 % with CLCDβ02-Pak. Another betasatellite associated with Okra leaf curl disease (OLCD) (OLCD β03-Pak) also seems to have evolved through recombination (Briddon et al. 2003).

9.2.5 Role of Betasatellite in Pathogenesis

Betasatellite has been found essential in the pathogenesis and expression of typical symptoms of enation, vein thickening, extreme leaf crinkling, twisting of petioles, etc. induced by the begomoviruses causing disease in ageratum,

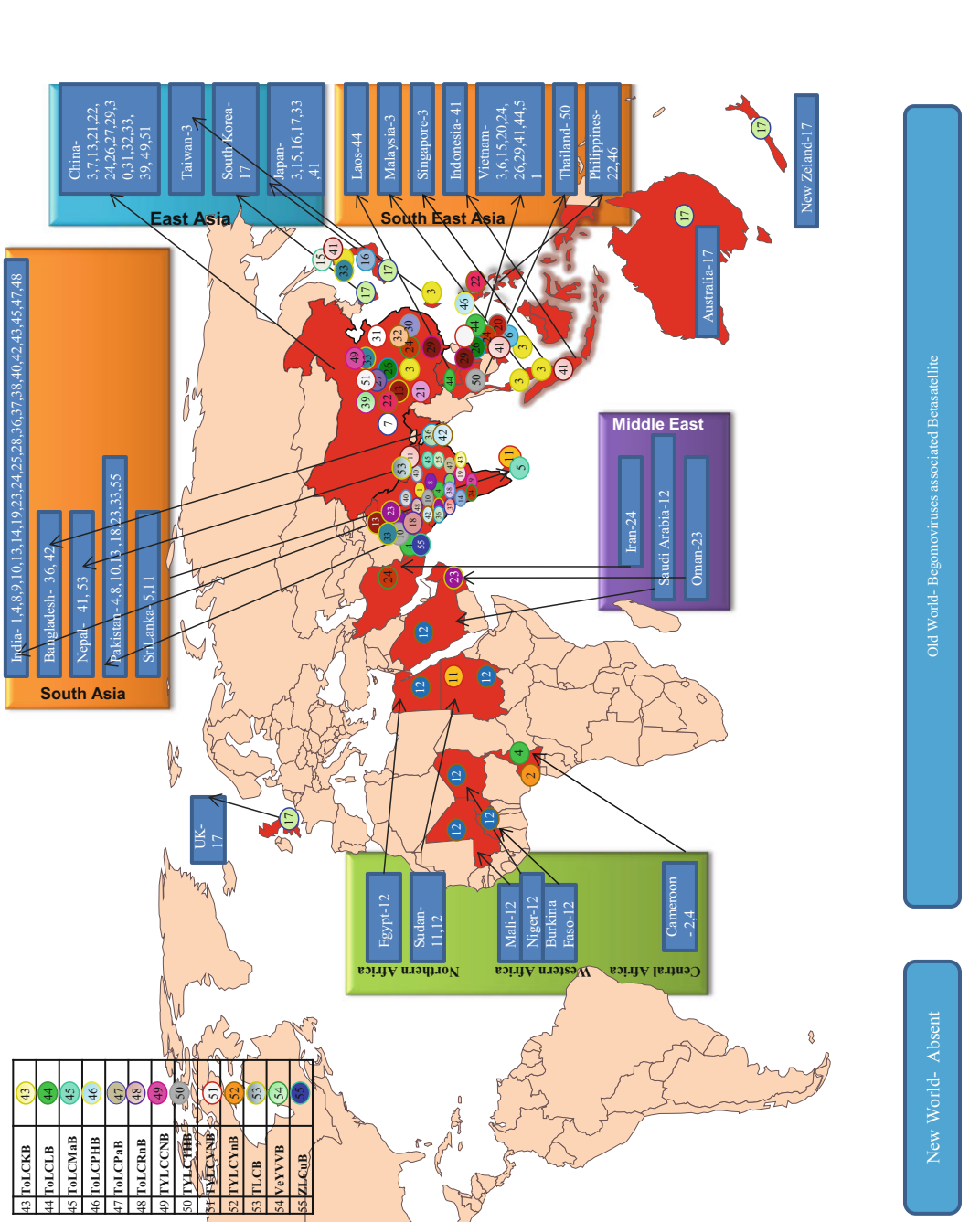

Fig. 9.1 World wide distribution of betasatellites associated with begomoviruses. Red color corresponds to countries all over the world from where betasatellites have been reported. Numbers on the map are indicative of specific area in the country from where they are reported and these number also correspond to betasatellite name in the Table 9.1

Table 9.1 List of begomovirus-associated betasatellites reported on different crops from various parts of the world

SN	Acronym	Betasatellite	Associated begomovirus	Crop	Year	Place	Country	Accession number	Reference
1	**ALCB**	Ageratum leaf curl betasatellite	Papaya leaf curl virus	Aster	2010	Lucknow	India	JQ408217	Srivastava et al. (2013a)
			Ageratum enation virus	*Amaranthus hypochondriacus*	2011	Lucknow	India	JX512904	Srivastava et al. (2014)
			Ageratum enation virus	*Amaranthus cruentus*	2008	Lucknow	India	JQ710745	Srivastava et al. (2013a, b)
			Ageratum enation virus	*Tagetes patula*	2012	Sikar	India	KC589700 NC_020889	Marwal et al. (2013)
			–	*Papaver somniferum*	2012	Lucknow	India	KJ948106	Unpublished
2	**ALCCMB**	Ageratum leaf curl Cameroon betasatellite	Ageratum leaf curl Cameroon virus	*Ageratum* sp.	2010	–	Cameroon	FR717141	Leke et al. (2012)
3	**AYVB**	Ageratum yellow vein betasatellite	Ageratum yellow vein virus	*Tobacco* sp.	2008	Fuzhou	China	EF527824	Unpublished
			–	*Ageratum conyzoides*	2003	Klang	Malaysia	AJ542497	Bull et al. (2004)
			Ageratum yellow vein virus	*Ageratum conyzoides*	1999	–	Singapore	AJ252072, NC_003403	Saunders et al. (2000)
			–	*Solanum lycopersicum*	2003	Changhua	Taiwan	AJ542495	Bull et al. (2004)
			Papaya leaf curl China virus	*Tobacco* sp.	2006	Hatay	Vietnam	DQ641709	Ha et al. (2008)
			Ageratum yellow vein virus	*Solanum lycopersicum*	2005	Okinawa	Japan	AB306522	Andou et al. (2010)
			–	*Ageratum* sp.	–	–	India	AJ316042	Briddon et al. (2003)
			Chili leaf curl India virus	*Mentha* sp.	2011	–	India	KF364485	Saeed et al. (2014)
			–	*Ageratum conyzoides*	2003	Madurai	India	AJ557441	Unpublished
4	**AYLCB**	Ageratum yellow leaf curl betasatellite	–	*Solanum nigrum*	2011	–	India	KJ028213	Unpublished
			Wheat Dwarf India Virus	Wheat	2011	Mohali	India	KC305092 KC305091 KC305089 KC305090 KC305088	Kumar et al. (2014)
			Ageratum enation virus	*Daucus carota*	2010	Mohali	India	JF728869	Kumar et al. (2013a, b)
			Tobacco curly shoot virus	Sunflower	2010	Karnataka	India	HQ407397	Unpublished
			–	*Ageratum* sp.	2003	Madurai	India	AJ557441	Unpublished
			–	*Ageratum* sp.	2008	Liongo	Cameroon	FM164738	Unpublished
			–	*Ageratum* sp.	2003	–	Pakistan	NC_005046 NC_011762	Briddon et al. (2003)
			–	*Hibiscus esculentus*	1997	Bahawalpur	Pakistan	AJ316031	Briddon et al. (2003)
			Alternanthera yellow vein virus	*Sonchus oleraceus*	2006	Lahore	Pakistan	AM412239	Mubin et al. (2010)
			–	*Ageratum* sp.	2003	–	Pakistan	AJ316026	Briddon et al. (2003)
			Ageratum enation virus	*Ageratum* sp.	2007	Lahore	Pakistan	AM698010	Tahir et al. (2015)
			–	*Ageratum* sp.	2003	–	Pakistan	NC_005046 NC_011762	Briddon et al. (2003)
5	**AYVSB**	Ageratum yellow vein Sri Lanka betasatellite	Ageratum yellow vein mosaic virus	*Ageratum* sp.	2004	–	Sri lanka	AJ542498	Bull et al. (2004)
6	**AlYVB**	Alternanthera yellow vein betasatellite	Alternanthera yellow vein mosaic virus	*Zinnia* sp.	2008	Hue	Vietnam	DQ641716	Ha et al. (2008)
7	**BLCCNB**	Bean leaf curl China betasatellite	Tomato yellow leaf curl China virus	Kidney bean	2005	–	China	DQ256459	Dong et al. (2007)
8	**BYVB**	Bhendi yellow vein betasatellite	Bhendi yellow vein mosaic virus	*Bhendi (Abelmoschus esculentus)*	2001	Madurai	India	AJ308425	Jose and Usha (2003)
			–	*Abelmoschus esculentus*	2008	Varanasi	India	HM590506	Unpublished
			–	*Abelmoschus esculentus*	2008	Dhanbad	India	HM590504	Unpublished
			–	*Abelmoschus esculentus*	2009	Aurangabad	India	KJ462079	Unpublished
			–	*Abelmoschus esculentus*	2011	Hyderabad	India	KC222955	Unpublished
			–	*Abelmoschus esculentus*	2014	Madurai	India	KR068483	Unpublished
			–	*Abelmoschus esculentus*	2007	Barrackpore	India	EF417919	Unpublished
			–	*Urena lobata*	2007	Barrackpore	India	EU188922	Unpublished
			–	*Hibiscus esculentus*	2001	–	Pakistan	AJ316030	Briddon et al. (2003)
9	**BYIVB**	Bhendi yellow India vein betasatellite	–	*Abelmoschus esculentus*	2006	Bangalore Aurangabad Tamil Nadu Guntur Karnataka Maharashtra Orissa Haryana	India	GU111991 NC_014846 GU111994 GU111993 GU111992 GU111988 GU111986 GU111962	Venkataravanppa et al. (2011)
			–	*Abelmoschus esculentus*	2009	Jalna	India	KJ462078	Unpublished
			–	*Abelmoschus esculentus*	2009	Coimbatore	India	KJ462077	Unpublished
			–	*Abelmoschus esculentus*	2009	Kalyani	India	KJ462076	Unpublished
			–	*Abelmoschus esculentus*	2009	Vijayawada	India	KJ462075	Unpublished

(continued)

Table 9.1 (continued)

10	**ChiLCB**	Chili leaf curl betasatellite	Chili leaf curl virus	*Petunia hybrida*	2014	–	India	KJ700655	Nehra and Gaur (2014)
			–	–	2005	Lucknow	India	DQ343289	Unpublished
			Pepper leaf curl Lahore virus	Capsicum	2007	Sahiwal	Pakistan	AM849549	Tahir et al. (2010)
			–	–		Meerut	India	JX193616	Unpublished
			–	*Solanum lycopersicum*	2009	Muskabad, Punjab	India	KJ605111	Unpublished
			–	Chili	2004	Jodhpur, Rajasthan	India	JF706231	Unpublished
			–	Capicum	2008	Palampur	**India**	FM877803	Unpublished
			–	Papaya	2008	Panipat	**India**	HM143904	Singh et al. (2012)
			–	*Abelmoschus esculentus*	2013	Moga	**India**	KJ614228	Unpublished
			–	*Gossypium hirsutum*	2005	Sindh, Tando Adam	Pakistan	FR751147	Tahir et al. (2011)
			–	*Capsicum annuum*	2001	–	Pakistan	NC_005048	Briddon et al. (2003)
			–	*Solanum lycopersicum*	2008	Multan	Pakistan	FJ515274	Unpublished
			–	*Solanum tuberosum*	2008	Punjab	Pakistan	FM179615	Mubeen et al. (2009)
11	**ChiLCSiB**	Chili leaf curl Sri Lanka betasatellite	Chili leaf curl Sri Lanka virus	Chili	2009	Nochchiyagama	Sri Lanka	JN555600 JN638445 JN638446	Senanayake et al. (2013)
12	**CLCuGB**	Cotton leaf curl Gezira betasatellite	Cotton leaf curl Gezira virus	Gossypium sp. Sida *Abelmoschus esculentus*	2005	–	Sudan	AY669329 AY077798 AY077799	Idris et al. (2005)
			–	Datura	2006	–	Sudan	DQ644564	Unpublished
			Cotton leaf curl Gezira virus	Okra	2007	–	Sudan	FJ868829	Unpublished
			Cotton leaf curl Gezira virus	Gossypium sp. *Abelmoschus esculentus*	2014	Jazan	Saudi Arabia	HG530542 KM279620	Idris et al. (2014)
			–	Gossypium sp.	2001	–	Egypt	AY044143	Unpublished
			Cotton leaf curl Gezira virus	*Abelmoschus esculentus*	2009	Sadore	Niger	FJ469629	Shih et al. (2009)
			Cotton leaf curl Gezira virus	*Abelmoschus esculentus*	2010	–	Burkina Faso	FN554579	Tiendrebeogo et al. (2010)
			Cotton leaf curl Gezira virus	–	–	–	Mali	–	Kon et al. (2009)
13	**CLCuMB**	Cotton leaf curl Multan betasatellite	–	*Gossypium* sp.	2010	–	Pakistan	HE963748	Unpublished
			–	*Gossypium* sp.	2010	–	Pakistan	HF564604 HF564603	Unpublished
			–	*Gossypium* sp.	2004	Multan	Pakistan	EU384598	Unpublished
			–	*Nicotiana benthamiana*	2009	–	Pakistan	FJ861372	Nawaz-ul-Rehman et al. (2012)
			–	Gossypium sp.	2010	–	Pakistan	FJ607041	Unpublished
			–	*Gossypium* sp.	2010	Multan	Pakistan	EU384587	Nawaz-ul-Rehman et al. (2010)
			–	Hibiscus	2008	Bhangha	India	EU825206	Paul et al. (2008)
			–	*Rumex nepalensis*	2015	Bandla, Palampur	India	LN810556	Unpublished
			–	Hibiscus	2004	Bhubaneswar	India	AY704663	Unpublished
			–	Mentha	2007	Ludhiana	India	EU862815	Borah et al. (2010)
			–	Hibiscus	2008	Haringhata	India	EF614159	Das et al. (2008)
			–	*Abelmoschus esculentus*	2009	–	China	FJ770371	
			–	Hibiscus	2007	–	China	EF465536	Mao et al. (2008)
			–	Gossypium sp.	2010	Nanning	China	GQ906588	
14	**CroYVMB**	Croton yellow vein mosaic betasatellite	Sweet potato leaf curl virus	*Ipomoea purpurea*	2012	New Delhi	India	JX050198	Geetanjali et al. (2013)
			–	*Benincasa hispida*	2014	Perambalur	India	KM588256	Unpublished
			–	Turnip	2014	New Delhi	India	KM229763	Unpublished
			–	Cucumber	2013	New Delhi	India	KC545814	Unpublished
			–	–	2013	Pune	India	JN663878	Unpublished
			–	Brassica	2012	New Delhi	India	JX270685	Unpublished
			–	Sunn hemp	2009	Barrackpore	India	GQ183866	Unpublished
			–	Radish	2008	–	India	FJ593630	Unpublished
15	**ErYMB**	Erechtites yellow mosaic betasatellite	Erechtites yellow mosaic virus	–	2008	HoaBinh	Vietnam	DQ641713	Ha et al. (2008)
16	**EpYVB**	Eupatorium yellow vein betasatellite	–	*Eupatorium makinoi*	2003	Fukuoka Prefecture	Japan	AJ438938	Saunders et al. (2003)
			Eupatorium yellow vein mosaic virus	Eupatorium	2003	Nishigoshi, Suya	Japan	AB300464	Ueda et al. (2008)
17	**HYVB**	Honeysuckle yellow vein betasatellite	Honeysuckle yellow vein mosaic virus	*Lonicera japonica*	2001	Ibaraki	Japan	NC_009571	Ogawa et al. (2008)
			Honeysuckle yellow vein mosaic virus	*Solanum lycopersicum*	2003	Masuda	Japan	AB236326	Ueda et al. (2008)
			–	Honeysuckle	2004	Kobe, Hyogo	Japan	AB182263	Unpublished
			–	Tomato	2012	–	South Korea	JQ728545	Unpublished
			– –	*Lonicera japonica* Tomato	1983 2012	Ayr, Queensland	Australia South Korea	JX416175 JQ728545	Unpublished Unpublished

(continued)

Table 9.1 (continued)

			Honeysuckle yellow vein mosaic virus	Honeysuckle	2003	–	UK	AJ316040	Briddon et al. (2003)
			–	*Lonicera japonica*	2003	Norfolk, Deopham	UK	AJ543430	Unpublished
			–	*Lonicera japonica*	2003	–	New Zealand	GQ131809	Unpublished
18	**KLCuB**	Kenaf leaf curl betasatellite	–	Holly hock	2007	Lahore	Pakistan	FN678779	Unpublished
			–	Alcea sp.	2014	–	Pakistan	LK028572	Unpublished
19	**LuLDB**	Ludwigia leaf distortion betasatellite	–	*Hibiscus cannabinus*	2008	Amadalavalasa Bahraich Kaisarganj	India	EU557374 EF614160 EF614162	Das et al. (2008)
			Mesta yellow vein mosaic Bahraich virus	Hibiscus	2009	Srikakulam	India	FJ159273	Roy et al. (2009)
			–	–		Bihar	India	JX315326	Unpublished
			–	Luffa	2004	Bihar	India	AY728262	Unpublished
			–	Bitter gourd	2004	–	India	AY817151	Unpublished
20	**LYVB**	Lindernia anagallis yellow vein betasatellite	Lindernia anagallis yellow vein mosaic virus	False pimpernel	2004	Hanoi	Vietnam	DQ641715	Ha et al. (2008)
21	**LuYVB**	Ludwigia yellow vein betasatellite	Ludwigia yellow mosaic virus	*Ludwigia hyssopifolia*	2006	Guangxi	China	AJ965541	Huang and Zhou (2006)
22	**MaLCB**	Malvastrum leaf curl betasatellite	–	Malvastrum	2011	Guangzhou	China	NC_023896	Unpublished
			Malvastrum leaf curl virus	*Ageratum Malvastrum*	2006	Guangxi	China	AJ971264	Huang and Zhou (2006)
			–	*Malvastrum coromandelianum*	2013	–	Philippines	NC_021929	Unpublished
23	**OLCuB**	Okra leaf curl betasatellite	–	*Abelmoschus esculentus*	2014	Ludhiana	**India**	KM116013	Unpublished
			–	*Abelmoschus esculentus*	2014	Sangrur	**India**	KM116012	Unpublished
			–	*Abelmoschus esculentus*	2004	Gurdaspur	**India**	KM108330	Unpublished
			–	*Abelmoschus esculentus*	2014	Barka	Oman	KF267444	Akhtar et al. (2014)
			–	*Abelmoschus esculentus*	2003	–	Pakistan	AJ316029	Briddon et al. (2003)
			–	*Abelmoschus esculentus*	2003	–	Pakistan	AJ316031	Briddon et al. (2003)
24	**PaLCuB**	Papaya leaf curl betasatellite	–	*Ipomoea purpurea*	2009	New Delhi	**India**	JX050199	Geetanjali et al. (2013)
			–	Papaya	2012	Vamban, Pudukkottai	**India**	KC959935	Unpublished
			–	Chili	2008		**India**	JN663874	Unpublished
			–	Papaya	2005	Chinthapalli	**India**	DQ118862	Unpublished
			–	*Solanum lycopersicum*	2009	New Delhi	**India**	GU370715	Unpublished
			–	Papaya	2003	New Delhi	**India**	AY244706	Unpublished
			–	Papaya	2014	Hainan	China	KJ642219	Unpublished
			–	Papaya	2015	–	Iran	KJ397536	Unpublished
			Papaya leaf curl China virus	Papaya	2008	Hatay	Vietnam	NC_009555	Ha et al. (2008)
25	**RaLCB**	Radish leaf curl betasatellite	–	Chili	2008	Salem	**India**	JN663873	Unpublished
			–	Crotalaria juncea	2008	Pratapgarh	**India**	GQ200445	Unpublished
			–	Chili	2011	Goa	**India**	JN663858	Unpublished
			–	Capsicum annuum	2010	Varanasi	**India**	JF513201	Unpublished
			Radish leaf curl virus	Radish	2006	Varanasi	**India**	NC_010239	Singh et al. (2012)
			–	Okra	2008	Vellanad	**India**	JN663877	Kumar et al. (2013)
26	**SiLCuB**	Sida leaf curl betasatellite	–	Abutilon	2000	Thanhhoa	Vietnam	NC_009557	Ha et al. (2008)
			Sida leaf curl virus	Sida	2006	Hainan	China	AM050733	Guo and Zhou (2006)
27	**SiYMCNB**	Sida yellow mosaic China betasatellite	*Sida yellow mosaic China virus*	Sida sp.	2004	–	China	AJ810095	Xiong et al. (2005)
			Sida yellow mosaic virus	Sida sp.	2006	–	China	AM048833	Xiong et al. (2006)
28	**SiYVB**	Sida yellow vein betasatellite	–	Sida sp.	2005	Madurai	**India**	AJ967003	Unpublished
			–	*Sida cordifolia*	2007	Barrackpore	**India**	EU188921	Unpublished
29	**SiYVVNB**	Sida yellow vein Vietnam betasatellite	–	*Sida acuta*	2004	Guangdong	China	KF990602	Unpublished
			–	*Sida rhombifolia*		–	Viet nam	DQ641712	Ha et al. (2008)
30	**SibYVB**	Siegesbeckia yellow vein betasatellite	Siegesbeckia yellow vein virus	*Siegesbeckia glabrescens*	2007	Guangdong province	China	NC_008237	Wu et al. (2007a, b)
			–	Siegesbeckia	2014	–	China	KF499590	Unpublished
31	**SibYVGxB**	Siegesbeckia yellow vein Guangxi betasatellite	Siegesbeckia yellow vein guangxi virus	Siegesbeckia	2007	Guangxi Province	China	AM238695	Wu et al. (2007a, b)
32	**TbCSB**	Tobacco curly shoot betasatellite	Tobacco leaf curl virus	Tobacco	2003	Yunan	China	AJ457822	Zhou et al. (2003)
			–	Ageratum sp.	2006	Yunan	China	AM260736	Unpublished
33	**TbLCB**	Tobacco leaf curl betasatellite	–	Tobacco	2011	Nawab Shah	Pakistan	NC_013800	Unpublished
			–	China bean	2008	Nawab Shah	Pakistan	FM955608	Unpublished
			–	–	1999	–	Pakistan	AJ316034	Unpublished
34	**TbLCJB**	Tobacco leaf curl Japan betasatellite	–	–	2005	–	Japan	AB236326	Ueda et al. (2008)
35	**TLCYuB**	Tobacco leaf curl Yunnan betaatellite	–	Tobacco	2012	Yunan	China	KC699043	Unpublished
36	**ToLCBB**	Tomato leaf curl Bangalore betasatellite	Tomato leaf curl Bangalore virus	*Solanum lycopersicum*	2010	Hessarghatta (Bangalore)	India	GU984046	Tiwari et al. (2010)
			–	–		Coimbatore	India	AY438560	Unpublished

(continued)

Table 9.1 (continued)

37	**ToLCBDB**	Tomato leaf curl Bangladesh betasatellite	–	*Solanum lycopersicum*	2001	Gazipur	Bangladesh	AJ542489	Bull et al. (2004)
			–	–	2003	Rajsthan	India	AY438558	Unpublished
			–	–	2005	Lucknow	India	DQ343289	Unpublished
			–	Brinjal	2012	Varanasi	India	JX311469	Unpublished
			–	Chili	2012	–	India	KF188707	Unpublished
			–	Chili	2008	–	India	JN663875	Unpublished
38	**ToLCGaB**	Tomato leaf curl Gandhinagar betasatellite	Tomato leaf curl Gandhinagar virus	*Solanum lycopersicum*	2012	Gandhinagar	India	NC_023038 KC952006	Rathore et al. (2014)
39	**ToLCCNB**	Tomato leaf curl China betasatellite	–	*Ageratum conyzoides*	2003	Guangxi	China	AJ704618	Unpublished
			–	Tomato	2002	–	China	AJ704609	Unpublished
			–	*Solanum lycopersicum*	2003	Yunan	China	AJ457820	Zhou et al. (2003)
40	**ToLCHJB**	Tomato leaf curl Hajipur betasatellite	Cotton leaf curl Burewala virus	*Solanum lycopersicum*	2010	Hajipur	India	NC_018614	Kumar et al. (2013)
41	**ToLCJB**	Tomato leaf curl Java betasatellite	Tomato leaf curl Java virus	Ageratum sp.	2007	–	Japan	AB162142	Kon et al. (2007)
			–	–	2003	Java	Indonesia	AB100306	Kon et al. (2006)
			–	*Carica papaya*	–	–	Nepal	KC282642	Shahid et al. (2013)
42	**ToLCJoB**	Tomato leaf curl Joydebpur betasatellite	–	Hibiscus cannabinu	2008	Ponduru	**India**	EU880233	Paul et al. (2008)
			Tomato leaf curl Joydebpur virus	*Solanum lycopersicum*	2013	Kalyani, West Benga	**India**	JN176566	Tiwari et al. (2013)
			Tomato leaf curl Joydebpur	*Solanum lycopersicum*	2006	–	Bangladesh	AJ966244	Maruthi et al. (2006)
			–	Chili	–	Nagpur	**India**	JN663863	Unpublished
43	**ToLCKB**	Tomato leaf curl Karnataka betasatellite	–	*Solanum lycopersicum*	–	Gujarat	**India**	KF515611	Unpublished
			–	*Solanum lycopersicum*	2012	Anand	**India**	KF515610	Unpublished
			–	–	2006	Janti	**India**	NC_008523	Unpublished
44	**ToLCLB**	Tomato leaf curl Laos betasatellite	–	–	2001	Savannakhet	Laos	AJ542491	Bull et al. (2004)
			Mimosa yellow leaf curl virus	Mimosa sp.	2006	–	Vietnam	DQ641710	Ha et al. (2008)
45	**ToLCMaB**	Tomato leaf curl Maharashtra betasatellite	–	*Solanum lycopersicum*	2009	Pune	India	AY838894	Unpublished
46	**ToLCPHB**	Tomato leaf curl Philippines betasatellite	Tomato leaf curl Philippines virus	*Solanum lycopersicum*	2011	–	Philippines	NC_009570	Sharma et al. (2011)
47	**ToLCPaB**	Tomato leaf curl Patna betasatellite	Tomato leaf curl Patna virus	*Solanum lycopersicum*	2008	Patna	**India**	NC_012493	Kumari et al. (2010)
			–	*Mirabilis jalapa*	2013	Palampur	**India**	LK054803	Unpublished
			–	Tobacco	2007	Pusa	**India**	HQ180393	Singh et al. (2011)
48	**ToLCRnB**	Tomato leaf curl Ranchi betasatellite	Tomato leaf curl Ranchi virus	*Solanum lycopersicum*	2007	Ranchi	**India**	JN663872 GQ994096	Kumari et al. (2010)
49	**TYLCCNB**	Tomato yellow leaf curl China betasatellite	–	Artemisia carvifolia	2007	–	China	EU365687	Unpublished
			Tomato yellow leaf curl China virus	Tomato	2008	–	China	AJ781301	Tao and Zhou (2008)
			–	Malva parviflora	2012	Sichuan	China	JX679253	Unpublished
			Tobacco leaf curl Yunan virus	*Solanum lycopersicum*	2004	Yunnan	China	AJ536627	Cui et al. (2004)
			Tomato leaf curl china virus	Malvastrum	2009	Zhejiang	China	AM980512	–
			–	*Solanum lycopersicum*	2009	–	China	GU199589	Unpublished
50	**TYLCTHB**	Tomato yellow leaf curl Thailand betasatellite	–	*Solanum lycopersicum*	2004	–	China	NC_004903	Li et al. (2004)
			–	*Solanum lycopersicum*	2004	–	China	AJ566748	Li et al. (2004)
			–	*Solanum lycopersicum*	2008	Dhanbad	**India**	EU573713	Unpublished
			–	Amaranthus sp.	2011	Banswara	**India**	KF471033	George et al. (2014)
51	**TYLCVNB**	Tomato yellow leaf curl Vietnam betasatellite	Tomato yellow leaf curl Thailand virus, Tomato yellow leaf curl Vietnam virus	*Solanum lycopersicum*	2008	–	Vietnam	EU189148	Blawid et al. (2008)
			Tomato yellow leaf curl Vietnam virus	*Solanum lycopersicum*	2006	–	Vietnam	NC_009560 DQ641714 EU189148	Ha et al. (2008)
52	**TYLCYnB**	Tomato yellow leaf curl Yunan betasatellite	–	–	2002	–	China	AJ421620	Zhou et al. (2003)
53	**TLCB**	Tomato leaf curl betasatellite	–	*Solanum lycopersicum*	2003	–	Nepal	AJ542492	Bull et al (2004)
54	**VeYVVB**	Vernonia yellow vein betasatellite	Vernonia yellow vein virus	*Vernonia cinerea*	2009	Madurai	**India**	NC_013423	Packialakshmi et al. (2010)
55	**ZLCuB**	Zinnia leaf curl betasatellite	Zinnia yellow leaf curl virus	Zinnia	2003	–	Pakistan	AJ316028	Briddon et al. (2003)
			Zinnia leaf curl virus	Zinnia	2004	Pattaya	Thailand	AJ542499	Bull et al. (2004)

((–) – Not Found)

bhendi, cotton, etc. when betasatellite is co-inoculated with DNA- A of helper virus (Briddon et al. 2001; Mansoor et al. 2003; Zhou et al. 2003; Jose and Usha 2003).

In bipartite begomoviruses DNA-A and DNA-B have specific iteron sequence in a species-specific manner. This assumes replication of cognate DNA-B by the Rep encoded by corresponding DNA-A. Similarly, betasatellite associated with different diseases like Agretum yellow vein disease (AYVD), Agretum yellow leaf curl diseases (AYLCD), Cotton leaf curl diseases (CLCuD), Honeysuckle yellow vein mosaic disease (HYVMD), Okra leaf curl disease (OLCD), Okra yellow vein mosaic disease (OYVMD), Tobacco leaf curl disease (TbLCD) and Tomato leaf curl disease (ToLCD) have been shown to be replicated by Rep protein encoded by DNA-A of different begomovirus species (Briddon et al. 2003; Bull et al. 2004). It indicates that the betasatellite has relaxed specificity for Rep to get replicated. Inoculation of DNA-A of *Sri Lankan cassava mosaic virus* (SLCMV) with betasatellite associated with AYVD produced typical yellow vein symptoms in *Ageratum conyzoids*, but symptoms develops when DNA-A alone is inoculated. These results indicate biological activity and requirement of betasatellite in pathogenesis and its relaxed specificity for Rep encoded by SLCMV (Saunders et al. 2002a, b). The betasatellite associated with CLCuD in Pakistan is also shown to replicate with the help of DNA- A of monopartite begomoviruses like CLCuMV, *Cotton leaf curl Kokhran virus* (CLCuKV), *Cotton leaf curl Alabad virus* (CLCuAV) and *Papaya leaf curl virus* (PaLCV) (Mansoor et al. 2003). ToLCV –sat from Australia has been shown to replicate not only by other begomovirus species but also by other genus; Curtovirus (*Beet curly top virus*) (Dry et al. 1997). So the specificity for Rep to replicate TLCV-sat DNA appears to be more relaxed. Mutation experiments have shown the role of ORF βC1 in the pathogenesis of TYLCCNV-y10 DNA-A; DNA-A when co-inoculated with betasatellite having in-frame AUG mutated ORF βC1 produced milder symptoms, which were comparable with DNA-A alone inoculated plants; however the replication of betasatellite plays an important role in symptom development but which is not essential for replication of betasatellite (Zhou et al. 2003 and Cui et al. 2004a, b).

Two major phenotypic symptoms are observed in disease complex with betasatellite like the vein yellowing as found in Ageratum yellow vein and leaf curling, vein swelling, vein darkening, ectopic enations, etc. as in CLCuD. Vein darkening is caused by replacement of spongy parenchyma by palisade parenchyma. Mansoor et al. 2003 have suggested that abnormal cambium activity in phloem parenchyma leads to formation of secondary vascular elements leading to downward leaf curling.

The role of βC1 gene of betasatellite in symptom development has been confirmed in transgenic *N. benthamiana* plants having βC1 of betasatellite associated with AYVD. These plants develop malformed leafy structure; severely distorted stems and leaves, vein greening, etc. as in diseased plants (Saunders et al. 2004 and Cui et al. 2004a, b). Leaf distortion and severe curling symptoms were produced in and transgenic *N. benthamiana* and *N. tabacum* βC1 gene associated with TYLCCNV-Y10.

Betasatellite has also been shown to contribute to expansion of host range of the associated begomoviruses. SLCMV, a bipartite begomovirus does not infect ageratum, but, when SLCMV DNA-A is co- inoculated with betasatellite of AYVD in ageratum, is infected producing typical yellow vein symptoms (Saunders et al. 2002a, b). The role of betasatellite in host range determination has also been demonstrated for TYLCCNV-Y10. Co-inoculation of TYLLCNV-Y10 DNA-A with associated betasatellite having deletion, infection was obtained in *N. benthamiana* and *N. glutinosa* but not in *N. tabacum* and *Lycopersicum esculentum* plants (Qian and Zhou 2005).

Co-inoculation of begomoviruses with the associated betasatellite produces more severe symptoms than in inoculation with the respective begomovirus alone. The co-inoculated plants also have high level of accumulation of helper begomovirus DNA-A. Earlier the enhanced severity in disease symptoms was considered to

be due to the infection of betasatellite on DNA-A replication (Saunders et al. 2000). But the recent evidences show that *β*C1 protein of betasatellite of TYLCCNV-Y10 could interact with host factor(s) to induce symptoms. They either act as suppressor of gene silencing or interfere with host defense system thereby allowing more efficient systemic infection of the plants (Cui et al. 2004a, b, 2005; Saunders et al. 2004) demonstrated that *β*C1 protein of DNA-β of TYLCCNV-Y10 and TbCSV-Y35 could bind both single-stranded and double-stranded DNA in size and sequence nonspecific manner. They could find that *β*C1 protein accumulation is the key requirements for symptom induction and silencing suppression.

Infectivity assays of Tomato leaf curl New Delhi virus (ToLCNDV) and Chili leaf curl betasatellite (ChLCB) were conducted by Akhtar et al. 2014, DNA-A and DNA-B of ToLCNDV isolated from chilies and tomato were found to be infectious and produced leaf curl symptoms when inoculated on *Nicotiana benthamiana* by biolistic gun method. Co-inoculation of ToLCNDV with ChLCB resulted in the severity of disease symptoms.

Association of betasatellites with bipartite begomoviruses is rare and has only been reported in India, where the role of a betasatellite in pathogenesis of a bipartite begomovirus, *Tomato leaf curl New Delhi virus* (ToLCNDV), was investigated (Sivalingam and Varma 2012). Tomato leaf curl New Delhi virus (ToLCNDV) DNA-A alone could infect tomato and *Nicotiana benthamiana* and induces mild symptoms. When these two hosts were co-inoculated with ToLCNDV DNA-A and ToLCNDV DNA-B or ToLCNDV DNA-A and CLCuMuB, typical leaf curling symptoms developed, but co-infections with all three components resulted in much more severe disease symptoms.

In addition, ToLCNDV DNA-A and DNA-B accumulated to six to eightfold higher levels in plants co-inoculated with all three components than in plants co-inoculated with only DNA-A and DNA-B. Like many other viral pathogenicity determinants, the βC1proteins can function as RNA silencing suppressors. The reported βC1 suppressors include TYLCCNB-βC1, CLCuMuB-βC1, and βC1 proteins of betasatellite associated with *Bhendi yellow vein mosaic virus* (BYVMV), *Tomato leaf curl Java virus* (ToLCJAV), and *Tomato leaf curl China virus* (ToLCCNV) (Cui et al. 2005; Gopel et al. 2007; Sharma et al. 2011). Unlike most geminiviruses studied, *Tomato yellow leaf curl China virus* (TYLCCNV) is susceptible to cytosine methylation and is not effective in suppressing TGS of a green fluorescent protein transgene in plants. In contrast, βC1 from TYLCCNB is able to mediate TGS suppression (Sunter et al. 1994).

Betasatellites enhance the accumulation of their helper begomoviruses by increasing the symptoms induced in some host plants (Briddon et al. 2001; Nawaz-ul-Rehman and Fauquet 2009; Patil and Fauquet 2010; Saunders et al. 2000), it is most probably due to the βC1 protein gene's silencing suppressor activity (Cui et al. 2005; Saeed et al. 2005).

9.2.6 Evolutionary Relationship

Origin of betasatellite is still unknown; however, certain speculations have been made based on available information. Bipartite begomoviruses might have evolved from an ancestral monopartite virus by component capture and duplication along with acquired novel genetic material. DNA-A donated its origin of replication to DNA-B while evolving and acquired additional genetic material. Alternatively, the monopartite virus donated origin of replication to betasatellite originating from an unknown progenitor and adopted along with DNA-A by component capture mechanism; component capturing led to extension of the host range, adaptation in the new environmental condition producing novel disease. Variability arose by recombination mechanism which provided enormous scope for diversification and modification of biological properties to allow adaptation to new ecological niches (Mansoor et al. 2003). Recombination, a powerful tool for evolution of betasatellite has been shown in several betasatellite associated

with AYVD and CLCuD by different research groups (Zhou et al. 2003; Briddon et al. 2003).

9.2.7 Betasatellite Used as Vector

*β*C1 gene of betasatellite associated with TYLCCNV-Y10 isolate was replaced with multiple cloning site (MCS) facilitating insertion of gene such as proliferating cell nuclear antigen (PCNA), phytoene desaturase (PDS) and sulfur (Su) gene or green fluorescent protein (GFP) in the MCS of betasatellite. Such constructs were inoculated with TYLCCNV-Y10 DNA-A separately on *N. benthamiana*, *N. glutinosa*, *N. tabacum* and tomato plants. Silencing of the above mentioned gene was found in all the plants. Results showed that this was due to *β*C1 gene which might have acted as a suppressor of gene silencing. It opens the possibility of the use betasatellite mediated vector system in functional genomics (Tao and Zhou 2008).

9.2.8 Emerging Betasatellites

Betasatellites are reported from South Asia, East Asia, Southeast Asia, Africa, the Middle East, the UK, Australia and New Zealand. That is from the Old World; there is no report of betasatellites from the New World. A number of betasatellites reported along with host plants are given in the Table 9.1 and also illustrated in the Fig. 9.1.

9.3 Alphasatellites

In 1999 a Nanovirus-like DNA component associated with Yellow vein disease of *Ageratum conyzoides* was reported (Saunders and Stanley 1999) which was later called as Agretum yellow vein alphasatellite. This was the first evidence of association of satellite like particles with geminiviruses. Thereafter number of alphasatellites are being reported from all over the world from different crops, viz., okra, cassava, chili, ageratum, sunflower, cotton, croton, hollyhock, Malvastrum, tobacco, tomato, and mesta. In 1999 from Pakistan, a novel circular ssDNA associated with cotton leaf curl disease was reported (Mansoor et al. 1999).

Alphasatellite genomes are approximately1,375nts and encode a single ORF (alpha-Rep). The alpha-Rep ORF encodes a 315 amino acid protein of ~ 37 kDa that resembles nanovirus Reps. Alpha-Rep sequences are more conserved than the full-length alphasatellite sequences (Briddon et al. 2004; Xie et al. 2010). A-rich region ~150- to 200-nt has an A content between 46 % and 58 % and is the only feature that can be used to distinguish begomovirus alphasatellites from nanovirus Rep-encoding components. It has been suggested that the A-rich sequences may only function to increase sizes of alphasatellite molecules to half the size of the begomovirus components (Briddon et al. 2004). The predicted alphasatellite hairpin structure has a loop containing a nonanucleotide, TAGTATTAC, common to nanoviruses that is also similar to the analogous TAATATTAC nonanucleotide sequence in begomovirus loop structures.

Alphasatellites have no contribution to symptoms induced by begomovirus-betasatellite disease complexes and appear to affect betasatellite replication but do not affect helper virus replication. Some alphasatellites can attenuate disease symptoms caused by begomovirus-betasatellite complexes in the early stages of infection (Nawaz-Ul-Rehman et al. 2010).

Alphasatellite was identified in Ageratum in Singapore (referred to as DNA-2) (Saunders et al. 2002a, b). DNA-2 type alphasatellite members have been identified in Oman (Idris et al. 2011) and India (Zaffalon et al. 2012). Although all these members contain conserved alphasatellite genome features, the DNA-2 type molecules are less homogeneous and have less than 50 % nucleotide sequence identity with each other. The DNA2 type alphasatellite identified in Oman can attenuate begomovirus symptoms and reduce accumulations of betasatellites. Alphasatellites were though discovered almost 16 years ago, still very less information is available about their function(s). Begomovirus-associated defective satellites have also been identified in malvaceous plants in Cuba and whiteflies in Florida, thus

indicating the natural occurrence of this type of satellite molecule (Fiallo-Olivéa et al. 2012).

9.3.1 Origin and Evolution of Alphasatellites

The alphasatellites most likely originated from nanoviruses through adaptation of a nanovirus component by becoming encapsidated in the begomovirus coat protein for whitefly transmission after vector feeding on plants co-infected with their begomovirus and nanovirus progenitors (Patil and Fauquet 2010). A related class of alphasatellites has also been found associated with viruses in the *Nanoviridae* family. Most alpha Reps are highly conserved, but alphasatellites found in the New World are more diverse and clarification of their evolution requires additional sequence studies of a wider range of isolates (Briddon et al. 2014).

9.3.2 Emerging Alphasatellites

Unlike betasatellites, the presence of alphasatellites, as in the case of Ageratum yellow vein Singapore alphasatellite (AYVSGA), Tobacco curly shoot alphasatellite (TbCSA), *Gossypium darwinii* symptomless alphasatellite (GDarSLA), and *Gossypium mustelinum* symptomless alphasatellite (GMusSLA) in plants infected with begomovirus-betasatellite complexes, reduces the accumulation of betasatellite (Wu and Zhou 2005; Idris et al. 2011; Nawaz-ul-Rehman et al. 2010) and viral symptoms (Wu and Zhou 2005; Idris et al. 2011). The coding region is most closely related to those of nanoviral Reps. Though alphasatellites can replicate autonomously, but they require helper virus for insect transmission and systemic spread in plants (Saunders et al. 2000; 2002a, b; Saunders and Stanley 1999). Alphasatellites are being reported from all over the world from number of crops. In mixed infections where both alphasatellite and betasatellite particles were used with helper begomovirus, alphasatellite modulated the begomovirus-betasatellite pathogenicity by interfering with βC1 a key virulence factor (Idris et al. 2011).

Occurrence of alphasatellites in different countries is given in Fig. 9.2 and detailed list of alphasatellites and crops on which they are reported is mentioned in Table 9.2.

9.3.3 Role of Alphasatellites

Alphasatellites were first detected when they were found associated with disease complexes of DNA β and helper virus (Briddon and Stanley 2006; Mansoor et al. 2003; Stanley 2004). It is speculated that after co-infection of begomoviruses and nanoviruses to host plants which are common host of both viruses these might have adapted to whitefly transmission (Mansoor et al. 1999; Saunders et al. 2002a, b; Saunders and Stanley 1999).

Alphasatellites can replicate autonomously, but a helper virus is required for insect transmission and for systemic spread in plants. (Saunders et al. 2000; 2002a, b; Saunders and Stanley 1999). In case of alphasatellites no effect has been reported on the development of symptoms (Briddon et al. 2004). In some reports an unusual class of alphasatellites has been shown to attenuate begomovirus-betasatellite symptoms by reducing betasatellite DNA accumulation (Idris et al. 2011). The alpha-Rep proteins encoded by two nonpathogenic alphasatellites: Gossypium darwinii symptomless alphasatellite, *Gossypium mustelinium* symptomless alphasatellite (GDarSLA and GMusSLA) associated with Cotton leaf curl Rajasthan virus (CLCuRaV) can interact with CLCuRaV Rep proteins (Nawaz-Ul-Rehman et al. 2010).

Alphasatellites possess a gene encoding a protein most closely related to the replication initiator (Rep) protein of nanoviruses. Consequently, they are capable of autonomous replication in plant host cells but require the helper begomovirus for movement within the plant and for insect transmission (Briddon et al. 2004). Alphasatellite molecules have been shown to ameliorate symptoms (Idris et al. 2011; Nawaz-ul-Rehman et al. 2012; Wu and Zhou 2005). Their presence has been reported not only in cultivated plants (tomato, cotton, okra, tobacco, watermelon), but also in ornamental and wild plants (*Althea rosea*,

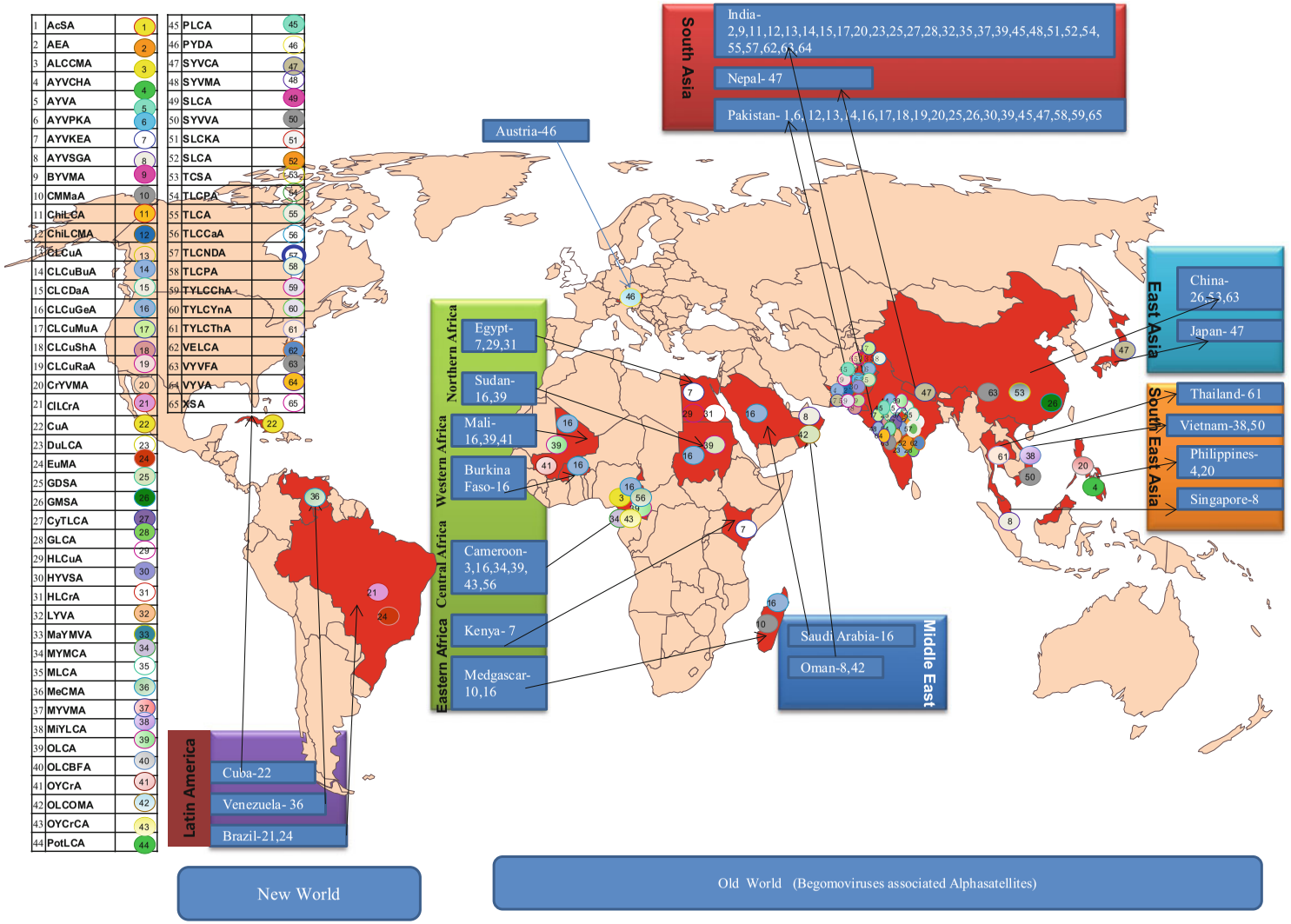

Fig. 9.2 World wide distribution of alphasatellites associated with begomoviruses. Red color corresponds to countries all over the world from where alphasatellites have been reported. Numbers on the map are indicative of specific area in the country from where they are reported and these number also correspond to alphasatellite name in the Table 9.2

Table 9.2 List of begomovirus-associated alphasatellites reported on different plants from various parts of the world

SN	Acronym	Alphasatellite	Associated begomoviruse	Crop	Year	Place	Country	Accession number	Reference
1	**AcSA**	Ageratum conyzoides symptomless alphasatellite	–	*Abelmoschus esculentus*	2012	Pano Aqil, Sindh	Pakistan	HG518788, HG518789, HG518790, HG518791, HG518792, NC_024710	Unpublished
2	**AEA**	Ageratum enation alphasatellite	–	*Papaver somniferum*	2012	Lucknow	India	JX913532, NC_019547	Unpublished
			–	*Amaranthus cruentus*	2011	Lucknow		JQ012793	Srivastava et al. (2013a, b)
			–	*Synedrella* sp.	2013	Palampur		LN558518	Unpublished
3	**ALCCMA**	Ageratum leaf curl Cameroon alphasatellite	Ageratum leaf curl Cameroon virus	*Ageratum conyzoides*	2010 2010 2008 2008 2008 2008	Cameroon	Cameroon	NC_014744, FR717143, FN675294, FN675292, FN675295, FN675293	Leke et al. (2012)
4	**AYVCHA**	Ageratum yellow vein China alphasatellite	Ageratum yellow vein China virus	*Syndrella nodiflora*	2012	–	Philippines	NC_023443, KF785752	She and He (2015)
5	**AYVA**	Ageratum yellow vein alphasatellite	Ageratum yellow vein virus	*Ageratum conyzoides*	2001	–	Singapore	AJ416153	Saunders et al. (2002a, b)
			Ageratum yellow vein virus	*Ageratum conyzoides*	1999	–	–	Y14168	Saunders and Stanley (1999)
6	**AYVPKA**	Ageratum yellow vein Pakistan alphasatellite	–	Ageratum conyzoides	2001	–	Pakistan	AJ512949	Briddon et al. (2004)
7	**AYVKEA**	Ageratum yellow vein Kenya alphasatellite	–	*Ageratum conyzoides*	2002	–	Kenya	AJ512963	Briddon et al. (2004)
			–	–	–	–	Egypt	AJ512960	Briddon et al. (2004)
8	**AYVSGA**	Ageratum yellow vein Singapore alphasatellite	Tomato yellow leaf curl virus	*Solanum lycopersicum*	2005	–	Oman	FJ956707	Idris et al. (2011)
			–	*Ageratum conyzoides*	–	–	Singapore	AJ238493 AJ416153	Briddon et al. (2004)
9	**BYVMA**	Bhindi yellow vein mosaic alphasatellite	–	Bhindi/Okra	2014	Madurai Ludhiana	India	NC_026947 KR068484 KJ843306	Unpublished
10	**CMMaA**	Cassava mosaic Madagascar alphasatellite	Cassava mosaic virus	*Manihot esculenta*	2013	Diana	Madagascar	HE984148 HE984149 NC_018628	Harimalala et al. (2013)
11	**ChiLCA**	Chili leaf curl alphasatellite	–	*Capsicum frutescens*	2012	Rajasthan	India	KF584013	Unpublished
			–	Amaranthus sp.	2011	Banswara	India	KF471047 NC_024374	George et al. (2014)
12	**ChiLCMA**	Chili leaf curl Multan alphasatellite	–	*Solanum tuberosum*	2008	Punjab	Pakistan	NC_013103 FM179614	Mubin et al. (2009)
			–	*Sonchus arvensis*	2010	Faisalabad	Pakistan	FN432360	Mubin et al. (2010)
13	**CLCuA**	Cotton leaf curl alphasatellite	Cotton leaf curl virus	Gossypium sp.	1999	–	Pakistan	AJ132344, AJ132345	Mansoor et al. (1999)
			–	*Luffa aegyptiaca*	2012	Bhatinda	India	KF584010	Unpublished
			–	*Gossypium hirsutum*	2012	Bhatinda	India	KF584012	Unpublished
				Gossypium hirsutum	2013	New Delhi	India	KM070823	Unpublished
14	**CLCuBuA**	Cotton leaf curl Burewala alphasatellite	–	*Gossypium hirsutum*	2007	Punjab	India	FN658727 FN658728, FN658729 FN658730 FN658733 FN658734	Zaffalon et al. (2012)
			–	*Gossypium hirsutum*	2012	Burewala	Pakistan	HF567947	Hameed et al. (2014)
			–	Gossypium sp.	2010	Sri Ganga nagar	India	GU992936 GU992937 HQ316180	Unpublished
			–	Gossypium sp.	2014	Punjab	India	KM923999	Unpublished
			–	*Triticum aestivum*	2011	Mohali	India	KC305094	Kumar et al. (2014)
			–	*Abelmoschus esculentus*	2010	Bihar	India	HM004548	Unpublished
			–	*Abelmoschus esculentus*	2009	Hajipur	India	HQ728354	Unpublished
			–	*Solanum lycopersicum*	2010	Hajipur	India	JX262389	Kumar et al. (2013a, b)
			–	Gossypium sp.	2013	Haryana	India	KM103526	Unpublished
			–	*Alcea rosea*	2006	Lahore	Pakistan	FR772089 FR772090 FR772091	Unpublished
			–	*Gossypium hirsutum*	2011	Burewala	Pakistan	HF567947	Hameed etr al. (2014)
			–	Gossypium sp.	2011	–	Pakistan	HE965670–HE965677, HE965681,HE965682, HE965683,HE965685–HE965689, HE966425, HE979545,HE972284, HE972283, HE972282,HE972281, HE972280, HE972279, HE972278, HE972277	Unpublished

(continued)

Table 9.2 (continued)

15	**CLCuDaA**	Cotton leaf curl Dabwali alphasatellite	–	*Gossypium hirsutum*	1995	Rajasthan Dabwali	India	AJ512957	Briddon et al. (2004)
16	**CLCuGeA**	Cotton leaf curl Gezira alphasatellite	Cotton leaf curl Gezira virus	*Solanum lycopersicum*	2011	Wad Maidani	Sudan	KC763634	Fialo olive et al. (2013)
			Tomato leaf curl Sudan virus	*Solanum lycopersicum*	2011	Wad Maidani	Sudan	KC763633 KC763632 KC763631 KC763630	Fialo olive et al. (2013)
			Cotton leaf curl Gezira virus	*Abelmoschus esculentus*	2010 2008 2008	–	Burkina faso	NC_013593 FN554583 FN554582 FN554581 FN554580	Tiendrebeogo et al. (2010)
			–	Hollyhock	2010	Shambat	Sudan	HM446369	Unpublished
			–	–	–	–	Mali	–	Kon et al. (2009)
			East African cassava mosaic Kenya virus	–	–	–	Madagascar	–	Harimalala et al. (2012)
			–	*Abelmoschus esculentus*	2007	Njomba	Cameroon	HE858192	Leke et al. (2011)
			–	–	2013	Jazan	Saudi Arabia	HG530547	Idris et al. (2014)
			–	*Abelmoschus esculentus*	–	–	Saudi Arabia	HG530546 HG530545 HG530543	Idris et al. (2014)
			–	*Gossypium hirsutum*	2012	–	Pakistan	KC763631	Unpublished
17	**CLCMuA**	Cotton leaf curl Multan alphasatellite	–	*Gossypium hirsutum*	2012	–	Pakistan	HF564605 HF564602 HF564600 HE965684	Unpublished
			–	–	2011	–	Pakistan	HE979548 HE979546 HE978348 HE978347 HE978346 HE978345 HE966424 HE965684 HE965680 FR873573 FR873571 HE599399 HE599398 FR877532	Unpublished
			–	*Gossypium hirsutum*	2011	–	Pakisatan	HF567946	Hameed et al. (2014)
			–	Wheat	2012	–	India	KC305093	Kumar et al. (2014)
			–	*Solanum nigrum*	2011	Mohali	India	KJ028212	Unpublished
18	**CLCuShA**	Cotton leaf curl Shahdadpur alphasatellite	–	–	–	–	–	–	Amrao et al. (2010)
19	**CLCuRaA**	Cotton leaf curl Rajasthan alphasatellite	–	*Digera arvensis*	–	–	Pakistan	–	Mubin et al. (2009)
20	**CrYVMA**	Croton yellow vein mosaic alphasatellite	–	*Gossypium hirsutum*	2014	–	India	KM103524 KM103523	Unpublished
			–	*Gossypium hirsutum*	2013	Hissar	India	KM103524 KM103523	Unpublished
			–	*Malvastrum coromandelianum*	2012	–	Philippines	KC577541	Unpublished
			–	*Acalypha* sp.	2007	Haryana	India	–	Zaffalon et al. (2012)
			–	*Gossypium hirsutum*	2010	Faisalabad	Pakistan	FR877534	Unpublished
21	**ClLCrA**	Cleome leaf crumple alphasatellite	Cleome leaf crumple virus	–	–	–	Brazil	NC_014646 FN436007	Paprotka et al. (2010)
22	**CuA**	Cuban alphasatellite 1	Sida yellow mottle virus (SiYMoV), tomato yellow distortion leaf virus (ToYDLV), sida golden mosaic Florida virus (SiGMFV), sida golden mosaic Liguanea virus (SiGMLV)	–	2014	–	Cuba	NC_021708 HE806451	Jeske et al. (2014)
23	**DuLCA**	Duranta leaf curl alphasatellite	–	–		Rajasthan	India	KC206077	Unpublished
24	**EuMA**	Euphorbia mosaic alphasatellite	–	–			Brazil	–	Paprotka et al. (2010)
25	**GDSA**	Gossypium darwinii symptomless alphasatellite	–	*Gossypium hirsutum*	2012	Multan	Pakistan	HE972276 HE965679 HE965678 NC_013013	Unpublished
			–	Luffa	2011	Burewala	Pakistan	HF567944	Unpublished
			–	*Alcea rosea*	2006	Lahore	Pakistan	FR772092	Unpublished
			–	*Gossypium davidsonii*	2008	Multan	Pakistan	EU384626	Unpublished
			–	*Gossypium hirsutum*	–	–	–	EU384651 EU384647 EU384646 EU384645 EU384644	Unpublished
			–	*Gossypium hirsutum*	2013	Haryana	India	KM103525	Unpublished
			–	*Abelmoschus esculentus*	2014	Ludhiana	India	KJ843307 KJ843305	Unpublished
26	**GMSA**	Gossypium mustelinum symptomless alphasatellite	–	–		Lahore	Pakistan	FR772087	Unpublished
			–	*Gossypium mustelinum*	2009	–	Pakistan	NC_013012 EU384662 EU384661 EU384660 EU384659 EU384658 EU384657 EU384656	Unpublished
			–	*Alcea rosea*	2006	–	Pakistan	FR772087	Unpublished
			–	*Gossypium hirsutum*	2008	–	Pakistan	EU384663	Unpublished

(continued)

Table 9.2 (continued)

			Vernonia yellow mosaic virus	–	–	–	China	–	Zulfiqar et al. (2012)
27	**CyTLCA**	Cyamopsis tetragonoloba leaf curl alphasatellite	Gaur leaf curl virus	Guar	2010	Lucknow	India	GU385877.1	Kumar et al. (2010a, b)
28	**GLCA**	Guar leaf curl alphasatellite	Guar leaf curl virus	Guar	2010	Lucknow	India	GU385877	Kumar et al. (2010a, b)
			Wheat dwarf India virus *(WDIV)*	Wheat	2014	Mohali	India	KC305095 NC_020502 KC305096	Kumar et al. (2014)
			–	*Gossypium hirsutum*	2011	–	Pakistan	HE979547 HE599397 HE599396	–
29	**HLCuA**	Hibiscus leaf curl alphasatellite	–	*Abelmoschus esculentus*	–	–	Egypt	AJ512961	Briddon et al. (2004)
30	**HYVSA**	Hollyhock yellow vein symptomless alphasatellite	Hollyhock yellow vein virus	*Hollyhock*	2010	Lahore	Pakistan	FR772086.1	Unpublished
31	**HLCrA**	Hollyhock leaf crumple alphasatellite	–	*Alcea rosea*	–	–	Egypt	AJ512962	Briddon et al. (2004)
32	**LYVA**	Lantana yellow vein alphasatellite	Lantana yellow vein mosaic virus	Lantana	2012	Sirsa	India	KC206075.1	Marwal et al. (2013)
33	**MaYMVA**	Malvastrum yellow mosaic alphasatellite	Malvastrum yellow mosaic virus	Malvastrum	2008	–	–	AM236764 AM236763 AM236766	Guo and Xie (2008)
34	**MYMCA**	Malvastrum yellow mosaic Cameroon alphasatellite	Tomato leaf curl Cameroon virus	*Solanum lycopersicum*	2010	Lysoka	Cameroon	NC_014907 FN675298 FN675303 FN675297	Leke et al. (2011)
35	**MLCA**	Marigold leaf curl alphasatellite	–	Honeycomb	2012	Lakshmangarh	India	KC206078	Unpublished
36	**MeCMA**	Melon chlorotic mosaic alphasatellite	Melon chlorotic mosaic virus	Melon	2010		Venezuela	KF670682	Romay et al. (2015)
			–	*Cucumis melo*	2010	–	–	KF670681 KF670680 KF670681	Unpublished
37	**MYVMA**	Mesta yellow vein mosaic alphasatellite	–	*Abelmoschus esculentus*	2014	Ludhiana	India	KM108329	Unpublished
			–	*Abelmoschus esculentus*	2014	Hoshiarpur	India	NC_024372	Unpublished
38	**MiYLCA**	Mimosa yellow leaf curl alphasatellite	Mimosa yellow leaf curl virus	Mimosa	2008	–	Viet nam	NC_009564 DQ641719	Ha et al. (2008)
39	**OLCA**	Okra leaf curl alphasatellite,	–	*Gossypium hirsutum*	2012	–	Pakistan	HE972285 HE966418	Unpublished
			–	*Abelmoschus esculentus*	–	–	Mali	NC_010620	Kon et al. (2009)
			–	*Abelmoschus esculentus*	–	–	Cameroon	FM164740 FM164739	Unpublished
			–	*Abelmoschus esculentus*	2007	–	Sudan	FJ868830	Unpublished
			–	–	–	–	Oman	–	Akhtar et al. (2014)
			–	*Gossypium hirsutum*	2011	–	Pakistan	HE979544 HE966420 HE966417	Unpublished
			–	*Abelmoschus esculentus*	2007	Haryana	India	FN658718	Unpublished
40	**OLCBFA**	Okra leaf curl Barkina Faso alphasatellite	–	–	–	–	–	–	Leke et al. (2013)
41	**OYCrA**	Okra yellow crinkle alphasatellite	Okra yellow crinkle virus	–	–	–	Mali	–	Kon et al. (2009)
42	**OLCOMA**	Okra leaf curl Oman alphasatellite	Okra leaf curl virus	*Abelmoschus esculentus*	2014	–	Oman	KF267445	Akhtar et al. (2014)
43	**OYCrCA**	Okra yellow crinkle Cameroon alphasatellite	Okra yellow crinkle virus	*Abelmoschus esculentus*	2011	Lysoka	Cameroon	NC_014906 FN675288	Leke et al. (2011)
44	**PotLCA**	Potato leaf curl alphasatellite	Alternanthera yellow mosaic vein virus	*Sonchus arvensis*		–	–	–	Mubin et al. (2010)
45	**PLCA**	Papaya leaf curl alphasatellite	–	Papaya	2014	–	Pakistan	NC_023292	Unpublished
			–	Papaya	2010	–	India	JQ322970	Unpublished
46	**PYDA**	Pea necrotic yellow dwarf alphasatellite	–	*Pisum sativum*	2010	–	Austria	KC979052 KC979051	Unpublished
47	**SYVCA**	Sida yellow vein China alphasatellite	–	–	–	–	–	–	–
			–	*Cucurbita maxima*	2014	–	Japan	KJ466050	Shahid et al. (2015)
			–	*Carica papaya*	–	–	Nepal	–	Shahid et al. (2013)
			Ageratum yellow vein virus (AYVV) Tomato yellow leaf curl virus (TYLCV)	*Solanum lycopersicum*	2014	–	Pakistan	KC677736 NC_023484	Shahid et al. (2015)
48	**SYVMA**	Sida yellow vein mosaic alphasatellite	–	–	–	Ludhiana	India	KJ843304	Unpublished
49	**SLCA**	Sida leaf curl virus-associated alphasatellite	–	–	–	–	–	–	Ha et al. (2008)
50	**SYVVA**	Sida yellow vein Vietnam alphasatellite	Side yellow vein Vietnam virus	*Sida rhombifolia*	2006	Hanoi	Vietnam	NC_009563 DQ641718	Ha et al. (2008)
51	**SLCKA**	Sunflower leaf curl Karnataka alphasatellite	–	*Solanum lycopersicum*	2012	–	India	NC_019499	Unpublished
52	**SLCA**	Synedrella leaf curl alphasatellite	–	*Synedrella nodiflora*	2014	PortBlair	India	NC_025244	Unpublished
53	**TCSA**	Tobacco curly shoot alphasatellite	Tobacco curly shoot virus	*Nicotiana benthamiana*	2004	Yunan	China	NC_005057	Xie et al. (2004)
54	**TLCPA**	Tobacco leaf curl PUSA alphasatellite	Tobacco leaf curl Pusa virus	*Nicotiana tabacum*	2011	Pusa–Bihar	India	NC_014597	Singh et al. (2011)
55	**TLCA**	Tomato leaf curl alphasatellite	–	*Solanum lycopersicum*	2013	Bhatinda, Punjab	India	KF584011	Unpublished
56	**TLCCaA**	Tomato leaf curl Cameroon alphasatellite	–	*Solanum lycopersicum*	2012	Cameroon	Cameroon	NC_014747	Leke et al. (2012)

(continued)

Table 9.2 (continued)

57	**TLCNDA**	Tomato leaf curl New Delhi alphasatellite	–	*Solanum lycopersicum*	2011	New Delhi	India	JQ041697	Unpublished
58	**TLCPA**	Tomato leaf curl Pakistan alphasatellite	–	*Euphorbia pulcherrima*	2008	–	Pakistan	NC_012789	Unpublished
59	**TYLCChA**	Tomato yellow leaf curl China alphasatellite	Tomato leaf curl China virus	*Duranta* sp.	2007	Faisalabad	Pakistan	AM749494	Unpublished
60	**TYLCYnA**	Tomato yellow leaf curl Yunnan alphasatellite	–	*Solanum lycopersicum*	2003	–	China	NC_005058	NC_005058
61	**TYLCThA**	Tomato yellow leaf curl Thailand alphasatellite	Tomato yellow leaf curl Thailand Virus	*Solanum lycopersicum*	2003	–	Thailand	NC_005059	Unpublished
62	**VELCA**	*Verbesina encelioides* leaf curl alphasatellite	*Verbesina encelioides* leaf curl virus	*Verbesina encelioides*	2011	–	India	NC_015631	Prajapat et al. (2011)
63	**VYVFA**	Vernonia yellow vein Fujian alphasatellite	Vernonia yellow vein Fujian virus	*Vernonia cinerea*	2012	Fujian	China	JF265670	Zulfiqar et al. (2012)
			Mung bean yellow vein mosaic virus	–	–	–	India	–	Satya et al. (2014)
64	**VYVA**	Vinca yellow vein alphasatellite	–	Vinca	2013	Sikar, Rajasthan	India	KF992228	Unpublished
65	**XSA**	Xanthium symptomless alphasatellite	–	Xanthium	2009	–	Pakistan	HF547408	Unpublished

((–) – Not Found)

Ageratum conyzoides, *Cleome affinis*, *Euphorbia* spp., *Hibiscus rosa-sinensis*) infected by monopartite (Mubin et al. 2009; Idris et al. 2011; Tiendrebeogo et al. 2010) and bipartite (Paprotka et al. 2010, Romay et al. 2010) begomoviruses. Saunders and Stanley (Idris et al. 2011) first reported that alphasatellites can systemically infect *Nicotiana benthamiana* in the presence of cassava mosaic geminiviruses (CMGs), particularly *African cassava mosaic virus*.

9.4 Conclusion

Over the past 15–16 years, associations of betasatellites with begomoviruses have emerged as serious threats to a wide range of crops in the whole world. Betasatellites can be trans-replicated by several different begomoviruses, and trans-replication by true monopartite begomoviruses has the potential to form new disease complexes through acquisition of various begomovirus betasatellites from mixed infected plants. Moreover, betasatellites play important roles in determining begomovirus host ranges and hence could lead to emergence of new complexes that can cause severe crop epidemics.

Alphasatellites were first identified in association with monopartite begomovirus infections in the Old World that are known to harbor betasatellites and more recently in plants infected with bipartite begomoviruses. The biological functions of alphasatellites are still obscure. These satellites have emerged due to several reasons most important being the movement of infected material and vectors due to poor quarantine facilities. Also vector *Bemisia tabaci* has also extended its host range and thus transmitting virus to wide range of crops.

Monopartite begomovirus encode all the genes which are required for a successful infection. Most of these genes code for multifunctional proteins which adds further complexity in their interaction with host proteins, and their de novo creation. This shows the ability of begomoviruses and their associated satellites to rapidly evolve in response to selection pressures such as host plant resistance.

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Epidemiology of Begomoviruses: A Global Perspective

10

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"Epidemiology is the science of disease in populations. Sometimes one needs to distinguish between the study of disease in populations and the study of populations of pathogens".

Van der Plank JE (1963) Plant Disease: Epidemics and Control

Abstract

Geminiviridae, particularly the *Begomovirus*, represents a family of DNA viruses that emerged as one of the most successful viral pathogens causing severe economic losses to dicotyledonous plants widely used as food, fiber, and ornamentals in tropical and subtropical agroecosystem. Diseases caused by the *Begomovirus* in cassava, cotton, tomato, okra, and mung bean are severe and have attained international status because of their loss and spread. Significant work has been done and reviewed on the epidemiological aspect of the diseases; however, it has mainly focused on the molecular diversity of the pathogen in the environment. Various factors affecting epidemics, viz., variation in the vector, virus, and environment, are most important in the outbreak and spread of the disease. Source of primary inoculum for most of the *Begomovirus* is still not completely elucidated. Recently, there was a report on the role of seeds in the indirect transmission of the *Begomovirus* in mung bean. In the present review, a holistic up-to-date view encompassing both studies of the disease in the populations and population of pathogens is discussed.

Keywords

Begomovirus • Satellites molecules • Epidemics • Vectors • Indirect seed transmission

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10.1 Introduction

Among biotic factors affecting plants, viruses are the potent biotic enemies, causing significant losses, and a precise technology for its effective management is yet to come. Mainly, plant-infecting viruses utilize an RNA genome, suggesting that the plant has imposed strict constraints on the evolution of DNA viruses. Geminiviruses, particularly the *Begomovirus*, represent a family of DNA viruses that have circumvented these impediments to emerge as one of the most successful viral pathogens causing severe economic losses to dicotyledonous plants widely used as food, fiber, and ornamentals in tropical and subtropical agroecosystem. The first documentary proof of existence of any viral disease on the plant was given by Japanese Empress Koken in 752 A.D. in a poem and translated by T. Inouye as "In this village, it looks as if frosting continuously, for the plant, I saw in the field of summer, the colours of the leaves were yellowing." The plant mentioned in the poem was identified as *Eupatorium lindleyanum*, found to be susceptible to the *Begomovirus* (Matthews 1991). Intensive research has been done to understand the host virus interaction in a particular environmental condition in depth. However, available epidemiological reviews discussing the research findings are narrowed to molecular context, focusing much on the pathogen diversity and its occurrence on main and collateral hosts. Review covering both the sides of population dynamics can provide a better insight into the host and pathogen behavior to understand the biological association to come up with concrete strategies for its better management. Therefore, the present paper has been compiled to provide a holistic view encompassing both "study of the disease in the populations and population of pathogens" accounting global perspective.

10.2 *Geminiviridae*: The Family of *Begomovirus*

Geminiviruses were earlier divided into four genera on the basis of their genome organizations and biological properties: (1) *Begomovirus*: The most important member of the group formed of viruses transmitted by the whitefly, *Bemisia tabaci* (*Gennadius*) (Hemiptera: Aleyrodidae), to dicotyledonous plants, with *Bean golden yellow mosaic virus* (now *Bean golden mosaic virus – Puerto Rico*) as the type species. (2) *Mastrevirus*: Virus with monopartite genome and transmitted by leafhopper vectors, primarily in monocotyledonous plants, represented by *Maize streak virus* as type species. (3) *Curtovirus*: Viruses that had monopartite genomes distinct from those of the mastreviruses and are transmitted by leafhopper vectors to dicotyledonous plants, having *Beet curly top virus* as the type species. (4) *Topocuvirus*, recognized late, having monopartite genome and transmitted by a treehopper to dicotyledonous plants with only a single species, *Tomato pseudo-curly top virus* (also classified the type species) (van Regenmortel et al. 1997; Fauquet et al. 2003, 2008). Further, the International Committee on the Taxonomy of Viruses (ICTV) created a *Geminiviridae* Study Group for assessing the taxonomic proposal of geminiviruses under the chairmanship of Prof J. K. Brown, who proposed three more genera, viz., *Becurtovirus, Eragrovirus*, and *Turncurtovirus* with their type species *Beet curly top Iran virus*, *Eragrostis curvula streak virus*, and *Turnip curly top virus*, respectively (Fig. 10.1). Among the different genera, *Begomovirus* is the largest genus (288 species) followed by *Mastrevirus* (29 species), *Curtovirus* (3 species), and *Becurtovirus* (2 species). The remaining three genera, viz., *Topocuvirus, Eragrovirus*, and *Turncurtovirus*, have only one species each (Anonymous 2015).

10.3 Genome Organization

A vital character of the virus is its genome organization. Members of the family *Geminiviridae* characteristically have circular single-stranded DNA genomes packaged within twinned (so-called geminate) particles. The bipartite genome comprises two single-stranded DNA (ssDNA) components of similar size (2.5–2.8 kb), arbitrary referred to as DNA-A and DNA-B. The nucleotide sequences of DNA-A and DNA-B are quite different, except for a short common region of

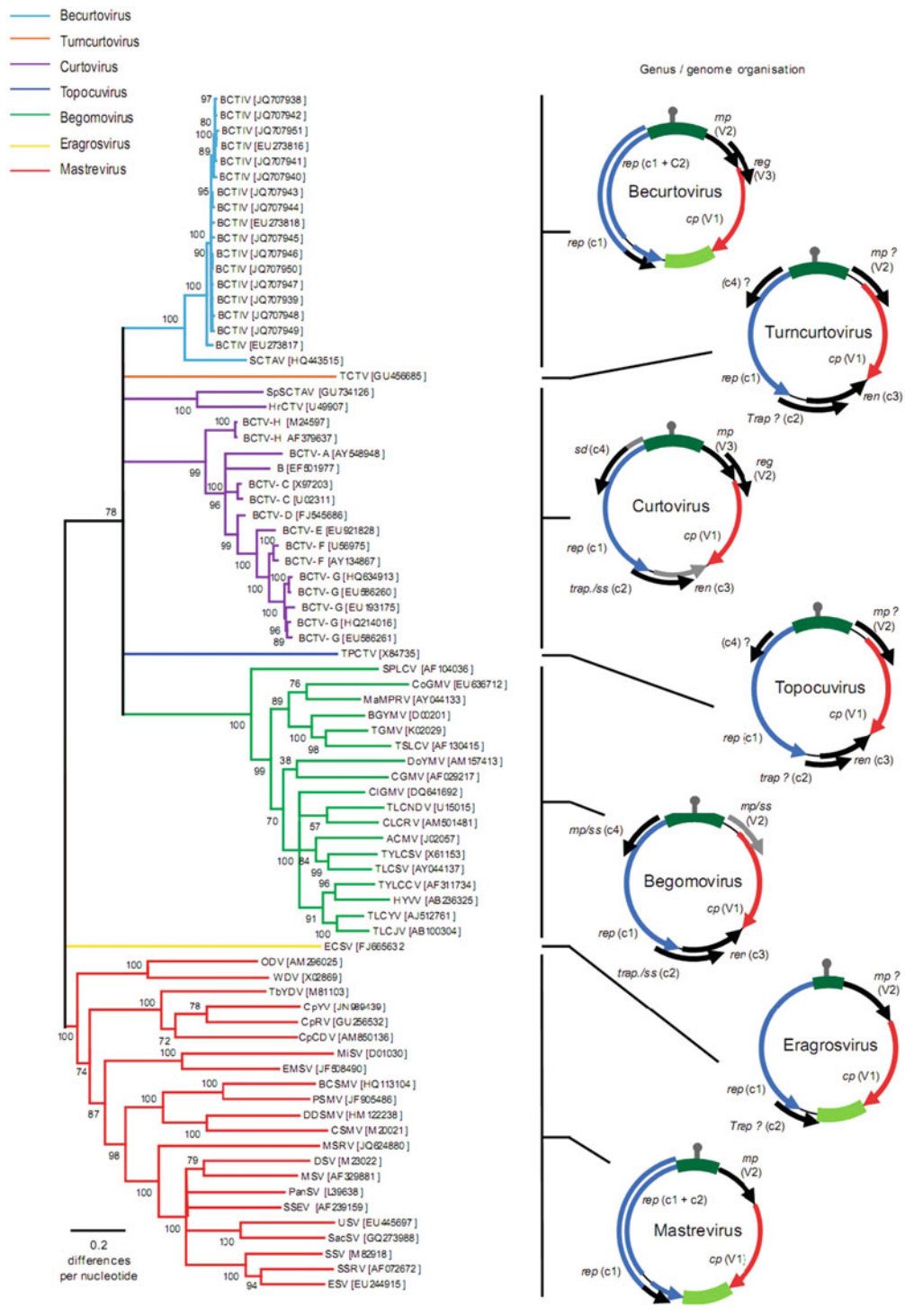

Fig. 10.1 Maximum likelihood tree (based on alignment of complete genome sequences) of becurto-, turncurto-, eragros-, curto-, topocu- viruses and other representative geminiviruses constructed using PhyML with GTR+I+G4. The genome organization for each genus is shown (Courtesy J. K. Brown study group, Anonymous 2015)

~200 nucleotides found to be very similar in the two DNAs. The common region includes a stem–loop structure, with the loop containing the nonanucleotide TAATATTAC, conserved across the genomes of all seven geminivirus genera. It also includes the origin for rolling-circle replication (Eagle et al. 1994; Laufs et al. 1995; Padidam et al. 1996; Orozco et al. 1998; Harrison and Robinson 1999, 2002; Zhou et al. 2003). *Begomovirus* genomes have a number of characteristics that distinguish Old World and New World viruses. All New World begomoviruses are bipartite, whereas in the Old World, most of the viruses are monopartite and the majority of these associate with recently identified satellite molecules. In addition, all Old World begomoviruses, with an exception of two unusual viruses, have an extra ORF (AV2) on the genome of DNA-A components that is not present in New World begomoviruses (Rybicki 1994; Stanley et al. 2005). Both DNA components contain protein-coding regions in the viral as well as complementary strand. Six such genes are universally present. DNA-A contains two genes (*AV1*and *AV2*) in the viral strand and three genes (*AC1, AC2*, *AC3*, and *AC4*) in the complementary strand. DNA-B contains one gene (*BV1*) in the viral strand and one gene (*BC1*) in the complementary strand. Some of the known functions of those proteins are summarized in Table 10.1. Most monopartite begomoviruses are associated with the satellites known as betasatellites, and in many cases, these *Begomovirus*–betasatellite complexes are additionally associated with the satellite-like alphasatellites. The betasatellites have a single gene (bC1) in the complementary sense and a region of sequence highly conserved between all betasatellites, known as the satellite conserved region (SCR), and a region of sequence rich in adenine (A rich). The alphasatellites have a single large protein (Rep) and contain an A-rich sequence. For each component the conserved hairpin structure, containing the nonanucleotide sequence TAATATTAC (TAGTATTAC for most alphasatellites) within the loop structure, is shown at position zero (Sattar et al. 2013).

Table 10.1 Some known functions of the mature *Begomovirus* genes

Gene	Protein	Functions
AV1	CP	Whitefly-mediated transmission, virion capsid, entry in nucleus
AV2	AV2	Symptom development and viral DNA accumulation
AC1	Rep	Replication associated protein. Viral DNA replication
AC2	TrAP	Transcriptional activator for the virus-sense genes, suppresses RNA silencing and other host defense responses. Activates expression of the capsid protein gene
AC3	REn	Enable viral replication
AC4	NSP	Shuttling of viral DNA in nucleus
BV1	NSP	Transport of viral DNA between the nucleus and cytoplasm and host range, properties of the virus
BC1	MPB	Mediates the cell-to-cell movement and viral pathogenic properties

10.4 Infection Cycle of Begomoviruses

Begomoviruses are inoculated to plant cells by the vector *Bemisia tabaci*, but a precise virus–host interaction is needed for *Begomovirus* infection to occur (Lazarowitz 1999), followed by movement of the virus to the nucleus where the replication and transcription of the genome happen. The virus particle movement apparently depends upon coat protein (CP) interactions with the host transport network, and it was found that a complex of ssDNA and CP enters the nucleus (Gafni and Epel 2002) to initiate replication. Begomoviruses follow a rolling-circle strategy; viral proteins required for the process are encoded by the DNA-A component of genome (Gutierrez 2000). The *AC1* gene (*Rep*) is responsible for initiating DNA replication during the rolling-circle amplification stage; *AC3* (*REn*) has also been found to play important role in viral DNA replication (Fontes et al. 1994a, b; Sunter et al. 1994; Laufs et al. 1995; Settlage et al. 1996; Orozco et al. 1997). An alternate replication strategy recombination-dependent replication (RDR) has

been reported by Jeske et al. (2001) wherein the host factors, alone or in combination with the Rep protein, are necessary or sufficient for replication. The AV2 protein has a role in symptom development and regulating viral DNA accumulation in MYMIV (Rouhibakhsh et al. 2011). The process of cell-to-cell and systemic spread of the single-stranded form of the viral genome produced during replication is highly dependent on proteins encoded by the B component of the virus genome. Two movement proteins (MPs), NSP encoded by *BV1* and MPB encoded by *BC1*, are essential for virus movement and systemic infection over the host (Schaeffer et al. 1995; Gilbertson and Lucas 1996; Jeffrey et al. 1996; Sanderfoot and Lazarowitz 1996; Sanderfoot et al. 1996; Guevara-Gonzalez et al. 1999; Lazarowitz 1999; Gafni and Epel 2002; Hehnle et al. 2004). NSP forms a complex with the virus genome and transports it from the nucleoplasm to the cytoplasmic domains where it interacts with *BC1*, and they function cooperatively in cell-to-cell movement of the viral DNA through the plasmodesmata. Moreover, *BC1* has also been reported to be responsible for pathogenicity of bipartite begomoviruses. The next step occurs when, via a short-distance movement, a virus reaches the vascular system and the host plant becomes systemically infected (long-distance movement). In *squash leaf curl virus* (SLCV), *BV1* has been implicated in affecting the host range properties and *BC1* in affecting viral pathogenic properties (Ingham et al. 1995). *Begomovirus* infection produces virus-associated alterations in plant cells and organelles. These structures show phloem limitation. Some begomoviruses are restricted to vascular system cells, whereas others can invade mesophyll tissue (Morra and Petty 2000). The loss of tissue specificity could, in some cases, be due to coinfection of the *Begomovirus* with another virus (Brown 1997).

10.5 Major *Begomovirus* Diseases and Epidemics

It is very difficult to estimate the accurate loss due to different diseases at various locations seasonally. Losses even vary from field to field depending upon crop management practices followed. The majority of the begomoviruses diseases causes 10–30 % yield losses in different ecological conditions in different crops. Few pathogens are ferocious and do cause catastrophic diseases in extensive areas conceding the excellent management practices are marked as diseases of national importance. Few *Begomovirus* diseases in the varieties of crops, viz., cassava, cotton, grain legumes, and vegetables, of tropic and subtropics regions have attained international importance because of their presence, spread, and repeated emergence.

10.5.1 Cassava Mosaic Disease (CMD)

Cassava, a staple food crop predominantly grown in central and southern Africa, is highly susceptible to virus infection, and particularly the CMD is placed among the most destructive viral diseases in the history. The earliest report of CMD was from Tanzania (Warburg 1894) describing yellow chlorotic mosaic coupled with leaf deformation and rugosity, however, remained unnoticed till 1920 as it was restricted to the limited area. Later in mid-1920s reports were cited for CMD epidemics across Africa, including Sierra Leone (Deighton 1926), Uganda (Hall 1928), Cameroon (Dufrenoy and Hédin 1929), Ghana {formerly Gold Coast (Dade 1930)}, Ivory Coast (Hédin 1931), Nigeria (Golding 1936), and Madagascar (Francois 1937). CMD caused severe epidemics in the period 1933–1944, causing the first recorded famine due to any viral disease. At that time, knowledge about the viruses was very limited; however, we had learned about the handling of the infectious diseases. All the possible attempts were made and the disease was managed by cultivating resistant varieties and sanitation of infected plants (Otim-Nape et al. 1997). A region-wide program of phytosanitation was implemented, incorporating byelaws mandating growers to uproot diseased plantings before the introduction of any disease-free material generated from partially resistant varieties (Jameson 1964). The situation remained under control until 1988. Regrettably, the disease reappeared in serious proportion in northern Uganda

in 1988 by breaking the resistance of all the available varieties. The new emergent virus was subsequently characterized as *East African cassava mosaic virus-Uganda* (EACMV-UG) which was more severe and rapidly expanding. Plants were severely affected and resulted in such a low yield that local food shortage and starvation occurred. By 1989, a severe epidemic was reported in the West Nile region of northwestern Uganda. By April 1992, most of the fields were infected 80–90 % and cassava production was virtually ceased due to the poor yields from diseased crops. As cassava is vegetatively propagated, the virus got transmitted from parents to progeny through stem cuttings. Since farmers were aware of the futility of planting infected cuttings, 95 % drop was observed in cassava plantations. The epidemic was noticed in about 75 % cropping area of Uganda and later moved southwards at the rate of 10–20 km per year. Available data on the incidence of CMD are highly variable indicating the incidence ranging from 15 to 50 % (Thresh et al. 1997). On an estimate, 150 thousand ha of cassava-growing land was abandoned, equivalent to over 2.2 million metric tons of crop of US $440 million value. The continent-wide loss of fresh tubers and roots per year estimated was 12–13 million tonnes worth of US $ 1200–2300 million (Thresh et al. 1997). Huge food shortages and famine in a number of districts, particularly in the eastern and northern regions of Africa, were observed. In 1994, more than 3000 people died of famine-related illnesses. The disease expanded into the neighboring countries of Kenya, Tanzania, and Sudan (Legg 1999). The CMD pandemic, as it became designated, continued to spread both to the south and west, and the recombinant virus (EACMV-UG) associated with the pandemic was reported in far west regions of the Congo Republic by 1999 (Neuenschwander et al. 2002). It became a massive political problem, people were starving, and no solution was available. All cassava-growing areas in Africa were facing CMD problem. With time, it is now known to occur in all the cassava-growing countries of Africa and the adjacent islands, India and Sri Lanka, with varied incidence. A total of eight viruses, viz., *East African cassava mosaic virus* (EACMV), *EACM Cameroon virus* (EACMCV), *EACM Kenya virus* (EACMKV), *EACM Malawi virus* (EACMMV), *EACMV Zanzibar virus* (EACMVZV), *Indian cassava mosaic virus* (ICMV), *South African cassava mosaic virus* (SACMV), and *Sri Lankan cassava mosaic virus* (SLCMV), have been found associated with the disease at various locations.

10.5.2 Tomato Yellow Leaf Curl Disease (TYLCD)

Tomato yellow leaf curl virus causes most devastating epidemics in tomato crops stretched over a wide area of the Old as well as New World countries (Pico et al. 1996). TYLCD was first identified in Israel in 1959 and was isolated and sequenced in 1988. Presently, it is known as *Tomato yellow leaf curl virus Israel* (TYLCV-Is) (Navot et al. 1991; Czosnek and Laterrot 1997). In Israel the disease severity was found to be high, wherever whitefly populations were high, causing 100 % yield loss (Cohen and Antignus 1994; Polston and Lapidot 2007). Unknowingly, in the late 1980s from Israel, it spread to the Dominican Republic. A tomato producer introduced this virus by adopting a greenhouse cultivar that was only available as transplants from Israel. This was the first entry of the Old World *Begomovirus* into New World and the virus is popularly known as the virus of the New World. With time the virus spread to many Mediterranean and Caribbean countries. In 1992, it destroyed 90 % of the tomato crop in the Dominican Republic (Polston and Anderson 1997). TYLCV-Is was subsequently identified in Jamaica and Cuba (McGlashan et al. 1994; Ramos et al. 1997). In July 1997, one plant was found infected with TYLCV-Is in a field in Collier County, Florida, and almost at the same time, four tomato plants with symptoms typical of TYLCV-Is were found in a retail garden center in Sarasota, Florida. In late 1996 or early 1997, it appeared that TYLCV-Is entered in the USA in Miami-Dade County, Florida. Subsequently, infected tomato transplants produced for retail

sale at two Miami-Dade County facilities were rapidly distributed *via* retail garden centers throughout the state. Infected plants purchased by homeowners and placed in and around homes appeared to be the source of TYLCV-Is for nearby commercial nurseries and production fields. To have a check to the spread, a number of regulatory procedures, as well as field management practices, were implemented in the 1997–1998 production season to minimize the movement of TYLCV-Is within and out of the state (Polston et al. 1999). In spite of it, in Florida numerous reports came of crop failures due to TYLCV-Is; it ruined Florida's US $ 500 million tomato industry and spread throughout the southern regions. Since then, TYLCV has also been reported from Africa, Asia, Australia, the Caribbean, Europe, and North and Central America. In North America, TYLCV is known to occur in Florida, Georgia, and Louisiana and has recently been detected in California and Arizona also. Presently there are 11 species reported of TYLCV virus, viz., TYLCV, *TYLC Axarquia virus* (TYLCAxV), *TYLC China virus* (TYLCChV), *TYLC Guangdong virus* (TYLCGuV), *TYLC Indonesia virus* (TYLCIDV), *TYLC Kanchanaburi virus* (TYLCKaV), *TYLC Malaga virus* (TYLCMalV), *TYLC Mali virus* (TYLCMLV), *TYLC Sardinia virus* (TYLCSV), *TYLC Thailand virus* (TYLCThV), and *TYLC Vietnam virus* (TYLCVNV).

10.5.3 Cotton Leaf Curl Disease (CLCuD)

The CLCuD is another example, which has destroyed Pakistan's economy, causing devastating losses in India and has also entered into China. It was first noticed in Nigeria on *Gossypium peruvianum* and *G. vitifolium* (Farquharson 1912). In Pakistan the disease was first observed near Multan on few cotton plants in 1967. Initially, it was assumed as minor disease, till 1988 only 60 ha area in the Multan district was infected, but subsequently, the geographic distribution of CLCuD increased exponentially. In 1991–1992 Pakistan's cotton production was at peak figuring 12.822 million bales with 769 bales/ha as productivity. But, in the very next year 1992–1993 due to a severe epidemic of CLCuD, the production went down to 9.054 million bales with 543 bales/ha productivity. Situations further aggravated in 1993–1994 and cotton production further reduced to 8.041 million bales with merely 487 bales/ha productivity (Cororaton and Orden 2008). In 1997, CLCuD was also reported from Sindh province of Pakistan, which was free from this disease (Mansoor et al. 1998). Estimated crop loss due to CLCuD was US$ 5 billion during the year 1992–1997. On an estimate, more than 7.7 million bales of cotton loss were seen due to CLCuD from 1988 to 2002 (Akhtar et al. 2005). Urging upon the need to address the problem, CLCuD-resistant cotton varieties (CIM 448, CIM 443, CIM 446, CIM 473, CIM 435, and FH 900) were developed through conventional breeding in which no existing virus strain could be detected (Rahman et al. 2002). After the development, release, and rapid popularization of these resistant varieties, the disease severity and incidence of the disease reduced significantly and satisfactorily yield losses were recovered gradually. Production of cotton again reached to 11.24 million bales during 1999–2000 with 641 bales/ha productivity (Anonymous 2001). Unfortunately, during the 2001 growing season, the resistance of these varieties was broken down, and symptoms of CLCuD were observed on all the developed resistant varieties at Burewala, Vehari district, Pakistan. The incidence ranged from 15 to 50 % and by 2002 the virus spread rapidly and disease symptoms could be seen throughout the district. All these resistant varieties were still disease-free at the near area of Faisalabad about 150 km away, while susceptible varieties (S12 and CIM70) possessed typical symptoms of CLCuD. Scions of CLCuD-affected plants of resistant varieties collected from Vehari were grafted onto ten plants each of resistant genotypes at the National Institute for Biotechnology and Genetic Engineering (NIBGE); Faisalabad showed disease in 20–40 % plants, confirming a breakdown of resistance. This new strain was termed as Burewala strain, now recognized as *cotton leaf*

curl Burewala virus (CLcuBuV), which has transformed almost all the cotton resistant varieties susceptible (Mahmood et al. 2003; Mansoor et al. 2003). In India the disease was first reported in patches around the Sri Ganganagar district of Rajasthan on *G. hirsutum* in 1993, and later it spread to the entire northern India within a short span of 4–5 years. Most common cotton varieties in the northern states at that time, i.e., RST-9 in Rajasthan, F-846 in Punjab, and HS-6 in Haryana, were severely hit. Since, against virus, genetic resistance is the only reliable and cheaper method of management, several resistant/tolerant varieties, viz., RS-875, RS-810, RS-2013, F1861, LH-2076, H1117, H-1226, and H-1236 and hybrids like LHH144, CSHH198, CSHH238, and CSHH 243, were developed to control the damage significantly. But, in 2009–2010 CLCuD reappeared in a severe form in some areas of northern India. The hitherto known resistant varieties also showed susceptible reaction at hot spot areas. Accumulation of recombination events over the years, coupled with favorable environmental conditions, appeared to have knocked down the resistance (Bhatoa et al. 2009). In China, during 2008 and 2009 cotton fields of the suburb of Nanning city, Guangxi, were showing upward leaf curl symptoms accompanied by thickening and often thick dark greening of leaf veins, leafy venations, and stunting; further investigation showed it was virus and was having 99.7% nucleotide sequence similarity with CLCuMV (Cai et al. 2010). High sequence similarities strongly advocated that the virus must have been introduced from Pakistan or India, where it was predominant; however, the route is still not clearly elucidated. Seven diverse species of begomoviruses have been found associated with the disease in the Indian subcontinent, viz., *CLC Multan virus* (CLCuMV), *CLC Rajasthan virus* (CLCuRaV), *CLC Alabad virus* (CLCuAlV), *CLC Kokhran virus* (CLCuKoV), *CLC Bangalore virus* (CLCuBaV), *CLC Burewala virus* (CLCuBuV), and *Papaya leaf curl virus* (PaLCuV) (Mansoor et al. 2003; Amrao et al. 2010). Host range identified for CLCuV included *Abutilon theophrasti* (Nill), *Althaea rosea* (Cav.), *A. ficifolia*, *A. kurdica*, *A. nudiflora, A. pontica, A. sulphurea, G. barbadense, G. hirsutum, Hibiscus cannabinus* (L.), *H. esculentus (*L.*), H. ficulneus, H. huegelii, H. trionum, H. sabdariffa* (L.), *Lavatera cretica, Malva alcea* (L.), *M. silvestris* (L.), *M. moschata* (L.), *Malvaviscus arboreus Cav., Pavonia hastata* (L.), *Sida acuta (Burm.), S. alba* (L.), *S cordifolia* (L.), and *Nicotiana tabacum* L. (Tarr 1951, 1957; Bink 1975; Cauquil and Follin 1983; Fauquet and Thouvenel 1987). Similar to CLCuV symptoms were also reported in other plant species in Africa, but there is ambiguity whether the same virus is involved in these species or not. These include *Corchorus fascicularis* Lau. (*Tilliaaceae*), *Phyllanthus niruri* L. (*Euphorbiaceae*), *Clitoria ternatea* L. (*Fabaceae*), *Phaseolus vulgaris* (*Fabaceae*), *Sida urens* (*Malvaceae*), *Petunia* sp. (*Solanaceae*), and *Urena lobata* (Tarr 1951; Nour and Nour 1964; El-Nur and Abu Salih 1970). In Pakistan under field conditions, CLCuV symptoms were observed on alternate hosts like brinjal, cucurbits ("Tinda", "Kalitori"), *Convolvulus arvensis*, *Rumex dentatus*, watermelon, cowpea, and lily plants (Anonymous 1993; Farooq et al. 2011).

10.5.4 Yellow Mosaic Diseases of Different Crops

A group of yellow mosaic diseases caused by the different *Begomovirus* in different crops, viz., tomato, okra, and pulses, are endemic in many of the regions of India. Tomato leaf curl disease (TLCD) is an important disease of tomato and causing nearly 40–100% yield loss depending upon the stage of infection and severity (Chakraborty et al. 2003; Dasgupta et al. 2003). TLCD was first reported in northern India by Vasudeva and Raj (1948) and subsequently from central India (Varma 1959) and southern India (Govindu 1964, Sastry and Singh 1973). Presently TLCD has been spread in all the tomato-growing regions of the country. Two distinct categories of virus producing different types of symptoms, viz., leaf curl (LC) and yellow mottle (YM), are included in the TLCD. It is very difficult to recognize the symptoms of the viral

disease by the virus name as found in other viruses. Therefore, in the present discussion, for better understanding, the virus producing YM symptoms has been generalized and was written as *tomato leaf curl virus-yellow mottle* (ToLCV-YM) and the virus producing only LC symptoms has been written as *tomato leaf curl virus-leaf curl* (ToLCV-LC). ToLCV-YM is extended throughout India except in the Peninsular Deccan Plateau, which includes Hyderabad, Telangana, Karnataka, and Kerala. Yellowing of the lamina or yellow-colored mottling is the predominant symptom of the disease. ToLCV-YM in India is completely different than TYLCV of the New World. ToLCV-LC is expanded throughout India and many parts of the world. ToLCV-YM has been found to contain both DNA-A and DNA-B molecules, whereas ToLCV-LC contains only DNA-A molecule. Few members of the ToLCV-YM can be transmitted mechanically through sap (Chakraborty et al. 2003). However, none of the members of ToLCV-LC were found to be transmitted by the sap. Population of tomato leaf curl viruses (ToLCVs) in India is highly diverse (Borah and Dasgupta 2012). TLCD is caused by the 42 different species of *Begomovirus* throughout the world (Anonymous 2015). Four virus species, viz., *tomato leaf curl Gujarat virus* (ToLCGuV), *ToLC Bangalore virus* (ToLCBaV), *ToLC Karnataka virus* (ToLCKaV), and *ToLC Kerala virus* (ToLCKeV), has been reported from India. ToLCGuV is of YM category, whereas other viruses are of LC categories. Yellow vein mosaic disease (YVMD) in okra (bhindi) was first reported from Bombay (presently Mumbai) in India (Kulkarni 1924) and has been found to present throughout the India. Up to 96 % loss by the YVMD has been reported by Pun and Doraiswamy (1999). Mahatma et al. (2007) observed 1.2 million tonnes of fresh fruit losses to the tune of US $ 134 million due to YVMD in India in assessment year 2003–2004. The disease is caused by the *okra yellow vein mosaic virus* (OYVMV). Yellow mosaic diseases (YMD) of legumes are also another major constraint in improving the productivity of grain legumes in India. Yield loss per annum due to YMD was estimated to be $ 300 million taking black gram, mung bean, and soybean together (Varma and Malathi 2003). Patel et al. (2012) observed that the mung bean crop gets infected by YMD within 9 days of sowing. If favorable environmental conditions prevail, the incidence can reach to cent percent within a month. Potential yield losses due to the YMD in mung bean depend upon the stage of infection. If the plant is infected at the age of 9 days, the potential yield losses are around 74.68 %, whereas if the plant is infected at 79 days after the sowing, the potential yield losses are around 6.10 %. Similarly the yield losses at the different stages of the plant were observed, and it was found that if the plant is infected at 16, 23, 30, 37, 44, 51, 58, 65, and 72 days after the sowing, it causes 71.85, 59.52, 53.43, 50.42, 47.28, 41.07, 32.60, 12.72, and 10.32 % yield losses, respectively. In India, YMD was first reported in lima bean (*Phaseolus lunatus*) in western India in the 1940s. Later in 1950, YMD was seen in dolichos (*Lablab purpureus*) in Pune (Malathi 2007). Nariani (1960) observed YMD in mung bean (*Vigna radiata* L.) in the experimental fields at the Indian Agricultural Research Institute, New Delhi. Subsequently, this disease has been reported from other parts of India, Pakistan, Sri Lanka, the Philippines, Indonesia, and Thailand (Bakar 1981; Honda et al. 1983; Malik 1992). Yellow mosaic disease (YMD) of different legumes is caused by different strains and variants of two distinct *Begomovirus* species, viz., *mung bean yellow mosaic virus* (MYMV) and *mung bean yellow mosaic India virus* (MYMIV).

10.6 Factor Affecting Epidemics

Disease occurs when a susceptible host and aggressive pathogen interact in favorable environment. An epidemic will only ensue if all three factors continue to be present. Humans have contributed significantly to overlap all these three factors repeatedly over time, the present ecosystem is least diversified, and they do ensure the continuous availability of host by agricultural intensification and extensive monoculture-based

cropping pattern. Particularly with reference to the *Begomovirus*, availability of the host has helped vector to survive across seasons and assisted in evolution of improved biotype having high host range and fecundity. Rapid encounter of diverse host with different genetic makeup forced viruses to recombine and reassort to design into more aggressive pathogen. Precisely, the phenomenal potentialities of the entire *Begomovirus* group to emerge as the most successful pathogen in the diverse ecological niche strongly suggest that the evolution of the host, vector, and pathogen took place concurrently. Among the different factors affecting epidemic, variation in the vector, virus, and environment and source of primary inoculum are most important and are discussed hereunder.

10.6.1 Variation in Vector

Begomoviruses are transmitted by whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in a persistent manner. The first description of *B. tabaci* is on tobacco (*Nicotiana tabacum* L.) from Greece, and in the 1930s it was recognized as agricultural pest in Africa. As whiteflies were the most common and only insects present on cassava, hence, it was suspected as the vector for the virus (Dade 1930). The first firm evidence of transmission by a whitefly (*Bemisia* sp.*)* was obtained from the Democratic Republic of Congo (Kufferath and Ghesquie're 1932). After acquisition, whiteflies can transmit the virus for 5–20 days, or even for longer period and sometimes entire life span (Muniyappa et al. 2003; Rojas 2004). Whitefly breeds throughout the year between 8 and 35 °C temperature; the optimum temperatures for breeding ranges from 28 to 32 °C and relative humidity 62 to 92 %. Whiteflies lay eggs, 150–300 eggs per female during her lifetime, on the underside of the leaves; incubation period varies from 3 to 5 days. All nymphal stages are sedentary (except first-stage crawlers) found on the underside of leaves. Nymphs have four stages; the fourth one "puparium" (nonfeeding stationary stage) reaches in 9–14 days after hatching and retains for 2–8 days. Total life cycle is completed in 14–122 days with around 11–15 generations in a year depending upon the environmental conditions. These vectors required fresh tender foliage for feeding and oviposition. If the environment is conducive for whitefly and tender leaves are available, activities of the whiteflies increase and in turn enhance the possibilities of disease spread. These whiteflies being light can have a flight of 7 km over land and water surface with the help of wind (Cohen 1982; Byrne and Bellows 1991). For a positive virus transmission by these flies, approximately 15–30 min of feeding by an adult for acquisition followed by 6–12 h latent period is required. Optimum experimental transmission is accomplished with a 12–24-h acquisition access period (AAP) and a 24–48-h inoculation access period (IAP). There is no evidence that any whitefly transmission varies from these relative patterns, suggesting that the transmission pathway is conserved for all the begomoviruses because of specific cellular and molecular virus–vector interactions. These viruses are considered non-replicative in their vector and are not passed transovarially. However, replication of tomato yellow leaf curl virus (TYLCV) has been found in their whitefly vector and can be transovarially transmitted for at least two generations. Up to 20 % of the insects in each generation have been found to inoculate tomato plants (Ghanim et al. 1998). In another instance, Bosco et al. (2004) detected tomato yellow leaf curl Sardinia virus (TYLCSV) in eggs, nymphs, and to a lesser extent in adults of first-generation progeny. Inheritance of TYLCSV DNA was found until the third generation but not the infectivity. Most members of the *B. tabaci* group are capable of ingesting and transmitting begomoviruses to and from numerous host combinations. There are rare examples in which a *Begomovirus* host range is restricted by the range of its *B. tabaci* vector. The emerging nature of the virus diseases, especially in the New World, is mainly attributed to the worldwide spread of a new "silver leaf" or B biotype of the vector *B. tabaci*, which was earlier named as *Bemisia argentifolii*; however, it was not accepted by many of the taxonomists. The Indian subcontinent is believed to be the center of origin of *B.*

tabaci because of the numbers and types of natural enemies found in the region. However, the B biotype is recently recorded in the region (Banks et al. 2001). The origin of B biotype may be Northeast Africa to Middle East Peninsula region (De Barro et al. 2000). Host range of the biotype and ability to transfer the virus from the weeds to the main crop and vice versa have been the major reason of persistence of the virus in the field (Polston and Anderson 1997; Varma and Malathi 2003). Before the introduction of TYLCV, tomato mottle virus (ToMoV) was found widespread and damaging in all tomato-producing areas of Florida. Incidences were as high as 95 % in 1990–1991, and the virus was estimated to have reduced the value of southwestern Florida tomato crop by 125 million USD. Rapid spread of TYLCV by the biotype B of *Bemisia tabaci* has made the ToMoV of minor importance, and TYLCV emerged as most destructive disease in the Florida and other countries of the New World. The B biotype has, however, not been responsible for some of the most serious *Begomovirus* epidemics that have occurred in the Old World.

10.6.2 Variation in the Virus

Evolution and genetic variation in living forms are natural phenomenon; likewise in virus, high variation is observed. A combination of complex events in the ecosystem causes spontaneous variation in virus genome; these changes sometimes convert them into more virulent strains than their predecessors. These new virus strains emerge as a result of spontaneous mutation in few organisms from the base population resting on the host species acting as reservoirs. Spread from the reservoir into a new environment (including the host as a key component of a parasite's environment), and establishing productive infections and effective between-host transmission mechanisms are the steps required for emergence to occur (Elena et al. 2009; Elena 2011). Evolution of virus is comparatively rapid; therefore, the reemergence of the disease after the first remission in many crops at different geographical locations is quite common. The reemergence of cassava mosaic disease in Uganda during 1988 was because of the emergence of more virulent recombinant virus, referred to as East African cassava mosaic virus-Uganda (EACMV-UG) (Deng et al. 1997; Zhou et al. 1997). It was the first report of the recombination in begomoviruses. Symptoms elicited by the recombinant virus were more severe than those of the previously occurring ACMV, although plants infected with both EACMV-UG and ACMV showed the most severe symptoms (Harrison et al. 1997). The enhanced severity was shown to be the result of a synergistic interaction between ACMV and EACMV-UG (Harrison et al. 1997; Pita et al. 2001). Subsequent studies have shown that mixed ACMV- and EACMV-like virus infections are frequent wherever the severe form of CMD occurs (Berry and Rey 2001; Ogbe et al. 2003; Were et al. 2004). Synergism between an EACMV-like virus and ACMV has also been reported from Cameroon (Fondong et al. 2000). However, an association between mixed CMG infects ions, and an expanding pandemic of severe CMD has only been demonstrated in East Africa. The breaking of the resistance of existing resistant varieties and reappearance of the CLCuD during 2001 Burewala, Vehari district, Pakistan, were due to the emergence of a more virulent, CLcuBuV strain. These evidences strongly suggest that the variation in the virus due to their evolution and breaking of the resistance of plant in the ecosystem is most rapid and common phenomena to establish them as most destructive plant pathogen. Similarly the reappearance of the CLCuD in Rajasthan during 2009–2010 is also because of the emergence of the disease and was also due to entry of the more virulent strain of the virus.

10.6.3 Weather Parameter

Weather factor such as temperature, relative humidity, rainfall, and bright sunshine is important weather parameter affecting the activities of host, vector, and virus. Weather factor favorable for the outbreak of whitefly includes temperature above 33 °C associated with scanty rainfall. Ohnesorge et al. (1980) observed that adult

whitefly preferred young leaves than older for oviposition, which was impaired by rainfall and low temperature. High temperatures associated with scanty rainfall are conducive to rapid breeding, greater longevity, and heavy infestation of whiteflies. Incidence is largely controlled by rain particularly if the shower is heavy and well spread. The increased light intensity has been shown to increase activity of the whitefly vector. Field spread of CLCuD is affected by climatic conditions like rainfall, wind, and temperature. Rainfall prior to seedling may result in the development of an increased population of vector due to abundance in food source (Bink 1975). As cotton is grown only for part of the year, cultivated hosts and alternate weeds serve as virus reservoirs (Giha and Nour 1969). CLCuD infestation increases in the range of 33–45 °C (maximum temperature) and 25–30 °C (minimum temperature); however, it is not affected much by rainfall and humidity.

10.6.4 Source of Primary Inoculums

Information about the epidemiology of the disease helps in devising effective disease management strategies. The source of the primary inoculum plays most crucial role in the epidemics of any disease. Cassava is propagated by the stem cutting, and CMD is primarily transmitted through the propagating materials. Therefore, the disease could be efficiently managed in those days even when little was known about the viruses. Source of primary inoculum for many crop raised through seed is still not clearly identified. A matter of fact, cultivated crop does not remain in the field throughout the year; during the off period, the virus may remain in the reservoir host for the few months and then returns in the main crop by the activities of vector with the development of the crop. Interestingly, site of infection of by *Begomovirus* (TYLCV) and distance from source of primary inoculum are mostly found to be negatively correlated, whereas virus (TYLCV) intensity is positively correlated with whitefly population (Aboul Ata et al. 2000). This phenomenon is true for almost all the viral diseases. Activities of the whiteflies increase in the field immediately after the crop emergence as tender leaves, preferred sites for feeding and oviposition, are available abundantly. Microclimate of the crop also plays a key role for the vector to flourish well. Therefore, the spread of the disease in the field is rapid and can happen for miles if not checked in time. Movement of cotton leaf curl disease in Indian subcontinent is an excellent example of disease spread by the activities of whiteflies from Pakistan to its border states of India (Rajasthan, Haryana, and Punjab). Similarly, long-distance movement of TYLCV has occurred by accidental transportation of infected planting material. Few begomoviruses have well-defined host range; however, many of them lack it. Molecularly, it has been demonstrated that begomoviruses infecting the crop are entirely different than the expected species of the begomoviruses. Soybean crop at the National Botanical Research Institute, Lucknow (India), was found 80–90 % infected in 2005. Virus was expected to be the member of MYMV or MYMIV infecting soybean. However, a partial sequence of a 800 bp amplicon resembled only 57 % with MYMIV-Sb; however, it showed 95 % homology with CLCKV. This was an example of cross movement of the virus in the entirely different unrelated family of the crop. Molecular data indicated soybean as a new host of CLCKV (Raj et al. 2006). Theoretically, if this cotton *Begomovirus* strain can infect soybean, then, it should also infect cotton too. Similarly Venkataravanappa et al. (2013) observed typical upward leaf curling, vein clearing, vein thickening, and yellowing symptoms in okra from Bangalore rural district, Karnataka. Molecularly the virus showed 92.8 % sequence similarity with CLCuBV and was designated as a distinct strain (CLCuBV-[India: Bangalore: okra: 2006]). The host range of the virus was shown to be very narrow and limited to two species in the family Malvaceae, i.e., okra (*Abelmoschus esculentus*) and hollyhock (*Althaea rosea*), and four in the family Solanaceae which included different varieties of tobacco. Molecular data theoretically imply that the virus can infect cotton too; however, concrete practical evidence is not available. OYVMV recombined with another unidentified geminivirus in some coinfected host, perhaps okra, to produce a geminivirus variant capable of

infecting cotton systemically in Pakistan before the epidemics of CLCuD (Zhou et al. 1998). In India, cotton, okra, and soybean are grown in the states of Gujarat, Maharashtra, Madhya Pradesh, Karnataka, Andhra Pradesh, Telangana, and Tamil Nadu districts since long. Soybean and okra are severely infected by the *Begomovirus* in all these states, creating high virus pressure throughout the year. Aggressive biotype whitefly (B biotype) has also been observed in the southern part (Banks et al. 2001). Conditions are conducive for the evolution of virus to infect cotton, and its further spread by the vector is available in the middle and southern India. However, there is no report of economic loss due to CLCuD in middle and southern India. Even if the cross movement or the recombination of the virus will take place in the middle and southern India at a very least frequency, it will be a serious problem for the production of cotton in these area. During October 2009, vein and leaf yellowing symptoms were observed on more than 50% plants in *Gaillardia* (*Gaillardia pulchella*) in Udaipur (Rajasthan). Molecularly, virus was found to have 94% identity with *tomato leaf curl Bangladesh virus*-[Bangladesh:2] (ToLCBDV-[BD:2]) and accordingly was named as *tomato leaf curl Bangladesh virus-Gaillardia* [India:Udaipur:Gaillardia:2009] (ToLCBDV-Gaill [IN: UDR: Gaill: 2009]). The disease was transmitted by whiteflies, on susceptible plants (Mahatma and Mahatma 2012); however, the transmittance was not established on tomato cv. Arka Sourabh (unpublished information). All these facts and figure clearly reflect a gap in understanding the correlation between biological and molecular properties of *Begomovirus* and also direct to consider both molecular and biological data concurrently before reaching to a valid conclusion.

10.6.4.1 Role of Seed in the *Begomovirus* Transmission

There is no report of seed transmission of any of the *Begomovirus* in any of the crop. During 2008, it was observed that some of the plants of the mung bean (*Vigna radiata* L.), Gujarat, India, were found to infect the first trifoliate and have typical yellow-colored symptoms on the pod and seed coat (Fig. 10.2). PCR with the *Begomovirus* specific primers revealed presence of *Begomovirus* in the different part of the seeds, viz., pod, seed coat, and cotyledon; however, it

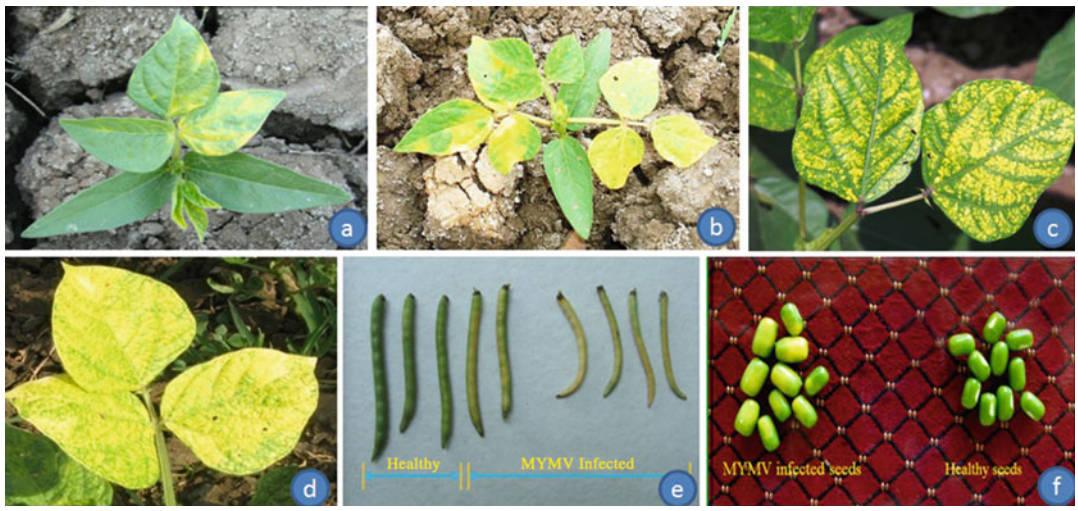

Fig. 10.2 Typical symptoms of MYMV: (**a**) on first trifoliate; (**b**) first and subsequent trifoliate; (**c**)green vein banding on mature leaf; (**d**) complete yellowing; (**e**) healthy and symptomatic pods; (**f**) symptomatic and healthy mung bean seeds (Courtesy Pawar et al. 2015)

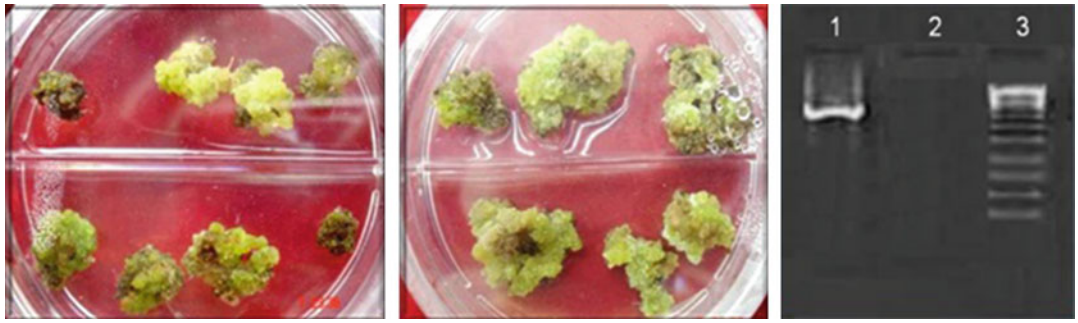

Fig. 10.3 Induction of callus from the cotyledon and detection of MYMV: (**a**) callus from the MYMV infected cotyledon; (**b**) callus from the healthy plant; (**c**) PCR amplification of MYMV using degenerate primers, lane 1- infected callus, lane 2- healthy callus, DNA 100 bp ladder (Courtesy Pawar et al. 2015)

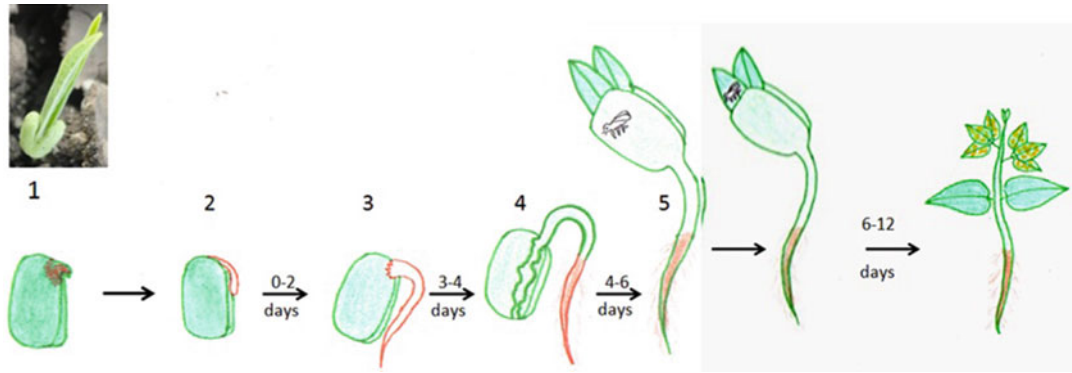

Fig. 10.4 Schematic representation of different events from the germination of mungbean seed and movement of MYMV by the vector whitefly from the cotyledon to true leaf: *1–4* = germination of seed; *5* = acquisition of virus by the whiteflies from the cotyledon; *6* = release of virus from the cotyledon to the true leaf; *7* = representation of symptoms of MYMV on the developing seedling. In inset above-emerged seedling with the cotyledon as seen 5 days after sowing (Courtesy Pawar et al. 2015)

was absent in embryo (Fig. 10.3). Callus developed by using the seed cotyledon as explant confirmed that the virus remains viable in the cotyledon of the seed (Pawar 2010). Accordingly the route of movement of *Begomovirus* indirectly through the seed from one crop season to another was traced (Pawar et al. 2015). Germination of mung bean is epigeal and the seedling emerges within 4–6 days, rupturing the crust of the soil. Critical examination suggests that cotyledon softens enough and becomes suitable for the feeding of whiteflies by imbibing water. Few whiteflies that feed on the emerging cotyledon acquire virus which is subsequently transmitted to the developing seedling on feeding (Fig. 10.4). Division of cotyledonary cells and the virus does not take place at the stage; therefore, it does not get transmitted from cotyledon to emerging seedling; however, seed indirectly serves as a source of primary inoculum for the initiation of the disease in the population, which rapidly spreads in the entire field if the vector is available (Pawar et al. 2015). There is only one report of this kind and it needs to be validated in other crops, especially having epigeal germination like that in cotton, okra, etc. The information can help in tracing the movement of the virus and also in the management of the disease to much extent.

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Current Knowledge of Viruses Infecting Papaya and Their Transgenic Management

11

Ritesh Mishra, Rajarshi Kumar Gaur, and Basavaprabhu L. Patil

Abstract

Papaya (*Carica papaya* L.), native to the South American continent, is an important horticultural crop cultivated across the tropical and subtropical regions of the globe. Papaya is rich source of vitamin-C and globally it is ranked fourth in total fruit production, next only to bananas, oranges and mangoes. India is the leading producer of papaya and both India and Brazil put together account for more than 50 % of global papaya production. Multiple pests and pathogens are known to inflict damage to papaya, of which viral diseases are the most damaging ones. Of all the viral diseases, papaya ring spot virus (PRSV) belonging to the *Potyviridae* family is most important one, followed by the viruses belonging to the *Geminiviridae* family causing leaf curl disease in papaya. Other viral diseases of papaya are *Papaya meleira virus* (PMeV), *Papaya mosaic virus* (PapMV), *Papaya lethal yellowing virus* (PLYV) and several other viruses are known to infect papaya, but may not be of economical significance. Management of viral diseases in papaya is very crucial to accomplish a good harvest, and of all the management practices, genetic engineering papaya for virus resistance is most promising and successful. The PRSV resistant transgenic papaya varieties "SunUp" and "Rainbow" developed by the University of Hawaii and extensively cultivated in the Hawaii islands of United States is the most successful field application of transgenic technology. Since there is significant sequence variation in the PRSV strains from different parts of the world and many more diverse range of viruses

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are known to infect papaya, there is an urgent need to develop region specific virus resistant papaya.

Keywords
Papaya • Papaya ring spot virus • Geminiviruses • Leaf curl disease • Virus resistant transgenics

Papaya (*Carica papaya* L.) is widely cultivated in tropical and sub-tropical regions of the five major continents of the world (Manshardt 1992). But the major share of its total production of 11.57 million metric tons (m MT) from 433,057 ha of cultivated area in 2010 came from Asia, Central America, and Africa. India, Brazil, Nigeria, Indonesia, and Mexico are among the major papaya-producing countries (FAOSTAT 2012). Figure 11.1 shows the percentage contribution of different regions for Papaya production. Worldwide, India leads in the production of papaya, followed by Brazil, Indonesia, Dominican Republic, Nigeria, Mexico, DR Congo, China, Thailand, Guatemala, Cuba, Philippines, Colombia, Venezuela, Peru, Bangladesh, Kenya, Costa Rica, El Salvador, and Ghana (FAOSTAT). The commercial cultivation of papaya is not able to achieve its full yield potential due to its low production as a result of widespread incidence of viral diseases. Papaya fruit is rich in vitamin A, B1, and B2 and contains valuable proteolytic enzyme papain, which helps in digesting protein rich food, with several pharmaceutical applications.

Papaya grows best in the warm and humid tropical regions of the world. The center of origin of Papaya is located in the tropical regions stretching from South Mexico to the Andes in South America. According to their botanical characteristics papaya is perennial herbaceous plant, and not a tree. Papaya grows to a height of 6–9 m and has a single stem (Fig. 11.2a). These plants remain productive for up to 10 years, however in most commercial plantations the plants are replaced every 3 years. Depending on the variety of papaya and its surrounding conditions, fruit formation can start anywhere between 6 and 12 months after planting. The fruits then need 5–9 months to completely develop. It is therefore possible to harvest papaya within a year and to replant them every year. Papaya is a major horticultural crop of India and many largest markets for the fruit are available in Delhi, Haryana, Gujarat, Andhra Pradesh, Kerala, Madhya Pradesh, Chhattisgarh, Assam, Karnataka, and Uttar Pradesh.

Papaya plants are infected by several different viruses, such as papaya ringspot virus (PRSV) (Fig. 11.2b), papaya leaf curl virus (PLCV) (Fig.

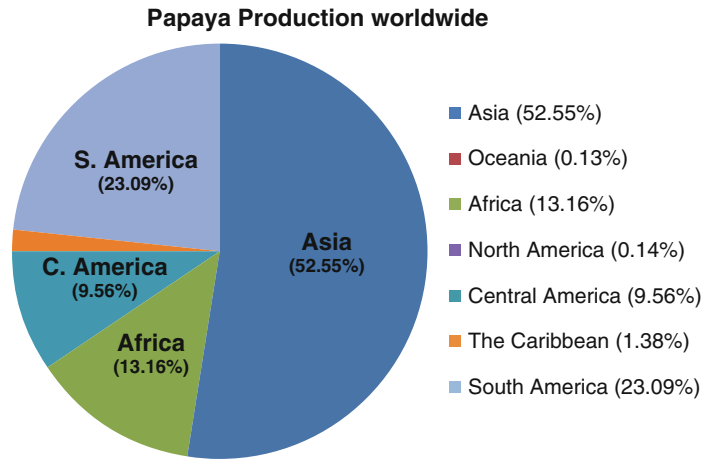

Fig. 11.1 Venn diagram depicting the percentage contribution/share of different continents/ regions, in the production of papaya

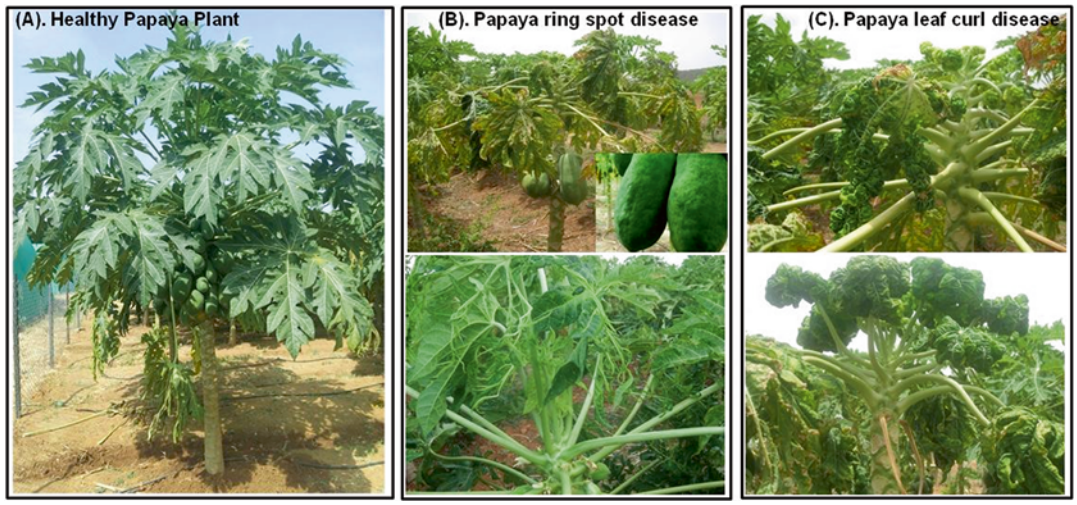

Fig. 11.2 (**a**) Healthy and virus free papaya plant with good fruit set under drip irrigation in Karnataka state of India. (**b**) Papaya plant affected by ring spot disease, ring spots are visible on the papaya fruit, and in *lower panel*, the leaves become very much narrowed like rat tails. (**c**) Papaya leaf curl symptoms (*upper* and *lower panel*)

11.2c), papaya mosaic virus (PMV), and papaya lethal yellowing virus (PLYV), which reduces the development and production of papaya plant as well as the fruits. These plant viruses are mainly transmitted though vector by both persistent and non-persistent mode (Martinez et al. 2010).

11.1 Viruses Infecting Papaya

Several different viruses pose a serious threat to papaya cultivation. These include potyviruses, such as *Papaya ringspot virus* (PRSV) (Tripathi et al. 2008), *Papaya leaf distortion mosaic virus* (PLDMV) (Maoka et al. 1996), and *Zucchini yellow mosaic virus* (ZYMV) (Ferwerda-Licha (2002); the potexvirus, *Papaya mosaic virus* (PapMV) (Wang et al. 2013); the geminiviruses, *Papaya leaf curl virus* (PaLCV) (Chang et al. 2003), *Papaya leaf crumple virus* (PaLCrV), *Chilli leaf curls virus* (ChiLCuV) and *Tomato leaf curl New Delhi virus* (ToLCuNDV), and *Croton yellow vein mosaic virus* (CYVMV), which are associated with different betasatellites (Singh-Pant et al. 2012; Pramesh et al. 2013); the rhabdoviruses, *Papaya droopy necrosis virus* (PDNV) (Wan and Conover 1983) and *Papaya apical necrosis virus* (PANV) (Hernandez et al. 1990); the tospovirus, *Tomato spotted wilt virus* (TSWV) (Gonsalves and Trujillo 1986); and the sobemovirus, *Papaya lethal yellowing virus* (PLYV) (Amaral et al. 2006; Pereira et al. 2012). Among the known papaya viruses, PRSV, PLDMV, and PapMV have been detected in Hainan Island, China (Wang et al. 2013; Tuo et al. 2013; Lu et al. 2008). Currently, PRSV is considered the most widespread and destructive disease damaging papaya production in China (Lu et al. 2008). However, the presence of PLDMV was confirmed recently in commercialized PRSV-resistant transgenic papaya in Hainan and Taiwan (Tuo et al. 2013; Bau et al. 2008), indicating a potential threat to papaya in China and other countries. However, PapMV has been considered as of minor importance because it is rarely found in the field (Cruz et al. 2009; Noa-Carrazana et al. 2006). These three viruses cause similar symptoms in papaya, such as mosaic, yellow-green leaf discoloration and distortion of leaves, water-soaking streaks on petioles, and ring-spots on fruits, making it difficult to distinguish among PLDMV, PRSV, and PapMV without further diagnostics. Furthermore, mixed infections of PRSV and PLDMV or PapMV have been reported in papaya in Taiwan and the Philippines, respectively (Bau et al. 2008; Cruz

et al. 2009). Thus, to make rapid diagnosis and to check the spread of disease, it is necessary to develop an effective and rapid detection method for these viral infections in papaya (Tuo et al. 2014). Brief descriptions of some papaya infecting viruses are given below.

11.2 Papaya Ringspot Virus

Papaya ringspot virus (PRSV) is the causal agent of ring spot disease in papaya and the characteristic symptoms of which are mottling, blister-like patches, and distortion of leaves associated with ring spots on papaya fruits. It transmits from plant to plant through mechanical activities like pruning and biologically by numerous aphid species such as *Myzus persicae*; however, no seed transmission has been reported. The infection cycle of a Potyvirus begins when the entry of viral particle in the cell via a wound (or during feeding by its vector aphid) (Mishra et al. 2014). There are mainly two biotypes of PRSV (P and W) that are recognized; in which P biotype infects papaya and several members of the melon family, whereas the W biotype infects only cucurbits such as watermelon. These two types of PRSV are serologically indistinguishable, and because of their high sequence similarities, they are now considered to be the same virus species.

Because of PRSV infection, the cultivation of papaya has been limited in many parts of the world. Worldwide papaya ringspot disease is the major obstacle to large-scale commercial production of papaya (Yeh and Gonsalves 1984). PRSV was first reported in the Hawaii islands of United States in the 1940s (Jensen 1949a) and later became prevalent in Florida (Conover 1964), Caribbean countries (Adsuar 1946; Jensen 1949b), South America (Herold and Weibel 1962), Africa (Lana 1980), India (Capoor and Varma 1948; Singh 1969), the Far East (Wang et al. 1978), and Australia (Thomas and Dodman 1993). To date, most of the major papaya plantation areas across the globe suffer from devastation by this obnoxious virus.

PRSV belongs to the genus *Potyvirus* and the virus family *Potyviridae*. The virus is a non-enveloped, flexuous rod-shaped particle that is between 760 and 800 nm long and 12 nm in diameter (Fig. 11.3). It contains approximately 2000 copies of coat protein (CP), which encapsidate a single stranded, positive-sense RNA (+ ssRNA). Its genome size is approximately 10 kb in length, which has a 5′ terminal-linked protein (VPg) and a 3′ poly-A tail. Because of its positive-sense RNA genome, it can act directly act as a messenger RNA and directly code for proteins. The RNA genome contains one long open reading frame expressed as a 350 kDa polyprotein precursor. This polyprotein is proteolytically processed by viral and host proteases into ten smaller functional proteins. The exact mode of processing and function of these proteins is still controversial, but it is believed that they may be multifunctional. According to previous studies, these proteins are P1, helper component proteinase (HC-Pro), P3, cylindrical inclusion (CI), nuclear inclusion A (NIa), nuclear inclusion B (NIb), coat protein (CP), and two small putative proteins 6 K1 and 6 K2. Both CP and HC-Pro are required for the aphid transmission of a potyvirus. The viral genome encodes a large polyprotein that is processed by

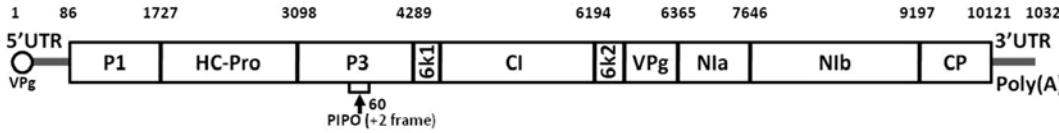

Fig. 11.3 *Genome organization of Papaya ring spot virus (PRSV): P1*, serine proteinase, the first protein (63 kDa); *HC-Pro*, helper component cysteine proteinase (52 kDa); *P3*, third protein (46 kDa); *PIPO*, a short ORF embedded within the P3 (46 kDa), but translated in the +2 reading frame, *6 K1* and *6 K2*, two 6 kDa proteins; *CI*, cylindrical inclusion protein (72 kDa); *VPg*, viral protein genome-linked (21 kDa); *NIa*, main viral proteinase (48 kDa); *NIb*, replicase (59 kDa); and *CP*, coat protein (35 kDa). The PRSV genome has untranslated regions (*UTR*) on either end of its RNA genome, which are termed as 5′ UTR and 3′ UTR and the 3′UTR has a Poly (A) tail. The relative nucleic acid positions are indicated on the *top*

three different virus encoded proteinases to yield the mature functional proteins. Two proteinases, P1 and the helper component proteinase (HC-Pro), catalyze only autoproteolytic reactions at their respective C termini. The remaining cleavage reactions are catalyzed by autoproteolytic mechanisms by the small nuclear inclusion protein (NIa-Pro) (Fig. 11.3).

Most studies in India have focused on examining sequence variation in the CP gene of PRSV and these studies suggested that there was a little sequence variation among the CP genes from different PRSV isolates within the country (Chakraborty et al. 2015; Akhter et al. 2013). However, recent sequence data of CP gene of PRSV isolates from India and Mexico suggest greater sequence variation among the local populations of PRSV isolates in other countries. Currently, 22 complete genome sequences (isolate P only) of well-characterized PRSV are available in the NCBI GenData Bank, of which one each from Hawaii and Mexico, two each from Brazil (two W isolates) and Thailand (one P isolate and one W isolate), five isolates from Taiwan (four P isolates and one W isolate), and three from India (two P isolates and one W isolate) are available, and a phylogenetic analysis of all these whole genome PRSV sequences is done by MEGA 5.2 (Fig. 11.4). Phylogenetic analysis reveals the closeness between the viral sequences, sequence diversity among isolates of PRSV and their distribution understanding the viral origins, development, dispersion as well as disease etiology. This information could be useful in developing effective virus disease management strategies.

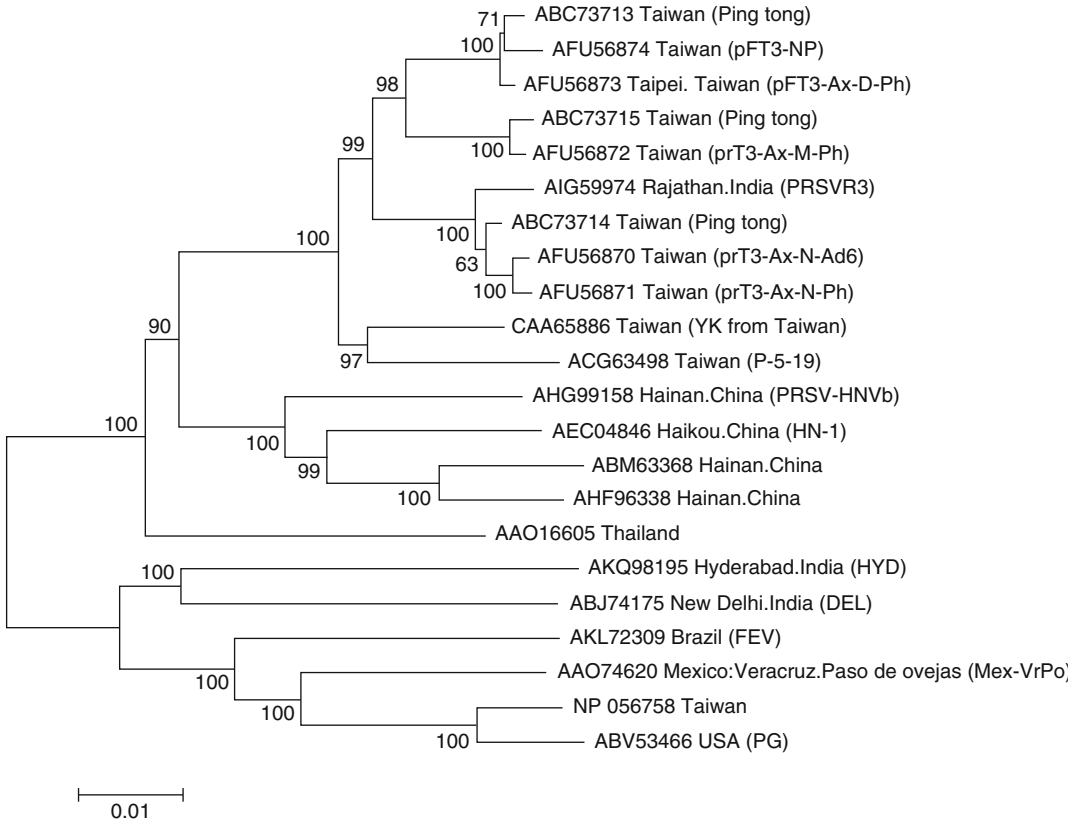

Fig. 11.4 Phylogenetic analysis of the whole genome sequences of different isolates of Papaya ring spot viruses from different regions across the globe. Each isolate is indicated by its accession number, followed by the location and the isolate name is given in the parenthesis. Bootstrap values were obtained from 1000 replicates

11.3 Papaya Leaf Curl Viruses

Papaya leaf curl virus (PLCV) and other begomoviruses belonging to the family *Geminiviridae* are the main causal agents of papaya leaf curl disease. The symptoms of which are curling/rolling of the leaves downward and inward in the form of an inverted cup and the thickening of veins as well as reduction in the production of papaya. Papaya leaf curl disease first reported by Thomas and Krishnaswami (1939) is caused by begomoviruses such as *Papaya leaf curl virus* (PaLCuV) (Singh-Pant et al. 2012), *Chilli leaf curls virus* (ChiLCuV), *Tomato leaf curl New Delhi virus* (ToLCuNDV), and *Croton yellow vein mosaic virus* (CYVMV), which are also associated with diverse betasatellites (Singh-Pant et al. 2012; Pramesh et al. 2013). In advanced stages of the disease, defoliation takes place and the growth of the plant is arrested. PLCV belongs to the family *Geminiviridae*, genus *Begomovirus*. It is not transmitted mechanically and the virus vector is whitefly (*Bemisia tabaci*) and often associated with satellites, such as betasatellites and alphasatellites and produce characteristic symptoms, including leaf curl, that adversely affect the photosynthetic tissues (Singh-Pant et al. 2012).

The family *Geminiviridae* includes plant infecting circular single-stranded DNA viruses that have geminated particle structure. Both monocotyledonous and dicotyledonous plants are infected by members of this family and have a global presence. The family *Geminiviridae* is represented by seven different genera: *Eragrovirus*, *Curtovirus*, *Begomovirus*, *Becurtovirus*, *Topocuvirus*, *Turncurtovirus*, and *Mastrevirus*. *Becurtovirus*, *Eragrovirus*, and *Turncurtovirus* accommodate various recently discovered geminiviruses that are highly divergent and, in some cases, have unique genome structures (Varsani et al. 2014).

The largest genus, *Begomovirus*, currently contains more than 192 species (Rey et al. 2012) that have either bipartite genomes (DNA-A and DNA-B) (Fig. 11.5) or monopartite genomes (DNA-A), associated with satellite molecules (betasatellite or alphasatellite). The DNA-A component typically has six open reading frames (ORFs): *AV1*/*V1* (coat protein, CP) and *AV2*/*V2* (pre-coat protein) on the virion-sense strand, and *AC1*/*C1* (replication associated protein, Rep), *AC2*/*C2* (transcriptional activator, TrAP), *AC3*/*C3* (replication enhancer, REn) and *AC4*/*C4* (AC4/C4 protein) on the complementary-sense strand

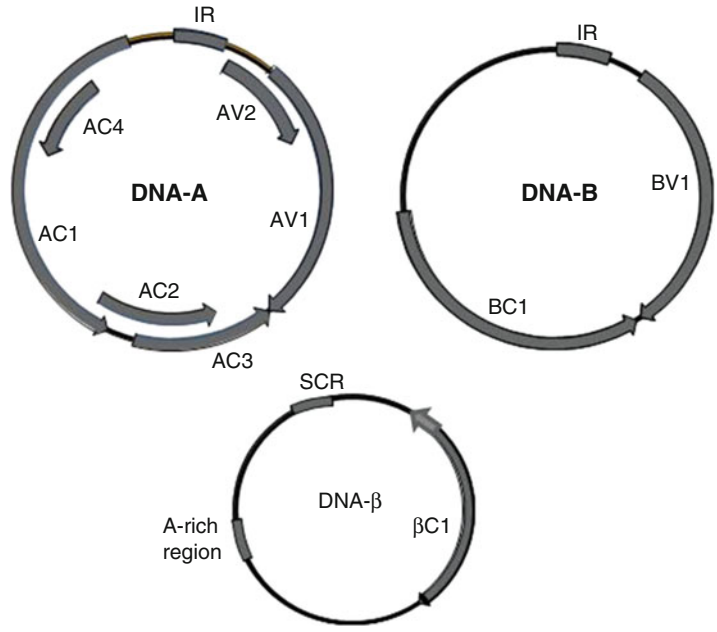

Fig. 11.5 Organization of the genomic components (DNA-A and DNA-B) of a bipartite begomovirus (Genus: *Begomovirus*, Family: *Geminiviridae*) and the betasatellites (DNA-β) associated with the monopartite begomovirus

of the viral genome (Patil and Fauquet 2009). Whereas the DNA-B component has two ORFs encoding for movement proteins: *BV1* (nuclear shuttle protein, NSP) on the virus-sense strand, which helps in nucleo-cytoplasmic transport and *BC1* (movement protein, MP) on the complementary-sense strand, taking care of cell to cell movement (Rojas et al. 2005; Seal et al. 2006). The opposing transcription units of begomovirus DNA-A and -B components are separated by an intergenic region (IR) that generally shares a highly conserved region of approximately 200 nt, named the common region (CR) (Lazarowitz 1992). The CR contains an origin of replication (ori) that includes a stem-loop structure encompassing a nonanucleotide sequence (TAATATTAC) whose T7–A8 site is required for cleaving and annealing of geminiviral DNAs during replication (Arguello-Astorga and Ruiz-Medrano 2001; Fontes et al. 1994a, b). The conserved iterated sequences (iterons) are required for specific recognition and binding by Rep during replication. Till now multiple whole genome sequences are submitted in NCBI GenBank from India as well as other countries. Figure 11.6 represents the phylogenetic analysis of papaya leaf curl viruses from the Indian subcontinent.

11.3.1 Papaya Meleira Virus

Papaya meleira virus (PMeV) is the causal organism of papaya sticky disease, characterized by a spontaneous exudation of fluid and aqueous latex from the papaya fruits and leaves. The latex oxidizes after atmospheric exposure, resulting in a sticky feature on the fruit from which the name of this disease originates.

PMeV, the causal agent of Papaya sticky disease or "meleira", was reported from Brazil in the 1980s (Rodrigues et al. 1989) and from Mexico in 2008 (Perez-Brito et al. 2012). Although PMeV is considered as one of the important viruses infecting papaya in Brazil and Mexico (Abreu et al. 2015), knowledge of the sequence and genomic organization of this virus is meager. PMeV is not yet sequenced and has also not been classified by the International Committee on the Taxonomy of Viruses (ICTV). Phylogenetic analysis of a ~560 bp sequence amplified from the replicase gene of a PMeV isolate from Brazil indicated that it possesses a high similarity with the mycoviruses of the family *Totiviridae* (Daltro et al. 2014; Araújo et al. 2007). However, a conclusive taxonomic classification will be possible only when the full genome is sequenced. PMeV is an isometric virus particle that has a double-

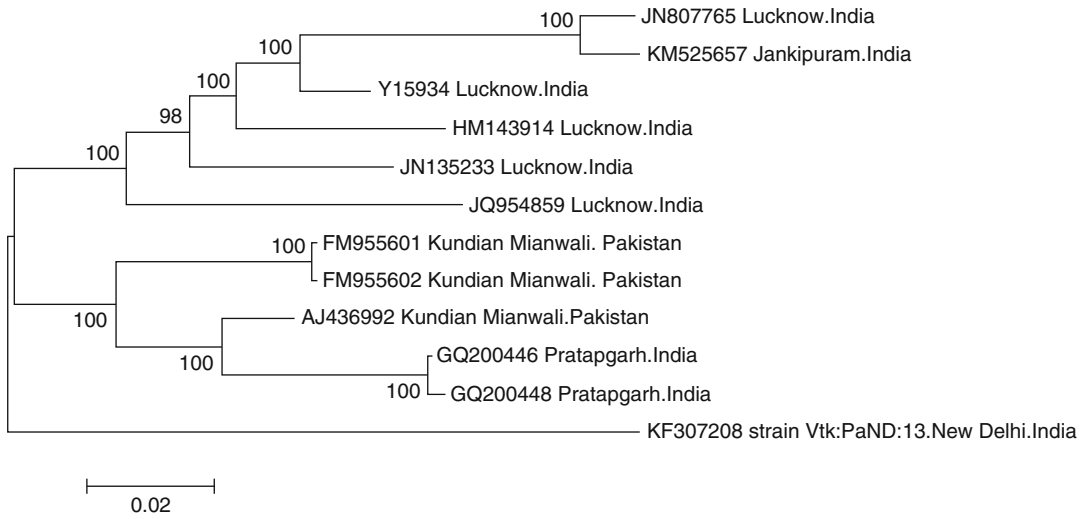

Fig. 11.6 Phylogenetic analysis of whole genome sequences of different isolates of Papaya leaf curl viruses from different regions of Indian subcontinent. Each isolate is indicated by its accession number, followed by the location and the isolate name is given in the parenthesis. Bootstrap values were obtained from 1000 replicates

stranded RNA (dsRNA) genome of 12 Kb (Kitajima et al. 1993; Maciel-Zambolim et al. 2003). Papaya fruits with sticky disease symptoms are commercially unacceptable as the disease compromises with the texture and flavor of the fruits, making them unfit for consumption, which prevents their export in the international market (Ventura et al. 2003).

11.3.2 Papaya Mosaic Virus

Papaya mosaic virus (PapMV) is a member of the genus *Potexvirus* and family *Alphaflexiviridae*. PapMV is a filamentous, flexuous rod, with 500 nm in length and it is a monopartite, positive-sense, single-stranded RNA surrounded by capsid proteins (Babin et al. 2013). The genome has been completely sequenced and is 6656 nucleotides long. This virus is transmitted by mechanical inoculation and also by seeds; however there is no report of an insect vector involved. Mature virions form banded inclusions that can be seen in leaf strips of infected plants when stained with either the Azure A nucleic acid stain or the Orange-Green protein stain.

Papaya (*Carica papaya*) is main host of PapMV, although PapMV was able to infect a few other plants including the ornamental snapdragons (*Antirrhinum majus*). It is also reported to naturally infect the leafy vegetable *Ullucus tuberosus*. PapMV was first reported in papaya in 1962 along with another virus that was later called as *Papaya ringspot virus*, a potyvirus. The two viruses were differentiated in 1965 and 1967 using particle lengths, serology, host range, inclusions, and aphid transmissibility. PapMV causes mild mosaic symptoms on papaya leaves and stunting of the plant. PapMV has also been reported from South America (Bolivia, Peru, and Venezuela) and the USA.

11.3.3 Papaya Lethal Yellowing Virus

Papaya lethal yellowing virus (PLYV) causes lethal yellowing disease in papaya plants. Lethal yellowing was first described in the early 1980s. The disease has been detected in several states of north eastern Brazil but has never been reported elsewhere (Pereira et al. 2012). Initial infection manifests as yellowing of the younger leaves, which later progresses to more severe symptoms of curled leaves, wilting, and senescence. PLYV is readily sap-transmitted and can be found in the soil, but a vector has never been identified. Virus particles are isometric with a diameter of ca. 30 nm, and the genome is comprised of one single-stranded, positive-sense RNA molecule reported to be 4800 nucleotides (nt) long. The sequence of a 1100-nt fragment that encompasses parts of the RNA dependent RNA polymerase (RdRp) and coat protein (CP) coding regions has indicated a significant degree of sequence identity to viruses in the genus *Sobemovirus* (Nascimento et al. 2010).

11.4 Genetic Engineering for Virus Resistant Papaya

Traditional breeding of papaya cultivars for resistance to viral diseases had limited success. The rapid advancement in genetic engineering technologies has made it possible to introduce selected genes into plants to control plant pests and diseases. Crop improvement to solve disease problems of a tree species like papaya has been enhanced by gene transfer techniques.

At the initial time, many studies indicated that resistance was primarily to virus particles, due to likely interference in the initial events during the infection process. Subsequent studies using *in vitro* encapsidated TMV genomic RNA encompassing reporter genes also suggested that virion disassembly was primarily reduced in transgenic plants (Beachy et al. 1990). However, CP-mediated resistance does not appear to work for plant-infecting DNA viruses (E.g. geminiviruses). However, the success of CP-mediated protection has resulted in the commercialization of squash lines resistant to *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), and *Watermelon mosaic virus* (WMV), and transgenic papaya resistant to *Papaya ringspot virus* (Tricoli et al. 1995).

Genetic engineering of plants has generated huge interest in developing methods for crop improvement (Bonfim et al. 2007; Prins et al. 2008; Grumet 1990). Many transgenic plants have been successfully produced with remarkable results such as resistance to herbicides, pests, and diseases (Azad et al. 2013). The success of any genetic transformation protocol depends on three key steps such as reproducible shoot regeneration system, establishment of an inoculation system, and a method for transformed shoot selection (Hamama et al. 2011). *Agrobacterium*-mediated transformation protocols differ from one plant species to another, within species and from one cultivar to another (de la Riva et al. 1998). *Agrobacterium*-mediated transformation has great advantages over direct transformation method as it reduces the copy number of transgene, potentially leading to fewer constructs with transgene co-suppression and instability (Hansen et al. 1997). Genetic transformation of papaya by using either *Agrobacterium* or microprojectile/particle bombardment methods have been successful (Fitch et al. 1990, 1993; Cheng et al. 1996). Fitch et al. (1990) developed transgenic papaya plants from somatic embryos after co-cultivation with *Agrobacterium tumefaciens* strain C58-Z707. Fitch and Manshardt (1990) transformed and regenerated transgenic plants using microprojectile bombarded from immature zygotic embryos and hypocotyls of Sunrise Solo and Kapoho Solo. Manshardt (1992) reported that papaya mosaic virus resistant variety can be developed through genetic transformation system using viral Coat Protein (CP) gene (Manshardt 1992). Pang and Sanford (1988) transformed papaya leaf discs, stems, and petioles of Sunrise Solo and Kapoho with *A. tumefaciens* strains GV3101, but they could not regenerate plantlets from these calli. Tennant et al. (1994) reported that the transgenic papaya expressing the coat protein gene showed high levels of resistance against the severe papaya ringspot virus in Hawaii. Type of explants and genotype are important factors for successful genetic transformation of papaya, using *A. tumefaciens*.

Developing virus resistant/tolerant plant cultivars is one of the most effective and economical way to control plant viral diseases. Natural sources of virus resistance have been extensively exploited to develop virus-resistant crop plants by conventional breeding.

Cross-protection is another conventional approach of virus control, also known to as "coat protein mediated protection or mild strain mediated protection". During cross-protection, when a plant is first inoculated by a mild strain of a particular plant virus, becomes immune/protected against subsequent infection from a severe strain of the same virus (Folimonova 2013). Although the actual mechanism of cross-protection is not yet understood, however it is thought that at least in a few cases the coat protein plays an important role.

The development of genetically engineered resistance to plant viral diseases is an outcome of efforts to understand the plant-virus interactions involved in "cross-protection". Historically, the expression of the *Tobacco mosaic virus* coat protein (CP) gene in transgenic tobacco plants is the first example of transgene-mediated virus resistance (Beachy et al. 1990). CP-mediated protection refers to the resistance obtained by the transgenic expression of the viral CP gene in the crop plants. This accumulation of the CP in transgenic plants will result in resistance to infection from the virus from which the CP gene is derived. Later, sequences derived from different gene/s of several other plant viruses were shown to confer virus resistance in experimental and/or natural host plants. Transgenic approaches have also been employed successfully to confer virus resistance by engineering virus-derived genes, defective interfering RNA, non-coding RNA sequences, and proteases into the susceptible crop cultivars. Non-viral genes, such as R genes, microRNAs, ribosome-inactivating proteins, protease inhibitors, and scFvs, have also been successfully used to engineer virus resistance in plants (Sudarshana et al. 2007; Reddy et al. 2009). However, transgene-mediated gene/RNA silencing by generation of small interfering RNAs (siRNAs) appears to be the prime mechanism to confer resistance to viruses (Waterhouse et al. 2001; Patil et al. 2011; Patil et al. 2016). The concept of pathogen-derived resistance has

also been employed for the development of transgenic papaya, using a coat protein-mediated, RNAi-based method, and replicase gene-mediated transformation for effective PRSV disease management (Azad et al. 2014).

With the development of the CRISPR/Cas9 technology, the genome editing in plants has received a major boost. This powerful tool allows substantial improvement of plant traits in addition to those provided by classical breeding. Engineering resistance to different plant viruses has been demonstrated by employing CRISPR-Cas9 technology, particularly by disrupting the recessive eIF4E gene function for control of potyviruses (Zlotorynski 2015).

There is a little or no source of genetic resistance to PRSV and PLCV available in the papaya germplasm. Large collections of papaya germplasm and cultivars representing the world's major production have been screened, but resistance has not been found. However, a close relative of Papaya, *Vasconcellea cauliflora* is resistant to PRSV and is used in the breeding program for PRSV resistance (Haireen et al. 2014).

11.5 PRSV Resistant Transgenic Papaya

In one of the study, to develop PRSV resistant transgenic Papaya, it was transformed with four different constructs containing either the modified or unmodified coat protein (CP) gene from a PRSV isolate from Florida, by Agrobacterium-mediated transformation (Davis and Ying 2004). The above four constructs had CP in the sense orientation (S-CP), antisense orientation (AS-CP), sense orientation with a frame-shift mutation (FS-CP), or sense orientation mutated with three-in-frame stop codons (SC-CP). All the 256 putative transgenic lines obtained with the above four constructs were subjected to mechanical inoculation with the H1K isolate of PRSV. Although, none of the transgenic plant lines were immune to the PRSV, there were transgenic lines showing higher resistance derived from all the four constructs. The PRSV resistant transgenic resistance was introgressed into six papaya genotypes through breeding technologies. The transgenic lines derived from the constructs FS-CP and SC-CP were highly fertile, whereas those derived from the constructs S-CP and AS-CP were infertile. Plants derived from 54 crosses and representing 17 transgenic lines were planted in the field. The field trial experiments showed that the 23.3 % of introgressed lines became naturally infected with PRSV, whereas 96.7 % of the non-transgenic control plants were infected by PRSV. Overall, the incidence of infection by PRSV in the R1 progeny was determined by both the transgenic line and the non-transgenic parent (Davis and Ying 2004).

Similarly, when a local variety of papaya from the Andean foothills of Venezuela was transformed independently with two different coat protein (CP) genes from two different PRSV isolates, designated VE and LA (Fermin et al. 2004; Mishra et al. 2010). Both the CP genes of both PRSV isolates (VE and LA) had a nucleotide and amino acid sequence similarity of 92 % and 96 %, respectively. Later, the progenies derived from the four PRSV-resistant R_0 plants were tested for resistance against the two homologous isolates VE and LA, and also two heterologous isolates HA (Hawaii) and TH (Thailand) in greenhouse conditions. The studies showed that the virus resistance was affected by sequence similarity between the transgenes and the viruses used for challenge inoculation: resistance was higher for plants challenged with the homologous isolates (92–100 % nucleotide similarity) than for the Hawaiian isolate (94 % similarity) or the Thailand isolates (88–89 % similarity) (Azad et al. 2014; Mishra et al. 2010). This strain-specific resistance limits the cultivation of these transgenic lines in other parts of the world, which represented by diverse PRSV sequences. In another transgenic study, the CP gene of a local PRSV strain from Taiwan, designated PRSV-YK was transformed into papaya and a total of 45 putative transgenic lines were obtained (Bau et al. 2004). When the plants of transgenic lines were mechanically challenged with PRSV-YK, different levels of resistance ranging from delay of symptom development to complete immunity was obtained. Further molecular characterization of nine selected trans-

genic lines exhibiting varying levels of resistance revealed that the expression level of the transgene was negatively correlated with the level of resistance. This suggested that the resistance was manifested by a RNA mediated mechanism (Bau et al. 2004). Seven selected transgenic lines were screened further for resistance to three PRSV heterologous strains originating from Hawaii, Thailand, and Mexico. Six of the seven transgenic lines displayed varying levels of resistance to the heterologous strains, and one particular line 19-0-1 was immune not only to the homologous YK strain but also to the three heterologous strains of PRSV. Thus, these PRSV CP expressing transgenic papaya lines with broad-spectrum virus resistance have great potential for use in Taiwan and other geographic regions to control PRSV (Gonsalves et al. 2004). Further, four transgenic papaya lines expressing PRSV CP gene were subjected to field evaluation, for PRSV resistance and fruit yield during 1996–1999. The papaya plants were exposed to natural virus inoculation by aphids in two adjacent fields in four different plantings at the same sites and none of the transgenic papaya showed severe symptoms whereas the control non-transgenic plants were 100 % severely infected by PRSV in 3–5 months after planting. In the initial two trials, 20–30 % of the transgenic plants showed mild symptoms with confined mottling or chlorotic spots on the leaves and the resistance levels or the rate of PRSV infections showed seasonal variation. However, in the third field trial, the incidence of the mild PRSV symptoms was enhanced, mostly due to the outbreak of root rot fungi in the rainy season. Interestingly, there was no visible adverse effect on the fruit yield and the fruit quality of the transgenic plants with mild PRSV symptoms. The transgenic lines yielded 10.8–11.6 and 54.3–56.7 times more marketable fruit with no ring spot symptoms, respectively, than controls in the first and second experiments (Bau et al. 2004).

In another study transgenic papaya with duel resistance to *Papaya ringspot virus* and *Papaya leaf-distortion mosaic virus* (PLDMV) were generated, since the later virus is emerging as a threat to PRSV-resistant transgenic papaya (Kung et al. 2009). Their studies also showed that double virus resistance in transgenic lines resulted from two or more copies of the transgene through the mechanism of RNA interference (Kung et al. 2010).

In addition to the use of transgenic technology for virus resistance, bacterially expressed double stranded can be used as a foliar spray/application on crop plants to control the invading plant viruses. Efforts have also been made to use this technology for the control of PRSV by targeting the CP, and significant levels of virus resistance were observed (Shen et al. 2014). Such studies have also raised the hopes for employing non-transgenic technologies for the control of plant viruses.

Replicase mediated resistance, a protein-based approach, has also been used to confer virus resistance and the resistance phenotype is influenced by mutations affecting the primary structure of the transgenically expressed replicase protein. Resistance created by the introduction of replicase gene was first demonstrated for the tobacco mosaic virus (TMV) in *Nicotiana tabacum* (Golemboski et al. 1990) and replicase genes with mutations are shown to confer virus resistance (Nunome et al. 2002). Chen et al. (2001) reported that replicase gene (RP) conferred resistance to PRSV in transgenic papaya, while Wei et al. (2007) reported that transgenic papaya with mutated replicase gene (RP) showed high resistance to PRSV.

11.6 Conclusion

Although significant progress has been made in developing transgenic papaya, tissue culture and regeneration protocols for papaya still remain challenging. Both biolistic and *Agrobacterium*-mediated transformation methods have been proved to be efficient for transformation and regeneration of transgenic papaya plants. The virus resistance accomplished by transgenic expression of coat protein gene is the most effective method for the control of PRSV. The transgenic papaya varieties Rainbow and SunUp developed by overexpressing PRSV-CP by Prof. Gonzalvez's group at University of Hawaii were deregulated and granted approval for their com-

mercialization/cultivation, thus representing the first transgenic fruit tree (Gonsalves 1998; Fuchs and Gonsalves 2007). The transgenic papaya lines showed varied levels of resistance, ranging from delay of symptom development to complete immunity. Use of chimeric gene constructs targeting multiple viral genes, such as CP, replicase, and the silencing suppressor HC-Pro, may prevent the emergence of a super virus strain that can break the transgenic virus resistance. This may also provide more stable and durable resistance against multiple viruses. Off late, the incidence of leaf curl disease has become a major concern and there are diverse geminiviruses involved in the leaf curl disease thus making it very difficult for the use of promising control strategies, which can counter the menace of diverse geminiviruses infecting papaya. It may be important to identify novel technologies such as CRISPR-Cas for the broad spectrum control of highly variable geminiviruses.

Until now very few virus resistant transgenic crop plants have been released for cultivation in farmer's field and none is available yet in the developing countries. However, a number of economically important virus resistant transgenic crops transformed with viral genes are of great interest in developing countries. The major issues hampering the production and deregulation of virus resistant transgenic plants are mainly socio-economic and related to bio-safety, intellectual property rights, and expenditure to generate transgenic crops. However, because of its real potential, we believe that transgenic technology will have more widespread and renewed interest in the near future.

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Potato Virus Y Genetic Variability: A Review

12

Rakesh Kumar Verma, Ritesh Mishra, and Rajarshi Kumar Gaur

Abstract

Disease caused by potato virus Y (PVY) infection is becoming a major constraint of sustainable potato production throughout the world and causes significant financial loss. Therefore, the improved scientific understanding about PVY population structure, genetic variability, and evolutionary dynamics is required for the development of management strategies against PVY infection. In this chapter we provide updated information about PVY genetic variability and factors that drive PVY evolution. The evolutionary dynamics of PVY seems to be robustly coupled to purifying selection in association with neutral evolution and some sporadic actions of positive selection. Recombination is a major driving force for evolution of PVY genome associated with natural selection and mutation. P1, NIb, CI, and HC-Pro regions of PVY genome showed higher sequence variability, dN/dS ratio, and higher number of mutation sites. Values of genetic distances in analyzed sequences revealed that the variations are distributed throughout of PVY genome. We also analyzed transition /transversion bias in 100 PVY complete genome sequences and obtain that the transitions are more favorable than the transversions. Recombination map of several PVY strains provides new insight in the emergence of new variants of PVY.

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Potato virus Y • Recombination • Natural selection • Evolution • Genetic variability

12.1 Introduction

In recent times, global threats of crop diseases caused by *Potyviruses*, such as *plum pox*, *onion yellow dwarf*, *papaya ringspot*, *turnip mosaic*, *soybean mosaic*, *tobacco vein banding mosaic*, and *watermelon mosaic viruses*, have led to remarkable economic losses (Garcia et al. 2014). *Potyviruses* are widespread in cultivated plants throughout all regions of the world, but as recent metagenomics studies have indicated, they are also found in high quantities in the wild (Roossinck 2012). *Potato virus Y* (PVY), together with *potato virus A* (PVA) and *potato leaf roll virus* (PLRV; genus *Polerovirus*), also causes serious damage to potato production worldwide and can reduce crop yields up to 90 % (Salazar 2003; Valkonen 2007; Visser et al. 2012). The study of genetic structure and their evolutionary history of plant viruses are crucial to designing stable and efficient control strategies as well as favors to understand the features of their biology such as changes in virulence and geographical ranges and their emergence as new epidemics (Garcıa-Arenal et al. 2003). *Potato virus Y* is a member of the genus *Potyvirus* of Potyviridae family and consists of a single-stranded, positive-sense RNA as a genetic material. The viral RNA encodes a single, large polyprotein that is cleaved by three virus-encoded proteases (P1, helper component proteinase, and the nuclear inclusion body A proteinase) into ten functional proteins (P1, HC-Pro, P3, 6 K1, CI, 6 K2, VPg, NIa-Pro, NIb, and CP) and additional peptide P3N-PIPO (Fig. 12.1) (Chung et al. 2008; Verma et al. 2015). Five main strains of PVY isolates are differentiated on the basis of their genome organization and hypersensitive response (HR) reactions in a set of potato indicators carrying different N resistance genes: PVY^O inducing a systemic mottle and eliciting HR in the background of *Ny* gene; PVY^C causes stipple streak and HR in the background of *Nc* gene; PVY^Z elicits HR in the presence of *Nz* gene but not in the presence of *Ny* and *Nc* genes; PVY^N causes a veinal necrosis and produces HR in the presence of all three resistance genes; and PVY^E produces HR in the presence of all three genes but indicating only mosaic and vein clearing in tobacco (Singh et al. 2008; Karasev and Gray 2013). PVY^O is the original

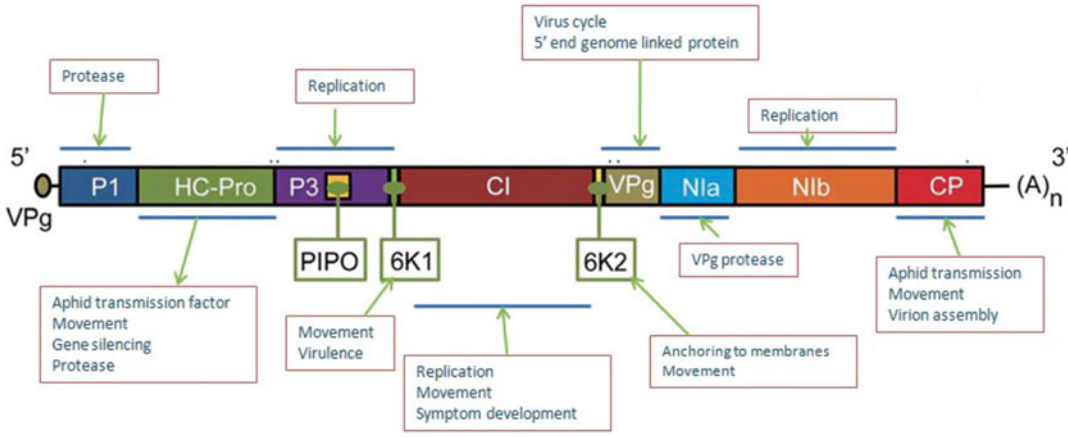

Fig. 12.1 Genomic organization of *potato virus Y*; different genes and their functions

wild strain of PVY. The ^{O} stands for "ordinary." The ^{N} strains cause a necrotic reaction on tobacco leaves but not on potato foliage (Singh et al. 2008). PVY^{NTN} is a PVY ^{N} type that causes necrosis on tobacco but can also cause necrotic flecking and ringspot symptoms in the tubers of some potato varieties. The ^{NTN} stands for "n-tuber necrotic" (Glais et al. 2002). PVY^{NO} are thought to be "recombinants," which means that they have some characteristics of both PVY^{O} and PVY^{N} (Upeksha et al. 2012; Whitworth et al. 2012). The origins of these prevalent recombinants and interrelations between diverse recombinant types have not been clear up to now (Hu et al. 2009; Karasev et al. 2011); however, at least two recombinant types, PVY$^{N\text{-}Wi}$ and PVY$^{N:O}$, have been considered to be almost identical, both biologically and genetically (Glais et al. 2002; Nie et al. 2004; Schubert et al. 2007). In this review we analyzed the genetic variations in *potato virus Y* by analysis of the 100 complete genome nucleotide sequences retrieved from NCBI GenBank. We also focused on the recombination breakpoints in different cistronic regions of PVY genome.

12.2 Sources of the Genetic Variations

Sequence variation in a population depicts the existence in that population of diverse alleles, or alternative forms, for a given gene, caused by variation in the order of bases in the nucleotides in genes. These variations consent flexibility and survival of a population in the face of changing environmental circumstances. RNA viruses flaunt a high degree of genetic variability that results from five major phenomena that affect their genome: mutation, recombination, natural selection, genetic drift, and migration (Moya et al. 2004). Rapid genetic changes, recombination, large population size, fast replication, and high mutation rates are characteristics of RNA viruses (Domingo et al. 2008; Cuevas et al. 2012a). Mutation and recombination are the major evolutionary forces in plant viruses generating genetic variability, afterwards structured by selection and genetic drift (Garcia-Arenal et al. 2001; Roossinck 2003). *Mutation* is the process that results in differences between the nucleotides incorporated into the daughter strand during nucleic acid replication and those in the template as a consequence of the lack of proofreading activity of RNA polymerases (Garcia-Arenal and Fraile 2011). RNA viruses show evidence of the highest mutation rates of any group of organisms, approximately one mutation for each genome, per replication (Malpica et al. 2002). Recombination and reassortment contribute to enhance genetic diversity and to produce novel genome that may have selective advantage over parental genomes (Nagy and Bujarski 1998). *Recombination* is the process by which segments of genetic information are exchanged between the nucleotide strands of diverse genetic variants during the process of replication. Recombination plays a key role to acquire sequence diversity and complex genotyping system of viruses and can be a dominant force in shaping the genetic architecture of organism and associated phenotypes (Posada et al. 2002) and also affects analyses of the molecular clock, demographic processes, or natural selection (Hon et al. 2008). The recent increase in viral sequences available and the improvement of methods of analysis have shown that recombination events can be traced in many viral families, predominantly in the family Potyviridae containing the genus *Potyvirus* (Chare and Holmes 2005). There are numerous reports on the genetic structure of *Potyvirus* populations, remarkably those on *potato virus Y* (PVY) (Visser et al. 2012; Ogawa et al. 2008, 2012; Karasev et al. 2011), *watermelon mosaic virus* (WMV) (Desbiez et al. 2011), *onion yellow dwarf virus* (OYDV) (Verma et al. 2015), *tobacco vein banding mosaic virus* (TVBMV) (Zhang et al. 2011), *zucchini yellow mosaic virus* (ZYMV) (Lecoq et al. 2009), *soybean mosaic virus* (SMV) (Seo et al. 2009), and *turnip mosaic virus* (TuMV) (Ohshima et al. 2002; Ohshima et al. 2007). These reports suggest that virus population has been fashioned by relative contribution of evolutionary forces such as recombination, selection, genetic drift, and mutation. *Natural selection* is one of the basic mechanisms of evo-

lution, along with migration, genetic drift, and mutation. The concept of fitness is central to natural selection. It is a gradual process by which variants that are more fit in a certain environment will have better potential for survival (positive or adaptive selection), whereas variants less fit decrease their frequency in population (negative and purifying selection) (Rubio et al. 2013). *Genetic drift* illustrated random fluctuations in the number of gene variants in a finite population due to random sampling from generation to generation (Masel 2011). It is a random process that can lead to large changes in populations over a short period of time and leads to fixation of alleles or genotypes in populations. It diminishes genetic variability of a population by decreasing the size of the population due to the *population bottlenecks* and *founder effects* (Ali and Roossinck 2008). Genetic drift can take place in different phenomena of the virus life cycle such as virus transmission between plants by vectors (Ali et al. 2006; Betancourt et al. 2008), movement between plant cells (Sacristan et al. 2003; Li and Roossinck 2004), and interaction between coinfecting viruses (Fraile et al. 1997). A final factor to consider in RNA virus evolution is migration (also known as *gene flow*) that is the transfer of genes from one population to another. Gene flow breaks down the geographical or other boundaries that could otherwise isolate populations. It is a unifying force that prevents populations from diverging and favors genetic uniformity between populations (Moya et al. 2004).

12.3 Genetic Variability in PVY Genome

Figure 12.2 shows the phylogenetic relationship between isolates of PVY, on the basis of complete genome sequences. The GTR + G + I nucleotide substitution matrix with the lowest Bayesian information criterion (BIC) was identified as the best-suited evolutionary model for our data set using MEGA 6.0 software (Tamura et al. 2013). Nucleotide sequences of different genes of 100 PVY geographical isolates were aligned and used to estimate mean nucleotide sequence diversity between each pair of isolates according to Kimura's two-parameter method. Nucleotide diversity is defined as the mean number of nucleotide substitutions per site that is the raw material for evolution via natural selection (Marco and Aranda 2005). It is one of the important aspects in determining whether organism can survive in novel environments (Dennehy et al. 2013). The overall mean value of nucleotide diversity for the polyprotein coding region of PVY was 0.053 ± .002 (Table 12.1). High nucleotide diversity values were found in P1, NIa-VPg, NIa-Pro, and NIb cistronic regions while other regions showed relatively low diversity values. 6K1 and P1 cistronic regions exhibited very low and very high nucleotide diversity, respectively (Table 12.1). Pairwise genetic differences at synonymous (dN) and nonsynonymous (dS) positions were ensured using the Pamilo-Bianchi-Li (PBL 1993) method. The dN/dS ratio can be used as an index exhibiting the pattern of selective constraint in evolutionary relationship (Nei and Gojobori 1986). The higher degree of variability in dN/dS ratio values of different cistronic regions consistent with different evolutionary pressures throughout the genome. The dN/dS ratio was significantly higher in P1 region suggesting that the P1 region is under looser evolutionary constraints than other cistronic regions. Potato virus Y is a right model of an RNA virus using elevated mutation rates and frequent recombination breakpoints to produce vast genetic diversity, and thus, it survives and succeeds in several hosts and in numerous environments (Karasev et al. 2011; Blanchard et al. 2008). The ratio (ω) of transition to transversion (amino acid varying) nucleotide substitution is a fundamental measure of the relative importance of selection and genetic drift and an important parameter to scrutinize sequence variations in a group of isolates (Kimura 1980). Rates of different transitional substitutions in PYV polyprotein are ranged from 14.87 to 26.78 and those of transversionsal substitutions are ranged from 1.87 to 3.11. The nucleotide frequencies are 31.07 % (A), 26.69 % (T/U), 18.66 % (C), and 23.58 % (G). The transition/transversion rate

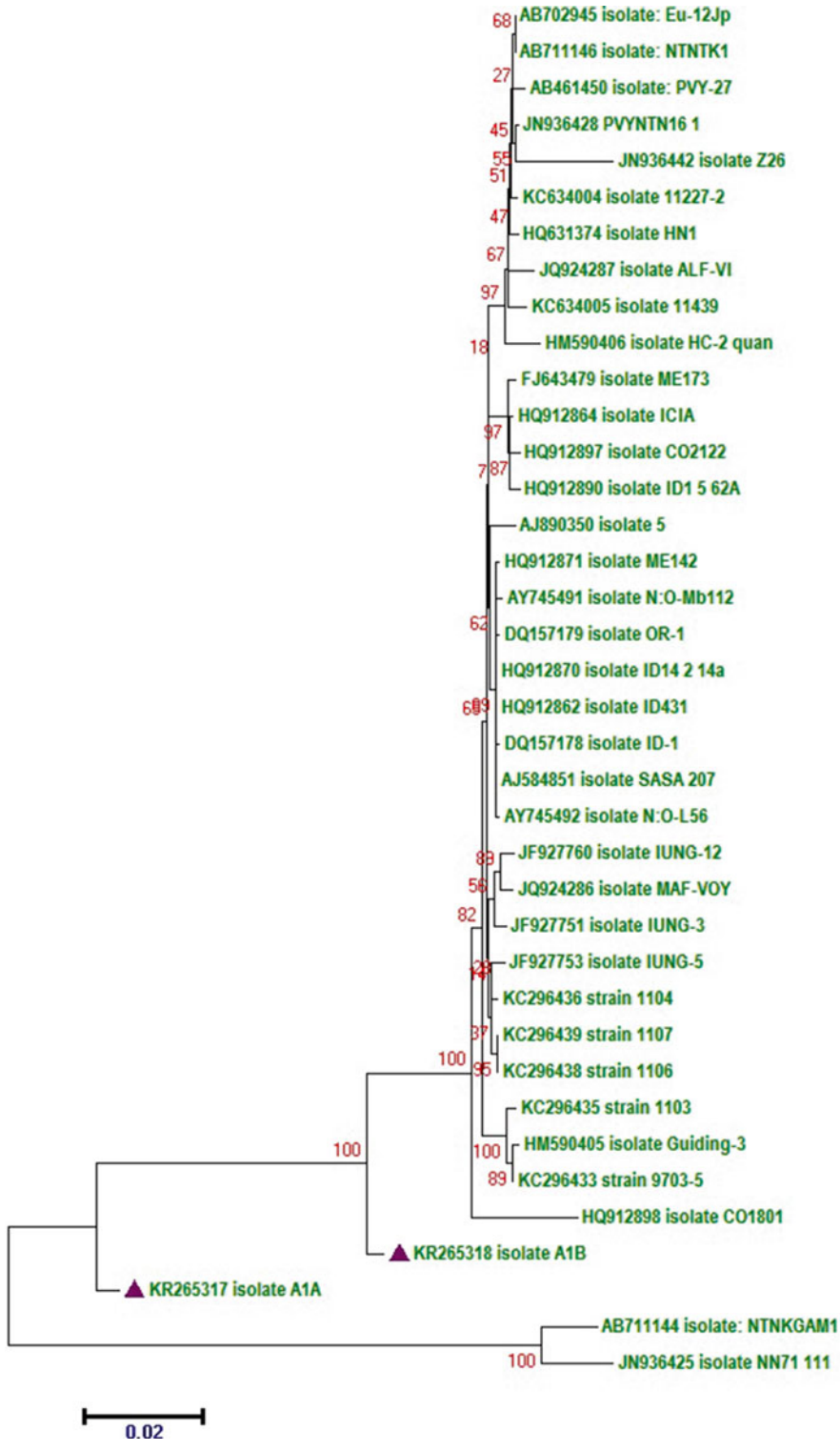

Fig. 12.2 Maximum likelihood phylogenetic tree of 100 PVY geographical isolates. The GTR+G+I nucleotide substitution matrix with the lowest Bayesian information criterion (BIC) was identified as the best-suited evolutionary model for analyzed sequences

Table 12.1 Nucleotide diversity for different genomic positions of PVY population

Genome regions	Nucleotide diversity			
	d	dN	dS	dN/dS
P1	0.025 ± 0.002	0.022 ± 0.002	0.032 ± 0.005	0.687
HC-Pro	0.008 ± 0.001	0.002 ± 0.00	0.022 ± 0.003	0.090
P3	0.007 ± 0.001	0.002 ± 0.0	0.018 ± 0.003	0.111
6K1	0.001 ± 0.000	0.00 ± 0.00	0.004 ± 0.002	0.00
CI	0.008 ± 0.001	0.001 ± 0.00	0.020 ± 0.002	0.05
6K2	0.004 ± 0.001	0.003 ± 0.002	0.008 ± 0.003	0.375
NIa-Pro	0.012 ± 0.002	0.008 ± 0.003	0.043 ± 0.006	0.186
NIa-VPg	0.019 ± 0.002	0.003 ± 0.001	0.032 ± 0.005	0.093
NIb	0.019 ± 0.002	0.005 ± 0.001	0.048 ± 0.005	0.104
CP	0.005 ± 0.001	0.002 ± 0.001	0.012 ± 0.003	0.166
Polyprotein	0.053 ± 0.002	0.068 ± 0.002	0.023 ± 0.002	2.95

d, nucleotide diversity estimated by Kimura's two-parameter method; dN and dS, nucleotide diversity at nonsynonymous and synonymous positions, respectively, estimated by the Pamilo-Bianchi-Li method; values are means ± SEM

ratios are k1 = 6.299 (purines) and k2 = 10.026 (pyrimidines). The overall transition/transversion bias is R = 3.877, where R = [A*G*k1 + T*C*k2]/[(A + G)*(T + C)]. The analysis involved 100 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + noncoding. All positions containing gaps and missing data were eliminated. There were a total of 9474 positions in the final dataset. The higher transition/transversion bias (R = 4.67) was consistent with the principle that transitions are biochemically more likely than transversions in the analyzed PVY genome sequences.

We assessed the recombination breakpoints in the 100 isolates of PVY genome using RDP, GENECONV, Bootscan, MaxChi, Chimaera, SiScan, and 3Seq algorithms implemented in RDP 4.0 program (Martin et al. 2010) and identified that the recombination is present throughout the genome. Our results were corroborated with previous findings on recombination in PVY genome (Lorenzen et al. 2008; Schubert et al. 2007; Ogawa et al. 2008; Hu et al. 2009; Ali et al. 2010; Cuevas et al. 2012b). The genomes of PVY^{O}, PVY^{N}, and PVY^{C} are nonrecombinant while PVY^{Z} and PVY^{E} are recombinant (Quintero-Ferrer et al. 2014). Recently Visser et al. (2012) describe the recombinant evolution map (Fig. 12.3) of PVY strains and confirm the single origin of recombinant PVY^{NTN} and PVY^{NW} strains, while at the same time providing evidence for recombination events within those strains. In Europe, North Africa, America, and Brazil, the nonrecombinant strains PVY^{O}, PVY^{N}, and PVY^{C} are less frequent but recombinant strains PVY^{NTN} and PVY^{NW} are spreading dominantly (Quintero-Ferrer et al. 2014; Lorenzen et al. 2006). Concomitantly the recombinant strains PVY^{E} and PVY^{Z} are very rare in Europe, North America, and Brazil with a limited number of isolates (Gray et al. 2010; Nie and Singh 2003; Visser and Bellstedt 2009; Sawazaki et al. 2009). The earlier study on the distribution of codon sites under selection pressures (purifying, negative, and control selection) for different cistrons of PVY isolates showed that P1, P3, 6K1, 6K2, and CP regions have more neutral codons, and a small number of codon positions were detected under positive selection (Cuevas et al. 2012a). In our analyzed PVY sequences, a higher number of mutation and polymorphic sites was observed in P1, NIb, HC-Pro, and CI regions rather than other cistrons.

12.4 Conclusion

Plant viruses and their vectors cause somber economic losses, reduce crop production, and have negative effects on the quality and security of food supplies. Understanding the factors that

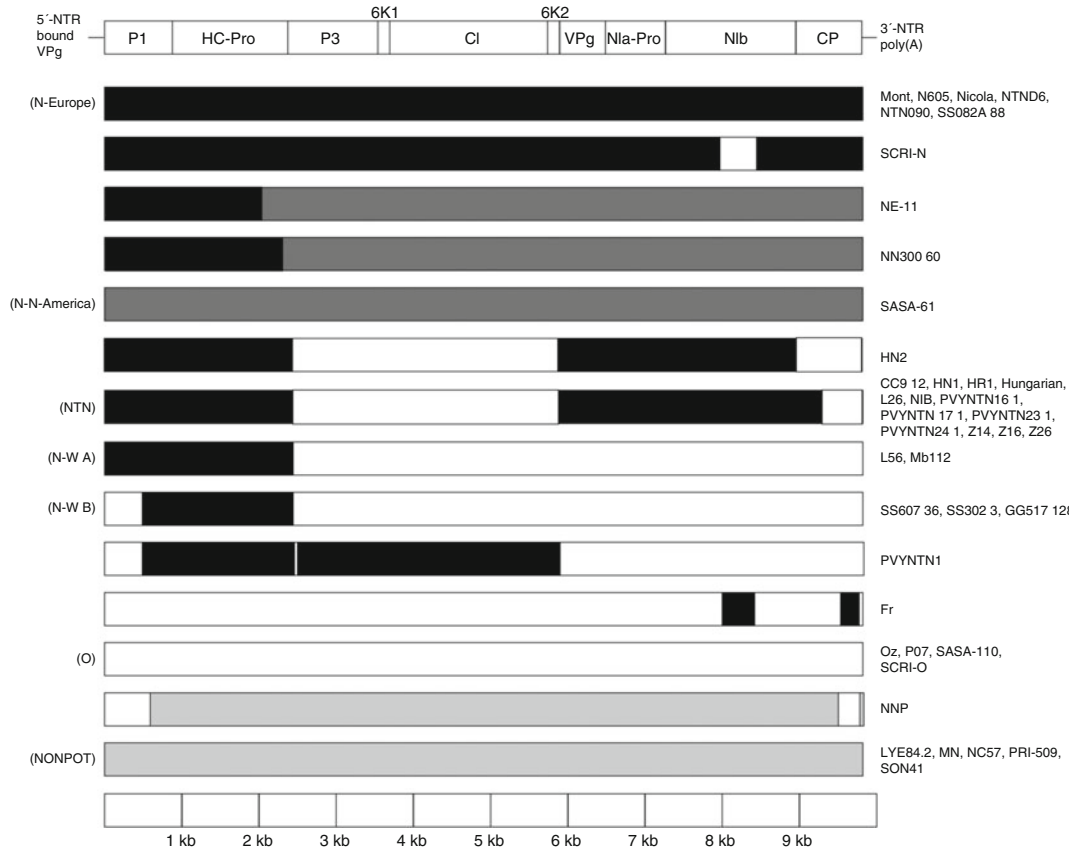

Fig. 12.3 Recombination map of PVY genome: Patterns of recombination between PVY strains PVY^{O} (*white*), $PVY^{N\text{-North America}}$ (*dark gray*), $PVY^{N\text{-Europe}}$ (*black*), and PVY^{NONPOT} (*light gray*) are illustrated (Visser et al. 2012)

establish the evolution of pathogens is an essential aspect of evolutionary biology with potentially central consequences for the control of pathogen-caused diseases (Moreno et al. 2004). Numerous approaches may be used to evaluate the genetic variation of plant viruses. Initially, variants were characterized by differences in biological properties such as the symptoms they caused in different host plant species, their host range, or vector transmission properties. Nowadays improvement in sequencing technology has resulted in the availability of many large datasets of genetic data, and computational methods have therefore become quite important in analyzing these data. Incongruent evolutionary relationships among isolates according to the genomic region considered have been studied for many *Potyvirus* species, and recombination events have been invoked to explain their evolution (Bousalem et al. 2000; Moury et al. 2002; Ohshima et al. 2002). Mutation, recombination, natural selection, genetic drift, and gene flow are the main descriptors of genetic variability in a population (Moya et al. 2004). These parameters can be evaluated in the form of sequence diversity, pairwise sequence distances, number of mutations, number of haplotype and polymorphic sites, ratio of synonymous to non-synonymous substitutions, and recombination breakpoints. Values of genetic distances between different isolates of PVY polyprotein ranging from 0.002 to 0.126 suggest that the sequence variation is distributed throughout the PVY genome, and P1, NIa-VPg, NIa-Pro, and NIb regions relatively have higher sequence divergence than other regions. P1, 6K2, NIa-Pro, P3, NIb, and CP cistrons also have higher dN/dS ratio compare to other genes. The ratio between

nucleotide diversities at nonsynonymous and synonymous positions (dNS/dS ratio) indicates the amount of variation in the nucleic acid that results in variation in the encoded protein as well as defines the degree of negative selection in genes and the degree of functional constraint for the maintenance of the translated protein sequence (Hughes, 2008; Cuevas et al. 2012b). The observation that, in all cistrons of PVY genome, the number of synonymous nucleotide substitutions per synonymous site *(d_S) exceeds the number of nonsynonymous substitutions per nonsynonymous site* (d_N) (Table 12.1) implies that past purifying selection has acted to eradicate nonsynonymous mutations to a much greater extent than synonymous mutations. Moury et al. (2002) have reported very few sites in 6K2 and CP (especially in N-terminal) protein undergoing positive selection while Cuevas et al. (2012a) describes a higher number of neutral selection in P1, P3, 6K1, 6K2, and CP regions. Covariation was also detected in P1, HC-Pro, P3, CI, NIa-Pro, and NIb regions (Cuevas et al. 2012a) with the P3 region showing the highest number of covarying sites (Mascia et al. 2010). P1, NIb, HC-Pro, and CI regions showed significantly higher mutation sites, and 6K1 and 6K2 regions showed absence of mutation sites in analyzed PVY sequences. These results support previous findings of PVY genome and suggest that the different cistrons of PVY genome are under different evolutionary pressure and exhibit diverse genetic variability as well as different modes of natural selection.

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13 Resistance Against *Papaya Ringspot Virus* in *Vasconcellea Species*: Present and Potential Uses

Sunil Kumar Sharma and Savarni Tripathi

Abstract

Papaya (*Carica papaya* L.) is cultivated in all continents producing 12.41 million tonnes from 434,785 ha, but major share of its production comes from Asia, Central America and Africa. Yield of papaya varies from place to place mainly due to the widespread incidence of viral diseases. The genus *Carica* is more vulnerable to diseases due to narrow gene pool. Among various viral diseases affecting papaya cultivation, *Papaya ringspot virus* type Papaya (PRSV-P), is the most devastating one in all major papaya-growing areas. The use of PRSV-P resistant transgenic papaya cultivars has been limited to certain geographical regions. Other approaches of managing PRSV-P have only limited success. Therefore, the approach of introgression of PRSV-P resistance in papaya from PRSV-P resistant wild relatives (*Vasconcellea species*) by conventional breeding has become the only viable option. All PRSV-P resistant *Vasconcellea species* attempted to be used as a source of resistant gene(s) had certain limitations. *V. cauliflora* rarely produced hybrids, and most of them were infertile. *V. quercifolia* produced resistant hybrids which developed mild virus symptoms with age. *V. cundinamarcensis* was reported to be consistently resistant against PRSV-P infection under many geographical conditions. But it was not possible to transfer PRSV-P immunity to *C. papaya* because F_1 hybrids were infertile females. The approach of bridge crossing is the latest strategy in developing PRSV-P resistance in *C. papaya. V. parviflora* which is cross-compatible with both *V. cundinamarcensis* and *C. papaya* was used as the bridge species. Stable homozygous hybrids of *V. cundinamarcensis* x *V. parviflora* were crossed with *C. papaya* to obtain PRSV-P resistant hybrid with marketable fruiting

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qualities. In addition to the genus *Vasconcellea*, other closely related genera of papaya (*Cylicomorpha, Horovitzia, Jacarantia* and *Jarilla*) should also be characterized and screened for variation in the PRSV-P resistance and their cross-compatibility with *C. papaya* to use them as wild sources in resistant breeding programme.

Keywords

Papaya ringspot virus type Papaya • *Vasconcellea species* • PRSV-P resistance • Resistance breeding • Bridge crossing • Embryo-rescue • Molecular markers

13.1 Introduction

13.1.1 Papaya

Papaya (*Carica papaya* L.) is cultivated in all continents producing 12.41 million tonnes from 434,785 hectare, but major share of its production comes from Asia, Central America and Africa. Asia produces 56 % of world papaya from 46 % of global area. Among major papaya-producing countries, India produced 42 % of world papaya from 30 % of area. The global average papaya yield is 29 tonnes/hectare (t/ha), while Asia produces it at 35 t/ha (FAOSTAT 2015). Yield of papaya in India varies from state to state. Top five papaya-producing states produced papaya at the average yield of 60 t/ha; however, the national average yield is 40 t/ha (Indian Horticulture Database 2014). The variability in papaya yield is mainly due to the widespread incidence of viral diseases. The genus *Carica* evolved in isolation in Central America away from other genera of the family Caricaceae. Consequently, it has only one species, *C. papaya*, and is more vulnerable to diseases due to narrow gene pool (Aradhya et al. 1999; Badillo 1993, 2000; Kim et al. 2002). Among various diseases affecting papaya cultivation, the viral disease, *Papaya ringspot virus* type Papaya (PRSV-P), is the most devastating one in all major papaya-growing areas in the world (Gonsalves 1998; Gonsalves et al. 2010). The natural spread of the disease is rapid; therefore, it may infect up to 100 % of plants. The disease is so devastating that farmers have stopped growing papaya in severely affected areas (Fig. 13.1). The use of PRSV-P-resistant transgenic papaya cultivars has been limited to certain geographical regions. In addition to the aggressive environmental activism, the main reason for the limited success of the transgenic cultivars was that they were resistant against homologous strain of PRSV-P. Other approaches of managing PRSV-P have only limited success (Sharma et al. 2010). Therefore, the approach of introgression of virus resistance in papaya from PRSV-P-resistant wild relatives (*Vasconcellea* species) by conventional breeding has become the only viable option. This chapter describes sources of PRSV-P resistance in *Vasconcellea* species and their present and potential uses in PRSV-P resistance breeding in papaya.

13.1.2 Papaya Ringspot Virus

The disease, caused by PRSV, was first described by Lindner et al. (1945) in Hawaii (USA), and later experiments by Jensen (1949) established that the causal agent was viral in nature. The name of the disease, ringspot, is taken from the appearance of rings on the fruit of the infected plants. Other symptoms are mosaic formation and chlorosis of the leaf lamina, water-soaked oily streaks on the petiole and upper part of the trunk, distortion of young leaves that sometimes results in shoe string-like symptoms and infected plants losing vigour and becoming stunted (Figs. 13.2 and 13.3). Fruits from infected plants are of poor quality with low sugar concentrations. Plants when infected with the severe strain of the virus at an early stage of growth usually do not

Fig. 13.1 General view of PRSV-P-tolerant (**a**) and PRSV-P-susceptible (**b**) papaya

Fig. 13.2 Healthy papaya plant (**a**) and plants with mild (**b**), moderate (**c**) and severe (**d**) symptoms

Fig. 13.3 General PRSV-P symptoms: Mosaic (**a**), leaf reduction (**b**), blisters (**c**), shoe string formation (**d**), oily spots on petioles and ringspots on fruits (**e**) and electron micrograph of the virus particle (**f**)

produce marketable fruits. The virus spreads in the field mainly by several species of aphid vectors in a non-persistent manner. Although papaya is the most important primary and secondary source for spread of the virus, it is also found in several species of the family Cucurbitaceae. PRSV is a member of the genus *Potyvirus* which is further classified into two types: type P (PRSV-P) which infects cucurbits and papaya and type W (PRSV-W) which infects cucurbits but not papaya (Purcifull et al. 1984). Both the biotypes are serologically indistinguishable. The virions are non-enveloped, flexious filamentous in shape and measure 760–800 × 12 nm (Gonsalves and Ishii 1980). Virus particles contain 94.5% protein and 5.5% nucleic acid. The protein component consists of the virus coat protein (CP) which has a molecular weight (Mr) of 36,000–36,500. Density of the sedimenting component in purified PRSV-P preparations is 1.32 g cm^{-3} in CsCl. The genomic RNA of the virus consists of 10,326 nucleotides long ssRNA with positive polarity and has the typical array of genes as present in potyviruses (Yeh et al. 1992). The genome is monocistronic and is expressed via a large polypeptide of 381 kDa that is subsequently cleaved by the virus-encoded proteinases to yield functional proteins (Yeh and Gonsalves 1985; Yeh et al. 1992). The genetic organization of PRSV RNA is VPg-5' leader–P1 (63 K)-HC Pro-P3 (46 K)-CI-P5 (6 K)-NIa-NIb-CP-3' noncoding region poly(A) tract (Yeh et al. 1992). Phylogenetic studies showed that PRSV-P coat protein gene sequences can diverge by 14% at nucleotide level and 10% at amino acid level in the Indian subcontinent (Jain et al. 2004). However, coat protein sequence of numerous strains have been analysed from various laboratories (Tripathi et al. 2008).

13.2 PRSV-P Resistance Breeding

13.2.1 Sources of Resistance

The pathogen diversity and the abundance of aphid vectors and that of alternate hosts are major challenges before effective PRSV-P management. A permanent solution of the problem is the development of resistant cultivars. To achieve this goal, identification of sources of resistance within *Carica* or related genera is the first step. *C. papaya* is reported to have low level of genetic variability of resistance for PRSV-P (Nishijima 1994). There is no established source of PRSV-P resistance in *C. papaya*. However, a papaya cultivar, 'Cariflora', was released as a tolerant to PRSV-P infection in Florida, USA (Conover et al. 1986). Similarly, 'Thapra' from Thailand (Prasartsee et al. 1995), 'Red Lady' and 'Known You No. 1' from Taiwan and 'Sinta' from the Philippines were other papaya cultivars claimed to be PRSV-P tolerant. These cultivars may become infected with the virus but remain symptomless or developed mild symptoms and able to produce economically viable yield (Fig. 13.3). Tolerance of these cultivars to PRSV-P varied with the virus isolates prevalent in different geographical locations. PRSV-P resistance has been reported in some species of the related genus, *Vasconcellea.* Initially, Horovitz and Jiménez (1967) reported PRSV-P resistance in some *Vasconcellea* species *(V. cauliflora*, *V. cundinamarcensis*, *V. stipulata* and *V. candicans*) and susceptibility in others (*V. monoica*, *V. quercifolia*, *V. microcarpa*, *V. horovitziana*, *V. goudotiana* and *V. parviflora*). Conover (1964) reported *V. cauliflora* susceptible and *V. quercifolia* resistant for PRSV-P in Florida (USA). Similarly *V. cauliflora* was reported to be susceptible to PRSV-P in Venezuela (Gonzalez and Trujillo 2005). PRSV-P resistance in *V. cauliflora* and susceptibility in *V. parviflora* have been established by other workers (Mekako and Nakasone 1975; Alvizo and Rojkind 1987; Magdalita et al. 1988; Amaral et al. 2006, Sharma and Tripathi, 2013). *V. cundinamarcensis* (also known as *V. pubescens*) is the only *Vasconcellea* species that has been reported to be consistently resistant to PRSV-P. While other resistant species, *V. cauliflora* and *V. quercifolia*, have been reported to be susceptible to PRSV-P under the same climatic condition (Table 13.1), distribution of PRSV-P resistance and susceptibility does not follow the logic of genetic affinities among various species of the genus *Vasconcellea*. It has been reported in the species from all three clades of the genus. The variation in the PRSV-P reaction in the same *Vasconcellea* species in different geographical locations is attributed to the genetic differences in

Table 13.1 Reaction of various *Vasconcellea* species to PRSV-P infection

Species	Place	Reaction to PRSV-P	References
V. cauliflora	Florida	Susceptible	Conover (1962, 1964)
	Venezuela	Resistant	Horovitz and Jiménez (1967)
	Mexico	Resistant	Alvizo and Rojkind (1987)
	Australia	Resistant	Magdalita et al. (1997b)
	Venezuela	Susceptible	Gonzalez (2000)
	India	Resistant	Sharma et al. (2015)
V. quercifolia	Venezuela	Susceptible	Horovitz and Jiménez (1967)
	Florida	Resistant	Conover (1964)
	Hawaii	Resistant	Manshardt and Wenslaff (1989b)
	Australia	Resistant	Drew et al. (2006a)
V. pubescens	Florida	Resistant	Conover (1964)
V. parviflora, *V. stipulata* and *V. goudotiana*	Venezuela	Susceptible	Horovitz and Jiménez (1967)
V. parviflora, *V. stipulata* and *V. goudotiana*	Australia	Susceptible	Magdalita et al. (1988)
V. stipulata, *V. pubescens*, *V. candicans* and *V.* x *heilbornii* nm. *pentagona*	Venezuela	Resistant	Horovitz and Jiménez (1967)
V. candamarcensis	Puerto Rico	Resistant	Adsuar (1971)

the virus strains and the environmental conditions. Some of the *Vasconcellea* species, namely, *V. cauliflora*, *V. quercifolia*, *V. cundinamarcensis* and *V. parviflora*, have been used extensively in the introgression of PRSV-P gene(s) to *C. papaya*.

13.2.2 Relationship Between *C. papaya* and *Vasconcellea* Species

Both genera *Carica* and *Vasconcellea* belong to the family Caricaceae. The genus *Carica* has only one species, *C. papaya*, while the genus *Vasconcellea* has 21 species (Badillo 2000, 2001).

The genus *Vasconcellea* has three species complexes:

- Clade 1: *V.* x *heilbornii*, *V. weberbaueri*, *V. stipulata* and *V. parviflora*
- Clade 2: *V. chilensis*, *V. candicans*, *V. quercifolia* and *V. glandulosa*
- Clade 3: All other taxa of the genus (d'Eeckenbrugge et al. 2014)

Both *Carica* and *Vasconcellea* are diploid and have 18 chromosomes (Manshardt and Drew 1998; Storey 1976). *Carica* has a one-celled ovary, while *Vasconcellea* has a five-celled ovary. According to the earlier understanding of the family Caricaceae, all species of the genus *Vasconcellea* were a section of the genus *Carica*. Therefore, in the literature prior to 2000, *Vasconcellea* species were referred to as the species of the genus *Carica*, and the crosses were called as 'interspecific', while they were, in fact, 'intergeneric' in nature (Sharma and Mitra 2014). Both the genera *Carica* and *Vasconcellea* are genetically divergent genera because of *Carica*'s earlier isolation from other wild relatives (Table 13.2). Among the genus *Vasconcellea*, the most closely related species to *C. papaya* were *V. stipulata* and *V. pubescens* (Jobin-Décor et al. 1997; Sharon et al. 1992) and *V. goudotiana* and *V. pubescens* (Kim et al. 2002), while the most distant species were *V. cauliflora* (Jobin-Décor et al. 1997) and *V. goudotiana* (Sharon et al. 1992; Kim et al. 2002).

Table 13.2 Description of species in various genera of the family Caricaceae

Genus	Species description
1. *Carica*	1. *papaya* L.
2. *Cylicomorpha*	1. *parviflora* Urban
	2. *solmsii* (Urban) Urban
3. *Horovitzia*	1. *cnidoscoloides* (Lorrence and Torres) V. Badillo
4. *Jacaratia*	1. *chocoensis* A. H. Gentry and Forero
	2. *corumbensis O. Kuntze*
	3. *digitata (Poeppig and Endl.) Solms-Laub.*
	4. *dolichaula* (J.D. Smith) Woodson
	5. *heptaphylla* (Vellozo) A. DC.
	6. *mexicana* A. DC.
	7. *spinosa* (Aublet) A. DC.
5. *Jarilla*	1. *caudata (Brandegee) Standley*
	2. *chocola* Standley
	3. *heterophylla (Cerv.) Rusby*
6. *Vasconcellea*	1. *candicans* (A. Gray) A. DC.
	2. *cauliflora* (Jacq.) A. DC.
	3. *chilensis* (Planch. ex A. DC.) A. DC.
	4. *crassipetala* (V. Badillo) V. *Badillo*
	5. *cundinamarcensis (Solms-Laub.)* V. Badillo
	6. *glandulosa* A. DC.
	7. *goudotiana* Triana and Planch.
	8. *horovitziana* (V. Badillo) V. Badillo
	9. *longiflora* (V. Badillo) V. Badillo
	10. *microcarpa* (Jacq.) A. DC. [four subspp.: Baccata, Microcarpa, Pilifera, Heterophylla]
	11. monoica (Desf.) A. DC.
	12. *omnilingua* (V. Badillo) V. Badillo
	13. *palandensis* (V. Badillo et al.) V. Badillo
	14. *parviflora* A. DC.
	15. *pulchra* (V. Badillo) V. Badillo
	16. *quercifolia* (St.-Hil.) A. DC.
	17. *sphaerocarpa* (García-Barr. and Hern.) V. Badillo
	18. *sprucei* (V. Badillo) V. Badillo
	19. stipulata (V. Badillo) V. Badillo
	20. *weberbaueri* (Harms) V. Badillo
	21. *V.* x *heilbornii* (V. Badillo) V. Badillo [different varieties – fructifragrans, chrysopetala 'Babacó']

Source: Sharma (2013)

13.2.3 Uses of *Vasconcellea* Species in PRSV-P Resistance Breeding

Use of *Vasconcellea* species for introgression of gene(s) of PRSV-P resistance in *C. papaya* is governed by genetic relatedness (particularly chromosome structural similarities), meiotic homology, recombination and generic compatibilities (Costa et al. 2008). PRSV-P-resistant *Vasconcellea* species available are genetically distant from *C. papaya*. Therefore, the biggest challenge before the PRSV-P resistance breeding is posed by the genetic cross-incompatibility between PRSV-P-resistant *Vasconcellea* species and *C. papaya*. Sawant (1958a, b) and Warmke et al. (1954) attempted a wide range of interspecific and reciprocal crosses between *Vasconcellea* species like *V. goudotiana*, *V. monoica*, *V. candamarcensis*, *V. cauliflora*, *V. grandis* and *V. erythrocarpa*. They reported variability in compatibility resulting into failure in fruit and seed development and hybrid seed germination. Fruits were dropped within few months after initial fruit set. Sawant (1958b) indicated that the genetic distance between species was likely the deciding factor for the fruit set and their retention. Similarly, Horovitz and Jiménez (1967) reported that hybrids between *C. papaya* and *V. stipulata* lacked vigour and viability. They often produced parthenocarpic fruits or nonviable seed. Post-zygotic barriers, like abnormal endosperm development or ovule and embryo abortion, limited the success of the crosses between *C. papaya* and *Vasconcellea* species (Mekako and Nakasone 1975; Manshardt and Wenslaff 1989a). Studies proposed that variable chromosome numbers, presence of univalents, lagging chromosomes at anaphase and meiotic irregularities were likely causes for the non-functional gametes and infertility among hybrids (Drew et al. 2006a). Aradhya et al. (1999) explained variability in fruit setting and their retention among hybrids on the basis of the genetic proximity among various species by using molecular taxonomy. Although progressing beyond F_1 generation in intergeneric crosses has been very difficult, some hybrids have been developed by crossing *Carica papaya* with *V.*

Fig. 13.4 *C. papaya* (**a**), *V. cauliflora* (**b**) and the hybrid (**c**)

cauliflora (Fig. 13.4), *V. cundinamarcensis*, *V. quercifolia*, *V. goudotiana* and *V. stipulata* (Manshardt and Wenslaff 1989b; Magdalita et al. 1997b; Drew et al. 1998; Siar et al. 2011; Sharma and Tripathi 2013).

13.2.3.1 *Vasconcellea cauliflora*

Unlike most of the *Vasconcellea* species, *V. cauliflora* is a lowland plant with its climatic requirements similar to those of *C. papaya*. Being resistant to PRSV-P infection, it was one of the earlier sources that were utilized widely for introgression breeding (Horovitz and Jiménez 1958; Sawant 1958a, b; Padnis et al. 1970; Moore and Litz 1984; Litz and Conover 1978; Magdalita et al. 1996; Manshardt and Wenslaff 1989a, b). Horovitz and Jiménez (1967) crossed *V. cauliflora* with the susceptible species *V. monoica* and *V. horovitziana* to understand the nature of PRSV-P resistance. The F_1 generation was resistant to PRSV-P, while the F_2 population showed segregation. No quantitative variation in F_1 population indicated that PRSV-P resistance was a single dominant trait. The use of *V. cauliflora* in the introgression breeding was severely constrained by its cross-incompatibility with the *C. papaya*. Varying successes of the hybridization between *C. papaya* and *V. cauliflora* have been reported by various workers. Horovitz and Jiménez (1967) lost the hybrids after transplanting them to the field. In the trial of Manshardt and Wenslaff (1989a), out of 25 hybrids of *V. cauliflora* x *C. papaya*, only one male plant was able to bear flowers. Even that plant had less than 1 % viable pollens. Magdalita et al. (1997a) reported successful development of 120 hybrids of *C. papaya* and *V. cauliflora*, out of which only five survived to the flowering stage. Pollen viability of the hybrids was drastically low and the backcrossing to *C. papaya* failed. Drew et al. (1998) reported failure of crossing between *C. papaya* and *V. cauliflora* in the climatic conditions of South East Queensland (Australia). Many plantlets raised from the rescued embryos died in vitro or in the glasshouse. Most of them died after 6–8 weeks in the pots and only a few survived till flowering. Among the other set of these hybrids raised in the tropical and humid climate of Los Baños (the Philippines), only a few of them grew up to maturity but produced infertile flowers. Genetic incompatibility between *C. papaya* and *V. cauliflora* was explained on the basis of their genetic distance (Jobin-Décor et al. 1997). Magdalita et al. (1997a) reported a high level of aneuploidy as one of the causes for the failure of the crosses. Some of the intergeneric hybrids had up to 48 % aneuploidy cells. The failure of hybrids to flower and the pollen infertility in the hybrids that flowered was reported to be caused by the chromosome imbalance related to incompatibility barriers and lack of homology between parental chromosomes (Magdalita et al. 1997a; Siar et al. 1998). There are reports of single-gene dominance regulating the PRSV-P resistance in F_1 intergeneric hybrids of *C. papaya* and *V. cauliflora* (Magdalita et al. 1997b). Vegas

et al. (2003) reported the effect of altitude on the survival and growth of the hybrids in Venezuela. Despite a considerable slower growth of hybrids at 500 m, the time from planting to flowering was comparable between the parent papaya plants and the hybrids. While at 1500 m, papaya performed inferior to the hybrids and did not fruit. The altitude of the experimental fields also influenced the sex expression of one of the hybrid clones. It had normal male flowers at 500 m, while at 1500 m, the hybrid developed andromonoecious plants.

Some successes in developing interspecific hybrids between *C. papaya* and *V. cauliflora* with PRSV-P resistance and normal fruit quality were reported from India in the 1980s (Khuspe et al. 1980). However, no confirmation of hybridity was demonstrated and no resistant papaya genotype has resulted from their work. The idea of breaking intergeneric crossing barriers by smearing stigma with sucrose solution (Iyer and Subramanyam 1984) was re-established recently in India. In a breeding programme of providing PRSV-P resistance to *C. papaya* 'Surya' by transferring resistant gene from *V. cauliflora*, Dinesh et al. (2007) got the maximum number of seed sets (13.37 %) when stigmatic surface of flower was smeared with 5 % sucrose solution. In a subsequent similar study in India involving crosses between *C. papaya* and *V. cauliflora*, true hybrids were produced by Jayavalli et al. (2011). The hybridity of the progenies was established by using inter-simple sequence repeat (ISSR) marker.

After repeated failures of developing successful crosses with *V. cauliflora*, the breeding efforts were expanded to other PRSV-P-resistant *Vasconcellea* species that were cross-compatible with *C. papaya* because of their genetic proximity (Jobin-Décor et al. 1997).

13.2.3.2 *Vasconcellea quercifolia*

V. quercifolia is a better choice as a source of gene(s) for PRSV-P resistance, for it is resistant to PRSV-P and cross-compatible with *C. papaya* (Drew et al. 2006a; Manshardt and Wenslaff 1989a). Manshardt and Wenslaff (1989b) reported first successful hybrids between three genotypes of *C. papaya* and *V. quercifolia*. From one of the genotypes, 28 embryos were obtained out of 136 crosses. The reciprocal crosses yielded no fruits. Later on, most of the PRSV-P resistance breeding work involving *V. quercifolia* was done at Griffith University, Brisbane, Australia, in the Philippines and in the Latin American countries. In a crossing programme involving *C. papaya* and *V. cauliflora*, Drew et al. (1998) obtained vigorously growing 300 hybrids. A highly embryogenic papaya genotype (2.001) was used to enhance the probability of producing the hybrid embryos in vitro (Drew et al. 2006a; Magdalita et al. 1996). A large number (>600) of F_1 hybrids were produced to facilitate selecting of F_1 male plants with pollen fertility. Among the hybrids, most plants were similar to *V. quercifolia* (branched), and few were similar to *C. papaya* (upright). Variable resistance to PRSV-P was reported when tested under the climatic conditions of Australia and the Philippines. Three-fourths of the hybrids showed PRSV-P resistance. The sex ratio of the hybrids was 2:49:49 of male/hermaphrodite/female (Drew et al. 2006a). Some pollen (up to 6 %) of all male plants and few of hermaphrodite plants were viable. Pollen from only male plants was able to backcross successfully with *C. papaya.* In their study, a highly embryogenic *C. papaya* genotype was used to increase the probability of producing useful hybrid embryos in laboratory, and a large population of plants was screened to produce a fertile first backcross (BC_1) plant. These hybrids had variable chromosomal counts and presence of univalents, laggards and meiotic aberrations and had very low pollen fertility (Drew et al. 2005). BC_1 plants showed high mortality and low vigour. Less than 8 % of the pollinated *C. papaya* flowers produced fruits in Australia. Of the rescued 63 embryos, only 50 developed into plants. Since pollen fertility was >80 % in male BC_1 plants, second backcross (BC_2) generation was easier to produce from BC_1 (Drew et al. 2006b). Among backcross populations, one plant showed high level of PRSV-P tolerance against multiple challenge inoculations in the glasshouse and under field conditions for Australian strain. However, it did not show resistance against the Philippine strain of PRSV-P (Drew et al. 2005). PRSV-P-

resistant BC_3, sib-crossed (SbC_3) and BC_4 plants were developed subsequently in the Philippines (Siar et al. 2011). Tropical climate of the Philippines proved to be more congenial for backcrossing than the subtropical climate in South East Queensland, Australia. The breeding sites and attendant ecological conditions are important factors for the success of *Carica* and *Vasconcellea* crosses. One BC_1 male plant in the Philippines, showing PRSV-P resistance, was obtained after dissecting a very high number of seed (114,839) from 940 fruits. The seeds produced less than 1 % embryos (1011). Out of which 733 were germinated in laboratory. Among these plants, 700 showed resistance against PRSV-P under the glasshouse and field conditions. The further population of the backcrossed and sib-crossed plants was raised from seed of the PRSV-P-resistant BC_1 plant, and they were challenge inoculated. Plants not showing PRSV-P symptoms after three challenge inoculations in the glasshouse were transferred to the field. The proportion of plants not showing PRSV-P symptoms after three inoculations in the glasshouse increased to 96.1 % in BC_4 and 91.3 % in SbC_3. However, these plants developed symptoms after field transplantation. The rate of symptom development was slower in these plants than the control (Davao Solo). Susceptibility of most of these plants to PRSV-P may be attributed to the preferential elimination of *V. quercifolia* chromosomes. Some plants of BC_2 and SbC_2 remained symptom-free up to 5–6 months. Some BC_4 and SbC_3 plants showed delayed symptoms and ability to produce symptom-free new growth from mildly infected plants. Few plants were free from infection up to 18 months under field conditions, after that they developed mild symptoms on leaves and fruits. They continued to produce fruits for 3 years under high disease pressure. Fruit quality of the hybrid plants was good (Siar et al. 2011). Presently, BC_4 and BC_5 generations are being evaluated in Thailand and Hawaii. Their resistance will also be tested against other strains of the virus from different geographical locations. Success of the breeding programme may be attributed to the use of modern biotechnological tools like the refined protocol for embryo rescue and in vitro culture of the resultant plants (Magdalita et al. 1996), and a reliable micropropagation technique used to multiply, maintain and conserve the resultant plants (Drew 1992). It is still not clear whether the resistant phenotype in *V. quercifolia* is controlled by a single dominant gene or multiple genes. It appears that PRSV-P resistance is controlled by more than one gene, for plants segregated into 3:1 ratio for resistant to susceptible in the F_1 generation of *C. papaya* and *V. quercifolia* (Drew et al. 1998). The identification of only one PRSV-P-resistant BC_1 plant when others showed varying susceptibilities supports multigene control theory (Drew et al. 2005). However, the incomplete meiotic pairing of chromosomes and the preferential elimination of *V. quercifolia* DNA do not support this interpretation. In the effort to produce plants homozygous for the PRSV-P-resistant gene(s), sib-crossing was attempted, however, with the inconsistent results. While SbC_3 generation showed higher proportion of resistant plants (91.3 %) compared to those of the BC_3 generation (65.3 %), the SbC_2 generation contained lower proportion of resistant plants (48.9 %) compared to the BC_2 generation (72.3 %) (Siar et al. 2011).

13.2.3.3 *Vasconcellea cundinamarcensis* with *V. parviflora* as the Bridge Species

V. cundinamarcensis (or *V. pubescens*) is consistent in resistance against varied strains of PRSV-P under different geographical conditions (Conover 1964; Horovitz and Jiménez 1967). In addition to PRSV-P resistance, it is also a source of genes for black spot (*Asperisporium caricae*) and cold tolerance. Several workers were successful in transferring gene(s) for PRSV-P resistance to the hybrid progenies (Horovitz and Jiménez 1967; Manshardt and Wenslaff 1989b; Drew et al. 1998). Earlier crosses between *C. papaya* and *V. cundinamarcensis* resulted into sterile F_1 progeny in tropical lowland conditions of Maracay (Horovitz and Jiménez 1967). While in Hawaii, several F_1 hybrids of *C. papaya* and *V. cundinamarcensis* were vigorous and

showed field resistance to PRSV-P. However, they were sterile, and backcrossing could produce only infertile sesquidiploids from unreduced megaspores (Manshardt et al. 1995). Some hybrids were produced by using embryo rescue and in vitro culture techniques. They were vigorous and resistant to PRSV-P. Their growth was slow during summer and exhibited better cold tolerance than *C. papaya*. All hybrids were female and had green flowers when immature and yellow when fully mature. The traits of flower colour and their shape were similar to those of *V. cundinamarcensis.* They occurred either singly on short peduncles (female) or as multiple flowers on long peduncle (male) similar to flowering pattern of *C. papaya*. All hybrids were resistant to PRSV-P when manually inoculated. It indicated a single-gene dominant control of the virus resistance (O'Brien and Drew 2009). Putative resistance genes for PRSV-P resistance in *V. cundinamarcensis* are being sequenced and characterized to transfer them to *C. papaya* to verify if they confer PRSV-P resistance in papaya (Drew 2014). PRSV-P resistance in *V. cundinamarcensis* was reported to be controlled by a single dominant gene or a group of genes at a single locus. The gene was mapped by Alamery and Drew (2014) and assumed to be a kinase gene.

V. parviflora is the most closely related to *C. papaya* among all *Vasconcellea* species (Jobin-Décor et al. 1997). Its climatic requirements are similar to those of *C. papaya* (Scheldeman et al. 2007). It is characterized by pink flowers, a dominant characteristic trait, that can be used as a physical marker to identify true crosses. It is susceptible to PRSV-P (Horovitz and Jiménez 1967; Drew et al. 1998) but cross-compatible with *C. papaya* producing up to 45 % fertile pollen in F_1 hybrids (O'Brien and Drew 2009). F_1 hybrids *V. parviflora* x *C. papaya* were produced by using embryo rescue technique and in vitro culture (Drew et al. 1998; O'Brien and Drew 2009). F_1 plants were grown vigorously in a glasshouse and slowly in the field. They retained dioecious nature of the parents. The dominant trait of pink colour of flower was visible in hybrids over the white colour of *C. papaya.* However, all F_1 hybrids were susceptible to PRSV-P (O'Brien and Drew 2009). Severe interveinal chlorosis was visible in the mature leaves.

The concept of bridge crossing was used by Drew and his group in Australia to overcome the incompatibility issues between *C. papaya* and *V. cundinamarcensis. V. parviflora*, being cross-compatible with both, was used as a bridging species for introgression of PRSV-P-resistant genes from *V. cundinamarcensis* to *C. papaya* (O'Brien and Drew 2009). Hybrids among *C. papaya*, *V. cundinamarcensis* and *V. parviflora* were produced. They were evaluated for PRSV-P resistance and other morphological properties. PRSV-P-resistant F_1 hybrids from the *V. cundinamarcensis x V. parviflora* were developed into F_2 and F_3 populations. PRSV-P-resistant homozygous plants (RR) were selected by the use of a codominant CAPS marker. They were either backcrossed to *V. parviflora* or to *C. papaya* (Dillon et al. 2005, 2006; O'Brien and Drew 2009). The disease resistance gene from *V. cundinamarcensis* was successfully backcrossed into *V. parviflora* from F_2 hybrids and subsequently in the F_1 hybrids between *C. papaya* and PRSV-P-resistant *V. parviflora.* The hybrids between F_2/F_3 plants and *C. papaya* were infertile. PRSV-P resistance was reported to be controlled by a single dominant gene in intergeneric hybrids between *V. cundinamarcensis* and *C. papaya* (Drew et al. 1998). Genetic mapping studies of *V. cundinamarcensis* x *V. parviflora* hybrids further supported the concept that PRSV-P resistance in *V. cundinamarcensis* is controlled by a single dominant gene (Dillon et al. 2006). A single resistance gene locus (prsv-1) was identified in *V. cundinamarcensis* (Dillon et al. 2005, 2006). The backcross generations (BC_1 and BC_2) between *V. cundinamarcensis* x *V. parviflora* F_3 RR plants and *V. parviflora* were also studied. The hybrids retained dominant genetic traits from *V. cundinamarcensis* (seven number of leaf vein), *V. parviflora* (pink flower colour) and *C. papaya* (red-green petiole colour and large plant size). Recessive traits (flower shape and fruit size) showed variations in the hybrids.

13.2.4 Modern Techniques Used in PRSV-P Resistance Breeding

13.2.4.1 Molecular Markers

Molecular markers have been used in the genetic improvement in papaya by determining the relatedness among the genotypes for germplasm resource management, parental choice (Eustice et al. 2008), identification of hybrids and as selection markers for many traits (Dillon et al. 2006; de Oliveira et al. 2010a). Molecular markers were a development over morphological and biochemical (isoenzymes) markers used to study hybridity of *C. papaya* and genetic relationships between *C. papaya* and its wild (*Vasconcellea)* relatives. They have reduced the time of testing parentage and their hybrids. The ideal molecular marker(s) for the gene(s) should be closely linked with the trait and should have dominant inheritance. DNA-based molecular markers are generally stable, unaffected by the environment and present throughout the plant life. The first use of DNA markers was reported to detect polymorphisms between *C. papaya* genotypes and the related species (Sharon et al. 1992).

The first PCR-based markers for papaya, randomly amplified polymorphic DNA (RAPD), were used to evaluate genetic relationships among papaya cultivars and between *C. papaya* and *Vasconcellea* species (Stiles et al. 1993). A modification of this technique, randomly amplified DNA (RAF), was used to identify markers linked to PRSV-P resistance in *V. cundinamarcensis* (Dillon et al. 2005). Amplified fragment length polymorphism (AFLP) markers, which were developed by the integration of AFLP and PCR, were used to learn the genetic relationship within the family Caricaceae (Kim et al. 2002; Van Droogenbroeck et al. 2002; Ocammpo Perez et al. 2007; Ratchadaporn et al. 2007). The nonspecific and dominant markers (RAPD, RAF, and AFLP) had the advantage of being used easily in papaya crop improvement, for they do not require DNA sequencing analysis. However, these markers were not reliable and stable; therefore, they had limited uses. Simple sequence repeat (SSR), also known as microsatellite, markers were an improvement over the dominant markers. They are locus specific and codominant because they flank sequences to amplify the tandem short repeat units dispersed throughout the genome. They may be highly polymorphic among individuals within a species due to conserved slippage. They have been widely used in the genetic diversity studies of papaya. Many SSR libraries have been developed (Perez et al. 2006; de Oliveira et al. 2010b; Eustice et al. 2008; Santos et al. 2003; Wang et al. 2008). Genetic relationships between *C. papaya* and *Vasconcellea* species were studied by comparing isozymes and RAPD techniques. Both techniques gave similar measures of genetic distance of 70 % dissimilarity between *C. papaya* and *Vasconcellea* species and approximately 50 % dissimilarities within *Vasconcellea* species (Jobin-Décor et al. 1997). RFLP marker-based phylogenetic relationship study of 12 cultivated and wild species of *Carica* confirmed the close association among South American wild *Carica* (*Vasconcellea*) species (Aradhya et al. 1999). AFLP marker-based genetic relationship study among 95 accessions of papaya and its wild relatives revealed that all papaya genotypes were placed in one cluster in addition to two other clusters comprising individuals either from *Jacaratia* or *Vasconcellea* (Van Droogenbroeck et al. 2002). AFLP markers detected only 12 % genetic variation among papayas and *Vasconcellea* species. However, it was not conforming to the wide range of morphological characteristics that were observed in the field (Kim et al. 2002). Seventeen RAPD primers were selected and screened to confirm hybridity of 120 putative intergeneric crosses of *C. papaya* x *V. cauliflora*. One to five primers confirmed that all 120 plants were genetic hybrids (Magdalita et al. 1997b). It may be noted that a single primer cannot guarantee accurate results; thus, more primers are necessary to confirm the hybridity. This is because chromosome elimination can occur during meiosis; thus, absence of a single marker may represent elimination of part of a chromosome. Molecular markers linked to the gene *prsv-1* have been used in the marker-assisted breeding programme because of their

dominant inheritance and the robust resistance to PRSV-P (Drew et al. 1998; Magdalita et al. 1997a). Five DNA markers which were developed by the use of RAF on bulked segregants of PRSV-P-resistant and PRSV-P-susceptible populations were identified in the cross of *V. parviflora* x *V. pubescens* (Dillon et al. 2006). The two candidate markers, Opa11_5R and Opk4_1r, were sequenced and converted to SCAR markers. The marker Opk4_1r was converted into a CAPS marker, *Psilk4*. The SCAR marker Opk4_1r detected similar band size for *V. pubescens*, *V. cauliflora* and *V. goudotiana* with the size of 360 pb, 360 pb and 361pb, respectively. The application of the SCAR and CAPS markers was confirmed in the interspecific populations of *V. pubescens* x *V. parviflora* for marker-assisted breeding (O'Brien and Drew 2010). The Opk4_1r SCAR marker amplified was in other *Vasconcellea* species (*V. quercifolia*, *V. goudotiana* and *V. cauliflora*). The CAPS marker was consistent in determining the allele of prsv-1 only in the crosses involving *V. pubescens* and *V. parviflora* (O'Brien and Drew 2010). Dillon et al. (2006) have developed two markers for PRSV-P resistance on *prsv-1 locus*. They were Opa5_11R with the primer sequences of:

- PBA115RF:CAATCGCCGTAGGAAAATTC
- PBA115RR:CAATCGCCGTAGAGGAGGAGG
- Opk4_1r PBK41R F: CCGCCCAAACTGCG GAACAC
- PBK41RR:CCGCCCAAACCCCCAACTAG

Recently, Alamery and Drew (2014) tested 16 primer pairs on the segregating populations; no SSR markers were linked to resistance gene. One marker, SP16, confirmed the hybridity of F_1 hybrids and suggested some chromosomal elimination from *V. quercifolia.* Fluorescent in situ hybridization (FISH) was used to explore genomic and chromosomal relationship among species of Caricaceae because the conventional karyotyping of papaya is difficult due to the small size of chromosomes, absence of heteromorphic pairs and lack of cytomorphological markers (Costa et al. 2008).

13.2.4.2 Embryo Rescue

Embryo abortion and lack of endosperm development were limiting the success of the hybridization programme of *C. papaya* with *Vasconcellea* species (Manshardt and Wenslaff 1989a). The protocol was required for the rescue of embryo before abortion. Rescue of the embryos of the crosses between *C. papaya* and *V. cauliflora* was successfully executed by Magdalita et al. (2001) when *C. papaya* was the female parent under subtropical climate. The protocol to rescue and germinate *C. papaya* x *V. cauliflora* immature embryos was developed in the mid-1990s (Magdalita, et al. 1996) which resulted into reduced losses of embryos and stronger plantlets in vitro. Later on, Drew et al. (1998) developed procedures for hybridization of papaya with related PRSV-P-resistant *Vasconcellea* species (*V. cauliflora*, *V. quercifolia* and *V. cundinamarcensis*). Hybrid plants were produced by forming embryogenic cultures of germinated embryos on hormone-free agar medium. The protocol has been standardized to produce hybrids between *C. papaya* and other PRSV-P-resistant species, *V. quercifolia* and *V. cundinamarcensis*. Few *C. papaya* x *V. quercifolia* hybrids produced some viable pollen. Using this technique, intergeneric hybrid plants were produced between *C. papaya* x *V. goudotiana* and *C. papaya* x *V. parviflora*. Razali and Drew (2014) optimized a protocol for papaya embryogenesis and regeneration for transformation of papaya. They compared De Fossard (DF), Murashige and Skoog (MS) and modified MS media as induction medium for embryogenesis. Zygotic embryos and hypocotyl explants produced embryogenic callus after 2–3 weeks of culture on MS and MS-modified media. The use of 200 mgL^{-1} carbenicillin and 143 mgL^{-1} adenine hemisulfate in modified MS media produced the highest frequency of callus formation. DF medium consisting of ½ strength of DF minerals, full-strength MS vitamins and 10 mgL^{-1} 2,4-D produced embryogenic callus in 4–5 weeks. The medium often produced somatic embryos without callus formation on the apical meristem of the immature embryos. There is a potential to use this protocol to evaluate both the efficiency of transformation of papaya and the

efficacy of the putative resistance genes when transformed from *V. cundinamarcensis* into papaya.

13.3 Conclusion

All PRSV-P-resistant *Vasconcellea* species attempted to be used as a source of resistant gene(s) had some limitations. *V. cauliflora* and *V. cundinamarcensis* are not cross-compatible with *C. papaya*; *V. quercifolia* is cross-compatible with *C. papaya* but the resistant hybrids were neither uniform in virus resistance nor stable. *V. cauliflora*, which was initially used as a source of resistance, was cross-incompatible with *C. papaya*. They rarely produced hybrids, and most of them were infertile. PRSV-P-resistant *V. quercifolia*, cross-compatible with *C. papaya*, produced resistant hybrids which developed mild virus symptoms with age. Further breakdown of the resistance was also observed with field exposure. The species, *V. cundinamarcensis*, was reported to be consistently resistant against PRSV-P infection under many geographical conditions. But it was cross-incompatible with *C. papaya*. It was not possible to transfer PRSV-P immunity to *C. papaya* because F_1 hybrids with *C. papaya* were infertile females. The approach of bridge crossing is the latest strategy in developing PRSV-P resistance in *C. papaya*. *V. parviflora* which is cross-compatible with both *V. cundinamarcensis* and *C. papaya* was used as the bridge species. *V. cundinamarcensis* and its hybrids with *V. parviflora* and *C. papaya* were symptomless and have consistently demonstrated resistance to PRSV-P. The hybrids were established to have heterozygous allele of the gene for PRSV-P resistance from *V. cundinamarcensis*. Stable homozygous hybrids of *V. cundinamarcensis* x *V. parviflora* were crossed with *C. papaya* to obtain PRSV-P-resistant hybrid with marketable fruiting qualities. However, they could provide a short-term solution to this virus until more durable virus-resistant plants have been developed or until there is a more universal acceptance of genetically modified fruit crops. The potential of remaining *Vasconcellea* species in imparting PRSV-P resistance has not been tested fully. To use these wild sources in imparting resistance in *C. papaya*, intraspecies variation for PRSV-P resistance within *V. cauliflora* is being studied in India (Sharma et al. 2016). In addition to the genus *Vasconcellea*, other closely related genera of papaya (*Cylicomorpha*, *Horovitzia*, *Jacarantia* and *Jarilla*) should also be characterized and screened for resistance to PRSV-P (Scheldeman et al. 2007). The genera *Jarilla* and *Horovitzia* are more closely related to *Carica* when compared with the genus *Vasconcellea*. Variation in the PRSV-P resistance and their cross-compatibility with *C. papaya* are required to be explored fully to use other wild sources in resistant breeding programme.

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Closteroviruses: Molecular Biology, Evolution and Interactions with Cells

14

A.A. Agranovsky

Abstract

Family *Closteroviridae* combines about 40 positive-strand RNA viruses infecting higher plants. Closteroviruses have several distinguishing traits: (i) semi-persistent mode of insect transmission (implying that a virus is retained within a vector for hours) and phloem-limited nature of virus infection; (ii) unique structure of elongated particles with spiral type of symmetry built of several virus proteins; (iii) large RNA genomes of up to 20 kb, a value close to the largest positive-strand RNA genomes of animal nidoviruses; (iv) the presence of a gene homologous to the HSP70 family of cell chaperones; and (iv) the presence of a number of duplicated and diverged genes (or parts thereof), e.g. the genes for the major and minor capsid protein (CP). The genome expression of closteroviruses occurs by proteolytic processing and +1 ribosomal frameshifting for the replication-associated products of the 5′ -proximal genes, whereas the 3′-terminal genes are expressed via a nested set of subgenomic mRNAs. This strikingly resembles the expression strategy of nidoviruses. Besides fundamental interest to the molecular biology and evolution of the large RNA genomes, it is important that some closteroviruses cause grave diseases in cultivated crops like citrus trees, sugar beet, and tomato. This makes the studies of closterovirus molecular biology and interactions with cells important in the applied aspect.

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14.1 Introduction

Family *Closteroviridae* (from κλωστερ, Greek for 'thread') combines about 40 positive-strand RNA viruses infecting higher plants (Table 14.1) (Agranovsky 1996; Bar-Joseph et al. 1979; Martelli et al. 2012). Closteroviruses have several distinguishing traits:

(i) Semi-persistent mode of insect transmission (implying that a virus is retained within a vector for hours) and phloem-limited nature

Table 14.1 Current taxonomic layout of the family *Closteroviridae*[a]

Genus	Vector	Genome sequence
Closterovirus	Aphids	
Approved species		
Beet yellows virus (BYV)		X73476.1, AF056575.1
Beet yellow stunt virus (BYSV)		U51931.1*
Burdock yellows virus (BuYV)		NA
Carnation necrotic fleck virus (CNFV)		GU234167
Carrot yellow leaf virus (CYLV)		FJ869862
Citrus tristeza virus (CTV)		CTU16304
Grapevine leafroll-associated virus 2 (GLRaV-2)		JX559644, AY881628
Mint virus 1 (MV-1)		AY792620
Raspberry leaf mottle virus (RLMoV)		NC_008585
Strawberry chlorotic fleck-associated virus (SCFaV)		DQ860839.1
Wheat yellow leaf virus (WYLV)		NA
Putative species		
Alligator weed stunting virus (AWSV)		NA
Clover yellows virus (CYV)		NA
Dendrobium vein necrosis virus (DVNV)		NA
Festuca necrosis virus (FNV)		NA
Fig leaf mottle-associated virus −1 (FLMaV-1)		AM279676*, KC914285*
Fig mild mottle virus (FMMV)		FJ611959.1
Crinivirus	Whiteflies	
Approved species		
Abutilon yellows virus (AbYV)		AY422070.1*, AY422069*
Bean yellow disorder virus (BYDV)		EU191904.1, EU191905.1
Beet pseudo-yellows virus (BPYV)		AY330918.2, AY330919.2
Blackberry yellow vein-associated virus (BYVaV)		AY776334.2, AY776335.2
Cucurbit yellow stunting disorder virus (CYSDV)		AY242077.1, AY242078.1
Cucurbit chlorotic yellows virus (CCYV)		AB523788, AB523789
Diodia vein chlorosis virus (DVCV)		GQ225585.2, GQ376201.2

(continued)

Table 14.1 (continued)

Genus	Vector	Genome sequence
Lettuce chlorosis virus (LCV)		FJ380118.1, FJ380119.1
Lettuce infectious yellows virus (LIYV)		U15440, U15441
Potato yellow vein virus (PYVV)		AJ557128, AJ557129, AJ508757
Strawberry pallidosis-associated virus (SpaV)		AY488137, AY488138
Sweet potato chlorotic stunt virus (SPCSV)		AJ428554, AJ428555
Tomato chlorosis virus (ToCV)		KC887998, KC887999
Tomato infectious chlorosis virus (TICV)		FJ815440, FJ815441
Ampelovirus	Mealybugs	
Approved species		
Subgroup I		
Grapevine leafroll-associated virus 1 (GLRaV-1)		JQ023131.1, AF195822*
Grapevine leafroll-associated virus 3 (GLRaV-3)		KJ174518.1
Little cherry virus 2 (LChV-2)		AF531505
Pineapple mealybug wilt-associated virus 2 (PMWaV-2)		AF283103.1
Subgroup II		
Grapevine leafroll-associated virus 4 (GLRaV-4)		NC_016416.1
Pineapple mealybug wilt-associated virus 1 (PMWaV-1)		AF414119
Pineapple mealybug wilt-associated virus 3 (PMWaV-3)		DQ399259.2
Plum bark necrosis stem pitting-associated virus (PBNSPaV)		EF546442
Velarivirus	Unknown	
Approved species		
Cordyline virus 1 (CoV-1)		HM588723
Grapevine leafroll-associated virus 7 (GLRaV-7)		HE588185
Little cherry virus 1 (LChV-1)		Y10237
Unassigned species		
Mint vein banding-associated virus (MVBaV)	Aphids	KJ572575*
Olive leaf yellowing-associated virus (OLYaV)	Unknown	AJ440010*

[a]Adapted from Martelli et al.(2012). Database accession numbers of partial sequences are marked by asterisk; the unmarked numbers correspond to complete genome sequences. NA, not available

of virus infection (with some closteroviruses being semi-phloem limited, whereas for others the phloem limitation is strict)

(ii) Unique structure of elongated particles with spiral type of symmetry built of several virus proteins

(iii) Large RNA genomes of 15–20 kilobases (kb), a value close to the largest positive-strand RNA genomes of animal nidoviruses

(iv) The presence of a gene homologous to the HSP70 family of cell chaperones

(v) The presence of a number of duplicated and diverged genes (or parts thereof), e.g. the genes for the major and minor capsid protein (CP)

Besides fundamental interest to the molecular biology and evolution of the large RNA genomes, it is worth to mention that some closteroviruses cause grave diseases in cultivated crops like citrus trees, sugar beet, and tomato (Duffus 1973; Falk and Duffus 1988). This makes the studies of

closterovirus molecular biology and interactions with cells important in the applied aspect.

Closterovirus cytopathology, ecology, relationships with vectors and disease control have been reviewed earlier (Bar-Joseph et al. 1979; Duffus 1973; Falk and Duffus 1988; Lesemann 1988; Lister and Bar-Joseph 1981; Murant et al. 1988; Tollin and Wilson 1988). The molecular organisation, evolution and taxonomy of closteroviruses have been discussed in several more recent reviews (Dolja et al. 2006; Karasev 2000; Martelli et al. 2012). This chapter provides an overview of the current advances in understanding of the molecular biology of closteroviruses and their interactions with host cells.

14.2 Taxonomy of Closteroviruses

The family *Closteroviridae* is divided into four genera: *Closterovirus* (type species, beet yellows virus (BYV)), *Crinivirus* (type species, lettuce infectious yellows virus (LIYV)), *Ampelovirus* (type species, grapevine leafroll-associated virus 3 (GLRaV-3)) and *Velarivirus* (type species, grapevine leafroll-associated virus 7 (GLRaV-7)) (Martelli et al. 2012; http://ictvonline.org/virusTaxonomy.asp). This latest version of the closterovirus taxonomy is used in this chapter (Table 14.1). It should be noted that, apart from biological patterns and serological properties, it is based on phylogenetic reconstructions of the protein domains conserved in the *Closteroviridae*, namely, the HSP70 homolog, RNA polymerase and major CP (Fig. 14.1). This approach, which has been made possible by accumulation of comprehensive sequencing data (Table 14.1), has clear evolutionary message. It is plausible that the closteroviruses evolved from a common ancestor and diverged along with adaptation to specific insect vectors: aphids (*Closterovirus*), whiteflies (*Crinivirus*), and mealybugs (*Ampelovirus*). The vector, if any, is yet to be identified for the members of the *Velarivirus* genus. There is a single exception for the aphid-borne mint vein banding-associated virus (MVBaV) which is phylogenetically distant from the members of the *Closterovirus* genus as well as from any other established genus within the family (Fig. 14.1; Martelli et al. 2012).

14.3 Genome Organisation and Gene Functions

14.3.1 Genus *Closterovirus*

14.3.1.1 Beet Yellows Virus

The genome of the Ukrainian strain of BYV consists of 15,480 nt (Agranovsky et al. 1991b, 1994a). The genomic RNA is 5′-capped and has no 3′-poly(A) (Karasev et al. 1989), the features apparently shared by all the *Closteroviridae* members. It contains ten ORFs, flanked by 5′- and 3′-untranslated regions (UTRs) of 107 and 141 nt, respectively (Fig. 14.2). The 5′-proximal ORF1a codes for the 295 kDa product (1a polyprotein) which encompasses the domains of papain-like cysteine proteinase (PCP), methyltransferase (Mtr) and RNA helicase (Hel) (Fig. 14.2). Mtr and Hel are conserved in the large subsets of positive-strand RNA viruses (Koonin and Dolja 1993; Rozanov et al. 1992). The Mtr domain is involved in the capping of viral mRNAs (Ahlquist et al. 1985; Dunigan and Zaitlin 1990; Mi and Stollar 1991; Rozanov et al. 1992). The Hel domain is likely to assist unwinding of RNA duplexes upon replication (Gorbalenya et al. 1989; Gorbalenya and Koonin 1989; Lain et al. 1990; Leitão et al. 2015). The ORF1b overlaps the last 40 triplets of ORF1a and codes for a product of ~53 kDa (Fig. 14.2) containing the domain of RNA-dependent RNA polymerase (Pol) (Kamer and Argos 1984; Koonin 1991). The array and conservation of the Mtr, Hel, and Pol domains clearly suggest that the BYV RNA replicase belongs to the alphavirus-like superfamily of replicases of positive-strand RNA viruses (Agranovsky et al. 1994a; Goldbach et al. 1991). The minimal BYV cDNA-based replicon consists of the ORFs 1a and 1b flanked by the 5′- and 3′-UTRs, thus proving that the proteins 1a and 1b are necessary and sufficient for the RNA replication (Peremyslov et al. 1998).

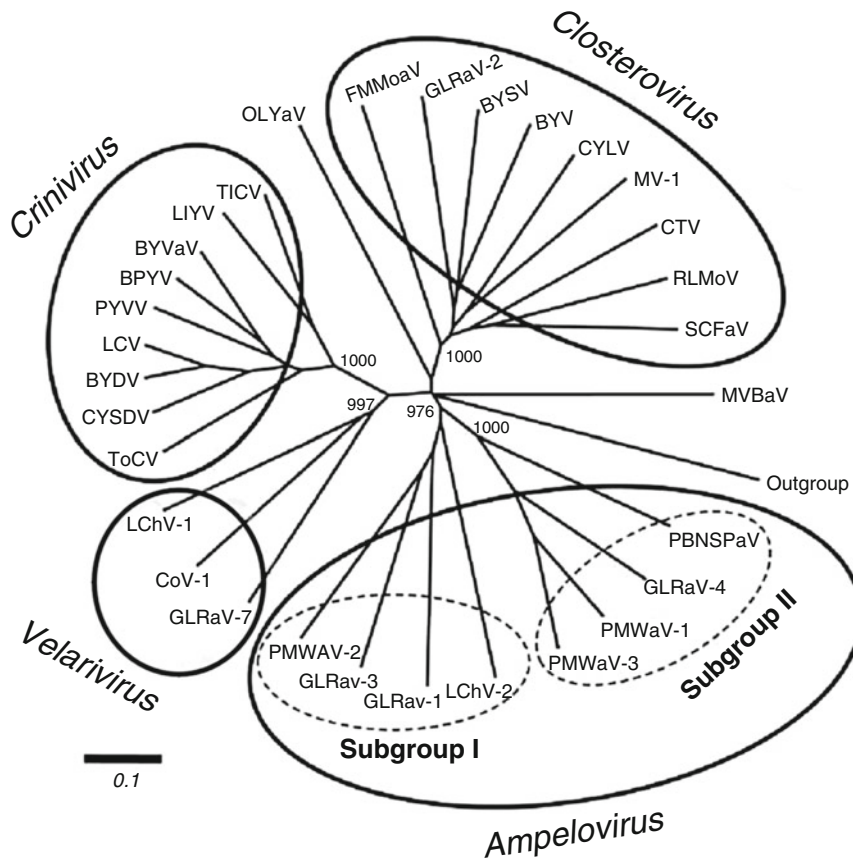

Fig. 14.1 Tentative phylogenetic reconstruction derived from aligned complete amino acid sequences of the HSP70h of members of family *Closteroviridae*. Distances are proportional to branch lengths. Bootstrap values are indicated at the main branch nodes. The bar represents 0.1 amino acid change per site. Abbreviations for viruses: genus *Ampelovirus*: grapevine leafroll-associated virus 1 (*GLRaV-1*), grapevine leafroll-associated virus 3 (*GLRaV-3*), grapevine leafroll-associated virus 4 (*GLRaV-4*), little cherry virus 2 (*LChV-2*), pineapple mealybug wilt-associated virus 1 (*PMWaV-1*), pineapple mealybug wilt-associated virus 2 (*PMWaV-2*), pineapple mealybug wilt-associated virus 3 (*PMWaV-3*), plum bark necrosis stem pitting-associated virus (*PBNSPaV*); genus *Closterovirus:* beet yellows virus (*BYV*), beet yellow stunt virus (*BYSV*), citrus tristeza virus (*CTV*), carrot yellow leaf virus (*CYLV*), grapevine leafroll-associated virus 2 (*GLRaV-2*), mint virus 1 (*MV-1*), raspberry leaf mottle virus (*RLMoV*), strawberry chlorotic fleck-associated virus (*SCFaV*), fig mild mottle-associated virus (*FMMoaV*); Genus *Crinivirus:* beet pseudo-yellows virus (*BPYV*), blackberry yellow vein-associated virus (*BYVaV*), cucurbit yellow stunting disorder virus (*CYSDV*), lettuce infectious yellows virus (*LIYV*), lettuce chlorosis virus (*LCV*), potato yellow vein virus (*PYVV*), tomato infectious chlorosis virus (*TICV*), tomato chlorosis virus (*ToCV*), bean yellow disorder virus (*BYDV*); Genus *Velarivirus*: little cherry virus 1 (*LChV-1*), cordyline virus 1 (*CoV-1*), grapevine leafroll-associated virus 7 (*GLRaV-7*); Unassigned mint vein banding-associated virus (*MVBaV*). Outgroup, heat shock protein 70 from *Arabidopsis thaliana* (acc. number NP_187864) (Adapted from Martelli et al. (2012), with permission)

The BYV leader proteinase (L-Pro) deserves a special comment. Experiments with the full-length cDNA of BYV showed that in-frame deletion of nine codons overlapping the PCP cleavage site abolishes RNA replication. More extended in-frame deletions in the N-terminal part of the L-Pro caused two- to fivefold reduction of RNA amplification levels (Peremyslov et al. 1998). Hence, the L-Pro, once released from the polyprotein, exhibits significant effect on replication, although it remains unclear whether it acts directly as a component of the replication

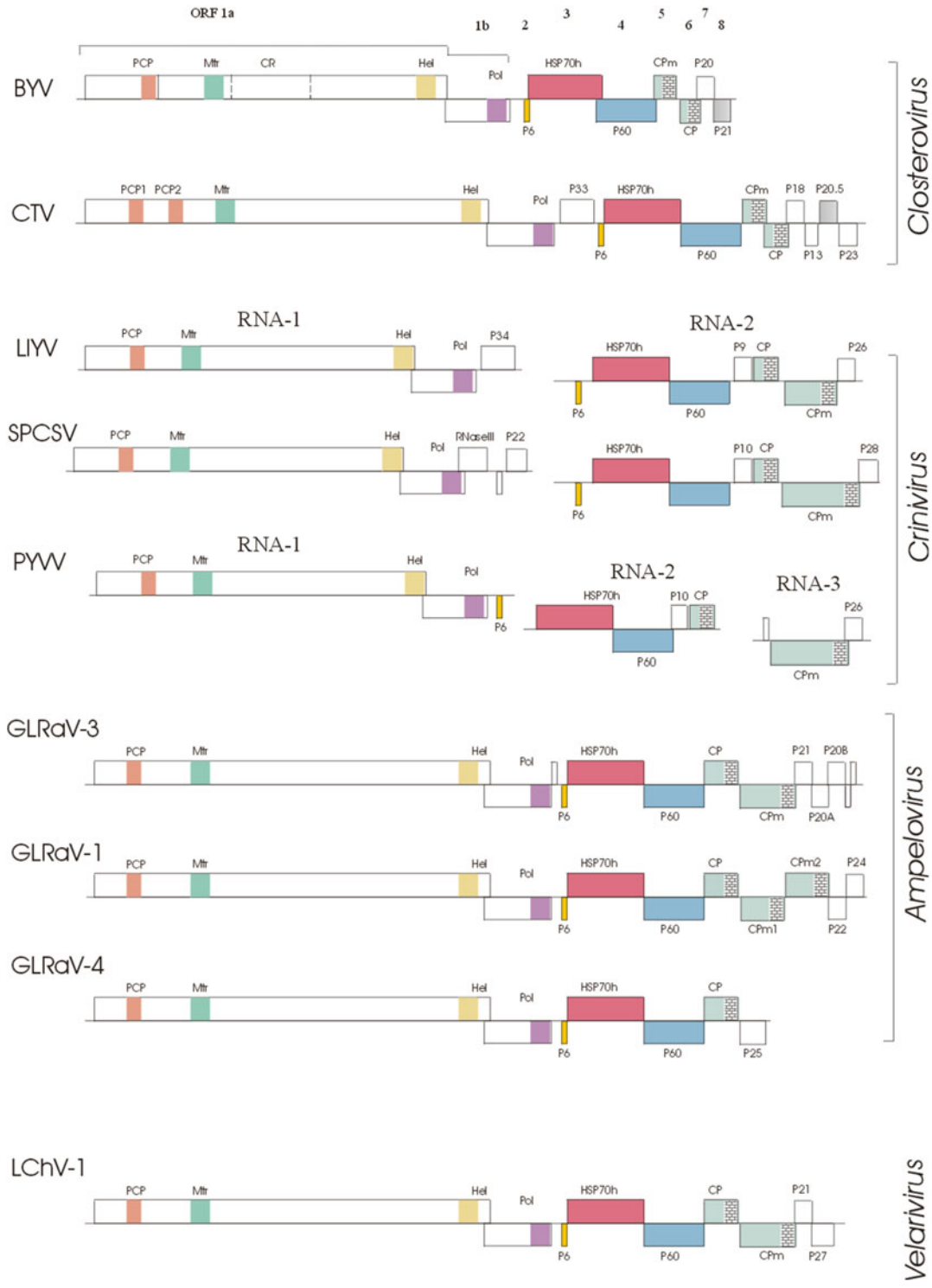

Fig. 14.2 Comparison of the genome maps of beet yellows virus (*BYV*); citrus tristeza virus (*CTV*) (genus *Closterovirus*); lettuce infectious yellows virus (*LIYV*); sweet potato chlorotic stunt virus (*SPCSV*); potato yellow vein virus (*PYVV*) (genus *Crinivirus*); grapevine leafroll-associated viruses 3, 1 and 4 (GLRaV-3, GLRaV-3 and GLRaV-4; genus *Ampelovirus*); little cherry virus 1 (LChV-1, genus *Velarivirus*). ORFs are shown as boxes,

complex or indirectly, e.g. by affecting the cell defence mechanisms (Peremyslov et al. 1998; Zinovkin et al. 2003). Apart from polyprotein processing and RNA amplification, the leader PCP is involved in long-distance transport of the BYV infection (Peng et al. 2003).

The BYV ORFs 2, 3, 4, 5, and 6 constitute a 'five-gene module' conserved in all closterovirus genomes (Fig. 14.2; Dolja et al. 2006).

ORF2 codes for a small hydrophobic protein P6 (Agranovsky et al. 1991b), a type III integral membrane protein associated with endoplasmic reticulum (ER) (Peremyslov et al. 2004b). The P6 is necessary for the cell-to-cell movement of the virus infection (Alzhanova et al. 2000).

The P65 product of ORF3 is closely related to the HSP70 family of cell heat shock proteins (Agranovsky et al. 1991a). HSP70s are ubiquitous molecular chaperones which assist proper folding and transport of other proteins (reviewed in Gething and Sambrook 1992). HSP70 molecule consists of the N-terminal ATPase domain and the C-terminal peptide-binding domain (reviewed in Craig et al. 1993). The BYV HSP70 homolog (HSP70h) contains an N-terminal domain whose sequence and tentative spatial fold are very similar to the HSP70 ATPases and a unique C-terminal domain that cannot be folded into the β4α structure typical of the HSP70 peptide-binding domains (Agranovsky et al. 1991a; Rippmann et al. 1991; F. Rippmann, personal communication). In line with these predictions, the bacterially expressed BYV P65 exhibits in vitro the magnesium-dependent ATPase activity which, in contrast to that of the cell HSP70 ATPases, is not stimulated by random peptides (Agranovsky et al. 1997). Several lines of evidence clearly show that the BYV HSP70h acts as a movement protein: (i) it is able to mediate the cell-to-cell movement of transport-deficient mutants of distantly related plant viruses (Agranovsky et al. 1998), (ii) a fraction of HSP70h is localised to plasmodesmata of infected plant cells (Medina et al. 1999) and (iii) knockout mutations of the HSP70h gene in the full-length BYV cDNA abolish the cell-to-cell movement of the virus (Peremyslov et al. 1999). The HSP70h is associated with actin cytoskeleton (Prokhnevsky et al. 2005) and is driven by myosin VII class motors along the actin filaments to plasmodesmata (Avisar et al. 2008). In earlier report, the BYV HSP70h was found to interact with microtubules (Karasev et al. 1992). Moreover, the HSP70h is necessary for the assembly of BYV particles (Alzhanova et al. 2007) and is present in mature particles, in about ten copies per virion, as an integral part thereof (Napuli et al. 2000).

The ORF4 product of 64 kDa (P60) is involved in the cell-to-cell movement and particle assembly; as is the HSP70h, the P60 is likely to be retained in the mature particles in non-stoichiometric amounts (Alzhanova et al. 2000, 2007; Napuli et al. 2003).

ORF5 and ORF6 code for the 24 kDa minor capsid protein (CPm) and 22 kDa major CP (Agranovsky et al. 1991b, 1995). ORF5 most likely arose from ORF6 by duplication and divergence (Boyko et al. 1992). The CPm encapsidates ~650 nt from the 5′-end of the genomic RNA, whereas the CP encapsidates the major part of the genome (Agranovsky et al. 1995; Zinovkin et al. 2003). Both capsid proteins are necessary for the cell-to-cell movement. This is supported by point mutagenesis of the respective start codons or the conserved arginine codons in ORF5 and ORF6 in the infectious cDNA clone of BYV. Translation arrest of either ORF 5 or 6, or the Arg/Asp substitution which rendered the encoded CP or CPm incapable of RNA coating, resulted in a movement-deficient phenotype (Alzhanova et al. 2000).

Fig. 14.2 (continued) with the related domains indicated in the same fill-in. *PCP* papain-like cysteine proteinase, *Mtr* methyltransferase, Fig. 14.2 (continued) *Hel* NTPase/helicase, *Pol* RNA-dependent RNA polymerase, *P6* small hydrophobic protein, *HSP70h* HSP70-related protein, *P60* ~ 60 kDa protein, *CP and CPm* major and minor capsid proteins, respectively. Vertical bar, the BYV PCP cleavage site; dotted bars, putative cleavage sites implicated from apparent molecular masses of the respective BYV 1a fragments. Drawn approximately to scale

ORF7 encodes a P20 protein which plays a role in long-distance transport. As shown in experiments with yeast two-hybrid system, P20 specifically interacts with the HSP70h (Prokhnevsky et al. 2002). This interaction is thought to bring the P20 to the particles, where it forms a tip at the end of the virion tail (Peremyslov et al. 2004a). The orthologs of the BYV P20 have not been found in the closterovirus genomes sequenced so far, except GLRaV-2 (Table 14.1).

The P21 product of the 3′-proximal ORF8 exerts the functions of replication enhancer and suppressor of RNA silencing (Reed et al. 2003). The P21 forms octameric rings that are able to bind RNA (Ye and Patel 2005). The orthologs of the BYV P21 are encoded in the genomes of several other members of the genus *Closterovirus*, but not in the other closterovirus genera (Reed et al. 2003; Ye and Patel 2005).

14.3.1.2 Citrus Tristeza Virus

Among plant RNA viruses, citrus tristeza virus (CTV) has the largest undivided genome (19,296 nt for the Florida T36 isolate) (Karasev et al. 1995; Pappu et al. 1994). The overall genome structure of CTV is similar to that of BYV, comprising the PCP, Mtr, Hel, and Pol domains, the small hydrophobic protein, the HSP70 homolog, the ~60 kDa protein related to the BYV P60, the 27 kDa CPm and the 25 kDa CP (Fig. 14.2). On the other hand, the CTV genome encodes some proteins or polyprotein domains that are not conserved in BYV, which accounts for the differences in coding capacities between the two closteroviruses. Thus, the PCP domain in the ORF1a product of CTV is duplicated, and the variable central region between the Mtr and Hel is larger than that in BYV (Fig. 14.2). Pairwise comparisons of the three leader proteinases, CTV L1-Pro (54 kDa), L2-Pro (55 kDa), and BYV L-Pro (66 kDa) revealed no sequence similarity among them apart from the C-terminal 150-residue portion encompassing the PCP domain. The gene-swapping experiments with the full-length cDNA of BYV showed that the CTV L1-Pro can substitute the BYV leader protein to rescue a replication-competent phenotype, thus indicating their similar roles in RNA amplification (Peng et al. 2001).

Apart from the conserved gene modules responsible for replication and encapsidation/movement functions, CTV has five additional genes as opposed to two in the genome of BYV. Among the products encoded by the 3′-proximal genes of CTV, the P20.5 is related to the BYV P21, whereas the P33, P13, P18, and P23 have no orthologs in current database (Karasev et al. 1995; Pappu et al. 1994). The P33 has a transmembrane domain at the C-terminus which mediates its insertion in membranes; this interaction mediates the ability of P33 to influence the virus host range (Kang et al. 2015). P33 is also involved in superinfection exclusion at the whole-organism level (Bergua et al. 2014). As is the P33, the P13 and P18 affect the host range and are necessary for infection of some CTV hosts, but not of the others (Tatineni et al. 2008). CTV genome encodes three distinct suppressors of RNA silencing, the CP, the P23, and the P20.5 (Lu et al. 2004). The P23 protein contains sequence motifs enriched in basic residues and a zinc finger domain. Besides its role as an anti-silencer, this protein is involved in regulation of asymmetric synthesis of the sense and antisense RNA strands (Satyanarayana et al. 2002; Lu et al. 2004; Flores et al. 2013).

14.3.1.3 Other *Closterovirus* Members

Grapevine leafroll-associated virus 2 (GLRaV-2) has a genome layout very similar to that of BYV, with the replicative module of overlapping ORFs 1a and 1b, the five-gene module, and two additional 3′-proximal genes. GLRaV-2 ORF1a codes for a tandem of leader proteinases as is the case of CTV (Meng et al. 2005). Experiments with GLRaV-2 full-length cDNA revealed that the two leader proteins have distinct functions in virus infection; L1-Pro is necessary for RNA amplification, whereas L2-Pro assists the viral systemic transport (Liu et al. 2009). Some closterovirus genomes, e.g. those of BYSV, carrot yellow leaf virus (CYLV) and SCFaV (Table 14.1), contain an ORF for a ~30 kDa protein localised between the replication module and the five-gene module,

as is the case of CTV. Notwithstanding, these proteins show no significant similarity to the CTV P33 (Fig. 14.2) or to any protein in current database, and their function is not known.

14.3.2 Genus *Crinivirus*

14.3.2.1 Lettuce Infectious Yellows Virus

The genome of lettuce infectious yellows virus (LIYV) is divided among RNA-1 and RNA-2 components of 8.1 kb and 7.2 kb, respectively (Klaassen et al. 1995). LIYV RNA-1 and RNA-2 show no similarity between their respective terminal UTRs, with the exception of the 5′-terminal pentanucleotide that is identical in both genomic components (Klaassen et al. 1995). LIYV RNA-1 has 5′-UTR of 98 nt, whereas RNA-2 has a 328-nt 5′-UTR with eight small upstream ORFs (uORFs) which may be expected to impair translation of the first gene in this RNA.

LIYV RNA-1 encompasses the overlapping ORFs 1a and 1b coding for the PCP, Mtr, Hel, and Pol, and the 3′-terminal ORF for a P34 protein (Fig. 14.2). P34 is an RNA-binding protein localised to the endoplasmic reticulum (ER) of the infected cells. Interestingly, P34 exerts transactivation of RNA-2 accumulation (Kiss et al. 2013; Wang et al. 2010; Yeh et al. 2000). LIYV RNA-2 contains genes for the small hydrophobic protein, the HSP70 homolog, the ~60 kDa protein related to the similarly encoded P60 proteins of BYV and CTV, the P9, the 28 kDa CP, the 52 kDa CPm and the P26 protein (Fig. 14.2). The functions of P9 and P26 are unknown. By analogy with the five-gene module proteins of BYV, the equivalent LIYV proteins P6, HSP70h, P60, CP, and CPm may be involved in cell-to-cell movement (Kiss et al. 2013).

The LIYV CP and CPm encapsidate the ‘body’ and ‘tail’ in the LIYV particles, whereas the HSP70h and P60 are present in virions in non-stoichiometric amounts (Tian et al. 1999). An important evidence for the role of the CPm in virus-vector interactions was obtained using LIYV as a model. A frameshift mutation in the LIYV RNA-2 full-length cDNA leading to the expression of a C-terminally truncated version of CPm (mutant p1-5b) rendered the virus incapable of whitefly transmission, but did not affect the systemic spread of the infection in plants (Stewart et al. 2010). It should be noted parenthetically that the truncated CPm was not detected in p1-5b virions by immunoelectron microscopy and immunoblot analysis (Stewart et al. 2010), and it is not clear whether the mutant protein devoid of the C-terminal ‘CP-like’ domain is at all competent in RNA encapsidation. Notwithstanding, the full-size LIYV CPm was found to bind specifically to the anterior foregut of the vector whitefly *Bemisia tabaci*, thus supporting the idea that the CPm serves to mediate the specific interaction between LIYV and its vector (Chen et al. 2011).

14.3.2.2 Sweet Potato Chlorotic Stunt Virus

Sweet potato chlorotic stunt viruses (SPCSV) RNA-1 and RNA-2 have sizes comparable to those of their counterparts in the genome of LIYV and show a similar organisation of ORFs (Kreuze et al. 2002). However, there are some notable genetic distinctions between SPCSV and LIYV (Fig. 14.2). Thus, SPCSV RNA-1 contains two 3′-proximal ORFs for the P27 and the P22 proteins which serve, in a concerted manner, as inhibitors of RNA silencing (Kreuze et al. 2002, 2005). P27 encompasses the conserved motifs of class 1 RNase III and displays a dsRNA endonuclease activity (Kreuze et al. 2005). Notably, only some strains of SPSVV contain the RNase III gene (Cuellar et al. 2008). SPCSV RNA-2 encodes the CPm with deduced molecular mass of 79 kDa (Kreuze et al. 2002).

14.3.2.3 Other *Crinivirus* Members

Among the criniviruses, a bizarre example is potato yellow vein virus (PYVV) which has a tripartite genome (Livieratos et al. 2004), where RNA-1 encompasses the replicative module and the small hydrophobic protein gene; RNA-2 encodes the HSP70h, the P60, the P10 and the 28 kDa CP; and RNA-3 encodes the P78 CPm and the P26 (Fig. 14.2).

In earlier works, the whitefly-transmissible cucumber chlorotic spot virus (CCSV) was

referred to as a virus closely related to LIYV and SPCSV, but having a monopartite genome (Agranovsky 1996; Woudt et al. 1993). However, partial genomic sequence of CCSV (L.P. Woudt, personal communication) is very close to those of the RNA-1 and RNA-2 of beet pseudo-yellows virus (BPYV; Tzanetakis and Martin 2004), suggesting that CCSV is in fact a strain of BPYV most likely having a bipartite genome.

14.3.3 Genus *Ampelovirus*

14.3.3.1 Subgroup I

The monopartite genome of grapevine leafroll-associated virus 3 (GLRaV-3; 18,498 nt) contains the replicative module (encoding a single PCP, Mtr, Hel, and Pol), the five-gene module (small hydrophobic protein, HSP70h, P60, CP, and CPm), and the 3′-terminal genes for nonconserved proteins P21, P20A, P20B, P4, and P7 (Fig. 14.2; Ling et al. 2004; Maree et al. 2013). P20B plays a role of RNA silencing suppressor (Gouveia et al. 2012). Further insight into the ampelovirus anti-silencing function was recently achieved for pineapple mealybug wilt-associated virus 2 (PMWaV-2), whose genome bears 3′-ORFs for the P22 and P20. It was found that the PMWaV-2 CPm, CP, P22, and P20 target local and systemic silencing in *Nicotiana benthamiana* (Dey et al. 2015).

A unique feature of the closely related grapevine leafroll-associated virus 1 (GLRaV-1) is the presence of two diverged CPm genes (Fig. 14.2; Fazeli and Rezaian 2000). It remains to be determined whether both the CPm-1 and CPm-2, along with the major 34 kDa CP, encapsidate the virus RNA. If this is true, the virions of GLRaV-1 would have the most peculiar structure among the *Closteroviridae*. The genome of little cherry virus 2 (LChV-2) encodes no detectable counterpart of the PCP domain and shows a shuffled arrangement of the five-gene module (Rott and Jelkmann 2005).

14.3.3.2 Subgroup II

A subset of ampeloviruses represented by GLRaV-4, plum bark necrosis stem pitting-associated virus (PBNSPaV), PMWaV-1 and PMWaV-3 (Table 14.1) is characterised by a 'genomic minimalism', as they have the smallest genomes among the *Closteroviridae* (~13,700 nt) encompassing only seven ORFs (Fig. 14.2). According to some reports, these viruses lack a recognisable gene for the CPm (Ghanem-Sabanadzovic et al. 2010; Martelli et al. 2012). Contradictory to this, some similarity was found between the CP and the ~25 kDa products of the 3′-terminal genes in PBNSPaV (Al Rwahnih et al. 2007) and PMWaV-1 (Melzer et al. 2008). The question as to whether the virion composition of the subgroup II ampeloviruses is distinct from that of the other *Closteroviridae* members awaits further study with immunoelectron microscopy and reverse genetics methods. As shown for PMWaV-1, the P60 protein serves as an inhibitor of RNA silencing (Dey et al. 2015).

14.3.4 Genus *Velarivirus*

A monopartite genome of little cherry virus 1 (LChV-1; Jelkmann et al. 1997; Keim-Konrad and Jelkmann 1996) consists of 16,934 nt. In the 5′–3′ direction, the sequence encompasses overlapping ORFs 1a and 1b coding for PCP, Mtr, Hel, and Pol (the replicative module); the genes for the small hydrophobic protein, the HSP70h, the P60, the 46 kDa CP and the 76 kDa CPm (the five-gene module); and the genes for nonconserved P21 and P27 proteins (Fig. 14.2; Jelkmann et al. 2012). The genomes of the related grapevine leafroll-associated virus 7 (GLRaV-7; Jelkmann et al. 2012) and cordyline virus 1 (CoV-1) (Melzer et al. 2011) display similar layouts.

14.4 Genome Expression

14.4.1 Proteolytic Processing

Proteolytic processing of the closterovirus replicase precursor has been extensively (and almost exclusively) studied using BYV as a model. Computer-assisted predictions and in vitro translation data have demonstrated that the BYV papain-like (thiol) proteinase domain mediates

1a autoproteolysis at the Gly-Gly bond to release a 588-residue (66 kDa) leader protein (Fig. 14.2; Agranovsky et al. 1994a). The catalytic Cys and His residues in the PCP active centre were confirmed by using point mutagenesis (Agranovsky et al. 1994a), and the identity of the BYV PCP cleavage site was directly proven by Edman degradation of the C-terminal proteolytic product purified from bacteria (Zinovkin et al. 2003). The release of the L-Pro is crucial for the BYV genome replication (Peremyslov et al. 1998).

Processing of the BYV 1a polyprotein in vivo is apparently complex, as indicated by immunoblot analysis of the infected plants with monoclonal antibodies. The L-Pro is expressed in vivo as 66 kDa protein (Zinovkin et al. 2003). More unexpectedly, Mtr and Hel reside in the respective 63 kDa and 100 kDa proteins, but not in a single 229 kDa product that could have been expected to result from the translation of ORF1a with concomitant release of the L-Pro (Erokhina et al. 2000). The 66 kDa, 63 kDa and 100 kDa proteins co-localise in membrane compartments specific to the closterovirus infection in cells, thus suggesting that they form a membrane-attached replication complex (Erokhina et al. 2001; Zinovkin et al. 2003). The mechanism of multiple BYV 1a cleavages is currently under study in our laboratory.

Hence, the BYV 1a and 1ab polyproteins are cleaved into the 66 kDa L-Pro, the 63 kDa Mtr, a ~70 kDa CR, the 100 kDa Hel and the putative ~150 kDa Hel-Pol fusion. It cannot be excluded that the CR and the Hel-Pol are processed into smaller fragments (cf. Cevik et al. 2008). Multiple cleavage of the replicase precursor may well be the case with other *Closteroviridae* members, yet this question awaits further study.

14.4.2 Ribosomal Frameshifting

Many RNA viruses utilise −1 translational frameshifting to express their polymerases, e.g. retroviruses (Jacks and Varmus 1985; Jacks et al. 1988), dsRNA-containing viruses (Dinman et al. 1991), and the diverse groups of positive-strand RNA viruses of animals and plants (Barry and Miller 2002; Brault and Miller 1992; Brierly et al. 1987; Godeny et al. 1993; Jiang et al. 1993; reviewed in Firth and Brierley 2012). The mechanism of −1 frameshifting relies on simultaneous slippage of two tRNAs bound to a 'shifty' mRNA consensus sequence X XXY YYZ, to decode it as XXX YYY, which is stimulated by a pseudoknotted secondary structure (reviewed in Firth and Brierley 2012). In the genomes of all closteroviruses sequenced to date (Table 14.1), the PCP-Mtr-Hel and Pol are encoded in overlapping ORFs 1a and 1b found in 0/+1 configuration, which is quite unusual for viral mRNAs. A few other examples are some dsRNA viruses of the family *Totiviridae* (Kim et al. 2005; Stuart et al. 1992) and the positive-strand RNA viruses chronic bee paralysis virus and Lake Sinai viruses 1 and 2 (Olivier et al. 2008; Firth and Brierley 2012). The closterovirus RNA polymerase is likely to be expressed via +1 translational frameshifting which, in BYV and most other family members, may occur on a conserved GUU_stop_C consensus at the ORF1a stop codon and involve a rightward slippage of the translating ribosome from GUU to UUU. The stop codon was proposed to serve as a slow-to-decode triplet promoting the frameshift (Firth and Brierley 2012). In the CTV genome, the putative +1 frameshift site GUU_CGG_C, predicted by analysis of the 1a and 1b protein sequence conservation profile, is found far upstream of the ORF1a stop codon. The rare arginine CGG triplet was suggested to be a slow-to-decode codon in CTV genome (Karasev et al. 1995), by analogy with +1 frameshift signals in yeast *Ty* retrotransposons (Guarraia et al. 2007; Farabaugh et al. 1993). We have produced an expression clone containing the region of BYV ORF1a/ORF1b overlap. In vitro translation of T7 transcript of this clone produced the major 1a fragment and the minor 1ab fusion product of expected size; the frameshifting efficiency was estimated at ~1 % (cf. Kim et al. 2005). Introduction of artificial in-frame stop codons in ORF1a upstream of the UAG and in ORF1b downstream of the UUU_AGC sequence blocked the synthesis of the fusion, suggesting the frameshift to occur within the CGGGUUUAG stretch. A point mutation of the stop codon (UAG to UAC) did not affect the synthesis of the 1ab fusion, thus suggesting that the

stop codon is not necessary for the frameshifting (M. Vitushkina and A. Agranovsky, unpublished data). What, then, serves a signal to impede the progress of the ribosome and to promote the +1 frame-switching in the BYV system? We have suggested that a putative pseudoknotted RNA fold overlapping the ORF1a stop codon might play such a role (Agranovsky et al. 1994a). However, this structure is not conserved in other closterovirus genomes and its significance has been questioned (Karasev et al. 1995; Klaassen et al. 1995). The mechanism of +1 frameshifting in closteroviruses still remains enigmatic and its unravelling requires experimental scrutiny.

14.4.3 Subgenomisation of 3′-Proximal Genes

Closterovirus genes located 3′-ward of the Pol gene (Fig. 14.2) are expressed via formation of 3′-coterminal subgenomic (sg) RNA species. In BYV-infected plats, six species of sgRNAs are produced, corresponding to the messengers for the P6, the HSP70h, the P64, the CPm, the CP, and the P21 (Agranovsky et al. 1994b; Dolja et al. 1990; Peremyslov and Dolja 2002; Vitushkina et al. 2007). A sgRNA for the BYV P20 has not been identified so far. Among the detected sgRNAs, those for the CP and P21 are the most abundant (Dolja et al. 1990; author's unpublished observations). In CTV infections, nine or ten sgRNAs are produced, so that each 3′-proximal gene likely receives a specific messenger (Hilf et al. 1995; reviewed in Dawson et al. 2013). The three smallest sgRNA species, followed by the sgRNA for CP, are the most abundant among the CTV-specific mRNAs (Hilf et al. 1995).

Closterovirus sgRNAs are of continuous template origin (Karasev et al. 1997), albeit it is not known whether these are transcribed from the full-length negative-strand or from 'anti-subgenomic RNAs' produced by premature termination of the negative-strand synthesis.

The transcription start sites have been mapped for the BYV sgRNAs for P6, HSP70h, P60, CPm and CP (Agranovsky et al. 1994b; Peremyslov and Dolja 2002; Vitushkina et al. 2007), as well as for some sgRNA species of CTV (Gowda et al. 2001; Karasev et al. 1997), crinivirus SPCSV (Kreuze et al. 2002), and ampelovirus GLRaV-3 (Jarugula et al. 2010; Maree et al. 2010). The sequence at the 5′-start sites of the BYV sgRNAs for CPm and CP is well conserved (CCAUUUYA; Y for pyrimidine) (Agranovsky et al. 1994b). Computer-assisted analysis of the nucleotide sequence upstream of the start sites of the BYV sgRNAs for the five-gene module unravelled two additional conserved motifs (Vitushkina et al. 2007). These motifs, which might constitute the subgenomic promoter elements, are conserved in the equivalent positions upstream of three orthologous genes of CTV (HSP70h, P60 and CPm) and two orthologous genes of BYSV (P60 and CP) (Vitushkina et al. 2007). The CTV transcription is regulated by the conserved promoter elements found in the 5′- and 3′-UTRs and by the internal subgenomic promoters; the secondary structure of these signals is apparently important for their function (Ayllón et al. 2004). On the other hand, in SPCSV (Kreuze et al. 2002) and GLRaV-3 (Jarugula et al. 2010; Maree et al. 2010), the RNA regions overlapping the sgRNAs start sites are nonconserved in sequence and secondary structure. Taken together, these findings support the notion of evolutionary plasticity of the closterovirus transcription regulation (Vitushkina et al. 2007).

14.4.4 Peculiarities of Closterovirus Genome Expression

For genome expression, closteroviruses combine autoproteolysis by one or two papain-like proteinases, ribosomal frameshifting and sgRNA formation, thus resembling the animal viruses belonging to the corona-like superfamily rather than any other known plant virus group (Agranovsky et al. 1994a; Agranovsky 1996; Dolja et al. 2006; cf. Gorbalenya et al. 2006). As closteroviruses and corona-like viruses represent evolutionarily disparate lineages (Koonin 1991; Koonin and Dolja 1993), it seems plausible that similar expression strategies in these groups have evolved

independently to confer an advantage in expression of large RNA genomes (Dolja et al. 2006).

Translation of the 5′-proximal genes in closterovirus genomes should produce the replicase components in unequal amounts. Thus, the synthesis of closterovirus RNA polymerase is obviously downregulated, as is the case with other virus systems employing translational frameshifting (Agranovsky et al. 1994a; Firth and Brierley 2012). The 1a and 1ab polyproteins undergo multiple processing in vivo. In BYV, these are cleaved into the 66 kDa L-Pro, the 63 kDa Mtr, a ~70 kDa CR, the 100 kDa Hel and the putative ~150 kDa Hel-Pol fusion. Regulated expression of the viral replicase as a set of distinct components is likely to provide the required flexibility in performing different enzymatic functions in RNA replication, namely, unwinding of duplexes, asymmetric synthesis of (+) and (−) strands, synthesis of subgenomic RNAs and RNA capping (Agranovsky et al. 1994a; Agranovsky 1996).

Expression of 3′-terminal genes in closterovirus genomes occurs via formation of a nested set of 3′-coterminal sgRNAs, which allows controlling the rate and time of synthesis of the proteins involved in movement, capsid assembly, silencing suppression and other accessory functions.

14.5 Particle Structure and Assembly

Closterovirus particles are very flexuous filaments 950–2000 nm in length, depending on the virus, and about 12 nm in diameter (Tollin and Wilson 1988). As demonstrated for BYV, CTV, and LIYV, the virions consist of a single RNA molecule coated by two capsid proteins, the CPm for the 5′-terminal 600–700 nt and the CP for the major part of the genome (Fig. 14.3a; Agranovsky et al. 1995; Febres et al. 1996; Zinovkin et al. 1999). The terms ‘rattlesnake structure’ and ‘morphologically polar structure’ have been coined to this unusual particle architecture (Agranovsky 1996; Karasev 2000; Dolja et al. 2006). The HSP70h and the P60, both indispensable for particle formation, are likely to be integral minor components of the virion tails (Napuli et al. 2000; Alzhanova et al. 2007; Satyanarayana et al. 2000, 2004).

The reverse genetics experiments with CTV full-length cDNA revealed that CPm, in the absence of other virion components, encapsidates the virus RNA from the 5′-terminus in a non-stop manner, whereas the HSP70h and the P60 force the CPm to form a proper ‘tail’ incorporating ~630 nt from the 5′-end, leaving the rest of the genomic RNA naked (Satyanarayana et al. 2004). Similarly, a concerted action of the BYV CPm, HSP70h, and P60 is necessary and sufficient to form the proper tail (Alzhanova et al. 2007; Peremyslov et al. 2004a). Although the arrangement of the HSP70h and P60 relative to each other and the CPm remains unclear, it is possible that the HSP70 and P60 form a ‘lock’ which stops the progress of CPm encapsidation (Fig. 14.3b). It would be interesting to see if this genomic region contains a distinct signal recognised by the HSP70h-P60 tandem (Fig. 14.3b). Notably, the closterovirus CP, CPm, and P60 contain the orthologous C-terminal domains conserved in all filamentous virus capsid proteins, indicating that the P60 may directly interact with RNA (Dolja et al. 1991; Napuli et al. 2003; Peremyslov et al. 2004a). The BYV CP, in the absence of CPm, HSP70h and P60, is able to encapsidate the entire genome, yet the packaging signal and the direction(s) of the CP assembly have not been determined (Alzhanova et al. 2001). It is tempting to speculate that the closterovirus CP encapsidates the RNA starting from the 3′-terminus or an internal origin of assembly and clings to the preformed CPm-P60-HSP70h tail, thus completing the particle formation (Fig. 14.3b).

In addition, the BYV P20 may form a morphologically distinct tip (most likely, RNA-less) on top of the virion tail (Peremyslov et al. 2004a). Being thus the fifth structural protein of BYV, the P20 is not necessary for the formation of the CPm tail (Peremyslov et al. 2004a; Prokhnevsky et al. 2002).

Closterovirus particles apparently serve as vessels for the cell-to-cell transport of virus infection, at least in the semi-phloem-limited infections characteristic of the *Closterovirus*

Fig. 14.3 (**a**) Immunoelectron microscopy analysis of the BYV particles in crude sap of infected *Tetragonia expansa* plants with mouse polyclonal antiserum against the CPm, followed by goat anti-mouse IgG conjugated to 15 nm gold beads, and negative staining with uranyl acetate (courtesy of D.-E. Lesemann). Arrows tag the distinct virion tail. Scale bar, 300 nm. (**b**) Tentative model of closterovirus particle assembly, assuming the existence of the 5′-terminal encapsidation signal 1 (enc-1) for the CPm, the 3′-terminal encapsidation signal 3 (enc-3) or an internal origin of assembly (OA) for the CP, and the encapsidation signal 2 (enc-2) at ~650 nt from the 5′-end, serving to discriminate between the CP and CPm whereupon they proceed to encapsidate the RNA in opposite directions. The HSP70h and P60 may form a 'lock' at the enc-2 and/or assist the capsid protein(s) in proper recognition of the enc-2. After completion of the assembly, the HSP70h and P60 are retained in the mature particle, possibly at the enc-2

genus members (Alzhanova et al. 2007; Dolja et al. 2006; Napuli et al. 2003). The movement of BYV particles through the plasmodesmata connecting the sieve elements with parenchyma cells, and the plasmodesmata between parenchyma cells, was demonstrated nearly 60 years ago in pioneering electron microscopy works by Katherine Esau's group (Cronshaw et al. 1966). The CPm tail also mediates the virus-vector interactions in criniviruses and perhaps other closteroviruses (Kiss et al. 2013).

14.6 Evolution of Closterovirus Genomes

Closterovirus genomes encompass the replicative module Mtr-Hel-Pol which is conserved within the alpha-like supergroup of positive-strand RNA viruses (Agranovsky 1996; Dolja et al. 2006; Goldbach et al. 1991; Koonin and Dolja 1993). The Mtr and Hel are spanned by a central region (CR) of marginal conservation (Agranovsky 1996; Dolja et al. 2006). It has been noted that among closteroviruses and the related alpha-like viruses of plants, the size of the CR grows almost linearly with the increase of the genome size, suggesting that evolutionary expansion of RNA genomes was attended by an increase in the replicase size and complexity (Agranovsky 1996).

Apart from the conserved replicative core that has been vertically inherited from an ancestor common with alpha-like viruses, closterovirus genomes bear genetic elements of apparently horizontal acquisition. This concerns the tandem of leader PCPs encoded in ORF1a of CTV and some other closteroviruses, the HSP70h closely related to the HSP70 family of cell chaperones, the CP and its diverged duplicates in CPm and P60, and the RNase III gene in SPCSV genome. The capture of foreign genes and intragenomic sequence duplication might be

driven by copy-choice RNA recombination (Kirkegaard and Baltimore 1986; Wang and Walker 1993). Upon evolutionary divergence of closteroviruses, some of these elements underwent further reassortment. Thus, the CPm gene was N-terminally extended and transposed with the CP gene in the five-gene modules of criniviruses, ampeloviruses and velariviruses compared to the members of the *Closterovirus* genus (Fig. 14.2). Some closteroviruses show extreme examples of gene shuffling fashion, such as the presence of triplicated CP gene (GLRaV-1), swapping of the five-gene module ORFs (LChV-2), and distribution of the five-gene module ORFs among the three genomic RNA components (PYVV).

Closterovirus genomes harbour additional variable genes which account for suppression of RNA silencing and other accessory functions. These genes are mostly found in the 3′-proximal genome portion, but in some cases are inserted downstream of the replicative module or within the five-gene module (Fig. 14.2). In some *Closteroviridae* members, the anti-silencing function is delegated to P60, CP, and CPm, possibly as a result of evolutionary adaptation of the variable portions of these proteins. Thus, expansion of the closterovirus genomes may be partly attributed to insertions and tandem duplications which occurred at both ends and within the conserved replicative core. The ampelovirus subgroup II members have relatively small genomes containing only the replicative and five-gene modules, being an exception from this trend.

An obvious problem in maintaining large RNA genomes is the accumulation of mutations in the progeny strands due to low fidelity of viral RNA polymerases (Holland et al. 1982; Steinhauer and Holland 1987). The closterovirus genomes do not encode equivalents of the proofreading exonuclease domain found in the largest RNA genomes of animal nidoviruses (Gorbalenya et al. 2006; Lauber et al. 2013), yet they maintain a relatively low mutation rate upon RNA replication (Rubio et al. 2013; Silva et al. 2012). The negative selection and homologous recombination between the virus RNA molecules to get rid of the incorrigible errors might serve to maintain viable progeny, unless this is provided by some intrinsic properties of closterovirus replicase yet to be discovered.

Evolution of closteroviruses towards increasing the genome size should have had to overcome packaging constraints. It has been suggested that acquisition of superflexible helical capsids by the ancestor of closteroviruses allowed their genomes to grow up to 20 kb (Dolja et al. 1994). The further evolution of capsid led to employment of the CPm, HSP70h and P60 (and possibly an additional tip-forming protein, like P20 in BYV) to confer their particles a structural complexity unprecedented among elongated plant viruses.

14.7 Cytopathology of Closterovirus Infections

Eukaryotic viruses from disparate groups, both DNA- and RNA-containing ones, induce in cells drastic rearrangement of the membranes leading to formation of 'virus organelles' or 'virus factories'. For positive-strand RNA viruses, it is suggested that these compartments protect the genome replication from nucleases and innate host defence mechanisms and help in creating sufficient concentration of interacting templates, replication proteins, and substrates (reviewed in den Boon and Ahlquist 2010; Gushchin et al. 2013; Netherton and Wileman 2011; Verchot 2011). Noteworthy, the intracellular transport of virus replication complexes and the related virus organelles is rather an active process than mere diffusion, as cytosol is a highly viscous matter where translocation of molecules or complexes exceeding a ~500 kDa limit is impeded (Luby-Phelps 2000; Greber and Way 2006).

Closterovirus infections induce in cells a number of cytopathic changes, of which the most characteristic are the 'BYV-type vesicles' represented by ~100 nm double-membrane vesicles (DMVs) and multivesicular complexes (MVCs; bunches of single-membrane vesicles surrounded by a common membrane) (Fig. 14.4; Lesemann 1988). The multivesicular complexes often neighbour with stacks of aligned filamentous BYV particles (Cronshaw et al. 1966; Lesemann 1988). The closterovirus DMVs

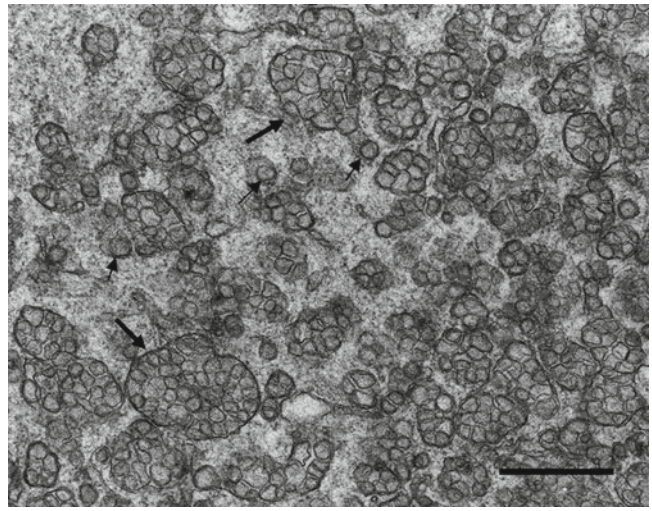

Fig. 14.4 Electron microscopy of the BYV-induced ultrastructures in leaf parenchyma cells of *Tetragonia expansa* (21 days p.i.). Double-membrane vesicles (DMVs, small arrows) and multivesicular complexes (MVCs, large arrows) on a tissue section embedded in Epon after fixation with glutaraldehyde and OsO_4. Scale bar, 0.5 μm

and MVCs resemble the membrane compartments produced in cells by infections of animal nidoviruses and flaviviruses (Gushchin et al., 2013; Knoops et al. 2008; Welsch et al. 2009). The membranes of DMVs and MVCs are likely to be derived from ER in the case of *Closterovirus* BYV (Gushchin et al., 2013) and *Crinivirus* LIYV (Wang et al. 2010) or mitochondria in the case of *Ampeloviruses* GLRaV-1 and GLRaV-3 (Faoro et al. 1992; Faoro and Carzaniga 1995). Inoculation of tobacco protoplasts with in vitro transcripts of the LIYV-specific full-length cDNA for RNA-1 (including a mutant bearing the ORFs 1a and 1b but devoid of the P33 gene) led to efficient replication and concomitant formation of the wild-type DMVs and MVCs (Wang et al. 2010). The BYV replication-associated proteins (L-Pro, Mtr, and Hel) co-localise with the DMV and MVC membranes in virus-infected cells, supporting the role of these ultrastructures as replication platforms (Erokhina et al. 2001; Zinovkin et al. 2003).

In search for the closterovirus determinants of membrane modification, we have studied the effects in cells of the putative membrane-binding domains predicted in the BYV 1a central region. Transient expression of a hydrophobic segment CR-2 (197 amino acids) in *Nicotiana benthamiana* led to reorganisation of the perinuclear ER into diffuse membrane reservoirs and formation of uniform globules ~1 μm in diameter mostly concentrated at the nucleus (Fig. 14.5; Gushchin et al., 2013). Some CR-2 globules were apparently motile; in line with this, a fraction of the globules co-localised with actin filaments (Gushchin et al., 2013). Deletion analysis of the CR-2 revealed that the ER transformation and the production of globules are two distinct phenotypes powered by different portions of

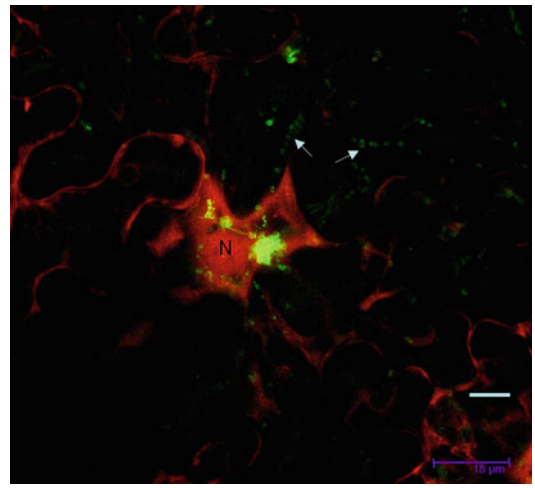

Fig. 14.5 Localisation of GFP-fused BYV CR-2 in epidermal cells of *N. benthamiana* leaves. The GFP/CR-2 was co-expressed with the red fluorescent marker protein mCherry, which localises to the cytoplasm and the nucleoplasm in plant cells (Lee et al. 2008). The proteins were visualised at 48 h post agroinfiltration by confocal laser scanning microscopy. Arrows indicate the motile CR-2 globules revealed in frame captures. Image represents the superpositions of series of confocal optical sections. N, nucleus

CR-2 (V. Gushchin and A. Agranovsky, unpublished data). It seems likely that creation of closterovirus replication platforms depends on the ER membranes and is accompanied by essential changes in perinuclear ER mediated by membrane-binding domain(s) in the 1a CR. It is tempting to speculate that the phenotypes induced by the BYV CR-2 segment might reflect some stages in the formation of closterovirus DMVs and VPs (Gushchin et al., 2013).

14.8 Conclusions

Closteroviruses evolved by surmounting the restraints imposed on the genome and particle structure of positive-strand RNA viruses. Colinearity and conservation of the main replicative domains clearly suggest the common ancestry of closteroviruses and other alpha-like viruses. However, closteroviruses have followed a distinct evolutionary pathway that has led to dramatic expansion of their genomes. Along with this, their evolution would have had to solve problems connected with replication and packaging of large RNA molecules. The packaging problem has been possibly resolved by evolutionary 'invention' of a superflexible capsid built of several proteins. Apparently, the expansion of closterovirus genomes has resulted from RNA recombination. It is possible that the horizontally acquired elements brought in novel enzymatic activities and structural elements advantageous for closterovirus adaptation to a distinct ecological niche, distinguished by the phloem-limited nature of infection and the semi-persistent mode of insect transmission.

The size and coding potential of the closterovirus RNA genomes may only be compared to those of the animal nidoviruses. The expression strategies and genome layouts developed in closteroviruses and nidoviruses are strikingly similar, despite the evolutionary disparity of these viruses. In both groups, (i) the 5′-terminal two-thirds of the genome are occupied by overlapping genes coding for replication-associated proteins, and the RNA polymerase is expressed by ribosome frameshifting; (ii) the 5′-proximal gene encodes one or two leader PCPs capable of self-cleavage; (iii) the processing of the polyproteins is apparently complex; and (iv) the 3′-proximal genes are expressed via a nested set of sgRNAs. Moreover, the structure of multivesicular complexes induced by closteroviruses in infected cells resembles those of nidoviruses rather than uniform 'vesicles with necks' characteristic of other alpha-like viruses. This may reflect independent adaptation to handling large RNA genomes in the two evolutionary distant lineages.

In the process of infection, closteroviruses induce in cells the specific multivesicular complexes, DMVs and MVCs, which apparently constitute the sites of RNA replication. The recent finding of a hydrophobic domain in the BYV 1a which exerts formation of motile globules connected to rearranged perinuclear endoplasmic reticulum and actin filaments may be a 'key to the highway' of closterovirus-cell interactions. This particularly concerns the virus-host interplay underlying the formation of closterovirus replication platforms in plant cells. Further studies of the functions encoded by the RNA genomes of closteroviruses are expected to provide a better understanding of the molecular mechanisms of their interactions with the genomes of their hosts and vectors.

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Major Virus Diseases of Groundnut in India and Their Management

15

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Abstract

Several virus diseases of groundnut have been reported in India based on symptoms, host range, and biological properties. Among those, *peanut bud necrosis virus* (PBNV), *tobacco streak virus* (TSV), *peanut mottle virus* (PeMoV), and *Indian peanut clump virus* (IPCV) are the economically important viruses of groundnut in India. *Peanut bud necrosis virus* belongs to the genus *Tospovirus*, transmitted effectively by *Thrips palmi*. PBND alone may cause 30–90 % yield losses. Necrosis of the terminal buds occurs which is a characteristic symptom of PBNV. Extensive field screening of the several genotypes, released varieties, and wild species at the hot spots has revealed the field tolerance of some of those genotypes. Peanut stem necrosis disease (PSND) is caused by the TSV of the genus *Ilarvirus* of the family *Bromoviridae*. Necrotic lesions on terminal leaflets, complete stem necrosis, and often total necrosis of entire plant are the characteristic symptoms of this disease. The PSND spreads mainly through the weed of crop species. A desired level of resistance of TSV has not yet been found in cultivated varieties of groundnut. The peanut clump disease of groundnut in India is caused by the IPCV of the genus *Pecluvirus*, family *Virgaviridae*. Symptoms are severe stunting of the plant appeared first on newly emerged leaves of two- to three-week-old seedlings. The host range of IPCV includes many monocot and dicot crop plants and weed species tested. IPCV was reported to be transmitted by the obligate fungal parasite (*Polymyxa graminis*) which is soilborne. Germplasm accessions,

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viz., NCAc 17099, NCAc 17133 (RF), and NCAc 17536, have been reported resistant to IPCV. Peanut mottle virus disease has been reported to occur on *rabi*/summer groundnut mainly in Andhra Pradesh, Maharashtra, and Gujarat. Newly formed leaves show mild mottling and vein clearing, whereas older leaves show upward curling and interveinal depression with dark green islands. The *peanut mottle virus* (PeMoV) occurs in nature on several important legume crops. Aphids are efficient vectors of PeMoV. Several lines of *Arachis* species like *A. glabrata* are reported to be resistant to this disease. *Peanut stripe virus* (PStV) is of quarantine significance to India and is almost eradicated from India. Since in most of the viral diseases sources with desired levels of genetic resistance could not be identified so far, transgenic approaches to engineer resistance to viruses by expressing the glycoproteins of tospoviruses in transgenic plants to block virus acquisition by thrips, by expressing truncated or modified forms of movement protein(s) of heterologous viruses, or by expressing virus-specific antibody genes may be adopted to tackle the viral diseases in groundnut.

Keywords
Groundnut • Viral diseases • Necrosis • Transgenic • Heterologous viruses

15.1 Introduction

One of the major production constraints of groundnut is the reduction in yield caused by the various diseases including those caused by viruses. Although natural infection of more than 30 plant viruses representing 14 groups has been recorded on groundnut from different countries (Sreenivasulu 2005), *groundnut bud necrosis virus* (GBNV), *tobacco streak virus* (TSV), *peanut mottle virus* (PeMoV), and *Indian peanut clump virus* (IPCV) are economically important in India (Table 15.1). The magnitude of losses caused by these virus diseases may vary from 1.2 to 100 % depending upon the extent of incidence and stage of crop growth.

The other viruses reported from groundnut fields in India are *tomato spotted wilt virus* (TSWV), g*roundnut rosette assistor virus* (GRAV), *groundnut rosette virus* (GRV, satellite RNA), *peanut stripe virus* (PStV), *peanut yellow spot virus* (PYSV), *peanut chlorotic leaf streak virus* (PCLSV), *groundnut yellow mosaic virus* (GYMV), *peanut green mosaic virus* (PGMV), *peanut crinkle virus*, and *groundnut ring spot viruses* (GRSV). PeMoV, PStV, IPCV, *peanut stunt virus* (PSV), *cowpea mild mottle virus* (CMMV), *cucumber mosaic virus* (CMV) and *cowpea aphid-borne mosaic virus* (CABMV) are seed transmitted and have quarantine importance.

15.1.1 Peanut Bud Necrosis Disease

The incidence of groundnut or peanut bud necrosis disease was reported in the annual report of Indian Agricultural Research Institute (IARI), India, in 1949 and later by Chohan (1972, 1974) and Ghanekar et al. (1979), and the name "bud necrosis" was given by Reddy et al. (1968). Initially, based on host range, vector transmission, virion morphology, and antigenic relationship, the causative agent was identified as *tomato spotted wilt virus* (Ghanekar et al. 1979). Later, in a reinvestigation on serological relationship and nucleocapsid sequence, a virus distinct from TSWV was identified and named as *groundnut bud necrosis virus* (Reddy et al. 1992).

Table 15.1 Groundnut viruses reported from India, their group, and their vectors

Virus	Group	Vector	References
Peanut bud necrosis virus	*Tospovirus*	*Thrips palmi* Karny (Thysanoptera)	Vijayalakshmi (1994) and Reddy et al. (1995)
Peanut stem necrosis virus	*Ilarvirus*	*Frankliniella schultzei*, *Scirtothrips dorsalis*,	Prasada Rao et al. (2009)
		Megalurothrips usitatus	
Indian peanut clump virus	*Pecluvirus*	*Polymyxa graminis* Ledingham (fungus)	Reddy et al. (1983)
			Nolt et al. (1988)
Peanut chlorotic leaf streak virus	*Caulimovirus*	*A. craccivora*, *Bemisia tabaci*, *M. persicae* (Homoptera)	Reddy et al. (1993)
Peanut stripe virus	*Potyvirus*	*A. craccivora*, *M. persicae*, *A. gossypii*, *Rhopalosiphum maidis*, *A. glycines*,	Prasada Rao et al. (1988)
		A. citricola, *Hysteroneura setariae* (Homoptera)	
Cowpea mild mottle virus	*Carlavirus*	*Bemisia tabaci* (Homoptera)	Iizuka et al. (1984)
Groundnut yellow mosaic virus	Geminivirus	*B. tabaci* (Homoptera)	Sudhakar Rao et al. (1980)
Peanut green mosaic virus	*Potyvirus*	*M. persicae*, *A. gossypii* (Homoptera)	Sreenivasulu and Demski (1988)
Peanut mottle virus	*Potyvirus*	*A. craccivora*, *M. persicae*, *A. gossypii*,	Rajeshwari et al. (1983) and Reddy et al. (1978)
		Rhopalosiphum padi (Homoptera)	
Peanut yellow spot virus	*Tospovirus*	*Scirtothrips dorsalis* (Thysanoptera)	Wongkaew (1986)

15.1.1.1 Distribution

Until the mid-1960s, bud necrosis was a minor disease in groundnut (Reddy 1988) which later occurred in epidemic proportions (Reddy et al. 1991; Vijayalakshmi 1994). The disease incidence of was reported to range from 1.2 to 100 % (Chohan 1974; Ghanekar et al. 1979; Mayee 1987; Singh and Gupta 1989; Anonymous 1985–1998; Singh and Srivastava 1995; Dharmaraj et al. 1995). The hot spot locations for groundnut bud necrosis disease are Jagtiyal, Kadiri, and Hyderabad in Andhra Pradesh, Latur in Maharashtra, Tikamgarh in Madhya Pradesh, Raichur in Karnataka, Mainpuri in Uttar Pradesh, and Saurashtra in Gujarat. GBNV has been considered as one of the major virus diseases of groundnut in Andhra Pradesh, Uttar Pradesh, Madhya Pradesh, Tamil Nadu, Karnataka, Gujarat, and Maharashtra on *kharif* groundnut crop. It has also been occurring on post-rainy season crop of groundnut in Saurashtra region of Gujarat; Nizamabad, Nalgonda, and Mahaboob Nagar districts of Andhra Pradesh; and Northern and Vidarbha regions of Maharashtra and north-eastern parts of Karnataka. In India, bud necrosis disease may cause 30–90 % yield losses depending upon the time of infection on plant growth stage (Ghanekar et al. 1979; Basu 1995).

15.1.1.2 Symptoms

Initially, faint chlorotic spots and mottling occur on young leaflets which develop subsequently into chlorotic and necrotic rings and streaks. Petiole bearing fully expanded leaflets become flaccid and droops. Necrosis of the terminal buds occurs which is a characteristic symptom of GBNV-infected groundnut plant. If the infection is on a 1-month-old plant, the entire plant may die and pod setting will be absent. Mild ring spots and necrosis in the petiole and portion of stem

have been observed if it infects at the later stages of crop growth. Stunting and proliferation of axillary shoots, distortion of lamina, mosaic, mottling, chlorosis, decrease in size, rarely shoestring appearance of leaflets, and bushy appearance of the plant are the characteristic secondary symptoms in GBNV-infected groundnut plants. Infected plants produce small and shriveled seeds with testa in red or brown or purple mottling. Late infected plants may produce seed of normal size, but the testae on such seed are often mottled.

15.1.1.3 Host Range

GBNV infects a large number of leguminous and solanaceous plants in India which include groundnut, tomato, chili, potato, peas, sunflower, cotton, many pulses, carrot, and brinjal, various ornamental plants like zinnia and cosmos and weeds such as *Ageratum conyzoides*, *Cassia tora*, *Acanthospermum hispidum*, *Desmodium triflorum*, and *Lagasca mollis* (Reddy et al. 1991; Hemalatha et al. 2008). The first report of the natural infection of jute by GBNV was reported by Sivaprasad et al., (2011).

15.1.1.4 Causal Virus

Groundnut bud necrosis virus (GBNV) also called *peanut bud necrosis virus* is a single-stranded, negative membrane-bound RNA virus [(−) ssRNA] which belongs to the family *Bunyaviridae* and genus *Tospovirus* (Virus Taxonomy: 2009). On the basis of the virion morphology, genome organization, transmission by thrips, NP gene sequence, vector specificity, host range, and serology, tospovirus species belonging to five different serogroups (I, II, III, IV, and V) have been recognized. Groundnut bud necrosis virus along with watermelon silver mottle virus (WSMV) and the tomato isolate of groundnut bud necrosis virus (GBNV-To) belongs to the serogroup IV.

The virion is quasi-spherical in shape, is 80–120 nm in diameter, and contains a segmented genome of three single-stranded RNA molecules (large (L), medium (M), and small (S)) that are each bounded by a nucleocapsid protein to form ribonucleoproteins which are encased within a lipid envelope consisting of two virus-coded glycoproteins (G_1 and G_2) and a host-derived membrane. L, M, and S RNA segments were characterized based on nucleotide sequence, genome organization, and homology to other tospoviruses (Satyanarayana et al. 1996a, b; Gowda et al. 1998; Akram et al. 2004; Venkatesan et al. 2009; Lokesh et al. 2010).

The 8911-nucleotide L RNA contains a single open reading frame (ORF) in the virion complementary strand and encodes a protein of 330 K. At the 5′ and 3′ termini of the sense RNA, there were 247- and 32-nucleotide untranslated regions, respectively, containing an 18-nucleotide complementary sequence with 1 mismatch.

The M RNA is 4801 nucleotides in length, which comprised two ORFs in an ambisense organization and terminal inverted repeats. The 3′ large ORF (3363 nucleotides in the virus complementary strand) encoded a protein with a predicted size of 127.2 K which was identified as the glycoprotein precursor (GP) of the G1 and G2 glycoproteins. A comparison of the deduced amino acid sequence of GP revealed 37 % identity and 58–59 % similarity with that of tomato spotted wilt virus (TSWV, serogroup I) and impatiens necrotic spot virus (INSV, serogroup III) and 21–23 % identity and 44–47 % similarity with those of other members of the genus *Bunyavirus.* The 5' small ORF (924 nucleotides in the virus sense strand) encoded a 34.2 K protein which was identified as the nonstructural (NSs) protein based on 41–43 % identity and 60–63 % similarity with that of TSWV and INSV. Defective RNA molecules derived from the genomic M RNA were detected during continuous passage of the virus by sap inoculations (Satyanarayana et al. 1996a).

The S RNA is 3057 nucleotides in length and contains inverted repeats and two open reading frames (ORFs) with an ambisense coding strategy that are separated by an A-rich intergenic region. One ORF (1320 nucleotides in the viral sense strand) encodes a 49.5 K protein, identified as the nonstructural (NSs) protein based on similarity to published tospovirus sequences. The second ORF (831 nucleotides in virus complementary strand) encodes a 30.6 K protein

identified as the nucleocapsid (N) protein based on sequence similarities. Amino acid sequence comparison of N and NSs proteins revealed identities of 22–34 % with the reported tospovirus isolates of serogroups I, II, and III, whereas it had 82–86 % identity with viruses in serogroup IV, WSMV, and tomato isolate of GBNV. Also, two sub-genomic RNA species detected in PBNV-infected tissue corresponded to the predicted sizes (1.65 and 1.4 kb) of the NSs and N mRNAs. GBNV-To showed 99 % identity with the WSMV N protein sequence. The data obtained confirm earlier reports that PBNV should be considered as a distinct species belonging to serogroup IV, along with WSMV and GBNV-To (Satyanarayana et al. 1996a, b). Recently the function of nonstructural (NSs) protein encoded by the small RNA genome (S RNA) was studied as bifunctional enzyme (NTPase and dATPase activities and ATP-independent RNA/DNA phosphatase activity), which could participate in viral movement, replication, or suppression of the host defense mechanism (Lokesh et al. 2010).

15.1.1.5 Transmission

The virus is readily transmissible by sap (mechanical inoculation) under experimental conditions. Though virus has been detected in the shell and seed coat, it is absent in embryo and hence, not seed transmissible (Pappu et al. 1999). Amin et al. (1981) reported that the virus causing bud necrosis disease in India is transmitted by two species of thrips, *Frankliniella schultzei* and *Scirtothrips dorsalis*. However, subsequent investigations showed that *Thrips palmi* transmits GBNV and not *F. schultzei* or *S. dorsalis* (Vijayalakshmi 1994; Reddy et al. 1995). *T. palmi* adults transmit the virus to a maximum of 100 % when there were 10 adults per plant (Vijayalakshmi 1994). Cowpea was found to be the best host for rearing and multiplying *T. palmi* under laboratory conditions (Vijayalakshmi 1994). Lakshmi et al. (1995) reported that only *Thrips palmi* transmitted the virus, acquiring the virus during larval stage, with a 5 min acquisition period, but the virus is transmitted only by the adults. Adults needed an acquisition feeding period of more than 1 h, and the majority transmitted the virus throughout their life.

15.1.1.6 Management

Cultural Practices

The lower incidence of groundnut bud necrosis disease was reported in crop sown in early June than the crop sown in late June (Ghewande 1983; Reddy et al. 1983; Dharmaraj et al. 1995). The highest incidence of 90 % was recorded in the crop sown around the first of August, and dry weather during the crop growth period promoted the disease to a great extent. The incidence of bud necrosis disease in the crops that were sown at low densities was proportionately higher than in those sown at high densities (Reddy et al. 1991; Bhatnagar et al. 1995). Groundnut intercropped with pearl millet/sorghum and early sowing showed less incidence of the disease at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India (Ghanekar 1980; Reddy et al. 1991, 1995). Roguing of infected plants, especially during early stages of plant growth, should be avoided because this practice creates gaps in the field and can increase the disease incidence (Reddy et al. 1991, 1995). Reduction in the incidence of bud necrosis disease was reported with the chemical control of thrips (Amin 1988; Singh and Srivastava 1995).

Host Plant Resistance

Very good progress has been made in the identification of sources of field tolerance to groundnut bud necrosis disease by different groups of researchers. Extensive field screening of several genotypes, released varieties, and wild species at the hot spots has revealed the field tolerance of some of those genotypes (Tables 15.2, 15.3, and 15.4).

15.1.2 Peanut Stem Necrosis Disease

A disease characterized with the necrosis of the stem and terminal leaflets followed by death of plant was epidemic resulting in severe crop losses

Table 15.2 The genotypes identified as tolerant to groundnut bud necrosis disease in field screenings at hot spots

Sl. no.	Genotype	References
1.	Robut 33–1	Amin (1985)
2.	ICGV 86031	Dwivedi et al. (1993)
3.	Spanish 5512, Spanish 67–5, ICGS 18, ICGV 86699, J 14, R 8821, R 7015, R 9021, ICG 1703, ICG 2711, EC 2215, ICG 5042, ICGV 98304, RSG 1	Basu (1995)
4.	CSMG 12. ICG 869, ICG 6317, CSMG 15	Singh and Srivastava (1995)
5.	R 8806; R 8970; R 8976; R 9621; R 9251; R 9214; R 9227; R 9204; ICGV numbers 86029, 86030, 86031, 89304; ICG 2271	Dharmaraj et al. (1995)
6.	ICG 239	Ghewande and Desai (1995)
7.	*Arachis chacoense*, *A. glabrata*, *A. skp.* (PI 262848), *A. pusilla*	Kolte (1984) and Reddy (1988)
8.	ICGV 86430, 2192–8(50), and 2169-5(9)	Buiel et al. (1995)
9.	CSMG 12, EC 21070, ICG 98, GBFDS 92, GBPRS 4, GBPRS 15, U 4–7–7, 28/207, Ah 7215, Ah 7286, Ah 7913, BPG 511, Chandra, CSMG 5, CSMG 5–1, CSMG 9, CSMG 15, CSMG 17, EC nos. 1246, 20957, and 21161	Singh et al. (1998)

(~ rupees 300 crores) in about 225,000 ha in Anantapur district of Andhra Pradesh, India, during rainy season of 2000. It was presumed to be caused by *peanut bud necrosis virus* (PBNV), because of the characteristics necrosis of terminal buds. In subsequent studies, however, *tobacco streak virus* (TSV) was found to be associated with the disease (Reddy et al. 2002). The disease was named as "peanut stem necrosis disease." This was the first report of occurrence of TSV in groundnut in India. TSV was also shown to cause sunflower necrosis disease in sunflower (Prasada Rao et al. 2000; Ravi et al. 2001; Ramaiah et al. 2001; Bhat et al. 2002). Although groundnut bud necrosis disease and peanut stem necrosis disease are caused by two distinct viruses belonging to the *Ilarvirus* and *Tospovirus* groups, respectively, the symptoms produced by them on groundnut are very similar making it difficult to distinguish between these two diseases in the later stages based on symptoms alone (Table 15.5).

15.1.2.1 Distribution

Peanut stem necrosis disease is prevalent in the districts of Anantapur; Kurnool, Cuddapah, and Chittoor in Andhra Pradesh; and Raichur in Karnataka. Limited surveys carried out in Gujarat (Porbandar, Rajkot, and Junagadh) and Maharashtra (Jalgaon and Dhulia) did not show significant levels of incidence of this disease. However, more extensive surveys and monitoring are needed in Karnataka, Tamil Nadu, and

Table 15.3 Short-duration (100–110 days) groundnut bud necrosis disease-resistant/disease-tolerant varieties

S. no.	Name of variety	Recommended for areas in	Season suitable for cultivation
1.	TAG 24	Maharashtra	Both *kharif* and *rabi*/summer
2.	Kadiri 3	All India	Both *kharif* and *rabi*/summer
3.	Kadiri 4	Andhra Pradesh	*Rabi*/summer
4.	JCG 88	Andhra Pradesh	*Rabi*/summer
5.	K-134	Andhra Pradesh	Both *kharif*
6.	BSR 1	Tamil Nadu	*Rabi*/summer
7.	R 8808 (Apoorva)	Southern peninsular states	Both *kharif* and *rabi*/summer
8.	R 9251	Karnataka	Both *kharif* and *rabi*/summer

Table 15.4 Medium-duration (110–120 days) groundnut bud necrosis disease-resistant/disease-tolerant varieties

S. no.	Name of variety	Recommended for areas in	Season suitable for cultivation
1.	ICGS 11	North Maharashtra, Madhya Pradesh	*Kharif*
2.	ICGS 5	Uttar Pradesh	*Kharif*
3.	ICGS 37	Gujarat	*Kharif*
4.	B 95	Maharashtra	*Kharif*
5.	ICGS 44	North Maharashtra, Madhya Pradesh, Gujarat	Both *kharif* and *rabi*/summer
6.	CSMG 884	Rajasthan, UP, Punjab	*Kharif*

Maharashtra, where sunflower necrosis disease caused by TSV is prevalent in sunflower crop.

15.1.2.2 Symptoms

Initial symptoms appear as large necrosis lesion on young quadrifoliate leaves, which coalesce and cover the entire leaflet followed by the necrosis of the stem below the necrotic leaves. If young plants (less than 1 month old) are affected, the entire plant is often necrotized, whereas in the case of older plants, one or more branches will have necrosis. These plants are stunted and do not show any axillary shoot proliferation. Majority of pods also will have necrotic spots, and in severe infection, the pod size will be reduced and kernels will become not marketable. The symptoms, however, have been shown to vary among varieties of groundnut. Infection by groundnut bud necrosis and peanut stem necrosis viruses can be distinguished precisely by ELISA tests.

15.1.2.3 Host Range

Several crop and weed species including sunflower, cowpea, and marigold are infected by tobacco streak virus. TSV can survive on cowpea, black gram, green gram, marigold, and sunflower. *Parthenium hysterophorus*, a widespread weed, acts as a symptomless carrier, and virus is spread through pollen grains by the three species of thrips, namely, *Frankliniella schultzei*, *Scirtothrips dorsalis*, and *Megalurothrips usitatus*. In case of groundnut, *F. schultzei* acts as viral vector (Reddy et al. 2002). The natural host range of the virus extends from weed species to crop plants (Table 15.6).

15.1.2.4 Causal Virus

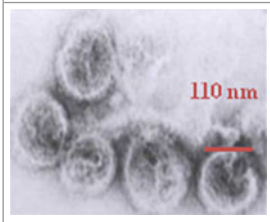
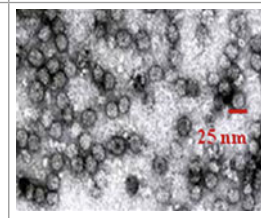
Tobacco streak virus (TSV) belongs to the genus *Ilarvirus* of the family *Bromoviridae*. The virus particles are spherical, in the range of 25–35 nm in diameter. Immuno- and nucleo-based assays for early and rapid diagnosis of the disease were also developed.

Peanut stem necrosis virus shares almost similar homology with the tobacco streak *Ilarvirus* (Prasada Rao et al. 2003). TSV contains a tripartite genome of 3.7, 3.1, 2.2 kilobases with a 0.9 kb sub-genomic RNA and all the three molecules of (+) sense RNA that are separately encapsidated in icosahedral particles. The virions are quasi-isometric and 27–35 nm in diameter. Coat protein molecules of TSV are approximately 25 K in mass. Genetic diversity of coat protein (CP) gene within TSV population originating from different hosts in India has also been addressed (Ravi et al. 2001; Bhat et al. 2002; Jain et al. 2003 and Bag et al. 2008). Coat protein (CP) gene (717 bp, 238 aa) is highly conserved in all isolates tested. Comparison of coat protein gene sequences revealed 98–100 % and 97–100 % identity at nucleotide and amino acid levels, respectively, suggesting that the TSV population is homogenous and perhaps has common origin in India. TSV coat protein production is dependent upon synthesis of sub-genomic RNA (RNA4) derived from RNA3. Replication of TSV requires CP or the sub-genomic RNA4. RNA1 and RNA2 encode proteins involved in viral replication, whereas RNA3 encodes a protein required for cell-to-cell movement. The viral coat protein is expressed by a sub-genomic RNA, designated RNA4, collinear with the 3′ end of RNA3.

15.1.2.5 Transmission

The peanut stem necrosis disease spreads mainly through the weed of crop species which are alternate host to the virus. The most commonly occur-

Table 15.5 Distinguishing features of groundnut bud necrosis and peanut stem necrosis diseases

Characteristics	PSND	PBND
Causal virus	*Peanut stem necrosis virus*	*Groundnut bud necrosis virus*
		
Group	*Ilarvirus*	*Tospovirus*
Groundnut plant showing characteristic symptoms of the disease on groundnut		
	Necrotic lesions on terminal leaflets, complete stem necrosis, and often total necrosis of entire plant	Chlorotic lesions on terminal leaflets, ring spots, and often necrosis of terminal bud
	Axillary shoot proliferation, restricted to apical portion, may occur	Axillary shoot proliferation with small and deformed leaflets. Infected plants remain stunted and seldom die
	Necrotic spots on pods. Testae are not discolored or mottled	No necrotic spots on pods and testae are discolored and mottled
Serological cross reaction	*Ilarvirus* in *Bromoviridae* reacts with many tobacco streak virus antisera	Distinct *Tospovirus* reacts only with peanut bud necrosis virus antiserum
Transmission	Seed transmitted in many hosts	Not seed transmitted in any of the hosts
Vectors	Transmitted by several thrips. Relationship is passive	Transmission by *Frankliniella schultzei* in a persistent manner
Primary spread	Mostly weed hosts	Weed and crop plants
Secondary spread	Selected weeds and crop plants	Selected weeds and crops plants

Modified from Prasada Rao et al. (2003)

ring weeds where the disease was epidemic were *Parthenium hysterophorus*, *Abutilon indicum*, *Ageratum conyzoides*, *Croton sparsiflorus*, *Commelina benghalensis*, *Cleome viscosa*, *Euphorbia hirta*, *Lagasca mollis*, and *Tridax procumbense*, all of which were infected. No symptoms were noticed in TSV-infected parthenium. Although the three thrips species (*F. schultzei*, *M. usitatus*, and *S. dorsalis*) were experimentally shown to transmit TSV in the presence of infective pollen, the flower inhabiting *F. schultzei* played a major role in the field spread of the virus. *F. schultzei* collected from flowers of infected *Parthenium* plants carried 8–10 pollen grains on their bodies. When these thrips attacked groundnut plants, the pollen grains got dislodged from their bodies and deposited on the leaves. Thrips, during their feeding, cause injury to both

Table 15.6 The natural hosts of TSV

Weed species	Crop plants
Abutilon indicum	Groundnut (*Arachis hypogaea*)
Acalypha indica	Safflower (*Carthamus tinctorius*)
Achyranthes aspera	Sunflower (*Helianthus annuus*)
Calotropis gigantea	Cotton (*Gossypium hirsutum*)
Cleome viscosa	Cow pea (*Vigna unguiculata*)
Commelina benghalensis	Black gram (*Vigna mungo*)
Croton sparsiflorus	Green gram (*Vigna radiata*)
Digera arvensis	Marigold (*Tagetes erecta*)
Euphorbia geniculata	
Euphorbia hirta	
Lagasca mollis	
Parthenium hysterophorus	
Tridax procumbense	

leaf tissue and deposited pollen and thus facilitate virus infection of the plant. Parthenium produces several flushes during its life cycle, thus ensuring continuous supply on pollen. Heavy westerly winds that occur during August and September can facilitate deposition of pollen on groundnut plants from infected parthenium as well as crop plants from sunflower and marigold. These pollens can facilitate virus transmission when plants are colonized by the thrips (Prasada Rao et al. 2003). In laboratory tests, transmission was achieved with pollen from sunflower, marigold, and parthenium deposited on the groundnut leaves that were colonized by the thrips.

Early infected groundnut plants do not flower. As it is a self-pollinated crop, groundnut is unlikely to contribute to disease spread. Sunflower is often grown adjacent to groundnut crop in peninsular India. Early infected sunflower plants usually produce malformed heads with few or no pollen. However, late-infected sunflower plants produce flowers that could severe as a source of inoculums. Groundnut crop grown adjacent to sunflower crop which was infected TSV invariably had shown stem necrosis disease. Although marigold can serve as an efficient source of inoculums, this crop is grown only under irrigation on a limited scale. Studies conducted on field-infected and mechanically inoculated plants of groundnut, sunflower, and parthenium have so far failed to show seed transmission of the virus. However, these studies were limited to a small number of seeds, and this aspect requires further investigation using large quantities of seed of more than one cultivar. Conditions congenial for disease epidemics are (i) pre-monsoon showers during late May or early June that encourage germination and growth of parthenium, (ii) sowing groundnut during July by which time parthenium is in full bloom, and (iii) normal rains, promoting good growth of groundnut as well as parthenium accompanied with one or two dry spells that encourage thrips multiplication and movement eventually resulting in virus spread.

15.1.2.6 Management

Resistant varieties; removal of weed host; border cropping of tall growing grasses or pearl millet or sorghum or maize (7–11 rows); maintaining optimum plant population thereby discouraging thrips landing on the groundnut crop; avoiding neighboring crops as sunflower, marigold, or other TSV susceptible crops; application of insecticides to manage the thrips, etc., were practices that could help to contain the disease. The several experiments conducted on the management of peanut stem necrosis disease remained inconclusive due to lack of high disease pressure.

Cultural Practices

During the survey of TSV epidemic on groundnut in Anantapur district of Andhra Pradesh, India, it was observed that natural barriers such as tall grasses in the field protected the adjacent crops from the disease. The tall grasses might obstruct not only wind-borne infected pollen from outside weeds but also wind-borne thrips. Raising pearl millet, sorghum, or maize (4–8 rows) as border crops around the field was effective in reducing the disease incidence.

More disease incidence has been recorded on groundnut in *kharif* season (rainy) than the *rabi* season due to plenty of virus inoculum by luxurious growth of parthenium. Early-sown groundnut crop suffers as compared to normal- and late-sown crops in *kharif* season (Prasada Rao et al. 2003). Further, optimum plant population should be maintained in the field as suboptimum population leaves bare patches in the field and facilitates thrips buildup due to discontinuation of green light reflectance.

Removal of weeds in fallow lands and roadside and on field bunds helps in reducing secondary inoculum. Among different weeds, *P. hysterophorus* is able to spread TSV-infected pollen more efficiently than other weeds. Hence, it has to be removed before coming to flowering. Moreover, sunflower and groundnut should not be grown side by side or at least avoid synchronization of flowering period of sunflower with groundnut crop as sunflower crop providing infective pollen inoculum with TSV.

Intercropping of red gram and bajra or red gram and castor with groundnut to reduces the disease intensity. Alternate cropping of sorghum, bajra, maize, castor, and sesamum with groundnut in endemic areas, at least once in 3 years, is recommended (Ghewande et al. 2002).

Host Plant Resistance

Various cultural and chemical management strategies evaluated in recent years have not been found satisfactory. A desired level of resistance of TSV has not yet been found in cultivated varieties of groundnut. In laboratory screening, 150 released cultivars of groundnut were found susceptible to TSV. Fifty-one wild *Arachis* accessions except *Arachis chacoense* (ICG 4983) were also found susceptible to TSV (Prasada Rao et al. 2003a). Kalyani et al. (2007) screened 56 groundnut germplasm accessions from 20 wild *Arachis* spp. in four sections (*Arachis*, *Erectoides*, *Procumbentes*, and *Rhizomatosae*), and no systemic infection was found in the following accessions, 8139, 8195, 8200, 8203, 8205,11550, CG 8144, and ICG 13210, even though 0–100 % infection was there on inoculated leaves. The following varieties were reported to have field tolerance of viral diseases from various institutes: AK 12–24, ALR 2, ALR 3, CSMG 84–1, DH 330, DRG 17 (Mukta), GG-7, ICGV 86590, JGN 3 (Jawahar Groundnut 3), JM 2, JM 24, JM 3, J-Mungfali −1, Jyoti, Kadiri 3, OG 52–1 (Smruti), R 8808 (Apoorva), R 9251, RSHY 1, TG 26, and Vemana (Ghewande et al. 2002).

At an agricultural research station, Kadiri, the following groundnut prerelease cultures were identified as tolerant lines against peanut stem necrosis disease caused by *tobacco streak virus*. All the lines were screened under high disease pressure (>80 %) using parthenium infector border (Vemana et al. 2015) (Fig. 15.1).

Spanish types: K-1535 (IPR), K-1470 (FDR)
Virginia types: K-1482 (FDR), K-1501 (LS), K-1504 (S) (LS), K-1504 (T) (LS), K-1563 (IPR), K-1574 (LS), K-1576 (LS), K-1577, K-1570 (TAF), K-1641 (LS), K-1643 (LS), K-1646 (LS), K-1648 (LS), K-1649 (LS), and K-1650 (LS)

Control of the Vectors

Seed treatment with imidacloprid (200SL) at 2 ml/l was very effective in controlling windborne thrips up to 20–25 DAS. Two foliar sprays using any systemic insecticide, namely, imidacloprid or dimethoate, at 25 and 45 DAS will be protect from thrips infection.

15.1.3 Peanut Clump Disease

The peanut clump disease of groundnut in India is caused by the *Indian peanut clump virus* (IPCV). The yield losses due to this disease may go up to 60 % in late-infected plants since such plants do not produce pods. The virus has a wide host range and several weeds commonly occurring in groundnut fields also get infected by the virus. IPCV are transmitted through seed (Nolt and Reddy 1984); soilborne fungus, *Polymyxa graminis*; and possibly nematodes (Ghanekar 1980).

Fig. 15.1 Screening block for peanut stem necrosis disease using *Parthenium hysterophorus* as infector rows

15.1.3.1 Distribution

Peanut clump disease has been reported from Punjab, Gujarat, Andhra Pradesh, Uttar Pradesh, and Rajasthan on crops grown in sandy soils. Wheat and barley crops were susceptible to *Indian peanut clump virus*-Hyderabad isolate (IPCV-H) under field conditions in Hyderabad, Andhra Pradesh (Delfosse et al. 1999).

15.1.3.2 Symptoms

The peanut clump disease appears in patches in the field and reappears in the same position in progressively enlarged patches, in succeeding years. The patchy appearance of the disease and its occurrence year after year in almost the same area of a field are due to the soilborne nature of the vector, *P. graminis*, and its survival as highly resistant resting spores. Typical symptoms are severe stunting of the plant apparent first on newly emerged leaves of two- to three-week-old seedlings. The newly emerged leaves show mottling and chlorotic rings. Later, the infected leaves turn dark green with faint mottling. Infected plants ultimately appear bushy and have small, dark green leaves and usually produce several flowers on erect petioles. The number and size of pods are reduced, resulting in small seeds. Root systems of infected plants get reduced in size and become dark. Their epidermal layer peels off easily (Nolt and Reddy 1984).

15.1.3.3 Host Range

The host range of IPCV includes many monocot and dicot plants which showed the highest incidence of weed species tested. The major hosts are wheat, barley, *Avena fatua*, *Cynodon dactylon*, *Digitaria ciliaris*, *Chenopodium murale*, *Cyperus rotundus*, *Sorghum halepense*, *S. bicolor*, *S. sudanensis*, *Dactyloctenium aegyptium*, *Cenchrus ciliaris*, *Eleusine coracana*, *Eragrostis ciliaris*, *E. tremula*, *E. unioloides*, *Pennisetum glaucum*, *Setaria italica*, *Triticum aestivum*, *Chenopodium album*, *Celosia argentea*, and *Oldenlandia corymbosa* (Delfosse et al. 1999;

Bhargava and Sobti 2000). Doucet et al. (1999) added *Eleusine coracana*, groundnut, maize, rice, sorghum, and finger millet to the list of host plants.

15.1.3.4 Causal Virus

Peanut clump virus (PCV) and *Indian peanut clump virus* (IPCV) belong to the genus *Pecluvirus*, family *Virgaviridae*. IPCV has predominantly two single-stranded RNA species with RNA1 and RNA2 (Reddy et al. 1985), and both were needed for production of lesion.

IPCV particle dimensions in leaf-dip preparations were 184 ± 8 by 24 ± 2 nm in uranyl acetate and 169 ± 5 and 239 ± 13 by 20 ± 1 nm in phosphotungstate (Thouvenel and Fauquet 1981). In IPCV-Ludhiana strain, 175 nm particles contain RNA2 and 235 nm particles contain RNA1, and both sizes of particles are needed to induce local lesions in bean tissues. IPCV isolates from India have been grouped into three distinct serotypes, viz., IPCV-H (Hyderabad), IPCV-D (Durgapura), and IPCV-L (Ludhiana).

Wesley et al. (1994) reported that the 5′-most open reading frame of the 4 kb RNA2 of IPCV encodes a protein of 208 amino acids. This protein is thought to be the coat protein of IPCV because its amino acid composition and M(r) closely resemble those reported for IPCV coat protein and because its amino acid sequence is 61 % identical to that of the coat protein of PCV from West Africa. The extent of the sequence identity between IPCV and PCV coat proteins confirms previous conclusions that the viruses are distinct rather than strains of one virus. The sequences of the coat proteins of IPCV and PCV were between 18 % and 26 % identical to those of other furoviruses and those of unrelated tobamoviruses and tobraviruses. In contrast, the coat protein sequences were 37 % (IPCV) and 36 % (PCV) identical to that of the coat protein of *barley stripe mosaic virus* (BSMV). This similarity between the coat proteins of viruses from different groups (=genera) is unusual but is consistent with previous reports of sequence relatedness in various genes between certain furoviruses and BSMV.

The RNA2 molecule of an isolate of the L serotype of IPCV consists of 4290 nucleotides with five open reading frames (ORF). The arrangement of the ORFs resembled that in RNA2 of PCV from West Africa. The proteins encoded by the ORFs in IPCV-L RNA are between 32 and 93 % identical to those encoded by PCV RNA. Partial sequence data for the RNA2 of isolates of the H and T serotypes of IPCV show that the coat and P40 proteins encoded by the 5′-most ORFs of RNA2 of IPCV-L, IPCV-H, and IPCV-T are as similar to each other as any is to the corresponding proteins of PCV. A conserved motif "F-E-x6-W" is present near the C-termini of the coat proteins of all three IPCV serotypes and of PCV, as it is in the coat proteins of other viruses that have rod-shaped particles, such as *tobacco mosaic virus* and *tobacco rattle virus*. The results support the distinction of IPCV and PCV as separate virus species (Naidu et al. 2000). The species are distinguished by different reactions with particular antisera (heterologous reactions are weak or undetectable). PCV occurs only in Africa, whereas IPCV occurs in the Indian subcontinent.

15.1.3.5 Transmission

The IPCV is transmitted through seeds up to 11 % in groundnut, pearl millet, wheat, and maize and therefore is of particular importance in germplasm collection (Reddy et al. 1998, 1999). IPCV was reported to be transmitted by the obligate fungal parasite (*Polymyxa graminis*) which is soilborne (Ratna et al. 1991). Natural virus transmission is highly influenced by the temperature (23–30 °C) during rainy season (Delfosse et al. 2002). Seed transmission in the field-infected groundnut plants ranged from 3.5 to 17 %, depending on the genotype. The transmission frequency was 48–55 % in seed collected from plants infected through seed (Reddy et al. 1998).

15.1.3.6 Management

Cultural Practices

Since the virus is seed borne, seed should be obtained from areas free from IPCV. It is possible to reduce the disease incidence by growing a crop non-preferred by *P. graminis*, the fungal vector, before raising groundnut crop in the rainy season. Such a crop would induce the germination of the resting spores, leading to a reduction in the inoculums of the fungus in the soil. Weed-free clean cultivation is very important in reducing the IPCV. As *P. graminis* multiplies intensively in monocots, those crops should be avoided in cropping systems with groundnut (Delfosse et al. 2002). *Cynodon dactylon* is a noxious weed commonly found in farmers' fields which can reproduce vegetatively through rhizomes and all the rhizomes of infected plant harbor the virus. Such rhizomes act as reservoirs of the virus, and the new roots arising from them can provide viral inoculum to the nonviruliferous *Polymyxa* and thus create a new nucleus of the disease.

Early sowing of the groundnut crop before the onset of monsoon rains and using judicious irrigation was a simple and effective cultural method of reducing incidence in irrigated areas. Peanut clump usually does not appear during the post-rainy season as IPCV transmission is correlated with temperature. Only negligible disease incidence has been observed when temperatures are below 25 °C (Reddy et al. 1988). During the rainy season, if sowing was delayed beyond the onset of the monsoon, most of the groundnut plants escape the disease. In summer, the soil temperature may easily reach 45 °C, and this dry heat is likely to break the dormancy of the spores of *Polymyxa* spp., and the first rains may induce the production of primary zoospores which infect even non-preferred hosts. However, preferred hosts of *Polymyxa* spp. such as sorghum, finger millet, pearl millet, and wheat can be infected throughout the year. Wheat was found to be infected even when the soil temperature varied from 17 to 24 °C, whereas the optimum temperature required for *Polymyxa* spp. was found to be between 25 and 30 °C for the IPCV isolates.

Host Plant Resistance

More than 8000 germplasm lines were tested under field conditions in Ludhiana and Bapatla, but none of them showed resistance or tolerance. Germplasm accessions, viz., NCAc 17099, NCAc 17133 (RF), and NCAc 17536, have been reported resistant to IPCV at ICRISAT. In field trials in India, the incidence of Indian peanut clump was reduced in groundnut plots where pearl millet had previously been grown as a bait crop (Delfosse et al. 1997).

15.1.4 Peanut Mottle

Peanut mottle virus (PeMoV) was first given its name in 1965 when it was isolated from peanuts in Georgia, USA. This virus was found to be seed transmitted in this host. In India, the virus was reported by Reddy et al. (1978). Subsequently, this disease has been reported to occur on *rabi*/summer groundnut from Andhra Pradesh, Gujarat, and Maharashtra.

15.1.4.1 Distribution

Peanut mottle disease has been reported to occur on *rabi*/summer groundnut mainly in Andhra Pradesh, Maharashtra, and Gujarat. Surveys conducted in *rabi*/summer of 1983 revealed that the incidence of the disease ranged from 3.7 to 40 % in cultivated bunch varieties (Ghewande 1983). The disease can cause up to 30 % loss in yield (Kuhn and Demski 1975).

15.1.4.2 Symptoms

Newly formed leaves show mild mottling and vein clearing, whereas older leaves show upward curling and interveinal depression with dark green islands. Infected plants are not severely stunted, and older plants seldom show typical disease symptoms. Some pods from plants infected with PeMoV may be smaller than normal and have irregular, green to brown patches. Seeds from such pods are discolored.

15.1.4.3 Host Range

PeMoV occurs in several important legume crops, including groundnut, bambara groundnut

and soybean, and weeds. The PeMoV occurs in nature on several important legume crops. The virus has been isolated from *Pisum sativum*, *Glycine max*, and forage legumes and a few weed hosts like *Cassia obtusifolia*, *C. leptocarpa*, *C. occidentalis*, and *Desmodium canum* in nature. Transmission through seed appears to be the most important source of PeMoV for groundnut. Most commercial peanut seed lots have a low frequency (<15.0 %) of seed infection. However, a frequency as low as 0.1 % will provide about two infected seedlings per 100 m^2 in a field. Aphids are efficient vectors of PeMoV and will transmit the virus rapidly to nearby plants (Kuhn and Demski 1984).

15.1.4.4 Causal Virus

PeMoV is a plant pathogenic virus of the family *Potyviridae*, genus *Potyvirus*. PeMoV is a flexuous, non-enveloped, filamentous virus with particles ranging from 723 to 763 nm in length and 12 nm in diameter (Pietersen and Garnett 1992). In infected plant cells, the virus makes characteristic *Potyvirus* cylindrical inclusions that are visible in the light microscope with proper staining.

15.1.4.5 Transmission

Infected groundnuts are considered to be the primary sources of PeMoV (Prasada Rao et al. 1993), and other nearby leguminous crops become infected from this crop. The virus is seed transmitted in a range from 0.1 to 3.5 % depending on the type of cultivar (Bashir et al. 2000). Adams and Kuhn (1977) reported that seed transmission is due to the presence of the virus in the embryo. PeMoV is transmitted in a stylet-borne (nonpersistent) manner by *Aphis craccivora*, *Myzus persicae*, *A. gossypii*, *Hyperomyzus lactucae*, *Rhopalosiphum padi*, and *R. maidis.*

15.1.4.6 Management

Cultural Practices

PeMoV can be managed by avoiding primary infection through seed by growing virus-free seeds, observing quarantine regulations, avoiding secondary infection by aphids through chemical sprays, sowing trap crop that attracts aphids, growing the crop barriers to minimize vector mobility for long distances, and controlling weed hosts.

Host Plant Resistance

Several lines of *Arachis* species like *A. glabrata* are reported to be resistant to this disease. Germplasm lines, viz., NCAc 2240 and NCAc 2243, have been reported tolerant to peanut mottle virus at ICRISAT, Patancheru, India.

15.1.5 Peanut Stripe Disease

A virus, first reported from China in 1983 as producing a mild mottle in peanut (*A. hypogaea*), was further characterized and named *peanut stripe virus* (PStV) in the USA. It has since been reported from major groundnut-growing areas in Southeast Asia including India and has been found to cause economically significant crop losses. In India, the PStV was observed in 1987 in eight entries originated from Junagadh, Gujarat, which had been grown for multi-locational evaluation under the All India Coordinated Research Project on Oilseeds. The disease incidence has been recorded up to 40 % (Varma et al. 1994). PStV infection has a highly variable effect on groundnut yield, depending on the test conditions, cultivar, and the virus isolate. In India, it is regarded as a quarantine disease. To contain the spread of the virus further and to eliminate chances of its establishment, all the genotypes in the varietal trials at all the 34 test locations in the country and most of the breeding materials developed at the National Research Centre for Groundnut were destroyed. This was followed by regular monitoring and destruction of all suspected samples. Thus, within 2 years after its first appearance, the virus was eradicated from all the groundnut research centers in the country.

15.1.5.1 Distribution

PStV is widespread in groundnut-growing areas throughout East and South Asia. It was first detected in India in 1987 and then in all groundnut-growing areas of Indonesia, Malaysia, Myanmar, Philippines, Thailand, and Vietnam (Demski et al. 1993).

15.1.5.2 Symptoms

In India, the initial symptoms appear as chlorotic flecks or rings on young leaves which persisted in the older leaves. The plants are slightly stunted. Subsequently, the older leaves show symptoms with mild mottle, blotch, stripe, chlorotic ring mottle, chlorotic line pattern, oak leaf pattern, or necrosis (Wongkaew and Dollet 1990). The symptoms normally persist throughout plant development. The "stripe" (V-shaped pattern) and "necrotic" islands, which are seen less often, can severely stunt the plants if they infect them early.

15.1.5.3 Host Range

Seed transmission frequency has been found to range from 12 % (JL 24) to 29 % (Kadiri 3). The host range include *Glycine max*, *Medicago sativa*, *Vigna radiata*, *V. unguiculata*, *Sesamum indicum*, and *Cassia tora*. The virus may be transmitted by *Aphis craccivora* and *A. glycines* in a nonpersistent manner. Higher incidence of the virus was observed in the post-rainy season than in the rainy season. This may be due to the presence of larger numbers of aphids during the post-rainy season.

15.1.5.4 Causal Virus

PStV particles are filamentous flexuous rods, approximately 752 nm long and 12 nm in diameter which belong to the genus *Potyvirus*. Each particle consists of single protein pieces of 33.5 K. The genome is single-stranded (ss) positive-sense RNA molecule of about 9500 nucleotides. The particles are relatively stable and can be stained with 2 % phosphotungstate or ammonium molybdate pH 6.5 (Demski et al. 1993).

The virus is serologically closely related to *blackeye cowpea mosaic virus* (BICMV), *azuki bean mosaic virus* (AzMV), and the serogroup B strains of *bean common mosaic virus* (BCMV) and distantly to *clover yellow vein virus* and *soybean mosaic potyvirus*. PStV was initially described as a new virus, but recent molecular studies have shown that it is a strain of BCMV which also includes BICMV and AzMV and three potyvirus isolates from soybean (Mishra et al. 1993).

15.1.5.5 Transmission

The virus is transmitted by several species of aphids in a nonpersistent manner, which is also the only means of disease spread under field conditions. *A. craccivora* is the major vector for the transmission of PStV. Apart from *A. craccivora*, *Myzus persicae*, and *A. gossypii*, *Hysteroneura setariae* have been shown to be highly efficient PStV vectors for the transmission of the disease. PStV transmission through groundnut seed can be as high as 37 % in artificially inoculated plants (Demski et al. 2004). Under natural conditions, however, the transmission frequency is up to 7 %. PStV seed transmission frequency can be influenced by the virus isolate, groundnut cultivar, and environment. The virus can be detected in both the embryo and the cotyledon, but not in the seed testa.

15.1.5.6 Management

Since the virus was of quarantine significance, all the efforts were to irradiate the pathogen by totally destroying the infected materials. On the management of the PStV disease, systematic studies were not reported from India.

15.1.6 Future Line of Research

To deal with the emerging and serious virus problems, an integrated approach, wherein the strength of the conventional approaches as well as the technological advancements in the field of biotechnology, has to be adopted. This includes the systematic screening of the genotypes and cultivation of virus-resistant plants developed through conventional breeding or transgenic technology. Adoption of appropriate cultural practices and effective management of vectors and alternate hosts are also equally important. Since in most of the viral diseases sources with desired levels of genetic resistance could not be identified so far, transgenic approaches to engineer resistance to viruses by expressing the glycoproteins of tospoviruses in transgenic plants to block virus acquisition by thrips, by expressing truncated or modified forms of movement protein(s) of heterologous viruses, or by express-

ing virus-specific antibody genes may be adopted. Transgenic lines of groundnut resistant to *Indian peanut clump virus* and GBNV and(or) TSV using coat protein (CP) and nucleocapsid protein (N) gene, respectively, have recently been developed at ICRISAT, Patancheru, and ICAR-DGR, Junagadh. Now it has become possible to develop antibodies specific to the viral nonstructural gene products, and thus it has become possible to differentiate transmitters from non-transmitters in vector populations. This approach can potentially be used to develop a disease forecasting system.

Once sources with desired level of genetic resistance to the viruses or the vectors were identified, the approaches like marker-assisted breeding can be exploited. Active collaboration between Indian laboratories with leading international virology lab may help in accelerating the developments.

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Status of Viruses Infecting Sunflower and Strategies for their Management

16

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Abstract

Among different diseases infecting sunflower (*Helianthus annuus*) worldwide, sunflower necrosis, sunflower mosaic, and sunflower leaf curl are important diseases in India. After thorough investigation, the causal organism was identified as *tobacco streak virus* (TSV) for sunflower necrosis disease (SND) in India. It belongs to the genus *Ilarvirus* and family *Bromoviridae*. It appeared as epidemic form from 1997 to 1999 in Southern India and caused yield loss up to 90 %. It is occurring in all the sunflower-growing states in India such as Andhra Pradesh, Karnataka, Tamil Nadu, and Maharashtra. It poses potential threat to the cultivation of sunflower in India. The early infection kills entire plants and produces characteristic symptoms, namely, necrosis of leaf, petiole, stem, and bracts and malformation of head with chaffy grains. TSV has wide host range infecting both crop and weed species growing in sunflower cropping system. TSV is pollen borne, and its seed transmission is not yet proved from any crop and weed plants. Infection is occurring in the presence of both infective pollen and thrips, and relationship of vector with virus is nonpersistent as there is no specificity between virus and vector. In India, effective screening was carried out against TSV in hot spots. However, complete resistant sources are not available in both germplasm and varieties/hybrids. Hence, effective breeding approaches should be developed to augment the resistance using resistant A line/CMS lines/R lines. Marker-assisted selection (MAS) has also not been exploited in this crop against TSV due to lack of resistant sources. Effective management practices such as late sowing (October sowing), border cropping (7–11 rows of sorghum), removal of weeds

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before flowering, intercropping with castor and red gram, treating the seed with imidacloprid (5 g/kg seed) or thiamethoxam (4 g/kg), and vector control using systemic insecticide at 15, 30, and 45 days after sowing were very effective to contain the disease. Systemic epidemiological studies were conducted in India and established the positive correlation of SND with thrips population and low temperature. So, effective disease forewarning system to be planned as the primary inoculum of the virus is provided by weed hosts prevalent in and round the sunflower fields. So far, coat protein (CP) gene of sunflower TSV isolate was very well characterized in India. Hence, CP gene-mediated resistance should be incorporated in the cultivated high yielders by genetic transformation using biotechnological tools. Combination of conventional breeding methods, different management practices, and transgenics should be adopted to tackle the SND menace in India.

Keywords
Sunflower • Viral diseases • *Tobacco streak virus* • Disease management

16.1 Introduction

Sunflower (*Helianthus annuus* L.) is an important edible oilseed crop in the country next to groundnut and soybean. The crop was introduced into India during 1969 which accounts for nearly 5 % of the current oil seed production. In India, the crop is cultivated in an area of 1.48 million hectares with production of 0.9 million tonnes (DOR Annual Report, 2010). Karnataka, Andhra Pradesh, Maharashtra, and Tamil Nadu are the major sunflower-growing states.

The crop is highly vulnerable to various diseases incited by viruses, bacteria, phytoplasma, and fungi (Kolte 1985) resulting in severe economic losses. Several viruses belonging to *Cucumo-*, *Ilar-*, *Poty-*, *Tospo-*, and *Umbravirus* groups are known to infect sunflower (Brunt et al. 1996). Several virus and viruslike diseases have been reported by various workers on sunflower, both in India and abroad (Table 16.1). Sunflower mosaic, chlorotic mosaic, yellow ring mosaic, yellow mosaic, yellow spot, chlorotic leaf, greening, cucumber mosaic, and mycoplasma-like organisms (strain of tomato big bud, aster yellows, and phyllody) have been reported.

16.2 Sunflower Mosaic Disease

16.2.1 Symptoms

The affected plants show mosaic patterns accompanied by ring spots or chlorotic spots that show tendencies to coalesce to form mosaic pattern, cupping, and malformation of leaves (Uppal 1933; Gupta 1981). The virus is restricted to sunflower only. *Nicotiana tabacum*, *N. glutinosa*, and *Capsicum annuum* are not infected by the virus. Another mosaic disease was reported which shows the presence of small circular chlorotic spots on leaves, which coalesce to form typical mosaic patterns (Venugopal Rao et al. 1987). Cupping and malformation of leaves, poorly developed root system, reduction in seed yield, seed viability, and pollen fertility are some of the associated symptoms.

16.2.2 Causal Organism

Sunflower mosaic virus (strain of *cucumber mosaic virus*)

Table 16.1 Viral diseases of sunflower and their transmission

S. No.	Disease	Virus	Genus	Transmission	References
1.	Sunflower mosaic disease	*Cucumber mosaic virus*	*Cucumovirus*	Seed and *A. gossypii*; *Myzus persicae*	Arnott and Smith (1967), Orellana and Quacquarelli (1968), Gupta et al. (1977), Gupta (1981), Gupta and Roy (1981), Venugopal Rao et al. (1987), Jitendra Mohan (1992), and Nagaraju et al. (1997)
2.	Sunflower ring spot disease	*Sunflower ring spot virus*	*Ilarvirus*	Thrips sp.	Behncken (1974)
3.	Yellow blotch disease (YBD) and leaf crinkle disease (LCD)	*Sunflower crinkle virus* (SuCV)	*Umbra virus*	*A. gossypii*	Theuri et al. (1987)
4.	Leaf curl disease	*Tobacco leaf curl virus*	*Begomovirus*	*Bemisia tabaci*	Chatterjee and Pandey (1977)
5.	Sunflower necrosis diseases (SND)	*Tobacco streak virus* (TSV)	*Ilarvirus*	Thrips sp.	Dijkstra (1983), Prasada Rao et al. (2000), Ravi et al. (2001), and Bhat et al. (2002b)
6.	Sunflower mosaic disease	*Sunflower mosaic virus* (SuMV)	*Potyvirus*	*Myzus persicae* and *Capitphorus elaegni*	Jindal et al. (2001) and Verma et al. (2009)
7.	Sunflower leaf curl disease	Sunflower leaf curl virus (SuLCuV)	*Begomovirus*	*Bemisia tabaci*	Govindappa et al. (2011)

16.2.3 Transmission and Host Range

The virus is reported to be sap transmitted (Battu and Pathak 1996). It is also transmitted through graft and seeds. The important vectors are aphid species, i.e., *Aphis gossypii*, *A. craccivora*, and *Myzus persicae*. The virus has a dilution end point of 1:500–1:1000, thermal inactivation point of 55–65 °C, and longevity in vitro for 66–72 h at room temperature (Nagaraju 1997). The host range of this virus is narrow infecting only one weed host (*Galinsoga parviflora*).

16.3 Yellow Ring Mosaic Disease

16.3.1 Symptoms

The disease shows severe mosaic accompanied by stunting and malformation of young leaves in the form of yellow rings (Gupta and Gupta 1977). Affected plants do not produce flowers or reduction of flower number.

16.3.2 Causal Organism

Yellow ring mosaic virus

16.3.3 Transmission and Host Range

The virus is transmitted both by mechanical sap and leaf grafting. The virus has limited host range producing chlorotic local lesions only on *Chenopodium amaranticolor*.

16.4 Yellow Blotch and Leaf Crinkle Diseases

This disease was reported from African countries (Theuri et al. 1987) and affected 80 % of the plants in certain fields.

16.4.1 Symptoms

In yellow blotch disease (YBD), initially short, irregular yellow vein bands appear, and later they coalesce to form distinct bright yellow blotches measuring 1–3 cm in diameter, but the plant height and leaf morphology are unaffected.

In leaf crinkle disease (LCD), irregular yellow bands appear as in yellow blotch, but also include severe leaf puckering, starting with the youngest leaves, reduced leaf size, and stunted plants.

16.4.2 Causal Organism

Virus particles are isometric, 26 nm in diameter, and serologically related to *beet western yellows virus*.

16.4.3 Transmission and Host Range

Both diseases can be transmitted by mechanical sap, and aphid (*A. gossypii*) was able to transmit YBD. Viruliferous aphids which transmit YBD include *Arachis hypogaea*, *Glycine max*, *Lactuca sativa*, *Nicotiana clevelandii*, and *Zinnia elegans*. There are no known sources of resistance against this virus.

16.4.4 Management

Clean cultivation by removing the weeds both inside the field and neighboring fields and removal and destruction of infected plants reduce further spread of the disease. Spraying of suitable insecticides to control the insect vectors as a prophylactic spray is recommended (Basappa et al. 2005).

16.5 Sunflower Mosaic Disease

In India, *sunflower mosaic virus* (SMV) incidence ranged from 5 to 10 %, and disease appears mostly in *kharif* season. It belongs to the genus *Potyvirus*. The disease will not have much impact on yield reduction. However, there are reports that SMV infection reduced the plant height, stem girth, leaf area, head size, and seed weight of sunflower hybrids (Jindal et al. 2001; Verma et al. 2009). So far, limited research work has been attempted with regard to sunflower mosaic disease since they do not cause much economic loss.

16.6 Sunflower Leaf Curl Disease

Recently, begomoviruses transmitted by whitefly *Bemisia tabaci* causing symptoms like leaf curl (Fig. 16.1), leaf thickening, enations, and stunting are emerging threat to sunflower cultivation (Govindappa et al. 2011). Sunflower leaf curl disease (SuLCuD) was observed to the extent of 40 % on "Sunbred 275" hybrid during *rabi* 2009 in the fields of University of Agricultural Science (UAS), Raichur, Karnataka. Phylogenic analysis of the core CP gene sequence of the virus with those of other begomoviruses clustered next to *tomato leaf curl virus* isolate and shared 97.5 % nucleotide identities. However, exact taxonomic status requires sequencing of the complete ssDNA viral genome. Tentatively, the virus name is given as sunflower leaf curl virus.

Fig. 16.1 Sunflower plants with marginal upward leaf curling and vein thickening

16.7 Sunflower Necrosis Disease (SND)

Sunflower necrosis disease (SND) has become a potential threat in all traditional sunflower-growing areas in India. SND was noticed in an epidemic form consecutively for 3 years (1997–1999), with the incidence ranging from 10 to 80 % and yield loss up to 90 % in most of the sunflower-growing regions of Southern India (DOR Annual Report 2001). The causal agent of SND was identified as *tobacco streak virus* (TSV) of genus *Ilarvirus* (Prasada Rao et al. 2000; Ravi et al. 2001; Bhat et al. 2002b).

16.7.1 Distribution

SND was reported from Netherlands, Australia (Sharman et al. 2008), and India. It was reported for the first time in India at Bagepalli village of Kolar district, Karnataka, in 1997 (Singh et al. 1997) which later spread to AP, TN, and Maharashtra. The disease was noticed in an epidemic form consecutively for the 3 years (1997–1999) in most of the sunflower-growing regions of Southern India (DOR Annual Report 2000). In AP, maximum incidence of SND (38 %) was reported during October 2003. Similarly, the highest average thrips population/head (16.3 thrips/head) was recorded during March 2004 on sunflower cv. Morden at Chevella, Narkhoda, and Kavvaguda villages of Ranga Reddy district during fortnightly survey (Upendhar et al. 2007).

The necrosis disease monitoring in Aland Taluk alone in Gulbarga district in Karnataka, India, indicated that out of 23,000 ha area planted, 12,142 ha (52.79 %) area suffered severe crop loss due to necrosis virus at early growth stage (45 days) during 2002–2003 (Narayana and Chandramohan 2007).

In Southern Karnataka, highest incidence of 22 % was reported on cv. KBSH-1 with mean thrips population of 2.42 per five plants at Bagepalli Taluk on May 2006 sown crop (Lokesh et al. 2008b), whereas in Northern Karnataka, highest incidence of SND (36 %) and thrips population (9.6 thrips/3 leaves) was reported in Raichur district (Swamy et al. 2010a).

16.7.2 Economic Importance

The intensity of disease ranged from 2 to 100 %. The seed yield losses are as high as 89 % under severe conditions (DOR Annual report 2001). The disease has significant impact on the crop as early infection either kills the plant or causes

severe stunting with malformed head or heads filled with chaffy seeds (Ramiah et al. 2001a; Ravi et al. 2001). Early infected plants remain stunted and develop malformed heads with poor or no seed setting, resulting in complete loss of the crop (Jain et al. 2003). There has been a continuous threat to sunflower production in India due to TSV and reduction of over 40% in the yield since 1997, amounting to annual loss of Rs.76 crores (Jain et al. 2003).

16.7.3 Etiology

The necrosis disease of sunflower is of recent origin. Initially, the causal virus of SND was reported as *tomato spotted wilt virus* (TSWV) of *Tospovirus* group and later identified as *tobacco streak virus* (TSV) of *Ilarvirus* group. Jain et al. (2000) and Venkata Subbaiah et al. (2000) reported an association of a *Tospovirus* with necrosis disease-affected sunflower samples collected from Bangalore, Dharwad, and Hyderabad that has serological affinities to *peanut bud necrosis virus* (PBNV). Jain et al. (2000) studied the serological relationship of sunflower necrosis virus with the antiserum of the members of "*Tospovirus*" serogroups at Indian Agricultural Research Institute (IARI), New Delhi, and reported a strong reaction of this virus with the antiserum of *watermelon silver mottle virus* (WSMV) and PBNV and concluded that the virus belonged to "*Tospovirus*" serogroup.

Later, on the basis of serological cross-reaction with *tobacco streak virus* antiserum, nucleic acid species, molecular weight of virus coat protein, and aphid non-transmissible nature, the causal virus of SND was confirmed as *tobacco streak Ilarvirus* (Prasada Rao et al. 2000). Serological relationship was demonstrated only with *tobacco streak virus* (TSV), which was confirmed by Western blot analysis and IEM decoration assays using SNV and TSV antisera in reciprocal tests (Ravi et al. 2001).

Bhat et al. (2002a) developed RT-PCR assay for the detection of TSV from sunflower using primers derived from coat protein (CP) gene of *tobacco streak virus*. On the basis of serological relatedness and sequence identity, it was proposed that the sunflower *Ilarvirus* from India should be considered as a strain of TSV belonging to subgroup I and designated as TSV-SF.

16.7.4 Viral Genome

Tobacco streak virus (TSV), first described by Johnson (1936), is the type species of the genus *Ilarvirus* of the family *Bromoviridae* that includes viruses having tripartite quasi-isometric particles of size 27–35 nm. The virus has three nucleoprotein particles designated as RNA-l (3.5 kb), RNA-2 (3.0 kb), and RNA-3 (2.3 kb) and a coat protein of c. 28 kDa. RNAs 1–3 are genomic and encode proteins la (119 kDa), 2a (91 kDa), and 3a (32 kDa), respectively, whereas RNA-4a (0.9 kb) and RNA-4 (1.0 kb) are subgenomic expressed from RNA-2 and RNA-3 which encodes 2b (22 kDa) and coat proteins (28 kDa), respectively. TSV genome is infectious only in presence of its coat protein or RNA-4.

16.7.5 Symptoms

The disease is observed at all growth stages starting from seedling to maturity. The characteristic field symptoms of the disease include extensive necrosis of leaf lamina, petiole, stem and floral calyx and complete death of seedlings (Fig. 16.2a–f). Early infection either kills the plant or causes severe stunting with malformed head or heads filled with chaffy seeds (DOR Annual Report 2000; Ramiah et al. 2001a; Ravi et al. 2001). Necrosis at bud formation stage makes the capitulum to bend and twist resulting into complete failure of seed setting (Chander Rao et al. 2002).

16.7.6 Host Range

Host range studies carried out by several workers revealed that the virus causing SND could infect members belonging to families Amaranthaceae, Chenopodiaceae, and Fabaceae (Ramiah et al.

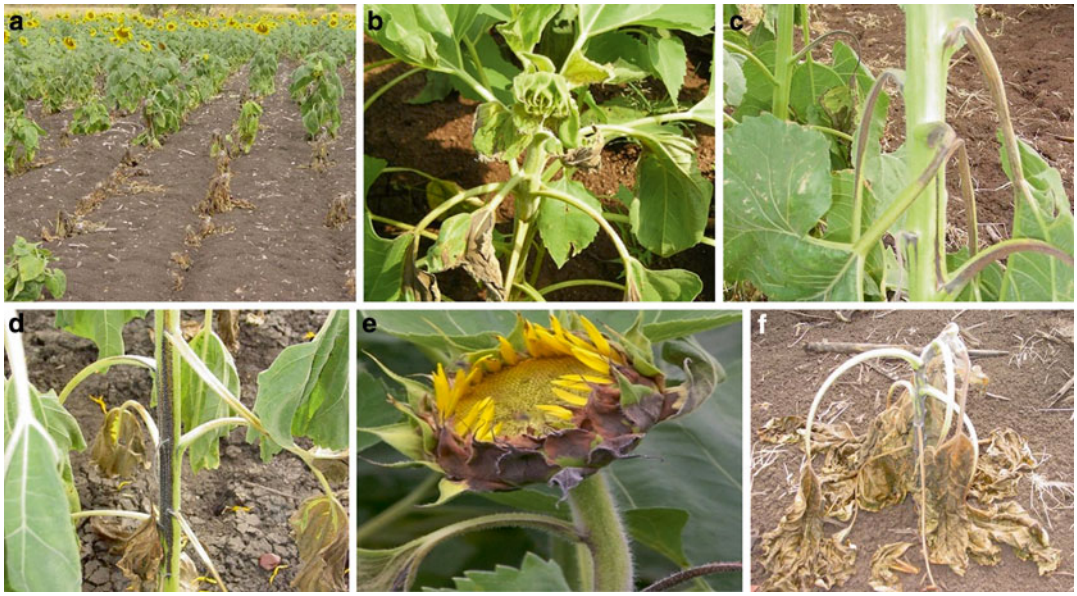

Fig. 16.2 (**a**) Sunflower seedling death, (**b**) leaf deformity, (**c**) petiole necrosis, (**d**) stem necrosis, (**e**) blackening of bracts, (**f**) complete death of plant

2001b); Asteraceae, Leguminosae, and Cucurbitaceae (Anjula and Nagaraju 2002); and Fabaceae, Malvaceae, Cucurbitaceae, and Solanaceae (Lavanya et al. 2005b). The weeds such as *Trianthema portulacastrum*, *Priva leptostachya*, *Digera arvensis*, *Clitoria ternatea*, *Solanum nigrum*, *Vernonia cineraria*, *Trichodesma indicum*, and many other species were found to serve as hosts for sunflower necrosis virus. Out of 43 plant species belonging to eight families tested for their reaction to SND by sap inoculation, 33 plant species from six families, viz., Asteraceae, Chenopodiaceae, Cucurbitaceae, Leguminosae, Malvaceae, and Solanaceae, expressed visible symptoms (Bharati bhat 2011).

Natural occurrence of TSV infection has also been recorded from other crops, such as cotton from Aurangabad, sunn hemp from Bangalore, mungbean from Coimbatore (Bhat et al. 2002b); groundnut from Hyderabad (Reddy et al. 2002); cucumber, gherkin, and okra from Karnataka (Krishna Reddy et al. 2003a, b); safflower from Maharashtra (Chander Rao et al. 2003b); chili from Uttar Pradesh (Jain et al. 2005); sunn hemp from Hyderabad (Santha Lakshmi Prasad et al. 2005); urd bean from Tamil Nadu (Ladhalaxmi et al. 2006); niger from Karnataka and soybean from Maharashtra (Arunkumar et al. 2007); cotton from Warangal district, AP (Prasada Rao et al. 2009b); and onion from Kurnool, AP (Sivaprasad et al. 2010). Vemana and Jain (2010) tested 70 plant species belonging to 18 families by sap inoculation with TSV – groundnut isolate and reported that 50 species belonging to Fabaceae, Asteraceae, Solanaceae, Cucurbitaceae, Malvaceae, Amaranthaceae, Chenopodiaceae, Umbelliferae, Aizoaceae, Commelinaceae, and Portulacaceae were susceptible to TSV.

The host range studies clearly indicated that several weed species occurring in sunflower fields and in crop plants such as groundnut, green gram, black gram, cowpea, and soybean were most common TSV hosts under field conditions (Table 16.2).

Table 16.2 Natural infection of TSV in crop and weed hosts

Cultivated species	Weed species
Capsicum annuum, *Gossypium hirsutum*, *Vigna unguiculata*, *Cucumis sativus*, *Cucumis anguria*, *Arachis hypogaea*, *Tagetes erecta*, *Guizotia abyssinica*, *Abelmoschus esculentus*, *Carthamus tinctorius*, *Glycine max*, *Helianthus annuus*, *Crotalaria juncea and Vigna mungo*, *Vigna radiate*	*Abutilon indicum*, *Acalypha indica*, *Achyranthes aspera*, *Acanthospermum hispidum*, *Calotropis gigantea*, *Cleome viscosa*, *Commelina benghalensis*, *Croton bonplandianum*, *C. sparsiflorus*, *Digera arvensis*, *Euphorbia hirta*, *E. geniculata*, *Lagasca mollis*, *Leucas aspera*, *Parthenium hysterophorus*, *Tridax procumbens*, and *Xanthium strumarium*

16.7.7 Transmission

16.7.7.1 Sap Transmission

The disease was transmitted by mechanical sap inoculation from sunflower to sunflower cv. Morden under laboratory conditions (Nagaraju and Hanumantha Rao 1999; Venkata Subbaiah et al. 2000). In general, *Ilarvirus* had wide host range as they were efficiently sap transmissible to many of the host plants belonging to Amaranthaceae, Chenopodiaceae, and Fabaceae under glasshouse conditions (Ramiah et al. 2001b; Jain et al. 2003).

16.7.7.2 Thrips Transmission

The major method of transmission of TSV is by infected pollen, which can be spread by wind or carried by insects. Thrips do not become infected with the virus, but transport the infected pollen on their bodies (Prasada Rao et al. 2003b). The causal virus of SND has been reported to be transmitted by various species of thrips in the presence of infected pollen grains of weed species. Thrips, while feeding, not only cause injury to leaf tissue but also deposit pollen and facilitate virus transmission to the plant. So far, no virus-vector specificity was established for this virus. Chander Rao et al. (2009) reported that pollen and thrips collected from TSV-infected *Parthenium* weed released together recorded 58.3 and 70 % disease incidence at vegetative and flowering stages of the crop. The results indicated that thrips along with the infected pollen together can spread the SND. So far, five species of thrips in AP, viz., *F. schultzei*, *S. dorsalis*, *T. palmi*, *M. usitatus*, and *H. gowdeyi* (Upendhar et al. 2007), and in Northern Karnataka, viz., *T. palmi*, *F. schultzei*, *B. melonicornis*, *H. gowdeyi*, and *T. hawaiiensis* (Swamy et al. 2010a), were recorded on sunflower.

The vector *T. palmi* successfully transmitted the virus to sunflower test plants, when an acquisition access period (AAP) of 2–3 days and inoculation access period (IAP) of 3–5 days were given (DOR Annual Report 2002; Aravind et al. 2005; Lokesh et al. 2005, 2008a; Swamy et al. 2010b).

16.7.7.3 Seed Transmission

Seed transmission was very well proved in different isolates of TSV worldwide both in seeds of naturally infected and artificially sap-inoculated plants belonging to different families. However, in India, seed transmission was not found in naturally or experimentally infected groundnut (Reddy et al. 2007; Vemana and Jain 2010), sunflower (Prasada Rao et al. 2009a; Bharati bhat 2011), urd bean, mungbean, soybean, French bean, marigold, *C. quinoa*, *G. globosa*, and *P. hysterophorus* (Prasada Rao et al. 2009a).

So far, TSV seed transmission was not proved from any crop or weed hosts in India as reported earlier by several workers. Hence, it is concluded that non-seed-transmissible strain of TSV might be existing in India. In the absence of seed transmission, primary inoculum of the TSV is provided by secondary hosts and weed hosts prevalent in and around the sunflower fields by thrips vector.

16.7.8 Epidemiology

16.7.8.1 Survival of the Virus

Studies on survival of TSV revealed the survival of virus throughout the year on several weeds, viz., *Parthenium hysterophorus*, *Tridax procumbens*, *Phyllanthus* sp., *Euphorbia geniculata*, and

Digera arvensis (DOR Annual Report 2002). Of the 35 common weed species collected from in and around the sunflower fields, 12 weeds showed natural infection of TSV such as *D. arvensis*, *A. aspera*, *Lagasca mollis*, *P. hysterophorus*, *A. hispidum*, *A. conyzoides*, *C. bengalensis*, *E. geniculata*, *Phyllanthus niruri*, *Malvastrum coromandelianum*, *Abutilon indicum*, and *Physalis minima* by back inoculation on assay host (Cowpea cv. C-152) and further confirmed by DAC-ELISA (Bharati bhat 2011).

TSV epidemiology in groundnut in Anantapur district was well studied (Prasada Rao et al. 2003a, b). Since virus is not seed-borne in groundnut, it has to be introduced from external sources. Weeds, viz., *Parthenium hysterophorus*, *Abutilon indicum*, *Ageratum conyzoides*, *Croton sparsiflorus*, *Commelina bengalensis*, *Cleome viscosa*, *Euphorbia hirta*, *Lagasca mollis*, and *Tridax procumbense*, were found to be the most common weed hosts for TSV under field condition. Of these, *Parthenium* is the most widely distributed and is a symptomless carrier of TSV and produces several flushes of flowers during its life cycle ensuring continuous supply of TSV-infected pollen. It harbors the virus as well as thrips and produces copious pollen throughout the season, acting as a primary source of inoculum initiating and sustaining the TSV infection during a crop season. Thrips colonizing flowers of these plants can become externally contaminated with pollen, and movement of these thrips to new hosts results in introduction of the virus into fields. Windblown pollen of *Parthenium* contaminates the leaves, and thrips arriving independently may well contribute to infection. Heavy westerly winds that occur during August and September facilitate deposition of virus-carrying pollen onto groundnut plants from the infected *Parthenium*, and other weed sources and virus transmission occur when such plants are colonized by the thrips. Cleistogamous species like groundnut are dead-end hosts.

16.7.8.2 Effect of Different Dates of Sowing

The disease incidence is higher in *kharif* and summer seasons, whereas it is low in *rabi* season. The sunflower cultivars sown during July and August had high necrosis incidence compared to post-rainy season, i.e., September onward (Shirshikar 2003).

Dry weather (July–August) with moderate temperature of 30–32 °C and 55–75 % relative humidity is conducive for thrips incidence. Higher population of thrips during *kharif* sowing was reported in sunflower cv. Morden (Singh 2005; Upendhar et al. 2006, 2009).

Epidemiological studies on SND indicated the positive correlation between thrips population and the weather parameters, viz., maximum and minimum temperature, sunshine, and dry spells, whereas negative correlation was observed with rainfall and relative humidity (DOR Annual Report 2006). Positive correlation of thrips population with maximum temperature and negative correlation with minimum temperature, RH-I, RH-II, and rainfall were established. Disease incidence showed a positive correlation with minimum temperature, RH I, RH-II, and rainfall. However, negative correlation with maximum temperature was observed. Besides, positive correlation between thrips population and disease incidence existed (Shivasharanayya and Nagaraju 2003; Upendhar et al. 2006, 2009).

16.8 Management Options

16.8.1 Host Plant Resistance

The most economical and convenient way to manage TSV is to grow resistant varieties. So far, complete resistant varieties/hybrids were not available in sunflower. Effective screening was carried out at the Regional Agricultural Research Station, Nandyal; Directorate of Oilseeds Research (DOR), Hyderabad; and University of Agricultural Science (UAS), Dharwad.

At DOR, sap inoculation technique has been optimized for large-scale screening of sunflower genotypes against SND. About 500 genotypes comprising the released cultivars, diverse inbreds, cytoplasmic male sterile (CMS) lines and restorer lines, germplasm accessions, and few derivatives of wild sunflower species were screened against

SND, and the degree of severity varied among the lines tested (Chander Rao et al. 2002). Twenty perennial wild *Helianthus* species were screened against SND, and none of the tested species produced the symptoms probably due to the indeterminate and perennating habit of the species studied. Systematic studies have been undertaken for identification of reliable sources of resistance to SND in wild sunflowers (Sujatha 2006). Babu et al. (2007) screened 30 hybrids along with their parents against sunflower necrosis disease under natural conditions, using 0–4 scale. Fourteen hybrids recorded resistant reaction, compared to check cv. Morden which showed highly susceptible reaction.

Mantur et al. (2002) took five improved cultivars, viz., KBSH-44, KBSH-1, Surya, PAC-1091, and Jwalamukhi, to evaluate the incidence of sunflower necrosis. The study revealed that the highest incidence was observed in Surya (16.44 %) followed by KBSH-44 (14 %), Jwalamukhi (8.2 %), and PAC-1091 (5.5 %), and least incidence was observed in KBSH-1 (3.9 %). Ranganatha et al. (2003) screened sunflower genetic stocks for necrosis disease in three sets during 2001–2002. The hybrids PCSH-245, KBSH-1 and exotic selection REC-430 and REC-435 recorded low necrosis (6.8–7.5 %). The populations DRSF-111 and GAUSUF-15 and selections REC-436 and Sel-Master recorded higher disease incidence (34.3–40.8 %). Among gene pool in hybrids, GP 9-217-4-4, 9-472-7-4, 9-163-8, 96-33E-4-2, 9-755-2, 9-709-4, 1–1737, 1–1880, 1334–21, and 2150–4 recorded very low disease incidence (3–5 %). However, GP9-152-5-4, 9-152-7, 1–1341, and 1–2087 inbreds recorded higher necrosis (42.5–47.5). In general hybrids indicated better tolerance than the populations and inbreds. Ajith Prasad (2004) reported that among the 96 genotypes screened during summer season, only eight (RHA-284, RHA-5D-1, RHA-265, RHA-859, RHA-297, RHA-365, CR-1, and R-214-NBR) were not infected by the disease, whereas the incidence in the field ranged from 0.0 to 16.66 %. In *kharif* 2003, among the 167 genotypes screened, 40 were not infected by the disease and the incidence ranged from 0.0 to 54.5 %. Bestar (2004) reported that out of 115 germplasm lines screened against SND, 27 germplasm lines were free from disease. The GMU 22 recorded the highest incidence of 24 % followed by 21 % in GMU 8 and 20.

16.8.2 Cultural Methods

Several cultural practices have been suggested for management of various virus diseases in crop plants that could help to prevent and contain the diseases. Cultural practices such as dates of sowing, border cropping, intercropping, roguing, optimum plant population, removal of weed hosts, etc., have been advocated by several workers to reduce disease incidence and intensity (Almeida and Corso 1991; Almeida et al. 1994; DOR Report 2002; Chander Rao et al. 2002; Prasada Rao et al. 2003b; Basappa and Santha Laksmi Prasad 2005; Lava Kumar et al. 2008).

16.8.2.1 Removal of Weed Hosts

Removal of virus sources (mostly weeds) germinated with early rains can reduce the TSV incidence. Similarly, removal of weeds in fallow lands and roadsides and on field bunds helps in reducing secondary inoculum. Moreover, sunflower and groundnut should not be grown side by side or at least avoid synchronization of flowering period of sunflower with groundnut crop as sunflower crop provides infective pollen inoculum with TSV. Removal of infected groundnut plants from the field will have no effect, as infected plants do not contribute to secondary spread. Similarly, removal of early infected sunflower will not reduce disease incidence as early infected sunflower does not produce flowers.

16.8.2.2 Border Cropping

Higher incidence of TSV (58–59 %) was recorded in tobacco crop, near the roadsides where the maximum weed population exists and infection reduced inside the field (Greber et al. 1991). During the survey of TSV epidemic on groundnut in Anantapur district of Andhra Pradesh, India, it was observed that natural barriers such as tall grasses in the field protected the adjacent

crops from the disease. The tall grasses might obstruct not only wind-borne infected pollen from outside weeds but also wind-borne thrips. Sowing 7–11 rows of fast-growing cereals (pearl millet, sorghum, or maize) as border crop around fields which obstructs the movement of thrips from landing on crop plants was found to reduce disease incidence in sunflower (DOR Report 2002; Chander Rao et al. 2002; Basappa and Santha Laksmi Prasad 2005) and groundnut (Prasada Rao et al. 2003b). Mesta et al. (2004) reported that the use of border crop-like sorghum reduced incidence of SND from 18 to 37 %.

16.8.2.3 Optimum Plant Population

Bare patches in the field attract thrips landing. Optimum plant population discourages thrips landing on the groundnut crop indicating maintenance of optimum plant population is one of the options for the management of TSV infection.

16.8.2.4 Dates of Sowing

Date of sowing of crops mainly depends on rainfall pattern and distribution. Early-sown groundnut crop was better than normal and late-sown crop in *kharif* season with special reference to TSV infection (Prasada Rao et al. 2003b). Shirshikar (2003) opined that SND could be minimized if sunflower is sown in the post-rainy season, i.e., from September onward. Upendhar et al. (2006 and 2009) reported that SND incidence was high during August (35.04 %) and September (49.93 %) and low during October (20.75 %).

16.8.2.5 Neighboring Crops

TSV susceptible crops like marigold and chrysanthemum should not be grown adjacent to sunflower fields.

16.8.2.6 Intercropping

Intercropping with red gram or castor was found to reduce disease intensity compared to monocropping of sunflower and groundnut (Prasada Rao et al. 2003b; Jain et al. 2006). Pearl millet, sorghum, and maize as intercrops in mung bean maintained their superiority in containing thrips population with 71.4, 61.5, and 57 % reduction and 68.9, 61.7, and 60.2% reduction over sole crop during *kharif* and *rabi* seasons, respectively. The same intercrops suppressed the disease incidence with 85, 83.5, and 79.2 % reduction and 65.8, 60.7, and 59.2 % reduction over sole crop during *kharif* and *rabi* seasons, respectively (Sreekanth et al. 2004).

16.8.3 Chemical Methods

Seed treatment with imidacloprid at 5 g/kg seed and imidacloprid (0.5 %) spray reduced disease incidence with higher yield compared with other treatments (DOR Annual Report 2006; Lokesh et al. 2008d). Management trial for SND at AICRP on oilseeds revealed that seed treatment either with imidacloprid at 5 g/kg seed or thiamethoxam at 4 g/kg seed followed by two sprays at 30 and 45 days found to reduce necrosis disease and increase seed yield significantly over untreated control (DOR Annual Report 2008; Shirshikar et al. 2009).

16.8.4 Antiviral Compounds

Use of various antiviral materials such as *Prosopis*, goat milk, and *Bougainvillea* as alone and in combination was used to induce the resistance in sunflower against TSV (Lavanya et al. 2005a). Among them, *Bougainvillea spectabilis* with goat milk, *Prosopis chilensis* with goat milk, *Bougainvillea spectabilis* alone, and *Prosopis chilensis* alone were found highly effective in inducing the resistance in sunflower against SND. The combination of treatments involving plant products with goat milk was more effective than the individual ones. Significantly enhanced PR proteins like β-1,3-glucanase and oxidative enzymes like peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase were observed in sunflower using above antiviral materials.

16.8.5 Biological Methods

Srinivasan and Mathivanan (2009) reported plant growth-promoting microbial consortia (PGPMC)-mediated biological control of SND under field conditions for the first time. Powder and liquid formulations of two PGPMCs (PGPMC-1, consisting of *Bacillus licheniformis* strain MML2501 + *Bacillus* sp. strain MML2551 + *Pseudomonas aeruginosa* strain MML2212 + *Streptomyces fradiae* strain MML1042, and PGPMC-2, consisting of *B. licheniformis* MML2501 + *Bacillus* sp. MML2551 + *P. aeruginosa* MML2212) were evaluated along with farmers' practice (imidacloprid + mancozeb) in farmers' fields. Significant disease reduction, increase of seed germination, plant height, and yield parameters with an additional seed yield of 840 kg ha^{-1}, an additional income of Rs. 10,920 ha^{-1}, and benefit-cost ratio of 6.1 were recorded using powder formulation of PGPMC-1.

16.8.6 Integrated Management

Management strategies such as altering sowing dates, seed treatment with imidacloprid to control thrips vector, barrier crops with fast-growing tall cereals to prevent vector movement, removal of TSV-susceptible weed hosts, and maintaining optimal plant population were found to significantly reduce disease incidence (Chander Rao et al. 2002; Prasada Rao et al. 2003b; Jain et al. 2006).

Six rows of sorghum as border crop and treating the seed with imidacloprid (5 g/kg seed) along with three sprayings with imidacloprid (0.5 %) at 15, 30, and 45 days after sowing minimized the incidence of SND in cv. KBSH-1 (Shirshikar 2008). Sunflower crop could be protected from heavy loss of yield due to necrosis virus disease by spraying of systemic insecticide, imidacloprid at 0.5 ml/l at 15, 30, and 45 DAS combined with seed treatment by imidacloprid (5 g/kg) along with border crop (sorghum) (Lokesh et al. 2008c). Seed treatment with imidacloprid at the time of sowing, spraying of oxydemeton-methyl at 45 days, growing border crop sorghum around the field and application of vermicompost + 50 % recommended dose of fertilizer, and application of NSKE (5 %) at 30 DAS were effective in reducing sucking pest complex and necrosis disease incidence (Katti et al. 2010). An integrated disease management strategy was worked out against SND at field level for two consecutive years during *kharif* season. Of 15 different treatments tested, seed treatment with thiamethoxam (4 g/kg seed) + three sprays of thiamethoxam (0.05 %) at 15, 30, and 45 DAS + three rows of border crop (sorghum) was found best in not only reducing the disease incidence and thrips population but also increasing the yield and yield attributes of sunflower cv. Morden (Bharati bhat 2011).

16.8.7 Transgenic Approach

A number of transgenic crops resistant to an infective virus have been developed by introducing a sequence of the viral genome in the target crop by genetic transformation. Virus-resistant transgenics have been developed in many crops by introducing either viral CP or replicase gene encoding sequences. So far, in India, not much effort was made to develop transgenics in sunflower crop due to lack of regeneration protocols and cross pollination nature of crop.

More than 95 % area under sunflower crop in India is covered by commercial hybrids based on the ***H. petiolaris* (*Pet 1*)** cytoplasm. At DOR, Hyderabad, attempts were made to identify new sources of CMS from wild *Helianthus* (Sujatha 2006). Interestingly, such a nuclear-cytoplasmic interaction had been identified in combinations, where *H. argophyllus* (designated as *Arg* cytoplasm) was used as ovule parent and several lines with the same cytoplasmic background and different phenotypic expressions have been developed. Also systematic studies were undertaken for identification of reliable sources of resistance to SND, and the respective gene has been cloned, but the limitations in plant regeneration and

transformation protocols for sunflower need to be circumvented so as to tackle the problem through transgenic approach.

16.9 Conclusion

Among different viruses infecting sunflower crop in India, *tobacco streak virus* is posing severe threat to sunflower production. Desired level of resistance to TSV both in cultivated species and in the germplasm of sunflower is not available as the screened cultivars/germplasm/CMS lines/R lines were found susceptible against TSV in laboratory screening. Therefore, there is an urgent need to search the resistance sources both in native and exotic germplasm which can be exploited to augment TSV resistance through breeding programs. As an alternative strategy, developing transgenic resistance is also desirable to combat this dreaded disease in sunflower. A combined effort of biotechnology and traditional breeding may further enhance opportunities for development of resistant varieties to SND. Effective screening should be carried under artificial epiphytotics using *Parthenium* infector border. Adoption of appropriate cultural practices and effective management of vectors and alternate hosts are also equally important. Moreover, marker-assisted selection (MAS) cannot be exploited in sunflower crop. To develop a marker, choose either DNA route or protein route (reverse genetics) using resistant or susceptible sources. Complete molecular characterization of virus associated with SND in order to identify the strains of TSV in different geographical regions of the country. Further studies are needed to ascertain the prevalence of TSV pathotypes/serotypes originating from different sunflower locations in the country.

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Viral Diseases of Banana and Their Management

17

Savarni Tripathi, Basavaprabhu L. Patil, and Raj Verma

Abstract

Banana (*Musa spp.*) is one of the most important staple food crops which provide nutrition and income for the millions of farmers worldwide, especially in tropical regions. Asia being the major continent for banana production contributes more than half of the world banana production (101.9 million tonnes). Banana is vegetatively propagated using suckers or through tissue culture plants which grow, mature and fruit without seasonality throughout the year. Viral diseases are considered a major concern for banana production because of their effects on yield and quality as well as limitations to germplasm multiplication and the international germplasm exchange. There are many (about 20) different viruses reported to infect banana worldwide. However, the economically most important viruses are: *Banana bunchy top virus* (BBTV), *Banana streak viruses* (BSV), *Banana bract mosaic virus* (BBrMV) and *Cucumber mosaic virus* (CMV). Among these, BBTV and BSV are major threats for banana production. Of the two, BSV exist as episomal and endogenous forms and more widely spread worldwide than BBTV, though later is so far most economically damaging virus contributing to a yield reduction of up to 100 %. Due to lack of durable virus resistance in the *Musa spp.*, measures such as phytosanitation, use of virus free planting material, strict regulation on movement of infected planting materials are effective means to control viral diseases in banana. Studies of several decades on the biology, epidemiology,

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survival, spread, sequence integration into the host genome of banana viruses and their integrated management strategies are summarized in this review.

Keywords
Banana bunchy top virus • Banana streak viruses • Banana bract mosaic virus, Cucumber mosaic virus • Musa Spp.

17.1 Introduction

Banana, a large herbaceous plant belonging to the genus *Musa* of family Musaceae, is among the world's most important staple food crops. Banana is cultivated in over 130 countries and is the major source of carbohydrate and income for millions of farmers in tropical regions of the world. In 2012, total banana production was 101.9 million tonnes from 4.9 million hectares. Asia being the major continent for banana contributes more than half of the world banana production. The total export value of banana was estimated to be US$ 894.6 million in 2011 (FAOSTAT 2015).

Most of the cultivated varieties of banana are sterile, parthenocarpic triploids, and derived from the two seedy species, *Musa acuminata* and *M. balbisiana*, contributing the A and B genomes, respectively (Ortiz 2013). Banana can have diploid, triploid, or tetraploid genomes, and cultivated banana includes hybrids within or between the two species. Originating in the Asia-Pacific region, *M. acuminata* was domesticated as early as 8000 BC, whereas hybridization with *M. balbisiana* probably occurred in India where the latter species originates (Price 1995). The banana germplasm moved as population migrated throughout the Asia-Pacific region followed by movement westward into the Middle East and Africa. Human migration and trade, especially in the Asia-Pacific, have played a major role in the wide dissemination of cultivars into Africa and Latin America (De Langhe and De Maret 1999; Perrier et al. 2011). More than 1000 *Musa* accessions have been identified in collections worldwide with a wide range of morphological traits and genome constitutions (Ortiz 2013; Pollefeys et al. 2004). Banana plants are vegetatively propagated which grow, mature, and fruit without seasonality throughout the year. Suckers spring up from the underground rhizome to replace the main shoot that withers after fruiting, and this process of succession continues indefinitely (Morton 1987). Farmers generally use young suckers removed from the old plantations to establish new fields. This practice has been among the major causes of outbreaks of several banana diseases and pests around the world (Jones 2002) especially viruses which are perpetuated along with the planting material.

About 20 different virus species representing five different families have been reported to infect banana worldwide. However, the most economically important viruses are *Banana bunchy top virus* (BBTV, genus *Babuvirus*, family *Nanoviridae*), *Banana streak virus* (BSV, genus *Badnavirus*, family *Caulimoviridae*), *Banana bract mosaic virus* (BBrMV, genus *Potyvirus*, family *Potyviridae*), and *Cucumber mosaic virus* (CMV, genus *Cucumovirus*, family *Bromoviridae*). Other viruses of minor significance are *Abaca bunchy top virus* (ABTV, genus *Babuvirus)*, abaca mosaic disease caused by a distinct strain of *Sugarcane mosaic virus* (SCMV) designated as SCMV-Ab (genus *Potyvirus*), *Banana mild mosaic virus* (BanMMV), and *Banana virus* X (BVX), the latter two being unassigned members in the family *Betaflexiviridae*.

Viral diseases are a major concern for banana production because of their effects on yield and quality, as well as limitations to the international germplasm exchange. This chapter summarizes the knowledge on the biology, epidemiology, survival, spread, sequence integration into the host genome of banana viruses, and their integrated management strategies.

17.2 Major Viral Diseases of Banana

17.2.1 Banana Bunchy Top Disease

Banana bunchy top disease (BBTD) is the most important and devastating disease of banana (Dale 1987). The disease was first recorded in 1889 in Fiji and has since spread to a number of countries in the South Pacific, Asia, and Africa (Magee 1927). BBTD is widespread in 36 countries of Asia, Pacific, and Africa (Blomme et al. 2013; Diekmann and Putter 1996; Jones 2013; Kumar et al. 2011) but there are no records of BBTD in the New World except in Hawaii (USA) (Conant 1992).

BBTD is a serious problem in many banana-growing areas. In Fiji, the first outbreak of BBTD was recorded in 1953, leading to a loss of 641,000 bunches (Magee 1953). In the 1920s, the Australian banana industry in New South Wales had collapsed, and the total area of production decreased by 90%. A similar situation also existed in Southern Queensland where more than 95% production was reduced (Magee 1927). In India, BBTD is reported to cause serious losses in many states involved in banana cultivation. An annual loss of about Rs. 4 million has been reported due to the disease in the state of Kerala alone. One of the most significant outbreaks of the disease in prized hill banana cv. Virupakshi was in the state of Tamil Nadu, India, which reduced the production area from 18,000 to 2000 ha (Kesavamoorthy 1980). Several bunchy top epidemics during 2007–2010 in Koduru, Andhra Pradesh, and Jalgaon, Maharashtra, India, caused an annual loss of US$50 million (Selvarajan and Balasubramanian 2014). A severe outbreak of BBTD was also reported from Pakistan where the production area was reduced to 26,000 ha from 60,000 ha (Khalid et al. 1993).

17.2.1.1 The Virus

Banana bunchy top virus (BBTV) is the causal virus for the BBTD (Magee 1940). BBTV is a multicomponent, circular, single-stranded DNA virus. BBTV is also the type member of the genus *Babuvirus* in the family *Nanoviridae* (King et al. 2012). The isometric virion measures 18–20 nm in diameter. BBTV genome contains at least six circular single-stranded (ss) DNA molecules and each component encoding for a protein from its single open reading frame (ORF) in the virion sense. These are designated as DNA-R (rolling circle replication initiation protein), DNA-S (coat protein peptide of 19.6 kDa), DNA-M (movement protein), DNA-C (cell cycle link protein), DNA-N (nuclear shuttle protein), and DNA-U3 (a protein of unknown function) (Beetham et al. 1999; Burns et al. 1995; Vetten et al. 2012). Each DNA components has two common regions: (1) common stem-loop region of 69 nucleotides with 62% conserved between viral components and (2) major common region of 92 nucleotides with 76% conserved between viral components (Burns et al. 1995).

BBTV isolates are grouped in two distinct subgroups, namely, Pacific-Indian Oceans (South Pacific) group and the Southeast Asian (Asian) group based on sequence analysis of DNA-R. The Pacific-Indian Oceans group includes isolates from Africa, Australia, Hawaii, South Asia, and Tonga, while the Southeast Asian group includes BBTV isolates from China, Indonesia, Japan, the Philippines, Taiwan, and Vietnam (Banerjee et al. 2014; Karan et al. 1994; Stainton et al. 2012; Yu et al. 2012). The sequence variation of DNA-R within isolates of each subgroup ranged from 1.9 to 3%, while variation between isolates of the two subgroups was as high as 10% (Karan et al. 1994). BBTV isolates, especially in the East Asian region, carry a satellite DNA component that encodes a protein homologous to Rep encoded in DNA-R (Horser et al. 2001).

17.2.1.2 Symptoms

The initial evidence of BBTV infection is noticed on the leaf veins, midrib, and petiole as discontinuous dark-green streaks of variable length. These symptoms are sometimes referred as "Morse code streaking" because the streaks are irregular and resemble a series of dots and dashes. Rubbing the waxy white coating on petiole and midrib makes it easier to see the streaking. Also, dark-green hooklike extensions can be seen in a narrow, light-green zone between the midrib and lamina. The short hooks point down along the midrib towards the petiole, and this can be seen

best by backlighting the leaf against the sky. At later stage in infected plants, new leaves emerge with difficulty and are narrower with wavy leaf lamina and yellow leaf margin. As the successive leaves of infected plants emerge, they are seen to be more and more abnormal. Leaves become progressively smaller in size with limited elongation of petiole. Leaves remain abnormally erect. When several abnormal leaves have emerged, an extreme congestion or bunching appears at the top of the plant (Fig. 17.1A), the symptom from which the disease is named. BBTV-affected and healthy leaves are different in texture. The petiole, midrib, and lamina of infected plants are harsh and brittle and can be easily snapped when bent or crushed in contrast to healthy ones being elastic and pliable in nature. Severely infected plants usually do not fruit, but if produced, the banana hands and fingers are likely to be distorted and twisted. The suckers that develop from infected mother plants have been infected with the virus and are severely stunted with the leaves that do not expand normally and remain bunched at the top of the pseudostem. These leaves are stiff and erect and are shorter and narrower. These infected suckers do not produce fruits. Occasionally, bracts of male flower buds turn to a leafy structure and exhibit dark-green dots and streaks (Thomas et al. 1994).

17.2.1.3 Disease Transmission, Host Range, and Epidemiology

Banana aphid (*Pentalonia nigronervosa*) is known to be the major vector of BBTV (Magee 1927). Banana aphid has worldwide distribution and transmits the virus in a persistent and circulative manner (Anhalt and Almeida 2008; Selvarajan et al. 2006). The primary spread of BBTV was reported to be through conventional planting materials including corms and suckers. All the infected suckers from infected stool eventually become infected. 100 % spread of BBTV was recorded in case of vegetative propagules including the suckers and corms (Magee 1927).

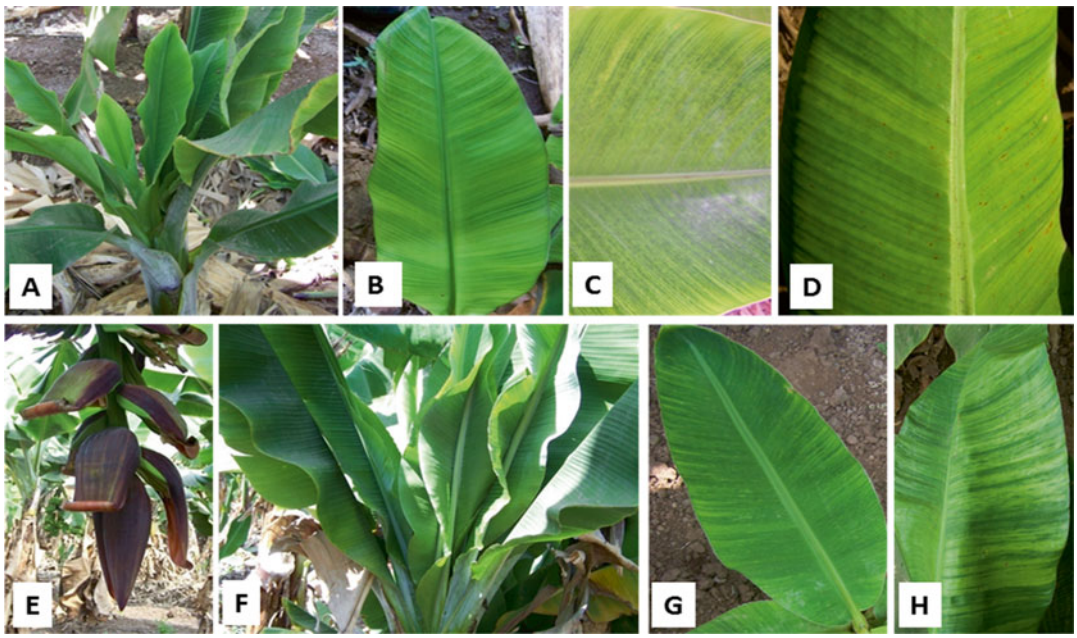

Fig. 17.1 Symptoms of viral diseases on banana plant. Progressive shortening and bunching of the leaves of banana plant infected by *Banana bunchy top virus* (**A**), chlorotic yellow dots and streaks (**B,C**) that sometimes turn necrotic (**D**) on banana leaves affected by *Banana streak virus*, distinctive mosaic pattern, spindle-shaped purplish streak on bract (**E**) and the appearance of the traveler's palm (**F**) of banana plant affected by the *Banana bract mosaic virus*, mild chlorotic (**G**) and severe chlorotic (**H**) streaks on leaf lamina of banana plant infected with *Cucumber mosaic virus*

BBTV is also known to be transmitted through micropropagated banana plants (Drew et al. 1989; Ramos and Zamora 1990; Wu and Su 1991); however, the rate of transmission is not always 100 %. Another species of banana aphid, *P. caladii*, has been shown to transmit BBTV at a lower efficiency than *P. nigronervosa* (Watanabe et al. 2013). *P. nigronervosa*, an efficient vector of BBTV, is known to be highly specific to *Musa* species (Robson et al. 2006). On banana, aphids are frequently observed near the base of pseudostem at the soil level and several centimeters below the soil surface (Robson et al. 2006).

The aphid vector can spread BBTV from a primary source to an estimated mean distance of 15.2 m in Australia (Allen 1987). A high rate of population growth of banana aphid, *P. nigronervosa*, was recorded at 25 °C (Robson et al. 2007). The transmission efficiency of BBTV by the aphid vector has been ranged from 46 to 67 %. The retention time of BBTV in vector after removal from the virus source was 13–20 days (Magee 1927; Hu et al. 1996). In Hawaii, the aphid was shown to transmit BBTV after the 4 h of minimum acquisition access period and required 15 min of minimum inoculation access period (Hu et al. 1996). However, in Australia, the minimum acquisition access period of 17 h and 30 min to 2 h minimum inoculation access period was reported for BBTV transmission (Magee 1927). There was no evidence of multiplication of BBTV in the aphid vector (Hafner et al. 1995). Anhalt and Almeida (2008) reported that adult aphids were more efficient in transmitting the virus as compared to the third instar nymphs.

BBTV is known to infect a range of *Musa* species (Thomas and Iskra-Caruana 2000). The virus infects natural and synthetic hybrids of *M. paradisiaca*, abaca (*M. textilis*) (Sharman et al. 2008), and *Ensete ventricosum* (Selvarajan and Balasubramanian 2013). However, plants belonging to the species *Alpinia*, *Heliconia*, *Canna*, and *Strelitzia* often found growing in the banana production zones which were found negative for BBTV infection (Geering and Thomas 1997). One report on BBTV detection in *Colocasia esculenta* in India (Ram and Summanwar 1984) was not proved unequivocally (Geering and Thomas 1997; Hu et al. 1996).

An infected plant and a vector in favorable environment are required to develop the disease epidemics. Infected planting materials (suckers) play a key role in the epidemic and long-distance spread of BBTD. The secondary spread or dissemination over short distance from these infection foci is by the aphid vectors. Allen (1978) estimated a distance of secondary spread of BBTV by aphids from initial source of infection to be 15.5–17.2 m in a commercial banana plantation. Nearly two-thirds of new infections were within 20 m of the nearest source of virus inoculums, and 99 % were within 86 m.

Epidemics can be a combination of several factors which include the presence of a large number of aphid vector population and distance of primary source of infection. A study in Australia reported that if the new plantation was located adjacent to diseased plantation, the chance of spread of BBTD into new plantation within the first 12 months was 88 %, and this chance was reduced to less than 5 % if the plantations were separated by 1000 m (Allen and Bemier 1977). Opina and Milloren (1996) also reported most of the new infections were either adjacent or of close proximity to the source of infection.

17.2.2 Banana Streak Disease

Banana streak disease is known to be the most widely distributed in banana plantations throughout the world. The disease was first observed in the Nieky Valley on the Ivory Coast in 1958 (Lockhart and Jones 2000b). Followed by, the disease was reported from Morocco where almost every established plantation was infected. The etiology of the disease was confirmed and first time identified the causal virus in Morocco (Lockhart 1986). The disease is now reported to occur in over 43 countries of Africa, Asia, Australia, Europe, Oceania, and tropical America (Diekmann and Putter 1996).

17.2.2.1 The Virus

Banana streak virus (BSV), a member of the genus *Badnavirus* of family *Caulimoviridae*, is the causal virus of the disease. The virions of BSV are bacilliform-shaped (120–150 × 30 nm), double-stranded circular DNA (dsDNA) genome approximately 7.2–7.8 kb long that uses a virus-encoded reverse transcriptase (RT) to replicate (Lockhart and Olszewski 1993). Harper and Hull (1998) were the first to describe the structure of the viral genome and named it as *Banana streak Obino l'Ewai virus* (BSOLV). The virus genome has three consecutive ORFs on one strand (King et al. 2012). ORF1 and ORF2 potentially encode for two small proteins of unknown function of 20.8 and 14.5 kDa. ORF3 is a large polyprotein of 220 kDa encoding at least four proteins encompassing a putative cell-to-cell movement protein, a coat protein (analogous to retroviral GAG), an aspartic protease, and a viral replicase consisting of RT and RNase H domains (Harper and Hull 1998; King et al. 2012). This polyprotein is cleaved into functional units by the aspartic protease once it has been fully translated.

BSV isolates show a high degree of heterogeneity and differ serologically, biologically, and at genomic level as well. This characteristic of BSV has created problem for reliable virus diagnosis (Lockhart and Olszewski 1993).

It has been shown that the BSV genomic sequences are integrated into the genome of banana (LaFleur et al. 1996). There are two infectious forms of BSV that exist: (i) the episomal form resulting from cells/plant infection following transmission by mealy bugs and (ii) endogenous forms which are endogenous viral sequences of BSV integrated within the banana genome. The development of episomal BSV infection from integrated sequences is associated with micropropagation and possibly other stress factors (Ndowora et al. 1999). Physical stresses have been associated to induce de novo viral particles (episomal form) from integrated BSV sequences possibly through intra-strand homologous recombination (Cote et al. 2010; Lheureux et al. 2003; Chabannes and Iskra-Caruana 2013; Iskra-Caruana et al. 2010). Both virus forms are able to infect plants systemically and cause disease (Harper et al. 1999; Harper et al. 2004; Iskra-Caruana et al. 2010), and the virus particles from both origins can be transmitted by mealybugs (Dahal et al. 2000; Kubiriba et al. 2001; Lockhart and Autrey 1988). More details of BSV sequence integration into banana genome are described in Sect. 17.4 of this chapter.

17.2.2.2 Symptoms

Symptoms of BSV vary from an inconspicuous chlorotic flecking to lethal necrosis depending on virus isolate, host cultivar, and the environmental conditions. However, the most common symptoms produced by most isolates are discontinuous sometimes continuous chlorotic or yellow dots or streaks that turn necrotic which run from the midrib to the leaf margin (Fig. 17.1B-D). In some cases, spindle or eye-shaped pattern or blotches are noticed. Sometimes the leaf lamina can be distorted. At later stage, the streaks darken to orange and often become brown or black. Necrosis has been observed to occur on the leaf midrib and petiole especially under low temperature and short-day conditions. Symptoms can be sparse or concentrated in distribution. Generally, symptoms are erratically distributed on the plant and not shown on all leaves. Some isolates are so severe that they immediately produce stunting with severe necrotic streaks, leading quickly to the death of the plant (Thangavelu et al. 2000). Other reported symptoms of BSV are streaks on pseudostem, falling away of the leaf bases from the pseudostem, narrow and thicker leaves, and constriction of bunch on emergence (Lassoudie're 1979). Stunting, reduced bunch size and distortion of fingers have also been reported (Gauhl and Pasberg_Gauhl 1994). Sometimes, broad yellow line in leaf lamina parallel to the midrib, purple margin on leaf lamina, leaf twisting, and an abnormal arrangement of the leaves similar to traveler's palm have also been observed in plant infected with BSV. Symptomatic and symptomless stages alternate in infected plants, but virus can be detected at all stages (Harper et al. 2002; Lassoudie're 1974; Lockhart and Jones 2000b).

17.2.2.3 Disease Transmission, Host Range, and Epidemiology

BSV fail to transmit by mechanical inoculations. The initial or long-distance spread occurs through vegetative propagation of infected planting materials such as suckers and micropropagated plantlets from infected banana plant. In field, spread of the virus from infected to healthy banana plant occurs by mealybug in a semipersistent manner. Field observations suggest that virus spread is slow (Daniells et al. 2001) with no difference in isolate transmission observed between two main vector species *Planococcus citri* and *Pseudococcus* spp. (Dahal et al. 2000; Kubiriba et al. 2001; Matile-Ferrero and Williams 1995). Other species reported to transmit BSV are *Dysmicoccus* spp. in West Africa and South America, *Planococcus musae* in Nigeria, *Ferrisia virgata* (striped mealybug) in India (Selvarajan et al. 2006), *D. brevipes* and *P. ficus* (Meyer et al. 2008), and *Paracoccus burnerae* (Muturi et al. 2013) in South Africa.

Unlike most *Badnaviruses*, BSV has a very limited natural host range. BSV infects most of the cultivated land races of cultivated banana. Moreover, the epidemiology of banana streak disease remains surprisingly unclear, and the role of host genome-integrated BSV still needs to be understood.

Pink sugarcane mealybug (*Saccharicoccus sacchari*) has been able to transmit the sugarcane bacilliform virus, a closely related virus, from sugarcane to banana experimentally and produced characteristic BSV symptoms (Lockhart and Autrey 1991). In many tropical regions, banana is found growing in close proximity to sugarcane, and it may be possible that sugarcane may act as source of inoculum for banana (Jones 1994; Lockhart 1994). Field reports suggest that natural spread of BSV by mealybugs on banana is limited and does not play a major role in disease epidemiology (Lockhart 1995).

17.2.3 Banana Bract Mosaic Disease

Banana bract mosaic disease, caused by the *Banana bract mosaic virus* (BBrMV), was first noted on several banana cultivars in the Philippines (island of Mindanao) in 1979 and thought to be different from all other recognized viruses of banana (Magnaye and Espino 1990; Rodoni et al. 1997). Later, BBrMV was found widespread throughout the Philippines. The disease was given the name bract mosaic at a meeting of banana virologists held in Los Baños in 1988 and includes a list of viruses of quarantine importance (Ferison and Putter 1989). Occurrence of the virus was discovered in other Asian countries including India, Samoa, Sri Lanka, Thailand, and Vietnam (Diekmann and Putter 1996; Rodoni et al. 1997; Rodoni et al. 1999). In Latin America, BBrMV occurrence was first reported from Colombia (Quito-Avila et al. 2013).

Limited reports are available on the economic impact of the disease. Kenyon et al. (1997) reported up to 40 % yield loss on Mindanao island of the Philippines. High rejection rate of marketable fruit was associated with the higher disease incidence which causes misshapen fingers. In Hawaii (USA), BBrMV was detected in ornamental ginger plants (*Alpinia purpurata*) but not in *Musa* (Wang et al. 2010). Yield losses between 30 and 70 % have been recorded in India and the Philippines (Cherian et al. 2002; Magnaye and Espino 1990; Thangavelu et al. 2000).

17.2.3.1 The Virus

BBrMV belong to the genus *Potyvirus* and family *Potyviridae*. Flexuous filamentous virus particles measuring 750×11 nm have been detected (Bateson and Dale 1995). Purified virions contain a major coat protein of 38–39 kDa. The virus genome consists of single-stranded positive-sense RNA of 1197 nucleotides long excluding the 3'-terminal poly(A) tail. The viral genome contains a typical large ORF of 9378 nucleotides coding for a polyprotein of 3125 amino acids with 9 protease cleavage sites, potentially yielding 10 matured functional proteins that have motifs conserved among homologous proteins of other potyviruses (Balasubramanian and Selvarajan 2012; Ha et al. 2008; Rodoni et al. 1997). The whole genome of BBrMV-TRY (India) and BBrMV-PHI (the Philippines) had 94 % nucleotide sequence identity and 88–98 %

amino acid sequence identities (Balasubramanian and Selvarajan 2012). Studies of genetic analysis of the CP gene of 49 isolates revealed a greater variation among them, and two of the isolates from Tamil Nadu were distinct with 18–21 % divergence (Balasubramanian and Selvarajan 2014).

17.2.3.2 Symptoms

The virus typically caused distinctive mosaic patterns on bracts. Spindle-shaped purplish streaks on bracts (Fig. 17.1e), pseudostems, midribs, peduncles, and even in fruits are characteristic symptoms of the virus (Rodoni et al. 1997; Selvarajan and Jeyabaskaran 2006; Thomas et al. 1997). In some cases, symptoms on the pseudostem are chlorotic on red background and reddish, yellow, or chlorotic on a green background. The symptom color may darken through red to brown and even black. Occasionally, chlorotic and spindle streaks appear on the leaves running parallel to the veins. Petiole and peduncles of Nendran banana become brittle, and fruits of infected plant rarely get to maturity. Bunches from infected plants unusually contain a long or very short peduncle, and in some cultivars, such as Nendran, the leaves appear as "traveler's palm" plant (Fig. 17.1f) (Balakrishnan et al. 1996). Necrotic streaks on fruits, leaves, pseudostems, and midribs have also been recorded (Selvarajan and Jeyabaskaran 2006).

17.2.3.3 Disease Transmission, Host Range, and Epidemiology

The primary source of infection occurs through virus-infected vegetative planting material. The BBrMV is transmitted by several aphid species (*P. nigronervosa*, *Rhopalosiphum maidis*, *Aphis gossypii*, *A. craccivora*) in a nonpersistent manner (Selvarajan et al. 2006). The host range of this virus is mainly restricted to *Musa* spp. including abaca (Sharman et al. 2000). Small cardamom in India (Siljo et al. 2011) and flowering ginger, *A. purpurata*, in Hawaii (Wang et al. 2010) are reported to be the natural hosts of BBrMV.

17.2.4 Banana Mosaic or Infectious Chlorosis Disease

The disease, first described in 1930 from Australia (Magee 1930), is one of the common viral diseases affecting banana and plantain worldwide. Banana mosaic is also known as infectious chlorosis, heart rot, sheath rot, and cucumber mosaic. Common strains of the virus have not been reported to cause severe epidemic or loss to banana plantation. However, severe strain (heart rot) of the virus is known to cause significant economic damage (Niblett et al. 1994). The disease is recognized in most of the banana-growing regions of the world (Lockhart and Jones 2000a).

17.2.4.1 The Virus

Banana mosaic disease is caused by the *Cucumber mosaic virus* (CMV) which is a member of *Cucumovirus* group (Yot-Dauthy and Bove 1996). Spherical virus particles of 28–30 nm in size have single-stranded positive-sense RNA as genome. Most of the CMV genome consists of three genomic and one subgenomic RNA species (Francki et al. 1979; Fauquet et al. 2005). In some isolates, fifth RNA species have been reported to be linked with symptom expression in some host plants. CMV isolates are grouped into two major subgroups I and II based on serology and molecular characters. Most of the CMV isolates from banana have been identified as subgroup I.

17.2.4.2 Symptoms

The virus causes variable symptoms from mild chlorosis to severe chlorotic streaks on leaf lamina (Fig. 17.1G and H) depending on the pathogen strain and the weather conditions. Symptoms are known to fluctuate during the growing season depending on the temperature and rainfall. Leaf deformation and curling are occasionally observed in the infected plants. This virus induces visible symptoms sporadically in the field, and majority of leaves did not show any symptom. Sometimes mosaic symptoms have been observed on fruits of infected plants. Generally, the symptoms

are more severe in winter time when temperatures fall below 24 °C in the tropics and subtropics. Symptoms are more pronounced which include necrosis of emerging leaves and internal tissues of pseudostem when banana plants are infected with severe strains of the virus. Fruits may show mosaic symptoms and bunches may bear malformed fruit or no fruit. Plant death may occur in very severe cases especially when plants get infected with severe strain soon after planting.

17.2.4.3 Disease Transmission, Host Range, and Epidemiology

The spread of the disease occurs in nature through vegetative planting material and by over 60 different species of aphid vector including *A. gossypii*, *A. craccivora*, *R. maidis*, *R. prunifolium*, and *Myzus persicae* (Rao 1980). CMV has a wide host range, infecting over 900 species in almost every region of the world. Aphids usually acquire the virus from diseased weed and other crops growing nearby and spread to banana plantation due to migration of viruliferous vectors from diseased areas. However, most of the aphid species do not colonize on banana, but they may be able to transmit the CMV with relatively less efficiency during their exploratory visit to banana. A higher incidence of CMV noticed in a newly planted field may be because of lack of alternative host for viruliferous aphid vectors within the field. However, a better understanding of disease epidemiology and aphid vector ecology is required.

17.3 Minor Viral Diseases of Banana

17.3.1 Abaca Mosaic Disease

This disease is caused by *Abaca mosaic virus* (AbaMV) which is a member of *Potyvirus* and first recorded in the Philippines in 1925 (Eloja and Tinsley 1963; Thomas et al. 1997). This disease is so far reported from the Philippines alone, where 25–50 % losses were observed in new plantings.

Whitish small dots later elongate and turn into spindle-shaped yellow chlorotic streaks on leaves, petioles, and midribs. The symptoms appear first parallel to minor leaf veins. As disease progresses, these chlorotic areas may turn in with rusty brown borders and extend from the midrib to the leaf margin. The infected leaves subsequently develop extensive yellow or pale-green stripes across the width of the leaf lamina.

The causal virus is a flexuous filamentous particle of about 680 nm long with single-stranded RNA as genome and closely related to sugarcane mosaic potyvirus (Eloja and Tinsley 1963). The host of this virus is mainly restricted to monocotyledonous plant. Natural hosts recognized so far include *M. textilis*, *Maranta arundinacea*, and *Canna indica*. The primary transmission of AbaMV is by vegetative propagation, and natural field spread of the virus occurs by aphids (mainly *R. maidis* and *A. gossypii*) in a nonpersistent manner (Diekmann and Putter 1996).

17.3.2 Banana Mild Mosaic Disease

The disease is caused by *Banana mild mosaic virus* (BanMMV) which appears to occur in Australia, Africa, Asia, Central and South America, and the Caribbean. The economic impact of this disease is not well known. The symptoms of this virus are uncertain and often symptomless infection occurs in *Musa* spp. Mild chlorotic mosaic and streaks have been observed on highly susceptible cultivars such as Ducasse (AAB, Pisang Awak) and Daluyano (AAB, plantain subgroup). Mixed infection with BSV, BBrMV, and CMV has been reported (Iskra-Caruana et al. 2008). Necrotic streaks have been reported in case of mixed infection with CMV in Guadeloupe (Iskra and Galzi 1998).

The causal virus particles are flexuous filamentous measuring about 580 × 14 nm with a coat protein of about 27 kDa. The genome of BanMMV is a single-stranded RNA of about 7.4 kb and contains five ORFs (Gambley and Thomas 2001). The virus is classified as an unassigned virus in the *Betaflexiviridae* (King et al. 2012).

BanMMV is transmitted through the vegetative propagation of planting materials. The spread of the virus in nature has not been identified. However, the high heterogeneity of the viral genome and the temporal increase in disease incidence within a field imply the natural transmission of BanMMV is occurring through some unknown mechanism (Teycheney et al. 2005).

17.3.3 Banana Dieback Disease

The disease was first reported from Nigeria in 1996. The purified virions from infected plants were 28 nm isometric particles. This virus has been shown to have some serological relationship with certain *Nepoviruses* (Hughes et al. 1996). Leaf chlorosis, wrinkling, marginal necrosis, and dieback of the cigar leaf are the symptoms induced by this virus. Infected suckers from the same mat become progressively more stunted and even the mother plant dies. Similar symptoms have also been noted in Ghana and Cameroon (Hughes et al. 1996). This disease can be mechanically transmitted to a limited range of herbaceous indicator plants (Hughes and Thomas 1999).

17.4 Viral Sequence Integration Within the Banana Plant and the Viral Evolution

Banana streak viruses (BSVs; genus *Badnavirus*) are members of the family *Caulimoviridae*, with a circular double-stranded (ds) DNA genome, replicating by reverse transcription (Hull 1999; Hull and Covey 1995). BSV naturally infects banana (*Musa* sp.) and is found in all banana-cultivating regions of the world (Lockhart and Jones 2000a, b). These are also called as plant pararetroviruses, which are phylogenetically related to *Metaviridae* (Ty3-Gypsy elements) (Malik and Eickbush 2001). Although the virus replication cycle has no genome integration step, the members of the *Caulimoviridae* family exist as both episomal (i.e., nonintegrated) and endogenous (i.e., integrated) sequences (endogenous pararetrovirus [EPRV]) in the host genome (Staginnus et al. 2009). The EPRVs have been described in both mono- and dicotyledonous plant families, and each of them has originated from independent integration events (Pahalawatta et al. 2008; Staginnus and Richert-Poggeler 2006; Su et al. 2007a). Though most EPRVs are generally eliminated from the plant genome, they are also retained by an endogenization phenomenon. The viral sequences initially integrate into the germinal cells, thus becoming the part of plant genome, and eventually the EPRVs are fixed in plant populations by different evolutionary forces (Hohn et al. 2008; Hull and Covey 1995). The process of genetic integration is mostly through illegitimate recombination between the plant and viral genomes (Staginnus and Richert-Poggeler 2006). Sequences of several pararetroviruses are known to be integrated into the nuclear genome of several different plant species.

The presence of EPRVs can result in the modification of host genome, modify methylation status of host genome and trigger chromosomal rearrangements in a way similar to the transposable elements (Bennetzen 2000; Hohn et al. 2008; Kidwell and Lisch 2000). The EPRVs may represent 100–1000 copies within the plant genome and are mostly found in the heterochromatin region (Gregor et al. 2004; Jakowitsch et al. 1999; Richert-Poggeler et al. 2003). Such large-scale amplification of EPRVs within the host genome is mediated by the transposable elements present in the vicinity of EPRVs (Matzke et al. 2004; Staginnus and Richert-Poggeler 2006). The EPRVs are known to produce transcripts, which are processed into small interfering RNAs (siRNAs), as reported in the case of petunia and tobacco, thus resulting in sequence-specific gene silencing of *Petunia vein clearing virus* (PVCV, genus *Petuvirus*) and *Tobacco vein clearing virus* (TVCV, genus *Cavemovirus*), respectively (Noreen et al. 2007; Staginnus and Richert-Poggeler 2006). A similar mechanism is known to occur in wild diploid *M. balbisiana* which is resistant to both endogenous BSV (eBSV) and BSV (Hohn et al. 2008). This resistance mechanism is also hypothesized to occur against the episomal and endogenous forms of several other members of the *Caulimoviridae* family. This gene silencing is mostly triggered by

the release of double-stranded RNA, due to the transcription of antisense RNA, which ultimately constitutes a selective force for the maintenance of tandem repeat structures found in the case of EPRVs (Gayral et al. 2008; Ndowora et al. 1999; Richert-Poggeler et al. 2003). Recent studies by Rajeswaran et al. (2014) have shown that despite the presence of different size classes of virus-derived small RNAs (21–24 nt), the viruses are able to evade siRNA-directed DNA methylation and thereby avoid transcriptional silencing. Such an evasion of transcriptional silencing most likely contributes to the persistence of episomal para-retroviruses within the banana plants, although they are not true retroviruses with obligatory genome integration step in their replication cycle.

The EPRVs can also get released as infectious and functional full-length viral genomes, thereby resulting in virus multiplication and development of viral symptoms in the host plant. Until now, such infectious EPRVs are reported for BSV in banana, PVCV in petunia, and TVCV in tobacco. Combination of novel genomes through interspecific hybridizations and subjecting them to tissue culture conditions or creating some stress conditions can result in activation of EPRVs (Dallot et al. 2001; Lheureux et al. 2003; Lockhart et al. 2000; Richert-Poggeler et al. 2003). In the case of banana plants, the eBSVs can be activated when two cultivars of diploid *M. acuminata* (AAAA genome) and *Musa balbisiana* (BB genome) are crossed to produce triploid interspecific hybrids with AAB genome. The infectious eBSV is transmitted by the diploid BB parent (*M. balbisiana*) alone, since it is reported to be present only in the case of B genome. The activation of infectious EPRVs is through two different mechanisms: (i) homologous recombination between the repeat regions surrounding them, resulting in the excision of a circular viral genome, and by (ii) transcription of EPRVs leading to a viral pregenomic RNA (Ndowora et al. 1999; Noreen et al. 2007; Richert-Poggeler et al. 2003). The presence of such infectious EPRVs may impact the virus evolution and thus represent an extreme case of virus transmission strategies. However, such an endogenous state of viruses can be disadvantageous for the virus, as it can result in formation of defective viral genomes, as a result of accumulation of deleterious mutations over the generations.

BSV is the generic name for several different species involved in the same disease, with a nucleotide sequence divergence of up to 30%. BSVs are classified into three major groups (BSV-1 (Bennetzen 2000), BSV-2, and BSV-3) based upon the phylogenetic analysis of RT/RNase H region of ORF-III (Harper et al. 2005). *Banana streak GF virus* (BSGFV) and *Banana streak Imove virus* (BSImV) belong to the BSV-1 group, while the BSV-2 and BSV-3 groups contain dozens of putative species (Bousalem et al. 2008; Gayral and Iskra-Caruana 2009; Harper et al. 2005), which are yet to be fully characterized with whole-genome data. The BSV-banana pathosystem is an excellent model to study the evolution of infectious EPRVs, mainly because of a well-studied phylogeny of BSV and their integrations in the *Musa* genome (Bousalem et al. 2008; Gayral and Iskra-Caruana 2009; Geering et al. 2005). In contrast to the EPRVs in the Solanaceae family, with several 100–1000 copies per genome (Gregor et al. 2004; Jakowitsch et al. 1999; Richert-Poggeler et al. 2003; Staginnus and Richert-Poggeler 2006), the integrations of BSImV (eBSImV) and of BSGFV (eBSGFV) are simpler models present as single copies in the *M. balbisiana* cv. Pisang Klutuk Wulung (PKW) genome (Gayral et al. 2010). The eBSImV (15.8 kb) is a monoallelic integration and eBSGFV is a diallelic integration, with two alleles eaBSGFV-7 (13.3 kp) and eBSGFV-9 (15.6 kb) of which only eaBSGFV-7 is infectious (Staginnus et al. 2009). The biology of the two infectious integrants eBSImV and eBSGFV is well studied and is able to reconstitute infectious viruses in interspecific hybrids between *M. balbisiana* cv. PKW and *M. acuminata* cv. "IDN 110 4x" (Iskra-Caruana et al. 2003; Lheureux et al. 2003). Both the genetic and environmental factors and their mechanisms that trigger the activation of eBSV are also well studied (Dallot et al. 2001; Lheureux et al. 2003; Gayral et al. 2010). Recently, the evolutionary history describing the presence and distribution of infectious eBSV within the *Musa* genus, and the phylogeny of *M. balbisiana*, and the host plant species has also been studied (Gayral et al. 2010). The early evolutionary

stages of infectious eBSV for two different BSV species, namely, BSGFV and BSImV, was investigated by studying their distribution, insertion polymorphism, and structure evolution among selected banana genotypes representative of the diversity of 60 wild *Musa* species and genotypes (Gayral et al. 2010). By using 19 different microsatellite loci, from the two chloroplast regions, *matK* and *trnL-trnF*, along with the nuclear genome, it was shown that both the BSV species integrated recently during banana evolution, circa 70,000 years ago (Su et al. 2007a). The studies revealed that these infectious eBSVs were subjected to diverse selection pressures and had different levels of genome rearrangement (Gayral et al. 2010; Fig. 17.2).

Recent studies showed that the three different BSV species, *Goldfinger* (eBSGFV), *Imove* (eBSImV), and *Obino l'Ewai* (eBSOLV), in the seedy *M. balbisiana* PKW exhibited extensive viral genome duplications and rearrangements (Chabannes et al. 2013). Segregation and fluorescent in situ hybridization studies on the F1 population of PKW showed that eBSImV, eBSOLV, and eBSGFV are each present at a single locus (Chabannes et al. 2013). These studies also revealed that both eBSGFV and eBSOLV had two distinct alleles, located on chromosomes 1 and 2 of the reference *Musa* genome, respectively, whereas the eBSImV had two structurally identical alleles (Chabannes et al. 2013). These studies also suggested for a sequential integration of eBSVs within the plant genome, and also the three loci displayed a differential evolution as inferred from the haplotype divergence analysis. However, the genotyping of both eBSV and viral particles expressed in the progeny showed that only one allele for each species was infectious (Chabannes et al. 2013).

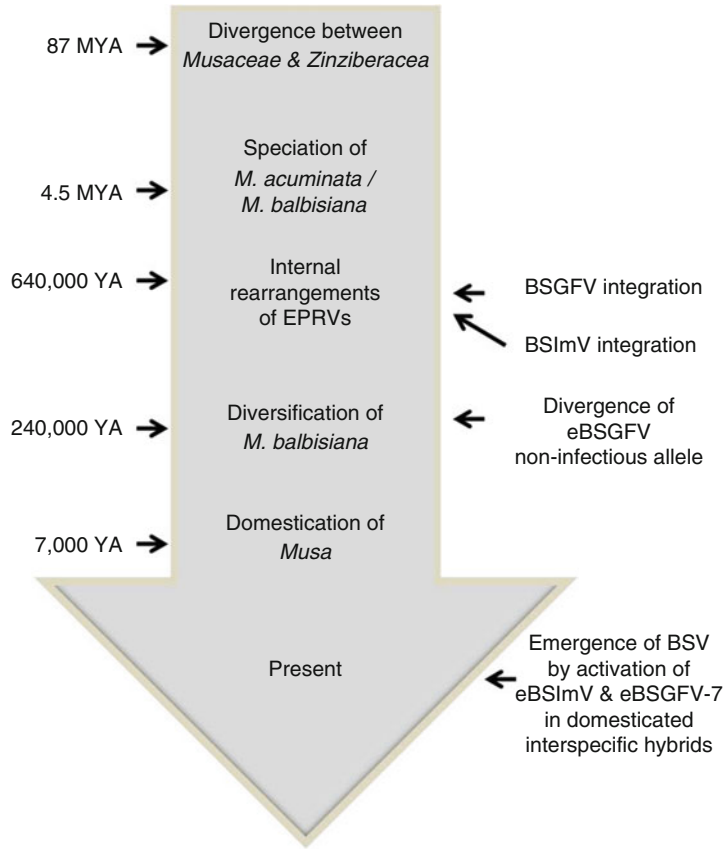

Fig. 17.2 Schematic diagram illustrating key events in the evolutionary history of eBSVs (BSImV and BSGFV), in association with estimated dates during the evolution of the *Musa* genus (Redrawn from Gayral et al. 2010)

17.5 Integrated Disease Management

In nature, the viral diseases in banana spread through planting materials and from plant to plant by insect vectors. Thus, a strict legislation to regulate the movement (quarantine) and certification program of banana planting materials are required to mitigate the impact of viral diseases on banana industries. At farm level, the damage by banana viruses can be minimized effectively by using integration of several methods such as use of resistant/tolerant cultivars, cultural practices (roguing and eradication), and vector control. Among several methods used, the exclusion of the disease by effective quarantine, use of healthy planting materials by a well-regulated certification program, and destruction of diseased stool are considered most effective in managing the banana viral diseases.

The use of disease-resistant cultivars offers convenient and effective means of disease management. Many banana cultivars evaluated in several countries indicate the lack of high levels of resistance to viral infections (Espino et al. 1993; Hooks et al. 2009; Hooks et al. 2008; Jose 1981; Magee 1948; Niyongere et al. 2011; Mwenebanda et al. 2007; Ngatat et al. 2013). However, some with the B genome (AAB and ABB) are tolerant or express symptoms more slowly than those with the A genome (AA and AAA) (Espino et al. 1993; Jose 1981; Ngatat et al. 2013). Recently, banana cvs. Tinawagan Pula and Tangongon with putative resistance to BBrMV were developed through in vitro mutagenesis by gamma irradiation (Dizon et al. 2012). These promising lines are yet to be evaluated for other horticultural traits at field level. However, the development of resistance through in vitro mutagenesis by gamma irradiation demonstrated the promise of mutagenesis in developing virus-resistant cultivars.

Although, for recovery of banana production in BBTD epidemic areas of Fiji, tolerant clones of banana have been utilized against BBTV (Magee 1948), for effective virus management at present, many countries utilize the integrated disease management which consists of several approaches, such as the eradication of infected plants by regular roguing, use of virus-free planting materials, and exclusion (quarantine) measures to prevent disease spread into other regions (Bouhida and Lockhart 1990).

With the advancements in virology, sensitive and efficient serological and molecular techniques are available for quick virus detection. By adopting the strategic virus detection system, the risk of free movement of infected planting materials to healthy area can easily be quarantined. In an affected area, one of the major limitations for banana growers is the availability of certified virus-free quality planting materials. Now meristem-tip culture combined with heat therapy (Lassois et al. 2013; Thomas et al. 1995) has been an established method to produce virus-free planting materials in the case of banana. The virus-free planting material developed in vitro can be utilized as mother stocks for mass multiplication of virus-free planting materials at commercial scale (Su et al. b). The mass production of virus-free planting material in vitro with proper virus indexing and certification program has been found useful for reducing the disease in several countries of Asia, Australia, and Hawaii. In India, a strict enforcement of national certification systems and commercial production units has been accredited by the Department of Biotechnology, Government of India, to produce certified tissue-cultured banana plantlets. In Australia, only pathogen-free stocks generated by the Queensland Banana Approved Nursery (QBAN) are allowed to be used as foundation stock in the TC industry (QPPR, 2002). Presently, virus-free TC plants are widely used to manage BBTV in Taiwan, the Philippines, and India (Molina et al. 2009; Selvarajan et al. 2011; Su et al. 2007b). However, many other banana-growing countries are yet to develop such guidelines or certification schemes for producing high-quality planting material.

In case of BSV, cryopreservation followed by apical meristem culture methods were developed that significantly reduce the virus titers (Helliot et al. 2002). It has been reported to eradicate episomal form of BSV in banana with the use of antiviral compounds such as adefovir, tenofovir,

and 9-(2-phosphonomethoxyethyl)-2,6-diaminopurine (PMEDAP) (Helliot et al. 2003).

For implementation of effective viral disease management, cultural practices such as regular surveillance, roguing, and eradication of diseased plants play an important role in minimizing the impact of disease. Early and efficient detection and subsequent eradication of infected plants are the key factors for reducing the disease. Control of weed and other alternate hosts (especially in the case of CMV), developing a banana-free buffer zone around a banana plantation, and diversified farming are important strategies to minimize viral diseases in banana. More occurrence of CMV outbreak has been reported in banana plantations near to cucurbits, tomato, or other vegetable fields (Magee 1940). The incidence was much less when banana was grown next to rice than when grown next to vegetable crops in Taiwan, and the disease was highest when intercropped with cucumber (Tsai et al. 1986). Regular surveillance and monitoring has been recommended to identify and rogue infected plants to reduce virus spread as early as possible (Allen 1987; Hooks et al. 2008; Magnaye 1994; Smith et al. 1998). Allen (1987) also recommended uprooting apparently symptomless plants within 5 m radius of symptomatic plants as a precautionary principle. This however has been shown to result in the elimination of a considerable number of uninfected plants for every infected plant uprooted. Removal of diseased plant and replant with virus-free banana contributed in managing the viruses in banana. Destruction of wild and unattended patches of banana is important to control the virus spread through vectors. Injecting herbicides, such as fernoxone or 2,4-D, together with systemic insecticide sprays, has been recommended to kill the infected plants and prevent the spread of vector from the treated plants (Regupathy et al. 1983; Robson et al. 2007). So far, intensive eradication of BBTV has been implemented only in Australia which showed marked reductions in the prevalence of the virus (Allen 1987; Cook et al. 2012). However, a similar program in the developing world could be highly challenging where plantation is mainly dominated by smallholder farmers.

Good horticultural practices with nutrition management in the plantation help to minimize the impact of disease. For example, application of proper fertilizer was reported to reduce the symptoms of BBrMV in certain commercial banana cultivars (Selvarajan et al. 2009).

Vector control by chemical methods has been tried to control viral diseases in banana which indicated limited success. Thus, using pesticides for vector control has so far received little attention. Pesticides for banana aphid control have been used mainly as a preventive measure to prevent their further spread from withering plants during eradication programs (Hooks et al. 2009).

17.6 Conclusions

Banana is one of the most important and high-priority food crops which provide staple food, nutrition, and income for the millions of banana farmers worldwide. That is why demand of banana at global level has been raised as revealed by increasing world banana production for the last decade. Moreover, banana fruits are particularly valued in the tropics because they yield irrespective of the seasons. Viruses are considered a major constraint to banana plantation as they not only cause yield reductions but are also a major limitation to the exchange of germplasm. Among viruses that have been reported on banana, BBTV and BBrMV and BSV are significant threats to banana production. Of these, BSV is more widely spread worldwide than BBTV, though the latter is so far the most economically damaging virus.

Introduction of virus-free planting material using tissue culture techniques, strict regulation on movement of infected planting materials, and development of transgenic resistant cultivars to these viruses are the most objective means to control these virus diseases in banana. However, all banana viruses cannot be eliminated through tissue culture and traditional breeding because most of the commercially important cultivars are (tetraploid) mostly sterile and develop fruit parthenocarpally.

The International Network for the Improvement of Banana and Plantain (INIBAP)

is a division of the International Plant Genetic Resources Institute (IPGRI) and has its mandate with the mission to improve the productivity and yield stability of banana and plantain. The development of virus resistance through transgenic approaches has promising potential to incorporate virus resistance in banana and currently gaining priority. RNAi-based resistance which is successfully employed for transgenic control of several plant viruses (Sudarshana et al. 2007, Reddy et al. 2009, Patil et al. 2011, Cillo and Palukaitis, 2014, Patil et al. 2016) has also been employed for transgenic control of BBTV in banana; however, there are no reports of any field trails.

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