Begomoviruses and Their Satellites Occurring in India: Distribution, Diversity and Pathogenesis

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V.G. Malathi, P. Renukadevi, S. Chakraborty, K.K. Biswas, Anirban Roy, P.N. Sivalingam, V. Venkataravanappa, and Bikash Mandal

Abstract

The begomoviruses (genus *Begomovirus*, family *Geminiviridae*) constitute the largest group of plant viruses causing devastating crop diseases in India. About 16% geminiviruses recorded worldwide occur in India. Begomovirus associated disease was recorded in India as early as 1924. During 1980s, begomovirus disease and the vector whitefly, *Bemisia tabaci* emerged as serious problems in many vegetable and pulse crops. The molecular characterisation studies on begomoviruses in India began during late 1980s and during last decade, a large number of begomoviruses were discovered causing diseases such as mosaic, yellow mosaic, yellow vein mosaic and leaf curl in numerous crops and wild plants. Currently, 322 begomovirus species have been officially recognised all over the world, of which about 82 begomovirus species are known to occur in India. The Indian subcontinent represents one of the important centres for begomoviruses origin and diversity. In this chapter, research work on identification and characterization of begomoviruses in diverse crops in India is presented. Geographic distribution, phylogenetic relationship, infectivities of cloned genome components, recombination, replication events, suppression of RNAi silencing, viral gene funtions as pathogenicity determinants and association of satellites and

V.G. Malathi (✉) • P. Renukadevi
Tamil Nadu Agricultural University, Coimbatore 641003, India
e-mail: vgmalathi@rediffmail.com

S. Chakraborty
School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India

K.K. Biswas • A. Roy • B. Mandal
Indian Agricultural Research Institute, New Delhi 110012, India

P.N. Sivalingam • V. Venkataravanappa
National Institute of Biotic Stress Management, Raipur 493225, India

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their role in disease development with reference to the Indian begomoviruses are discussed.

Kewords
Begomovirus • Betasatellite • Alphasatellite • Whitefly • Diversity • Begomovirus in India

5.1 Introduction

Begomoviruses (genus *Begomovirus,* family *Geminiviridae*) are highly prolific plant viruses causing significant economic losses in the agricultural produces in the tropical and subtropical environments of the world. The name of the genus *Begomovirus* is derived from the first two letters of the each word of the the type species, *Bean golden mosaic virus* (BGMV) causing golden mosaic disease in bean in Central America. The family name, *Geminiviridae* is derived from the zodiac sign 'Gemini', as the virion (22 × 38 nm) has characteristic twinned isometric morphology. The virion consists of two incomplete icosahedra (T = 1) containing a total of 110 coat protein subunits organized as 22 pentameric capsomers, encapsidating a circular single stranded DNA (ssDNA) genome of 2.5–2.9 kb (Harrison et al. 1977; Stanley 1985). Based on the genome organization, insect vector and host range, geminiviruses (family *Geminiviridae*) are classified into nine genera, *Becurtovirus, Begomovirus, Topocuvirus, Turncurtovirus, Capulavirus, Curtovirus, Grablovirus, Mastrevirus* and *Eragrovirus* (Zerbini et al. 2017). Begomoviruses are transmitted by only one vector species, whitefly (*Bemisia tabaci* Genn.). About 68.1% of geminiviruses belong to the genus *Begomovirus.* Presently, 322 virus species have been officially recgnised under the genus *Begomovirus*, which is the maximum number of members so far known in any genera of plant viruses. The virus species, *citrus chlorotic dwarf associated virus* and *mulberry mosaic dwarf associated virus* are included in the unassigned genus as their replication associated protein and genome are distinct from the other nine genera.

The diseases caused by begomoviruses are, yellow mosaic, veinal yellowing, leaf distortion, curling and stunting. Infection in early seedling stage, leads to poor fruit set and infertile seeds, resulting in severe yield loss. Begomoviruses affect a large number of dicotyledonous crops such as cassava, sweet potato, cotton, grain legumes and vegetables in the tropical and subtropical countries. Some of the diseases like cotton leaf curl and cassava mosaic are known for a century and cause huge economic loss (Varma and Malathi 2003).

All the eight genera of the family *Geminiviridae* have monopartite genome, whereas begomoviruses contain either monopartite or bipartite genome (Fig. 5.1). Almost all the begomoviruses of the New World are bipartite and have DNA A and DNA B component encapsidated separately in a geminate particle. Both DNA A

Fig. 5.1 Genome organisation of genera of the family *Geminiviridae* (Adapted from Zerbini et al. 2017)

and DNA B are essential for infectivity. The basic features of genome organization are the presence of coat protein gene (ORF V1/AV1-coat protein) in the viral sense strand (right half) and the replication associated protein gene (C1/AC1/Rep) in the complementary strand (left half). Both the ORFs diverge from a non-coding intergenic region, consisting origin of replication of viral sense strand and promoters of CP and Rep gene. The genera differ in number of genes encoded in the complementary strand. The complementary strands of mastre and becurtoviruses have only Rep which is translated from ORFs C1:C2 by transcript splicing in the complementary strand. However, three ORFs, transcriptional activator protein gene which also functions as silencing suppressor gene (C2/AC2/TrAP/*ss*), replication enhancer gene (C3/AC3/REn) and symptom determinant gene (C4/AC4/*sd*) are present in the complementary strand in curto, topocu, turncurto and begomoviruses. The genus *Eragrovirus* encodes a gene for homologue of C2 and the genera *Capulovirus* and *Grablovirus* encode for another gene whose function is not yet resolved. In DNA B, the gene encoding nuclear shuttle protein is present in the viral sense strand (BV1, NSP) and the gene encoding cell to cell movement protein is in the complementary sense strand (BC1, MP). Both the ORFs are separated by an intergenic region having origin of replication in viral sense strand. The DNA A component is dependent on DNA B for intracellular and intercellular movement. DNA B is dependent on DNA A for replication and encapsidation. In order to facilitate recognition of DNA B by the Rep encoded by DNA A, within the intergenic region is present a segment

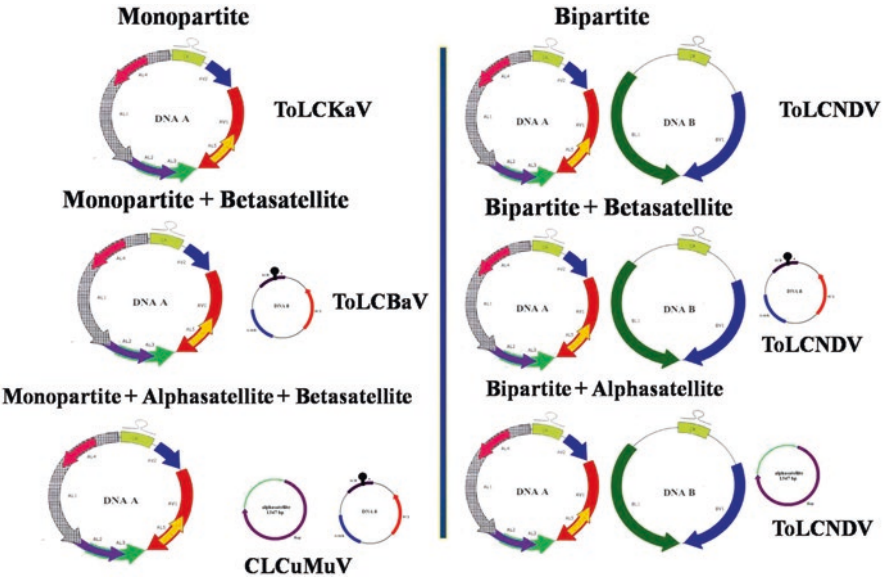

Fig. 5.2 Genomic components and genome organisation of the Old World begomoviruses

of 110–200 nt sequences which are highly conserved between the DNA A and DNA B component. This region labeled as common region (CR) is near identical between DNA A and DNA B component. The intergenic region consists of repetitive elements called iterons upstream of the highly conserved stem-loop structure. The iterons, represent the binding sites of Rep. The invariant nonanucleotide sequence TAA TAT TAC in the loop is conserved in all geminiviruses and the nicking between seventh and eighth nucleotide by Rep is proved to initiate replication.

Begomoviruses in the Old World have both monopartite and bipartite genome organization (Fig. 5.2). The monopartite begomoviruses in general and few of the bipartite begomoviruses of the Old World are associated with additional circular ssDNA components refered as satellites (1.3 kb). There are three types of satellites, alphasatellite, betasatellite and deltasatellite. The alphasatellites encode only one Rep gene having similarities with Rep protein of nanovirus. The betasatellite, shares the origin of replication sequence with the helper begomoviruses, it's replication is facilitated by the Rep protein encoded by DNA A of helper begomoviruses. There is one ORF (beta C1) encoded in the complementary sense DNA of betasatellite, which is the pathogenicity determinant and functions as silencing suppressor. All betasatellites have an extremely conserved region referred to as satellite conserved region (SCR), upstream of origin of replication which is essential for replication. Among monopartite begomoviruses, though DNA A alone can infect plant and systemically move, inoculation along with betasatellites lead to severe symptom production like enation, leaf malformation twisting and stunting. The alphasatellites replicate autonomously by it's own Rep. They are dependent on helper begomovirus

for their spread. A new set of non-coding sub viral molecules (633–750) designated as deltasatellites (Lozano et al. 2016) have been identified recently with begomoviruses infecting sweet potato (sweepoviruses). They are structurally similar to subgenomic betasatellite associated with tomato leaf curl virus (ToLCV) from Australia; they have the conserved stem and loop structure with nononucleotide sequence TAATATAC and SCR similar to betasatellites. The contribution of alphasatellites and deltasatellites to viral pathogenicity is yet to be understood.

The process of infection is initiated with the introduction of the virus in protophloem cell by the deep probing of vector whitefly. From the ssDNA/CP complex, ssDNA is released into the nucleus, where ssDNA becomes double stranded DNA. The replication is facilitated by the host DNA polymerase. Detection of primers with 5′ ribonucleotides complementary to SIR in mastrevirus are suggestive of initiation at the short intergenic region site; In a bipartitie begomovirus, African cassava mosaic virus (ACMV) ribonucleotides priming has been shown. The dsDNA is transcribed by the host RNA polymerase II and the earliest gene transcribed is the C1/AC1 or replication initiation/ associated protein (Rep). The replication is by a combination of rolling circle replication and recombination dependant replication. The Rep protein initiates replication by niking at the nononucleotide sequence TAATATT↓AC. The newly synthesized + strand is copied into dsDNA again by host DNA polymerase which may enter the replication cycle. Alternatively, the ssDNA may get encapsidated by the coat protein. The movement of viral DNA from the infection foci is mediated by the movement protein V2 in monopartite viruses or by BV1/BC1 in bipartite viruses. The viral DNA is transported out of the nucleus into the periphery of the cell from where they are docked on to plasmodesmata and transported into adjacent cells, finally the viral DNA (either ss or ds) enter into phloem parenchyma and companion cell. It is hypothesized that geminiviruses move as ss/ds DNA/movement protein complex, spread to young unfurling leaves from where they are acquired by the vector.

5.1.1 Begomovirus species in India

It is not an exaggeration to state that Indian subcontinent represents the key centre for the origin, diversity and spread of begomoviruses. Approximately 16% of world geminiviruses occur in India which tops the list of numbers of begomoviruses in different countries. Pakistan and China have nearly 13% and 8% of the geminiviruses recorded. There are approximately 1500 gene sequence entries in database which is the highest record worldwide. At present, the NCBI database has 810 full-length genome sequences of Indian geminiviruses. They belong to two genera, *Mastrevirus* (15 sequences) and *Begomovirus* (795 sequences). There are approximately 82 species of begomoviruses (Table 5.1), 2 species of mastreviurses, about 545 betasatellites and 146 alphasatellites.

Applying 91% identity as the threshold value for the demarcation of species in the DNA A nucleotide component using SDT analysis with MUSCLE option, the virus members of the genus *Begomovirus* have been reanalyzed and 322 species have been recognized by ICTV (Brown et al. 2015). Of these, 80 species are known

Table 5.1 Indian begomoviruses and their hosts

S.No	DNA-A Virus	Acronyms	Hosts
1.	Abutilon mosaic virus	AbMV	Abutilon
2.	Ageratum enation virus	AEV	Poppy, *Cleome gynandra,* Amaranthus, *Ageratum Crassocephalum*, Soybean, Carrot, Ornamental, Ageratum, Pointed gourd, Calendula, Tagetes, Tomato, Zinnia, Fenugreek, Papaya
3.	Ageratum leaf curl virus	ALCuV	Calotropis
4.	Allamanda leaf mottle distortion virus	All LMDV	Allamanda
5.	Alternanthera yellow vein virus	AlYVV	Rumex, Picrorhiza
6.	Andrographis yellow vein leaf curl	AYVLCV	Andrographis
7.	Bhendi yellow vein Bhubaneswar virus	BYVBhV	Bhendi
8.	Bhendi yellow vein mosaic virus	BYVMV	Bhendi
9.	Bhendi yellow vein Haryana virus	BYVHarV	Bhendi
10.	Chayote enation yellow mosaic virus	ChEYMV	Chayote
11.	Chilli leaf curl virus	ChiLCV	Chilli, Kenaf, Amaranthus, Tomato, *Solanum nigrum*, Petunia, Mentha, Papaya, Eggplant, *Phaseolus aureus*
12.	Chilli leaf curl Ahmedabad virus	ChiLCAV	Chilli
13.	Chilli leaf curl India virus	ChiLCINV	Chilli, Tomato
14.	Chilli leaf curl Kanpur virus	ChiLCKaV	Chilli
15.	Chilli Leaf curl Vellanad virus	ChiLCVV	Chilli
16.	Clerodendron yellow mosaic virus	ClYMV	Bougainvillea, Clerodendron
17.	Coccinia mosaic Tamil Nadu virus	CocMTNV	Ivy gourd
18.	Corchorus golden mosaic virus	CoGMV	*Corchorus capsularis*, Boehmeria.
19.	Corchorus yellow vein mosaic virus	CoYV	*Corchorus oletorius*
20.	Cotton leaf curl Alabad virus	CLCuAlV	Bhendi
21.	Cotton leaf curl Bangalore virus	CLCuBaV	Cotton, Bhendi, *Hibiscus cannabinus*
22.	Cotton leaf curl Barasat virus	CLCuBrV	*Malachra capitata*
23.	Cotton leaf curl Kokhran virus	CLCuKoV	*Cyamopsis tetragonoloba*, cotton
24.	Cotton leaf curl Multan virus- Rajasthan	CLCuMuV-Ra	Cotton, *Hibiscus rosa-sinensis, Hibiscus cannabinus*
25.	Croton yellow vein mosaic virus	CroYVMV	Croton bonplandianum
26.	Dolichos yellow mosaic virus	DoYMV	Dolichos
27.	Eclipta yellow vein virus	EYVV	Hibiscus
28.	French bean leaf curl virus	FbLCV	French bean
29.	Hemidesmus yellow mosaic virus	HemYMV	*Hemidesmus indicus*

(continued)

Table 5.1 (continued)

S.No	DNA-A Virus	Acronyms	Hosts
30.	Hollyhock leaf curl virus	HoLCV	*Andrographis paniculata*
31.	Hollyhock yellow vein mosaic virus	HoYVMV	Hollyhock, Bhendi
32.	Horsegram yellow mosaic virus	HgYMV	French bean, Lima bean, Horse gram
33.	Indian cassava mosaic virus	ICMV	*Jatropha curcus*, Cassava
34.	Jatropha leaf crumple India virus	JLCrIV	*Jatropha curcus*
35.	Jatropha leaf crumple virus	JLCrV	*Jatropha curcus*
36.	Jatropha leaf curl virus	JLCuV	*Jatropha integerrima, Jatropha multifida*, *Jatropha podagrica, Jatropha gossipifolia, Jatropha curcus, Ludwigia parviflora*
37.	Jatropha yellow mosaic India virus	JYMV	*Jatropha gossipifolia*
38.	Jatropha mosaic India virus Katarniaghat	JMINV	*Jatropha curcus, Jatropha gossipifolia*
39.	Malvastrum yellow vein virus	MaYVV	*Hibiscus cannabinus*
40.	Mesta yellow vein mosaic Bahraich virus	MeYVMBaV	*Hibiscus cannabinus*
41.	Mesta yellow vein mosaic virus	MeYVMV	*Hibiscus cannabinus, Hibiscus sabdariffa*, Bhendi
42.	Mirabilis leaf curl India virus	MiLCV	*Mirabilis jalaba*
43.	Mungbean yellow mosaic India virus	MYMV	Mungbean, Dolichos, Cowpea, Blackgram, Soybean, French bean.
44.	Mungbean yellow mosaic virus	MYMIV	Soybean, Blackgram, Mungbean, Moth bean,
45.	Okra enation leaf curl virus	OELCuV	Bhendi, Wild Bhendi, *Hibiscus cannabinus*
46.	Papaya leaf curl virus	PaLCuV	*Croton bonplandianum,* Acalypha, *Jatropha gossypifolia*, Cluster bean, Chilli, *Crambe abyssinica,* Turnip, Radish, Papaya, Sunnhemp, *N.glutinosa*, Aster, *Amaranthus cruentus*, Soybean, *Brassica rapa.*
47.	Papaya leaf crumple virus	PaLCrV	*Solanum nigrum*, *Andrographis paniculata, Cathranthus roseus*, Cowpea, Soybean, Papaya
48.	Pedilanthus leaf curl virus	PeLCV	Crape Jasmine, *Cestrum nocturnum*
49.	Pepper leaf curl Bangladesh virus	PepLCBV	*Solanum capsicastrum*, Chilli,
50.	Pepper leaf curl Lahore virus	PepLCLaV	Tomato
51.	Radish leaf curl virus	RaLCuV	Radish, Tobacco, Chilli, Bhendi
52.	Rhynchosia yellow mosaic India virus	RhYMIV	Rhynchosia
53.	Rhynchosia yellow mosaic virus	RhYMV	French bean

(continued)

Table 5.1 (continued)

S.No	DNA-A Virus	Acronyms	Hosts
54.	Rose leaf curl virus	RoLCuV	Rose
55.	Senna leaf curl virus	SeLCuV	Senna
56.	Sida leaf curl virus	SiLCuV	Sida
57.	Spinach yellow vein Sikar virus	SpiYVV	Spinach
58.	Squash leaf curl China virus	SLCCNV	Pumpkin, Ash gourd
59.	Sri Lankan cassava mosaic virus	SLCMV	Cassava
60.	Sunn hemp leaf distortion virus	SHLDV	Sunn hemp
61.	Sweet potato leaf curl virus	SPLCV	Sweet potato
62.	Synedrella leaf curl virus	SyLCuV	Synedrella
63.	Synedrella yellow vein clearing virus	SyYVV	Synedrella
64.	Tobacco curly shoot virus	TbCSV	Tomato, French bean, Wild sunflower
65.	Tobacco leaf curl Pusa virus	TbLCPuV	Tobacco
66.	Tomato enation leaf curl virus	ToELCV	Tomato
67.	Tomato leaf curl virus	ToLCV	Tomato, Mentha, Parthenium, Ocimum, sunflower, French bean, Papaya, Cherry tomato
68.	Tomato leaf curl Bangalore virus	ToLCBaV	Tomato, cotton
69.	Tomato leaf curl Bangladesh virus	ToLCBV	Gaillardia
70.	Tomato leaf curl Gujarat Virus	ToLCGuV	Tomato
71.	Tomato leaf curl Karnataka virus	ToLCKaV	Tomato
72.	Tomato leaf curl Joydebpur virus	ToLCJV	Chilli
73.	Tomato leaf curl Kerala virus	ToLCKeV	Tomato
74.	Tomato leaf curl New Delhi virus	ToLCNDV	Potato, Tomato, Bhendi, Chilli, Bittergourd, Eggplant, Ash gourd
			Cucumis, Ridge gourd, Chayote, Pumpkin, Sponge gourd, Papaya, Cotton
75.	Tomato leaf curl Palampur virus	ToLCPalV	Tomato, Rumex, Melon, Egg plant, Bitter cucumber, Pumpkin
76.	Tomato leaf curl Patna virus	ToLCPatV	Tomato, cotton, *Cassia tora*, Mentha, Tobacco
77.	Tomato leaf curl Pune virus	ToLCPuV	Tomato
78.	Tomato leaf curl Rajasthan virus	ToLCRaV	Tomato
79.	Tomato severe leaf curl virus	ToSLCV	Tomato
80.	Velvet bean severe mosaic virus	VBSMV	Velvet bean
81.	Vernonia yellow vein virus	VeYVV	*Vernonia cinerea*
82.	Vinca leaf curl virus	ViLCuV	*Vinca rosea*

Table 5.2 Proposed new virus species yet to be approved by ICTV

S.No	Name of the virus	Host	Isolation place/ state	Year
1.	Allamanda leaf mottle distortion virus isolate Al-K1, KC202818,	Allamanda	Kalyani/WB	2012
2.	Chayote enation yellow mosaic virus Adalur isolate-embryo AD1, KX.259336, KX.259339	Chayote	Adalur, Tamil Nadu	2015
3.	Chilli leaf curl Ahmedabad virus, KM880103	Chilli	Ahmedabad/Gu	2014
4.	Coccinia mosaic Tamil Nadu virus isolate TN TDV Coc 1, KM244719	Ivy gourd	Tindivanam/TN	2013
5.	Cotton leaf curl Barasat virus, LC080677	*Malachra capitata*	Barasat/WB	2014
6.	Eclipta yellow vein virus isolate WOK44, KT390456	Hibiscus	Mirzapur/UP	2014
7.	Hollyhock yellow vein mosaic virus isolate Alcea rosea:Lucknow, JQ911766 KT390462	Hollyhock	Lucknow/UP	2011
8.	Jatropha leaf crumple India virus, Jodhpur] isolate SKJ3, KM189819, KM189818, KM023146	*Jatropha curcus*	Jodhpur/RA	2011
9.	Mirabilis leaf curl India virus, LK054801	*Mirabilis jalaba*	Kangra/HP	2013
10.	Rhynchosia yellow vein mosaic virus clone pBdGn05, KP752090	French bean	Gandhinagar/GU	2014
11.	Senna leaf curl virus isolate Mohali, KU852742	Senna	Mohali/PU	2013
12.	Synedrella leaf curl virus, isolate Synd-1, KJ939345	Synedrella	Portblair/ Andaman	2009
13.	Synedrella yellow vein clearing virus, KX363443	Synedrella	Portblair/ Andaman	2013
14.	Tomato enation leaf curl virus isolate TC14, KP195260,	Tomato	Narasipura/KA	2008
15.	Tomato severe leaf curl virus isolate TC101, KP195267	Tomato	Kalakada/AP	2007

to occur in India (Table 5.1). The data entered in the NCBI database for the Indian begomoviruses after March 2015 to November 2016 were analyzed by the authors following the guidelines set by the study group on geminiviruses and 16 more new species were identified, which are listed in Table 5.2. The details on distribution of viruses in different states, the host range of some selected viruses and number of hosts Indian begomoviruses infect are given in Tables 5.3, 5.4, and 5.5.

Excellent reviews are available (Stanley 1985; Harrisson and Robinson 1999; Hanley-Bowdoin et al. 1999, 2013; Guiterrez 2000; Rojas et al. 2005; Briddon et al. 2003; Fondong 2013), which may be referred for understanding the geminivirus genome and gene functions. Begomoviruses being the most important viral pathogen in Indian agriculture (Varma and Malathi 2003), a great deal of attention has

Table 5.3 Number of begomoviruses recorded in the different states of India

S.No.	States	No. of viruses
1	Andhra Pradesh	8
2	Assam	1
3	Bihar	8
4	Chhattisgarh	1
5	Delhi	14
6	Gujarat	10
7	Haryana	10
8	Himachal Pradesh	10
9	Jharkhand	3
10	Karnataka	12
11	Kerala	9
12	Madhya Pradesh	6
13	Maharashtra	9
14	Meghalaya	1
15	Odisha	5
16	Punjab	10
17	Rajasthan	19
18	Tamil Nadu	16
19	Uttar Pradesh	33
20	Uttarakhand	1
21	West Bengal	11

Table 5.4 Natural infection of begomoviruses in major crop species in India

S.No.	Host (No. of viruses)	Viruses
1	Bhendi (11)	BYVMV, BYVBhV, ToLCDNV, OELCuV, OLCuV, CLCuAlV, CLCuBaV, RaLCuV, HoYVMV, MeYVMV, BVYMV-Har
2	Cassava (2)	ICMV, SLCMV
3	Chilli (11)	ChiLCAV, ChiLCV, ChiLCINV, ChiLCKV, ChiLCVV, PepLCBV, ToLCNDV, PaLCuV, RaLCuV, ToLCV, ToLCJV
4	Corchorus (2)	CoGMV, CoYMV
5	Cotton (6)	CLCuBaV, CLCuKoV, CLCuMuV, ToLCBaV, ToLCPTV, ToLCNDV
6	Cucurbits (6)	ChEYMV, CoMTNV, SLCCNV, ToLCNDV, ToLCPalV, AEV
7	Jatropha (7)	JLCrIV, JLCV, JMINV, JYMV, JLCrIV, ICMV, PaLCuV
8	Legumes (17)	AEV, MYMV, MYMIV, DoYMV, CLCuKoV, PaLCuV, FbLCV, VBSMV, ToLCNDV, ChiLCV, ToLCV, ToLCPtV, HgYMV, RhYMV, RhYMIV, TbSCV, PaLCrV
9	Mestha/Kenef (6)	ChiLCV, MeYVMV, MeYVMBaV, MaYVMV, OeLCuV, CLCuVaV
10	Papaya (5)	PaLCuV, PaLCrV, ChiLCV, ToLCNDV, ToLCV
11	Tobacco (3)	TbCSV, TbLCPuV, ToLCPtV
12	Tomato (18)	ChiLCV, ChiLCIV, PaLCuV, ToLCBaV, ToLCV, ToLCGuV, ToLCKaV, ToLCKeV, TbCSV, ToLCNDV, ToLCPalV, ToLCPtV, ToLCPuV, ToLCRaV, ToLCGaV, RaLCuV, AEV, PepLCULaV.
13	Radish (2)	RaLCuV, PaLCuV

Table 5.5 Host-range of the major begomoviruses in India

S.No.	Name of the virus	Hosts infected
1	AEV	Cleome, Amaranthus, Poppy, Ageratum, Carrot, Soybean, Zinnia, Tomato, Crassocephalum, Pointed gourd, Calendula, Tagetes, Fenugreek, Papaya
2	ChiLCV	Chilli, Kenaf, Amaranthus, *Solanum nigrum, Petunia,* Mentha, Egg plant, *Phaseolus aureus*, Papaya, Tomato
3	CLCuBaV	Cotton, Bhendi, Hibiscus
4	CLCuMuV	Hibiscus, Cotton, Hibiscus- rosa sinensis, *H.cannabinus*
5	MYMV	Blackgram, Mungbean, Soybean, Moth bean
6	MYMIV	Blackgram, Cowpea, Mungbean, French bean, Dolichos, Soybean
7	PaLCuV	Clusterbean, Papaya, Acalypha, Radish, Soybean, Tomato, Chilli, Amaranthus, Brassica, Aster, Jatropha, Crambe species, Turnip, Sunnhemp, Aster, Brassica
8	ToLCBaV	Cotton, Tomato
9	ToLCV	Tomato, Mentha, Chilli, Parthenium, Ocimum, Sunflower, French bean, papaya, cherry tomato
10	ToLCNDV	Potato, Luffa, Lagenaria, Pumpkin, Ash gourd, Cucumber, Chilli, Bhendi, Papaya, Cotton, Papaver, Egg plant, Bitter gourd, Chayote
11	ToLCPalV	Tomato, Pumpkin

been given to begomovirus research in India. As a result, a wealth of information has been generated. In the present chapter, efforts have been made to review the research work conducted on begomoviruses occurring in India. Details on full-length genome sequences of begomoviruses, discovery of new begomoviruses and recent changes in the nomenclature of the Indian begomoviruses are included. The investigations carried out to elucidate replication, suppression of RNAi and viral gene functions are briefly discussed. In this chapter, instead of crop based grouping, description of the work on the Indian begomoviruses are presented based on the four categories of disease symptoms: mosaic, yellow mosaic, yellow vein mosaic and leaf curl. The focus in the presentation is more on the significant biological and molecular features of the begomoviruses rather than the diseases.

5.2 Historical Developement

5.2.1 Global Scenario

The earliest recorded plant virus disease happens to be the one caused by whitefly transmitted geminivirus. The yellow vein virus symptoms in *Eupartorium chinense* was referred in a poem written by empress Koken in Manyoshu, a Japanese anthology prepared in AD 752. The virus described in the poem has been now identified as tobacco leaf curl virus belonging to the genus *Begomovirus* (Saunders et al. 2003). Almost thousand years later, the economically important geminivirus diseases were described in the nineteenth century such as African cassava mosaic

(Warburg 1894) and maize streak in Africa (Fuller 1901; Storey 1936), sugar beet curly top in western USA (Carsner and Stahl 1924), cotton leaf curl in Angola and Sudan (Tarr 1951) and bean golden yellow mosaic in South America (Costa 1976). In all these diseases, the role of whitefly or leafhopper as vector of the causal agent was established beyond doubt. However, the nature of the etiological agent remained elusive until 1980s. The studies on purification of the virus (Bock et al. 1974; Goodman et al. 1977; Larsen and Duffus 1984) revealed the consistent association of geminate particles with these diseases. The DNA genome was identified and on the basis of buoyant density gradient centrifugation, Francki and Hatta 1980 revealed the presence of one copy of circular DNA within the geminate particle. The viruses having ssDNA genome with the geminate morphology was proposed as a new group of plant viruses and named as geminivirus group (Harrison et al. 1977). On the basis of restriction mapping and infectivity dilution curve, Goodman 1977 predicted the genome of BGMV to be bipartite (Haber et al. 1981). However, other leafhopper transmitted viruses were found to be monopartite (Mullineaux et al. 1984). The bipartite nature of genome of the begomovirus was confirmed by complete nucleotide sequencing in the case of African cassava mosaic virus (ACMV) (Stanley and Gay 1983), tomato golden mosaic virus (TGMV) (Bisaro et al. 1982) and BGMV (Goodman 1977). Characterization of the Old World begomoviruses soon revealed the monopartitie nature of some begomoviruses such as tomato yellow leaf curl virus- (TYLCV) Thailand (Rochester et al. 1994) and TYLCV-Israel (Navot et al. 1991). The most innovative method of delivery of geminiviral genomic component into the host referred as agroinoculation was developed by Grimsley et al. (1986) for maize streak virus (MSV) in maize. This was picked up immediately and agroinoculation became the favoured technique of delivery of viral inoculum to seek answers to many questions on replication, viral gene function and suppression of RNAi defense and host resistance.

The absence of typical symptoms, when DNA A like components are inoculated in primary hosts for some begomoviruses such as Ageratum yellow vein mosaic virus (AYVMV) and cotton leaf curl viruses led to the discovery of alphasatellite and betasatellites associated with begomoviruses (Briddon et al. 2003; Saunders and Stanley 1999, 2000).

The difficulty in cloning the complete genomic component of unknown geminiviruses, especially those which are present in low concentration in plant tissues was overcome by the rolling circle amplification protocol (Haible et al. 2006). This method for enhancing the concentration of circular DNA molecule using Φ29 DNA polymerase made isolation and cloning of the geminiviral genomic components easy and thus the amplification technique revolutionized the geminivirus research.

5.2.2 Indian Scenario

The chronological development of begomovirus research can be catagorised into five distinct phases spanning nearly 100 years (Fig. 5.3). Yellow vein mosaic disease of bhendi occurring in Bombay, Maharashtra is the first whitefly transmitted

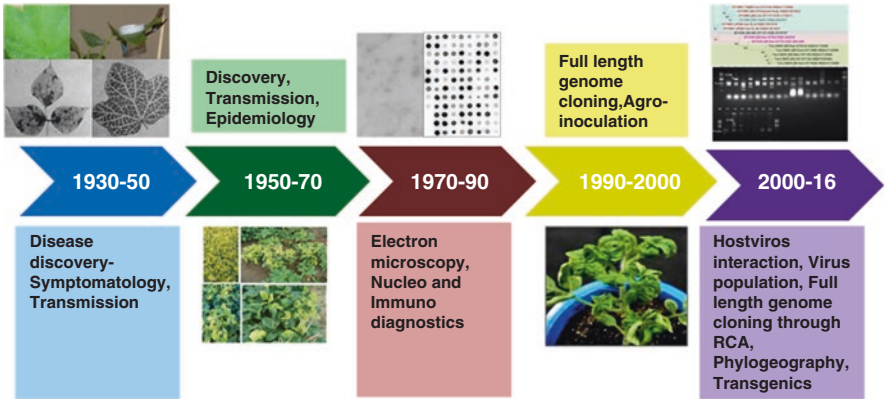

Fig. 5.3 Milestones in begomovirus research in India

geminivirus disease recorded in India (Kulkarni 1924). Subsequently, several diseases were described e.g., leaf curl disease of *Zinnia elegans* (Mathur 1932, 1933), tobacco (Pal and Tandon 1937; Pruthi and Samuel 1937) and tomato (Vasudeva and Samraj 1948) and yellow vein mosaic of pumpkin and yellow mosaic of limabean and dolichos (Capoor and Varma 1948a, b). While recording these diseases, the scientists also described the transmission characteristics of whitefly and host range of the virus. The period from 1950 to 1970, the investigations were focused on the vector, *Bemisia tabaci* and its management. To effectively manage the disease, the sources of resistance were identified such as *Carica cauliflora* for papaya leaf curl virus and *Abelmoschus manihot* var. *pungens* for yellow vein mosaic of bhendi. In this period, the most devastating chilli and papaya leaf curl disease (Mishra et al. 1963) and yellow mosaic disease (YMD) of grain legumes were recorded (Nariani 1960). It is interesting to note that, although yellow mosaic of limabean and dolichos was recorded as early as 1948, YMD of mungbean and blackgram was observed only in 1960s. In the year 1971, Seth et al. (1971) observed chlorotic stripes in self sown bajra plants. The virus could be easily transmitted by *Cicadullina mebila* and so they identified it as maize streak virus similar to one in Africa. The spread of yellow mosaic disease to different leguminous hosts was identified in northern and southern India. Muniyappa and Veeresh (1984) described whitefly transmitted viruses in several plant species, which indicated the potential of these viruses (Nene et al. 1971; Nene 1972) to emerge as serious pathogen.

The association of geminate particle with the disease was first demonstrated for Indian cassava mosaic virus (ICMV) (Malathi and Srinivasan 1983; Malathi et al. 1985). During the decade between 1980 and 1990, the major research area was diagnosis based on electron microscopy, serology and nucleic acid hybridization. Purification of horsegram yellow mosaic virus (HgYMV) (1987) and tomato leaf curl Bangalore virus (ToLCBaV), preparation of polyclonal antibody and its interaction with other begomoviruses were demonstrated (Muniyappa et al. 1991a, b). Immunosorbent electron microscopy was utilised to demonstrate the association of

begomoviruses in legumes, vegetables and other crops (Varma et al. 1989, 1993). As the picture on geminiviruses became clear globally, using the polyclonal antibody and DNA A probe to ACMV, geminivirus etiology in several plant species in India was confirmed (Harrison et al. 1991). Using monoclonal antibodies to ICMV and ACMV, the differential epitope profile of viruses were generated for ICMV, bhendi yellow vein mosaic virus (BYVMV), dolichos yellow mosaic virus (DoYMV) and mungbean yellow mosaic virus (MYMV) (Swanson et a1. 1992).

The molecular characterization of begomoviruses by then gained momentum in India and the begomoviruses associated with the yellow mosaic and tomato leaf curl were cloned and sequenced (Varma et al. 1991; Ramachandran et al. 1996; Srivastava et al. 1995). The infectivity of cloned components of the Old World bipartite begomovirus MYMIV was shown for the first time through agroinoculation (Mandal et al. 1997). More or less at this time (1989–1993), the cotton leaf curl epidemic emerged in western India and the involvement of a distinct begomovirus was estiblished (Varma et al. 1993). The period is also significant with the discovery of betasatellites in BYVMV infection in southern India (Jose and Usha 2003) and with cotton leaf curl virus in north western India (Radhakrishnan et al. 2004b). Betasatellites were further found to be ubiquitously associated with all the monopartitie and some bipartitie Old World begomoviruses. As more begomovirus disease problems emerged, there is a greater emphasis in begomovirus research in different Indian institutions; while on one side, molecular mechanism of viral pathogenicity was investigated, in parallel more begomoviruses from diverse plant species including crops and weeds were discovered and characterised.

5.3 Begomoviruses Causing Mosaic Diseases

The begomoviruses which are named after the mosaic symptom (Fig. 5.3) are Indian cassava mosaic virus (ICMV), Sri Lankan cassava mosaic virus (SLCMV), Jatropha mosaic India virus and Coccinia mosaic Tamil Nadu virus. Of these, ICMV and SLCMV are economically more important.

5.4 ICMV and SLCMV

5.4.1 Discovery and Distribution

The begomoviruses causing the severe cassava mosaic disease (CMD) in India were reported as ICMV (Malathi and Sreenivasan 1983; Hong et al. 1993; Dutt et al. 2005) and in Sri Lanka as SLCMV (Saunders et al. 2002b). Although, SLCMV was originally identified in Sri Lanka, the major characterization was carried out from the samples collected from Kerala and Tami Nadu, in India.

The CMD is a typical example of how crop introduced from another continent got infected by the virus occurring indigenously. Cassava, (*Manihot esculenta* Crantz) of the family *Euphorbiaceae* is a perennial shrub with tuberous roots and

its origin lies within the boundaries of Amazon centring around Brazil. Though, it was domesticated 10,000 years ago, it remained confined to Latin America until sixteenth century. The Portuguese rulers introduced the crop into Kerala state in the eighteenth and nineteenth century mainly to overcome the famine that existed between 1870 and 1920. The introduced crop was popularized by the erstwhile rulers of Travancore state. The resilient nature of the crop to survive in drought and in acid soil led to its widespread adoption. The CMD constitutes the earliest recorded disease as 'Krauselkranheit' by Warburg (1894) in Africa. As early as 1935, Storey established the viral etiology the disease. The CMD was noticed even in 1940s and Abraham (1956) mentioned this as a challenging threat to the cultivation of the crop. However the first published record of the disease happened only in 1966 by Alagianagalingam and Ramakrishnan (1966). The disease was mainly restricted to important cassava growing regions of India, *viz*., Kerala and Tamil Nadu. Manivasagam et al. (2006), surveyed and recorded more than 90% disease with disease severity ranging from 2.35 to 4.0. The crop was introduced in other states like Andhra Pradesh, North Eastern Indian States following which, incidence of the disease was noticed in these states too. The inadvertent distribution of virus borne setts has contributed to widespread of the disease where the crop is introduced. While both ICMV and SLCMV are present in Tamil Nadu and Kerala, their distribution in other states is not clear. There has been no report of these two viruses from other countries in Indian Subcontinent. Recently, in 2015, the cassava plants in Ratanakiri, Raunmonn, Cambodia were found infected by SLCMV (Wang et al. 2015).

5.4.2 Economic Loss

The yield loss due to the disease is dependent on the varieties infected. Yield loss ranges from 17 to 88%; in the highly susceptible cultivar Kalikalan, hairy roots like tubers were observed when the symptoms are severe. Malathi et al. (1985), reported upto 45% reduction in highly susceptible cultivars.

5.4.3 Symptomatology

The characteristics symptoms of the disease are discoloured pale green chlorotic area alternating with darker green tissue resulting in mosaic pattern; the leaves may be distorted and appear like leaf curl; in some cases even a shoe string like appearance is observed (Fig. 5.4e). The plant growth may be stunted and the cuttings from infected plants show deteoration.

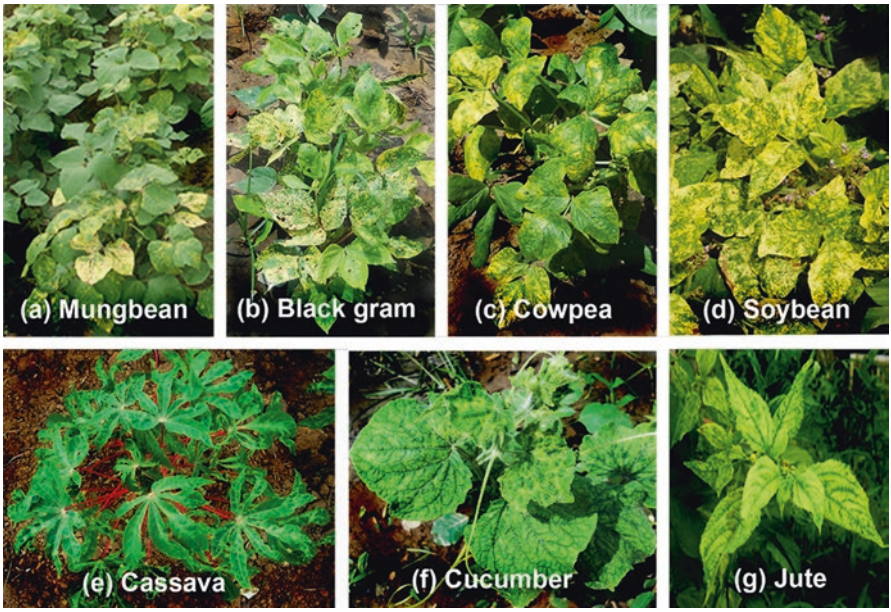

Fig. 5.4 Mosaic and yellow mosaic diseases in different crops caused by begomoviruses. (**a**–**d**) Mungbean yellow mosaic India virus causing yellow mosaic disease in grain legumes; (**e**) Indian cassava mosaic virus causing mosaic disease in cassava (**f**) tomato leaf curl New Delhi virus causing chlorotic blistering in cucumber; (**g**) corchorus golden mosaic virus associated with mosaic disease of jute

5.4.4 Transmission and Host Range

ICMV is transmitted from cassava to cassava by *B. tabaci*, with acqucition access period (AAP) of 24 h and IAP of 24 h on cassava seedlings (seedlings raised from true seeds) (Mathew and Muniappa 1993). The efficiency of transmission was about 11%. They also showed transmission of ICMV from cassava to *N. benthamiana* to be about 8% and from *N. benthamiana* to cassava about 20%, the overall transmission efficiency was 22%. The low efficiency of whitefly transmission was understood when Lisha et al. 2003 revealed that the population of whiteflies occurring on cassava is very distinct and only these populations successfully transmitted ICMV from cassava to cassava. They showed that the cassava reared whiteflies do not breed on sweet potato and neither the sweet potato reared whiteflies on cassava. However, eggplant and tobacco were common hosts for both biotypes. The cassava adopted haplotype of *B. tabaci* is referred to as Indian cassava biotype. Antony et al. (2006), further investigated vector transmission and demonstrated that after 8 h AAP/IAP, the presence of ICMV could be detected through PCR in the salivary glands, stylet and digestive tract, using specific primers. They obtained 71.43% transmission of ICMV from cassava to cassava. Typical chlorotic mosaic and distortion of leaves were observed 9 days post inoculation (DPI).

Duraisamy et al. (2013) performed transmission experiments using meristem derived virus-free plants of *cv*H226, using cassava strain whitefly by giving 48 h of AAP and IAP. They found that starvation for 3 h before AAP, 48 h AAP resulted in 80.5% of transmission. Typical symptoms were produced 25th DPI. They confirmed the presence of ICMV and SLCMV in the vector through PCR using specific primers for replicase gene. From above results, it appears that whatever might have been bottlenecks in achieving transmission earlier, had been resolved by using cassava strain specific whitefly haplotype.

Both ICMV and SLCMV are highly sap transmissible. ICMV was transmitted to four species of *Nicotiana*, *Petunia hybrid* and *Nicandra physoides*. In all these hosts, leaf curling, crinkling and chlorotic lesion, leaf deformations were produced 6–10 days after inoculation. Severe stunting and reduction in leaf lamina were also observed (Mathew and Muniyappa 1993). However ICMV was not sap transmitted from cassava to cassava, or from any other hosts to cassava.

Jose et al. (2008), performed sap inoculation of SLCMV on 75 plant species, and found that the virus was sap transmitted easily to 39 species belonging to *Solanaceae*. Chlorotic spots, leaf curling and vein clearing were some of the symptoms. SLCMV differed from ICMV in being infectious on *N. longiflora*.Some of the hosts like, *N. amplexicaulis*, *N. benavidesii*, *N. nudicaulis* which are easily infected by ICMV, are not hosts for SLCMV. In all these host species presence of the virus was confirmed by SLCMV specific primers.

Though the experimental host range of ICMV and SLCMV is wide, natural occurrence of these two viruses in other hosts is met with rarely. On the basis of PCR results, association of ICMV has been reported from bittergourd (Rajinimala and Rabindran 2007), mulberry (Sherry 2016) and Jatropha (Aswathnaryana et al. 2007; Gao et al. 2016).

5.4.5 Serology

The symptoms of CMD in India, were not distinguishable from the disease symptoms in cassava in Africa. The electron microscopic evidence for the association of geminivirus with the CMD in India was given by Malathi and Srinivasan (1983). Malathi et al. (1985) showed the positive serological reaction in gel diffusion test with the PAb raised against "T" strain of ACMV. Using the panel of monoclonal antibodies to ICMV and ACMV in TAS – ELISA tests, Harrison et al. (1986) and Harrisson and Robinson (1999) differentiated the Cassava mosaic virus isolates from Africa, India and Sri Lanka into three groups as A, B, C respectively. Mathews and Muniyappa (1992) purified ICMV from *N. benthamiana* and gave clear evidence of the presence of geminate particles by electron microscopy. They produced antibody by purifying coat protein which was used in detecting the ICMV in ISEM tests in cassava, ceara rubber and many hosts. The polyclonal antibody also reacted with other begomoviruses associated with weeds.

5.4.6 Genome Comparison

The complete genome of ICMV and SLCMV were sequenced and their entity as separate species was established (Saunders et al. 2002a, b). The occurrence of SLCMV in India was first noted by Dutt et al. (2005). Using abutting primers, they cloned full-length genomic components from infected cassava samples form Kerala. The genome of ICMV and SLCMV consists of 2.7–2.8 kb circular ssDNA encapsidated within geminate particles. They are bipartite and genome organization is similar to the Old World bipartite begomoviruses with two virion sense (AV1, AV2) ORFs and four (AC1, AC2, AC3, AC4) in complementary sense. In one of the isolates of ICMV, ICMV-KerI, there is 41 bp triple repeats insertion in CR which has extended the CR region upto 267 nucleotides in DNA A, 318 in DNA B. There is also an insertion of unrelated sequence of 21 bp in ICMV-Mah isolate. The iteron sequences of ICMV were identified as GGTACTCA, whereas that of SLCMV was TTGGAGACA similar to iteron sequences of ACMV.

In the GenBank database, full length DNA A sequences is available for 9 isolates of ICMV and 15 isolates of SLCMV. Of nine sequences of ICMV available, four are derived from Jatropha samples. On the contrary, all SLCMV sequences are derived only from cassava. There is nearly 80–84% nucleotide identity in DNA A component between SLCMV and ICMV (Dutt et al. 2005; Saunders et al. 2002b). Both ICMV and SLCMV exhibit 72–74% identitiy with ACMV and less than 69% identity in DNA A component with other begomoviruses. The interesting feature is SLCMV and ICMV share nearly 94% identity in DNA B component, however this identity is more pronounced in the coding region, outside CR, the identity is nearly 97%. Within the noncoding region, identity of SLCMV with ICMV is very less upstream of stem-loop region (57–59%) but more in downstream of stem-loop region (80%). In this case it is speculated that recombination event might have occurred where ICMV DNA B sequences up stream of stem loop might have been replaced with the same region from SLCMV DNA A. As a result of this recombination event SLCMV DNA A clones have transferred *cis* acting iteron motif required for replication.

Rothenstein et al. (2006) employed PCR-RFLP strategy to assess the variability among ICMV and SLCMV isolates. Full length DNA A types were restricted by *EcoRI*, *Hpa II* and *Sau3 A1* and their profiles were compared to identify ICMV and SLCMV isolates. On the basis of restriction profile they could decipher six genotypes, which on complete nucleotide sequencing revealed the presence of more number of SLCMV isolates than ICMV. When Patil et al. (2005) investigated the biodiversity using PCR-RFLP, and found that ICMV and SLCMV though present in mosaic affected areas, ICMV was restricted to certain regions, whereas SLCMV was widespread. On the basis of RFLP pattern, they also recorded high proportion (40%) of samples showing novel patterns different from ICMV and SLCMV. In their extensive survey, extent of mixed infection was low and the randomly distributed point mutation gave rise to novel RFLP patterns and not any recombination event.

5.4.7 Phylogenetic Relationship

Rothenstein et al. (2006) performed analysis taking recombination into consideration and showed that the tree topologies generated vary when partitioning of analysis is done between DNA A segments 2778–857, 857–1957 and 1958–2777 nucleotides. Different tree topologies obtained when they analysed the DNA A dissecting into three segments are indicative of recombinatorial events. Especially in the case of SLCMV (*CO*) and ICMV Mah isolates. When full length DNA A is considered without partitioning, all ICMV and SLCMV isolates get distinctly separated as two clades. Interestingly, when analysis is performed for DNA B components, the ICMV and SLCMV cluster together. Legg et al. (2015) analyzed the relationship between all cassava infecting geminiviruses and found that the ICMV and SLCMV representing South Asian groups of Cassava geminiviruses are well separated from other African CMGs.

5.4.8 Recombination

Nucleotides comparison between ACMV, ICMV and SLCMV clearly established that SLCMV show high nucleotide identity outside CR, the identity especially upstream of loop region is very less. This region is highly conserved among all SLCMV isolates. Saunders et al. (2002b) suggested that recombinations have occurred between SLCMV DNA A and ICMV DNA B. This phenomenon referred to as regulon grafting (transfer of intergenic region harboring replicational and transcriptional control elements) so as to enable SLCMV DNA A to capture ICMV DNA B and introduce *cis* acting iteron sequences to achieve replication. Rothenstein et al. (2005), Patil et al. (2005) also suggested several recombinational events occurring in DNA A component.

5.4.9 Establishment of Koch's Postulate

The cloned DNA components of ICMV (lDNA A and DNA B) and SLCMV when introduced either through mechanical inoculation or through *Agrobacterium tumefaciens* produced symptom readily in *Nicotiana benthamiana*, SLCMV produced severe stunting, leaf curl and chlorosis and inoculation with ICMV led to leaf curl symptoms. SLCMV was more virulent and severe than ICMV. Saunders et al. (2002b) demonstrated that ICMV clones were infectious on *N. clevlandi* and *N. glutinosa*. In the case of SLCMV, DNA A alone induced leaf roll and vein swelling symptom in *N. benthamiana.* The cloned components of SLCMV successfully induced symptom expression in cassava by biolistic inoculation. Leaf curl and mosaic symptoms were observed, 6 weeks after inoculation (Saunders et al. 2002b). Dutt et al. (2005) also obtained a mild mosaic symptom expression in meristem derived cassava plants cv Ebwanateraka 6 month post inoculation after biolistic delivery.

Symptom expression following inoculation of cloned ICMV component on cassava continued to be elusive. This bottleneck was overcome, when Rothenstein et al. (2005) infected cassava plants by delivering ICMV cloned components using a hand held particle gun. All the plants showed mild CMD symptom within 60 DPI. Mittal et al. (2008) proved the infectivity of SLCMV on cassava and the model host *Arabidopsis* by agroinoculation. They observed that post inoculation, DNA extracted from *Arabidopsis* was essentially multimers and showed slow electrophoretic mobility. In all these infectivity experiments, the presence of the viral DNA was demonstrated by analysis of replicative forms in Southern blots.

5.4.10 Exchange of Components

The cassava geminiviruses, ACMV, SLCMV and ICMV offer interesting experimentation system to study the complementation/ re-assortment of genomic components between begomoviruses. The pseudorecombination produced by re-assortment of components of ACMV and ICMV did not result in infection in *N. benthamiana* proving that these two are distinct virus species and exchange of component is not possible. Contrastingly pseudorecombinant produced by inoculation with ACMV DNA A and SLCMV DNA B, was infectious on *N. benthamiana*. This further suggests that iteron conservation seen between ACMV and SLCMV has promoted the *trans* replication of SLCMV DNA B by ACMV DNA A. Karthikeyan et al. (2016) demonstrated pseudorecombination between SLCMV and ICMV in *N. benthamiana* which resulted in symptom expression and the replication of ICMV DNA B by SLCMV DNA A.

Interestingly, SLCMV DNA A alone when inoculated on *N. benthamiana* produced symptom phenotype similar to the phenotype produced by the monopartite begomovirus. In addition Saunders et al. (2002b) showed that SLCMV DNA A replicated betasatellite, Ageratum yellow vein mosaic betasatellite and produced severe downward leaf curl symptom in *N. glutinosa* and yellow vein symptom in *Ageratum conyzoides*. The symptom produced in ageratum was like those produced by AYVMV and beet curly top virus (BCTV). Hence, Saunders et al. (2002b) suggested that SLCMV perhaps is a monopartite virus, which evolved to become bipartite by capturing ICMV DNA B.

5.4.11 Molecular Basis of Pathogenicity

The role of coat protein in symptom production was examined by Kelkar et al. (2016), taking SLCMV/*N. benthamiana* system. They generated CP null mutants, single, double triple and quadruple amino acid replacement mutations and tested the infectivity in comparison with wild type. The coat protein null mutants were not infectious, other mutation in combination or alone produced mild symptoms and reduced viral titre. The mutants like K129P/F152E led to the absence of leaf rolling,

which was restored when the mutant K128W was inoculated jointly. The viral titre as determined by qPCR was more than 100-folds reduced in some mutants like T128L, S134H, F152E. These changes in amino acid residues were predicted to affect the secondary structure of coat protein which may affect its other functions like interaction with V2.

Interestingly Resmi et al., (2014, 2015) observed a strange phenomenon that SLCMV, Rep gene induced high rate of transportation of IS426 elements in *Agrobacterium tumefaciens* when SLCMV – Rep gene under the transcriptional control of 35S promoter in sense orientation was introduced into *Agrobacterium tumefaciens* strain EHA105; the binary plasmid size increased in 15-folds. The sequences proximal to 35S promoter were rearranged, this region comprised of 1.3 kb IS426 element of *Agrobacterium tumefaciens*. Transfer of Rep gene in antisense orientation or non-functional version of Rep did not cause the transposition. They suggest that SLCMV Rep may act similar to RepA protein and may act along with transposans to trigger the transposition of 1S426.

Another dimension of interaction between *Agrobacterium tumefaciens* and begomoviruses was studied by Resmi et al. (2015), by raising transgenic *N. benthamiana* incorporated with vir E2 gene of *A. tumefaciens.*The three transgenic plants showed attenuated symptom after agroinoculation with SLCMV viral DNA and suggest that Vir E2 may be a good candidate gene to develop resistance against geminivirus.

5.4.12 Virus Prevalence in the Field

Survey conducted in Kerala (Jose et al. 2011) and Tamil Nadu (Rajinimala et al. 2011) clearly pointed out that, the disease incidence is very high, nearly 100% in some locations; in these locations there was high whitefly population of 17 or 15/plant. Jose et al. (2011) clearly showed that the infected planting material contribute to more disease spread than whitefly mediated transmission. In both the states, incidence of SLCMV infection was always higher than the ICMV, mixed infection of ICMV and SLCMV together were seen upto 12–16% that too only in two districts of Thiruvananthapuram and Malappuram. Jose et al. (2011) detected up to 33% infection of ACMV in the district Path in amthitta which needs to be carefully looked into. In the context of mixed infection Karthikeyan et al. 2016 made some interesting observation. The cassava plants infected by SLCMV collected from Malappuram and Thiruvananthapuram were maintained in glass house at MKU, India and University of Bazel, Switzerland. The plants from Thiruvananthapuram did not show persistent infection of SLCMV, exhibited symptom recovery phenomenon. The Malappuram isolate was persistent. Interestingly the SLCMV infected Thiruvananthapuram plant, exhibited the emergence of symptom after 6 month period, but the re emergent virus was found to be ICMV. All the three isolate, persistent SLCMV, non- persistent SLCMV and re-emerged ICMV were infective on *N. benthamiana.* Interestingly pseudorecombination occurred between SLCMV DNA A which transreplicated ICMV DNA B.

5.4.13 Resistance to SLCMV and ICMV

During the last five decades, continuous attempts are being made to identify resistant lines (Nair et al. 1998; Abraham et al. 2006; George et al. 2012). The cultivar SreePadmanaba (TMS30001 line Mnga-1 line) has been evaluated for resistance in field conditions and has been released for cultivation in Tamil Nadu. The open pollinated seedlings of Mnga-1 were evaluated from which 242 resistant lines have been identified. Two of three lines CMR-1 and CMR 129 are expected to perform well (Unnikrishnan et al. 2011). About 56 clones derived from West African land races having CMD-2 gene were crossed with inbred lines at CTCRI, India which are being evaluated (Sheela et al. 2012). Several interspecific hybrids involving species *Manihot glaziovii*, *M. caerulescens*, *M. peruviana* were generated of which, hybrids of *M. caerulescens* showed higher level of resistance. The hybrids are used as resistance donors to introgress the genes into elite Indian cultivars; One such derivative CMC-1 (Sheela et al. 2012) shows resistance. From above results it is not clear whether the resistance in Indian cassava cultivars to ICMV and SLCMV is governed by the polygenic recessive gene (CMD-1) or a major dominant gene (CMD-2).

5.5 Begomoviruses Causing Yellow Mosaic Disease

Begomoviruses causing yellow mosaic diseases are known to infect plant species of the families Leguminosae, Verbenaceae and Malvaceaeare/Tiliaceae in India. Mungbean yellow mosaic virus (MYMV) and mungbean yellow mosaic India virus (MYMIV) affect many grain legumes like cowpea, mungbean, blackgram and soybean (Fig. 5.4a–d). *Clerodendron inerme*, a common hedge plant grown in India is affected by a yellow mosaic disease, where Clerodendron yellow mosaic virus, a new begomovirus was identified (Sivalingam et al. 2011). The yellow mosaic disease of Jute (*Corchorus capsularis*) was described from West Bengal in 1978. Some of the elite cultivars of Jute like JRC 7447 and JRC212 showed nearly 50% of disease incidence (Ghosh et al. 2008). Corchorus golden mosaic virus, a New World begomovirus associated with yellow mosaic of Jute was characterized by Ghosh et al. 2008, 2012. Interestingly, the nonanucleotide sequence at the origin of replication of this virus was CATTATTAC instead of TAATATTAC, It is significant that a New World bipartite begomovirus has been described in a crop which has been cultivated since seventeenth century in India. MYMIV and MYMV are the two most important begomovirus causing causing widespread yield losses in grain legumes in India.

5.6 MYMV and MYMIV

The two virus species, MYMV (Morinaga et al. 1990; Ramachandran et al. 1996) and MYMIV (Varma et al. 1991) causing yellow mosaic disease in grain legumes constitute the most interesting group of viruses that are evolutionarily most diversed

from all the so far known begomoviruses in India. Considering that most of the pulse crops have the centre of origin in the Indian subcontinent, yellow msaic viruses may be considered as the virus of Indian origin. The begomoviruses causing yellow mosaic disease in legumes in Asian and South East Asian countries are designated as legumoviruses (LYMVs) due to their uniqueness. Currently, LYMVs consist of seven members, MYMV, MYMIV, dolichos yellow mosaic virus (DoYMV), horsegram yellow mosaic virus (HgYMV), Rhynchosia yellow mosaic virus (RhYMV), Rhynchosia yellow mosaic India virus (RhYMIV) and velvet bean severe mosaic virus (VbSMV). Of these LYMVs, MYMV and MYMIV are important as they have wide host range and cause considerable economic loss.

5.6.1 Discovery and Distribution

The earliest record of yellow mosaic disease is in 1948–1950, much later than the record of yellow vein mosaic disease of bhendi by Kulkarni (1924). Capoor and Varma (1948a, b, 1950) observed yellow mosaic symptoms in 1940 at Poona, in Dolichos and Lima bean (*Phaseolus lunatus*). They traced the yellow mosaic disease upto Gujarat, Khandesi and Deccan regions. The virus causing the disease was transmitted by whitefly and they rightly identified the viruses which occurred in dolichos and Lima bean as two distinct viruses which were not transmissible to other leguminous hosts. About 5 years later in 1955, Nariani observed yellow mosaic symptoms in mungbean at an experimental farm at IARI New Delhi and identified the virus distinct from dolichos and designated it as a mungbean yellow mosaic virus, as it was not transmitted either to dolichos or Lima bean (Nariani 1960). Subsequently yellow mosaic disease symptoms were observed in many pulse crops such as blackgram, cowpea, cluster bean, French bean, groundnut, horsegram, hyacinth bean, moth bean, mungbean, Lima bean, pigeonpea and soybean (Table 5.6). The disease was described mainly as yellow mosaic, occasionally as yellow flecks. Due to overlapping host range it was presumed that the disease in all these leguminous hosts is caused by mungbean yellow mosaic virus (Nariani 1960). Meanwhile the disease was also reported from Pakistan in cowpea and mungbean (Ahmed and Harwood 1973) in mungbean from Bangladesh (Jalaluddin and Shaikh 1981), Sri Lanka (Joseph et al. 1998) and Thailand (Shimizu et al. 1987). MYMIV was recently found associated with tomato leaf curl betasatellite in kidney bean in Oman (Shahid et al. 2017).

5.6.2 Economic Loss

Reduction in yield in grain legumes is highly dependent on the time of infection. The decrease in yield is marked when infection strikes at early stage. Infection of blackgram at four, five, six, seven and eighth week resulted in yield reduction upto 85%, 60%, 44%, 28% and less than 10% respectively (Nene 1972, 1973; Vohra and Beniwal 1979; Dhingra and Chenulu 1985; Suteri and Srivastava 1979). Singh et al.

Table 5.6 Crops and areas affected by mungbean yellow mosaic India virus and mungbean yellow mosaic virus in India

S.No.	Crop	Disease	Area	Reference
1.	Blackgram (*Vigna mungo*)	Yellow mosaic (yellow mottle and necrotic mottle)	Northern India	Nene (1973), and Singh et al. (1979)
			South India (Coimbatore)	Murugesan et al. (1977)
2.	Cowpea (*Vigna unguiculata*)	Yellow Mosaic	Northern India	Nene (1972)
		Yellow fleck	Northern India	Sharma and Varma (1976)
		Golden mosaic	India	Varma and Reddy (1984) and Srivastava and Varma (1988)
3.	Clusterbean (*Cyamopsis tetragonoloba*)	Yellow mosaic	Southern India	Rao et al. (1982)
4.	French bean (*Phaseolus vulgaris*)	Yellow mosaic	Uttar Pradesh (Pantnagar)	Singh (1979)
			Western India (Pune)	Unpublished result
5.	Groundnut (*Arachis hypogea*)	Yellow mosaic	Southern India	Sudhakar Rao et al. (1979)
6.	Horsegram (*Macrotyloma uniflourum*)	Yellow mosaic	Southern India	Muniyappa et al. (1975) and Muniyappa and Reddy (1976)
7.	Hyacinth bean (*Lablab purpureus*)	Yellow mosaic	Several part of India	Capoor and Varma (1948a, b), Muniyappa et al. (1975)
8.	Moth bean (*Vigna aconitifolia*)	Yellow mosaic	Rajasthan	Satyavir (1980)
9.	Mungbean (*Vigna radiata*)	Yellow mosaic	In all parts of India	Mishra et al. (1978), Nariani (1960), Varma et al. (1992), Bansal et al. (1984), and Nene (1973)
10.	Lima bean (*Phaseolus lunatus*)	Yellow mosaic	Western India	Capoor and Varma (1948a, b)
11.	Pigeonpea (*Cajanus cajan*)	Yellow mosaic	Northern India	Williams et al. (1968)
12.	Soybean (*Glycine max*)	Yellow mosaic	Madhya Pradesh	Keshwal et al. (1988) and Suteri (1974)

(1979) observed 19–21% reduction in blackgram and mungbean cultivation. In the blackgram cultivar T-49, yellow mosaic virus infection affects seed quality too as protein profile and nutrient content get altered. Varma et al. (1992) predicted that the yield loss due to YMD could be as high as $300 million in an epidemic year taking blackgram, mungbean and soybean together.

5.6.3 Symptomatology

The typical symptoms caused by infection of the yellow mosaic viruses as the name implies are the characteristic bright yellow/or golden mosaic. To start with, infection appears as small yellow specks on the veinlet of the young unfurling leaves which enlarge to form mosaic patterns with irregular green patches alternating with each other. The yellow area increases, coalesces and produces complete yellowing of leaves. Nene (1973) also recorded necrotic mottle symptoms in resistant cultivars of blackgram.

The affected plants produce fever flowers and pods, they turn yellow in colour and size of pods and seeds are heavily reduced. In French bean, when infected by MYMV and MYMIV downward leaf curl and stunting symptoms are produced; in the field, natural infection by HgYMV, bright yellow mosaic symptoms are expressed in French bean.

5.6.4 Transmission and Host Range

Both MYMV and MYMIV are transmitted by Whitefly, *Bemisia tabaci* Genn. in a persistent circulative manner. While yellow mosaic virus isolates of India are not sap transmissible, the mungbean isolate from Thailand is mechanically transmissible (Honda et al. 1983). *B. tabaci* is able to acquire and inoculate the virus in minimum acquisition and inoculation access periods (AAP and IAP) of 10–15 min each and optimum AAP and IAP are between 4–6 and −4 h, respectively. After an AAP of 30 min the virus requires a latent period of more than 3 h in the vector for transmission to occur (Nair and Nene 1973). However Chenulu et al. 1979 reported that latent periods have no effect. After AAP and IAP of 24 h each, single whitefly could infect 25% of plants and for 100% transmission, four to ten whiteflies per plant are required (Nair and Nene 1973). Female whiteflies are better transmitters and also retain the virus for a longer period (10 days) than the male whiteflies (3 days) (Rathi and Nene 1974). Neither female nor male adults can retain infectivity throughout the lifespan. Nymphs can acquire the virus from the infected leaves but not the first instar (Rathi and Nene 1974; Murugesan et al. 1977). The virus does not pass through the eggs of *B. tabaci*. The HgYMV was transmitted to several leguminous hosts and required only 30 min of IAP and 10 min of AAP. Incubation period in the vector was 6 h and infectivity was retained for 12 days (Muniyappa and Reddy 1976).

The most characteristic feature of YMVs is its very limited narrow host range. The two virus species MYMV and MYMIV are recorded only in legumes and earlier reports of its probable occurrence in *Brachiaria ramosa, Eclipta alba, Cosmos bipinnatus* and *Xanthium strumarium* (Rathi and Nene 1974) have been disproved after molecular characterization of viruses infecting those hosts has been accomplished. Within the leguminous hosts too, the four virus species exhibit difference in infectivity. MYMV, MYMIV and HgYMV infect majority of the crops such as blackgram, mungbean, soybean, horsegram, moth bean, French bean and pigeonpea. These two species MYMV and MYMIV do not infect cowpea and dolichos;

however through agroinoculation MYMIV isolate was shown to infect cowpea (Malathi et al. 2005) and a MYMIV isolate has been characterized from dolichos (Singh et al. 2006) which are suggestive that MYMIV can infect these two hosts too. There are differences in symptoms caused by HgYMV in French bean. While MYMV and MYMIV cause leaf curl like symptoms, HgYMV produces yellow mosaic. The virus species DoYMV has a very restricted host range that it infects only dolichos from which it has not been transmitted to any other host.

5.6.5 Seed Borne Nature of MYMV

The yellow discoloration of pods and seeds of infected plants and symptom emergence in the very first trifoliate leaf of the plants in the field were suggestive that MYMV may be seed borne, which was investigated by Satya et al. (2015). The distribution of the virus in various parts of the seeds of blackgram (*Vigna mungo* L. Hepper) plants naturally infected in the field was determined by polymerase chain reaction (PCR). Southern blot analysis and nucleotide sequencing of the PCR amplicons from the seed parts from groups of ten seeds revealed the presence of MYMV in the seed coat, cotyledon, and embryonic axes.The presence of virion particles was confirmed through double antibody sandwich enzyme-linked immune sorbent assay (DAS-ELISA) and immunosorbent electron microscopy (ISEM) even in a single whole seed. In confocal microscopy, positive fluorescent signals were obtained using coat protein gene-specific primers in the embryonic axes. However, in the grow-out tests performed with the same batch of seeds, therewas no symptom development in the seedlings though the virus (both DNA A and B components) was detected in 32% of tested seedlings.

5.6.6 Serology

Presence of geminate particles, reaction to polyclonal antibodies to ACMV and ICMV, positive hybridization with DNA A probe to ICMV gave clear indication that viruses causing the YMD are begomoviruses. Muniyappa et al. (1987) purified HgYMV and gave clear evidence for association of geminate particles and produced polyclonal antibody to HgYMV. Epitope profile generated on the basis of reaction to monoclonals to ACMV and ICMV differentiated the yellow mosaic viruses into two groups, one group comprising YMV in dolichos and another group including viruses infecting all other legumes (Harrison et al. 1991; Swanson et al. 1992). Genomic components of a mungbean isolate of YMV from Thailand were cloned by Morinaga et al. 1990. Varma et al. (1991) cloned the genomic component of blackgram isolate of MYMIV at IARI, in parallel a blackgram isolate of MYMV was cloned at Madurai by Ramachandran et al. (1996). Subsequently YMV isolates from mungbean, moth bean, pigeonpea, cowpea, soybean, dolichos and horse gram have been cloned and sequenced (Malathi 2007).

5.6.7 Genome Comparison

The genome of LYMVs comprises of two components DNA A and DNA B and their organization is similar to Old World bipartite begomovirus. The common region spans from 110 to 180 nucleotide length and has the Rep binding iteron sequences, promoters of Rep and CP gene and the characteristic stem-loop region having nono-nucleotide sequence. The comparison of complete nucleotide sequence of DNA A component of yellow mosaic viruses with other begomoviruses, led to clear differentiation of four species, MYMV, MYMIV, DoYMV, HgYMV on the basis of 91% species demarcation. Three other yellow mosaic viruses described from India are RhYMIV (Jyothsna et al. 2011) and VBSMV (Zaim et al. 2011), rhynchosia yellow mosaic virus (unpublished GenBank accession no. KP752090). The most distinct virus is DoYMV which shares only 61% identity with all other YMVs. The identity between the two species MYMV and MYMIV is 81%. RhYMIV and VBSMV exhibit 71–72% identity with MYMV and MYMIV. The relationship is more or less similar with respective isolates of MYMV and MYMIV from Thailand, Pakistan, Bangladesh, Nepal and Indonesia. In the DNA B component, the cognate DNA B of Thailand isolate MYMV-[TH-Mg1] and one blackgram isolate of Vamban MYMV-[KA 27] identity with DNA B of MYMIV-Bg3, MYMIV-Cp, MYMIV-Mg and MYMI-Sb is only 67%.

5.6.8 Multiple DNA B Components

The most unusual feature of MYMV is association of one DNA A with multiple DNA B. One blackgram isolate of MYMV from south India, MYMV-[IN:Vig] is associated with two distinct types of DNA B component. One type of DNA B MYMV-[KA 27] which shows 97% sequence identity with DNA B of Thailand isolate; and other set of DNA B's, KA22, KA28, KA34 which show only 71–72% identity with Thai isolate, but exhibited nearly 90–92% identity with DNA B of MYMIV (Karthikeyan et al. 2004; Balaji et al. 2004). Two types of DNA B components are also associated with a soybean isolate of MYMV [IN: Mad:Sb], one being closely related (96%) to DNA B of HgYMV.

Critical comparison of nucleotide sequence of the DNA B components of MYMV along with one DNA B variant cloned from Gujarat MYMIV-IN Anand 25 (John et al. 2008) revealed how the components have evolved. The four DNA B components, three associated with MYMV, one with MYMIV between themselves share 96% identity in the coding region ORF BVI and ORF BCI. However they differ in the non coding region. While DNA B of MYMV- KA22, KA28, KA34 exhibited similarity with CR of MYMV-[IN Vig], Gujarat isolate showed maximum identity with CR of MYMIV (John et al. 2008). These DNA B molecules referred to as DNA B variants may represent molecules generated by exchange of components between MYMV and MYMIV. Swapping of CR could have occurred from MYMV to MYMIV ("origin donation or regulon grafting" when both the viruses were present together in mixed infection. This is well borne out by the divergence observed between A and B components in the CR region in MYMV and MYMIV.

5.6.9 Association of Satellites

Analyses of LYMV infected samples by RCA let to unexpected identification of both beta and alphasatellites. Over the past few years, betasatellite has been found associated with MYMIV (Rouhibakhsh and Malathi 2005) and MYMV infected plants (Sathya et al. 2013). The symptoms in the presence of betasatellites are severe like crumpling, and severe leaf curl. In all these cases the betasatellite was identified as papaya leaf curl betasatellite. Sathya et al. (2013) found that samples of MYMV infected blackgram samples revealed the presence of alphasatellites, which was identified to belong to the vernonia yellow vein alphasatellite species. The importance of association of these satellites in LYMV pathogenicity is not understood, whether such tri/tetrapartite association is stable and contributes to viral pathogenicity needs to be looked into.

5.6.10 Divergence in CR

The most characteristic feature of MYMV/MYMIV is the divergence in CR between DNA A and DNA B component. Unlike the NW begomoviruses, wherein CR is near identical, 15–23% divergence was observed in whole CR and 23–29% in the origin of replication (Usharani et al. 2004a, b; John et al. 2008; Girish and Usha 2005). Compared to DNA B of Thailand isolate, there were deletion and mismatches in all the DNA B components. The most prominent deletion observed was deletion of 18 nucleotides from the nucleotide co-ordinate 2632–2649 compared to MYMV KA 27 and MYMV-NAM. While the rep binding iteron sequence is ATCGGTGT in MYMV/MYMIV with HgYMV it is GGTAT.

5.6.11 Phylogenetic Relationships

The phylogenetic analysis of YMV genome with other begomoviruses revealed that the viruses infecting legumes, (from Asian and South Asian countries) are distinct from and basal to all other begomoviruses; When other begomoviruses are clearly separated into Old World and New World viruses, the two group of viruses legumoviruses and sweepoviruses are distinct and do not group with either OW or NW viruses. Within legumoviruses, MYMV and MYMIV cluster together and DoYMV occupies a separate clade.

5.6.12 Recombination in LYMVs

The recombination events were considered to be low among LYMVs as mixed infection of LYMVs with other viruses within a single host species has not yet been met with; the multiple DNA B components though is suggestive of mixed infection of MYMV and MYMIV followed by component exchange, no recombination events

has been predicted. However, Ramesh and Chauhan (unpublished, personal communication) analyzed all the LYMVs and predicted four different recombination events in DNA A, of which the event 3, spanning from nucleotide co-ordinate 557–1074 in DNA A was detected in 48 isolates of MYMIV. Recombination in DNA A was higher in MYMIV (48 isolates) than MYMV and DoYMV. In DNA B component, three events were detected in MYMV and one in soybean isolate of MYMIV.

5.6.13 Establishment of Koch's Postulates

The technique of agroinoculation wherein *Agrobacterium tumefaciens* cells having more than one copy of DNA A and DNA B component in a binary vector are used to deliver the viral genome is widely used to prove the infectivity. For the first time, infectivity of OW bipartitie virus was demonstrated through agroinoculation. In the case of MYMV/MYMIV, the sprouting seeds are inoculated with slurry of bacterial cells, from which the replicating genome of the virus is rescued, which replicates actively resulting in systemic spread and typical symptoms (Mandal et al. 1997).

Adapting this technique Koch's postulates were established for cloned component of MYMIV isolates of blackgram (Mandal et al. 1997), mungbean (Biswas and Varma 2001), cowpea (Malathi et al. 2005), pigeonpea (Chakraborty 1996), soybean (Usharani et al. 2005); MYMV- blackgram isolate (Karthikeyan et al. 2004; Balaji et al. 2004; soybean isolate (Girish and Usha 2005) and HgYMV (Barnabas et al. 2010). It is interesting to note here that in all the clones of genomic components used, there was considerable divergence in CR between A and B components, but still, DNA A could *trans*-replicate DNA B and systemic symptoms were produced.

This technique was also used to prove that the yellow mosaic disease in cowpea is caused by a variant of MYMIV (Malathi et al. 2005) in north India. Normally MYMIV isolates of blackgram, mungbean, are not transmitted to cowpea by whitefly and *vice versa*. But Malathi et al. (2005) showed that MYMIV-cowpea isolate, agroinoculated onto blackgram and mungbean, produced typical symptoms from which it could be transmitted back to cowpea. The adaptation of cowpea isolate when inoculated on to blackgram and greengram plants was maintained by viral progeny which could be easily transmitted to cowpea. However the blackgram isolate could not infect cowpea, it only produced atypical leaf curl like symptoms. Though, blackgram and cowpea isolates of MYMIV shares 96% of sequence identity (Surendranath et al. 2005) reassortment of components (pseudorecombination) did not result in symptom production, suggesting, hindrance in the complementation both in replication and systemic movement of the components.

Jacob et al. (2003) suggested that presence of both DNA A and DNA B components in one strain of agrobacterium improved the infectivity of clones. Inoculations were performed with MYMV DNA A with different DNA B components (Balaji et al. 2004) and DNA B specific responses were observed. Inoculation of MYMV with cognate DNA B, KA27, which is closely related to DNA B of Thailand isolate of MYMV, resulted in severe symptoms of stunting and high viral titre in

mungbean. However, inoculation with KA22 DNA B (more closely related to MYMIV DNA B) showed intense mosaic and high viral titre in blackgram. Mahajan et al. (2011) further analysed the response of blackgram plants to inoculation with combinations of different DNA B. when DNA A was co-inoculated with KA27+KA22 DNA B, there was amelioration of severe stunting, reduction in appearance of yellow mosaic symptoms typical of KA22 in blackgram plants. These results were obtained even if KA22 were inoculated post inoculation of KA27. However, such yellow mosaic symptoms caused by KA22 could not be ameliorated by KA27 inoculation. By doing exchange of viral proteins, Mahajan et al. (2011), concluded that, the severe stunting phenotype obtained while inoculating KA27 DNA B is contributed by ORF BV1, the nuclear shuttle protein which seems to be the major symptoms determinant of MYMV DNA B. Kuruba et al. (2016) explained the differential symptom expression on the basis of ORF BV1 (NSP). They demonstrated by leaf tissue hybridization that in KA 27 infected mungbean plant and KA22 infected blackgram plants, mesophyll spread of the virus was observed. On the contrary, KA27 infected blackgram plants, virus did not spread to mesophyll cells. By exchanging NSP fragments of KA22 and KA27, they hypothesized that NSP determines the mesophyll spread.

5.6.14 Molecular Basis of Pathogenicity

5.6.14.1 Viral Replication and Reprogramming Cell Cycle Machinery

The viral DNA replication takes place either by rolling circle replication or by recombination dependent way. The geminiviruses encoded Rep initiates the replication by cleaving at the nonanucleotide site, but does not have polymerase activity. The host DNA polymerase and many host factors together referred to as host replisome take part in viral DNA replication. Three phases are recognized in viral DNA replication. They are initiation, elongation and termination.

Replication initiation protein or Replication associated protein (Rep) is a multifunctional protein, which is highly conserved in all geminiviruses. Rep executes ATP dependent isomerase I, ATPase and Helicase activities (Hanley-Bowdoin et al. 2013; Pant et al. 2001; Yadava et al. 2010). Mukherjee and his associates expressed the MYMIV protein and studied the events of replication (Singh et al. 2008; Yadava et al. 2010). Rep binds to the unique iteron sequence in a co-operative manner which results in conformational changes in DNA, like formation of cruciform structure. More than one molecule of Rep is required to perform both nicking and ligating activities, hence Rep oligomerizes and further hetero-oligomers are formed between Rep and REn.

Following the initiation by nicking activity of Rep resulting in free 3'OH end, elongation phase occurs. During the elongation phase, Rep, REn and many host fork proteins get assembled at the 5'3' direction. In order to synthesize new strand, the existing viral strand need to be dislodged by DNA unwinding. Yadava et al. (2010) showed that MYMIV possess characteristics motive of Helicase, with limited processivity, MYMIV Rep helicase translocates in 3′–5′ direction and requires

ssDNA of minimum six nucleotide length. It was also found that mutation in oligomerzation domain results in abolishment of helicase activity. At the end of elongation phase, the nascent concatenated DNA is subjected to Rep mediated nicking and ligation releasing the full length ss circular DNA. The newly synthesized ss DNA either re-enters the replication process resulting in a more number of copies of viral DNA or gets encapsidated. To facilitate active replication, Rep interacts with several DNA machinery protein; both Rep and REn interact with proliferating cell nuclear antigen (PCNA) which is a processivity factor of host-DNA polymerase and is involved with DNA replication and repair; with large unit of replication factor C complex and subunit of replication protein A. MYMIV Rep interaction with both PCNA and Rep C complex, down regulate nicking and ligating activity. The most interesting interaction was shown with RAD54, a protein involved in homologous recombination. MYMIV-Rep interaction with RAD54 was examined in vitro using yeast system and *in planta* in *Arabidiopsis thaliana*, which showed that contrasting to PCNA, interaction with RAD54 enhances ATPase and Helicase activities.

Geminiviruses invade cells which are fully differentiated, they have exited the S phase and do not have conducive environment for DNA synthesis. The characteristic features of geminivirus infection are the reentry of the cell into S phase. Transcriptomics analysis of geminivirus infection revealed upregulation of genes associated with late G1, S and early G2 phase and downregulation of those associated with early G1 and Late G2 phase. Reentry into S phase and sustaining the environment at increased DNA synthesis phase is brought about by Rep binding to the key molecule of the cell cycles, the retinoblastoma related protein (RBR). Rep binding to RBR ensures release of the transcription factor E2F, which activates genes encoding plant DNA polymerase and other accessory factor.

The Rep interacts with kinases, GRIMP and GRIKI, serine threonine kinase and with histones H3. The host protein with which MYMIV- Rep protein interacts was studied by phage display library and pull-down assay. The MYMIV replicon was cloned in YrP34 vector and the rolling circle replication was demonstrated in yeast (Yadava et al. 2010). This replicon was used to examine eukaryotic host factors needed for replication and more than 150 factors were isolated and about two dozen factors have been found to be required for MYMIV DNA replication in yeast system.

5.6.15 Interaction Between Rep and CP

MYMIV-CP was expressed by Malik et al. (2005) and its interaction with MYMIV Rep was demonstrated and they showed that MYMIV Rep binding with CP caused downregulation of nicking activity. The MYMIV-CP by blocking RCA initiation regulates ssDNA level. The pre coat protein/AV2 of MYMIV was also shown to affect the nicking activity of Rep which was further confirmed by *in planta* studies wherein inoculations of viral genome with mutations in AV2 ended up in reduction in super coiled DNA (Rouhibakh and Malathi 2011, 2012).

Guerra-Peraza et al. (2005), analyzed the MYMV coat protein and revealed that, it has two nuclear localizing signals (reresidue 3 KR and 41 KRRR) and interacts with nuclear import factor import in α. Thus CP may get imported into nucleus through importin alpha dependent pathway.

5.6.16 Bidirectional Transcription and its Regulation

Geminivirues genome is tightly packed and bidirectionally transcribed with overlapping genes on either side of the intergenic region. They utilize the host RNA polymerase II for transcription and the complementary sense gene (Rep) represents the early phase of transcription and the viral sense genes, coat protein and movement protein are expressed later. Typically multiple polysistronic RNA are produced with precised 5′ end and common 3′ end. The mapping of polyadenylated transcripts have been completed for MYMV and MYMIV.

The bidirectional transcription of geminiviruses is regulated by an interesting viral encoded protein referred to as AC2/ or C2 as Transcriptional activator protein (TrAP). It is a multifunctional 15 kDa Zinc binding protein which has a C- terminal acidic type of activation domain and N terminal nucleic acid binding domain. It binds to ssDNA in a sequence non specific manner and binding is facilitated through Zn finger motifs (Bisaro 2006) It *trans* activates the right ward promoters in both DNA A and DNA B. In the case of New World begomoviruses, Bisaro (2006) suggested that TrAP activates CP promoters in the mesophyll cells, and in phloem tissues, removes a suppressor element that inhibited TrAP independent activity. Trink et al. (2005) demonstrated TrAP mediated transactivation of MYMV AV1, BV1 as well as BC1 promoters by transciant expression studies in *Nicotiana plambaginifolia* protoplast. The MYMV-TrAP can also activate host gene transcription. Constitutive expression of TrAP under its own promoter does not produce lethal phenotype, whereas under 35S promoter toxic effects are observed.

Shivaprasad et al. (2005) mapped the viral transcripts from MYMV- infected blackgram plants using the circularized RNA as template which facilitate mapping of both 5′ start and 3′ poly adenylation site. They isolated two major rightward transcripts initiation (~1 kb), ORF AV1 could be translated from both the transcripts and ORF AV2 from longer transcripts.

Study on transcription units of leftward ORFs revealed interesting results. A single major transcripts start site was located at position 2649, which may represent dicistronic mRNA from which both ORF AC1 and AC4 may be translated. Additionally two more transcripts (0.6 kb and 0.65 kb with start sites, 23 and 26 nt upstream of ORF AC2 were mapped. It is inferred that both ORF AC2 and ORF AC3 may be translated from this transcript. Interestingly while mapping the transcript, Shivaprasad et al. (2005) found that RNA transcripts starting upstream of TATAA box in the intergeneic region. These start sites were located for both rightward and leftward transcription, suggestive of readthrough transcription on the circular DNA. Annealing between such sense and antisense transcripts may give rise

to dsRNA intermediates triggering RNAi silencing. This may also explain the siRNA derived from the MYMV promoter region by Pooggin et al. (2003).

In the DNA B component a 0.9 kb BV1transcription start site was mapped to A 410, A414 which is preceded by a short ORF. They also revealed that BC1 transcript (1.1 kb) exists in spliced and unspliced forms, having the consensus splice donor sites (AG/GU) and acceptor site (CAG/G). 123 nt length intron is located between 2359 and the BC1 start codon at 2117. This region also contains three shot ORFs which may facilitate in translation.

In MYMIV, Usharani et al. (2006) analyzed the transcripts of MYMIV infected French bean using 5′ and 3′ RACE and identified an additional transcript of 0.7 kb length on the rightward side, with start site mapping to 290. They speculated that ORF AV1 alone may be translated from such a transcript.

5.6.17 Promoters of LYMV

The intergenic region of DNA A and DNA B of begomoviruses share a common region of about 160–200 bp which contains *cis* elements that regulate the leftward transcription of Rep gene and rightward transcription of the CP gene. The TATAA box and G box present in CR constitute the core promoter elements for the transcription of Rep gene. Rep also functions as negative feedback regulator of its own transcription by binding to repeat sequences between the TATAA box and transcription start site. Unlike the Rep gene promoter the rightward CP gene promoter requires activation by TrAP which has been recorded by several workers (Hanley-Bowdoin et al. 1999).

The bidirectional promoter of MYMV in the intergenic region was studied by fusion of CAT reporter gene with putative promoter sequence. Shivaprasad et al. (2005) showed that except BV1 start site fusion, all other constructs expressed activity driven similar to what is driven by 35S promoter. Expression of CAT fused to start codon of AV2, AV1, BV1 and BC1 was enhanced 15 to 300-fold in the presence of MYMV- AC2. However AC2 activation on leftward ORFs was less pronounced (two to fourfold).

Sunitha et al. (2012) delineated a new 357-bp mono directional TrAP/Ren promoter. Besides stress- regulated motifs, several root specific motifs were also identified in this promoter which were further tested in transgenic tobacco lines. The transgenic plants having TrAP gene driven by its own promoters had normal phenotype, while toxicity was observed when TrAP gene was expressed under CaMV 35S promoter.

Usharani et al. (2006) studied the promoters of MYMIV by delivery of putative promoter constructs through *Agrobacterium tumifaciens* on *N. benthamiana* leaf and sprout seeds of French bean. They identified a TrAP independent activity of AV promoter and differential regulation of AC promoter. AV promoter had TATA box and initiator elements. Many transcription factor binding sites were also detected.

5.6.18 RNA Silencing Pathway and YMV Suppressors

RNA silencing refers to the highly conserved adaptive immunity response of the plants through siRNA to protect themselves from the invasion of viruses and transposans. The priming event is the formation of dsRNA intermediate which is cleaved into 21–24 nt siRNA by DICER like protein (DCLs).

The siRNA generated along with RISC complex target mRNA resulting in either its degradation or in translational arrest which is called as post transcriptional gene silencing. On the contrary the siRNA interact with argonaute 4 (AGO4), which directs the methylation of the promoter region resulting in transcriptional gene silencing. Upon geminivirus infection, it is presumed that the read through transcription at 3′ end of the virion sense and complementary sense transcripts produce dsRNA; however until now analysis of siRNA profiles upon geminivirus infection reveal abundance of 24 nt-siRNA (unlike RNA viruses) targeted to intergenic promoter region.

Countering this innate defense of the host plant, viruses encode proteins that interfere with the silencing pathway at any step and these diverse proteins are designated as viral suppressor (VSR). Geminiviruses encode three to four VSRs like AC2/C2/TrAp, C4/AC4/sd, AV2/V2/MP in monopartite viruses, and Beta C1 protein encoded by betasatellites.

Experiment conducted on the AC2 encoded by MYMV and MYMIV revealed interesting results contrasting to New World viruses. In NW virus TGMV, silencing activity is independent of transcription activation. Bisaro (2006) suggested that AL2 (AC2) protein interacts with Adenosine Kinase (ADK) which is required to maintain methyl cycles and S-adenosyl methionine dependent methyl transferase activity. AC2 interferes with ADK directed methylation and epigenetic modification of viral genome. They also suggested that AL2 interacts with SNF Kinase thereby limiting cellular AMP levels and basal defence of the plants.

The mechanism of action of AC2 protein of MYMV (Trinks et al. 2005) is different from that of NW viruses. Trinks et al. (2005) showed that silencing activity is dependent on transcriptional activation. They hypothesized that AC2 suppressor of MYMV activates expression of host endogenous gene like WEL-1 (WEL-1 3–5′ exonuclease). They suggest that AC2 may activate expression of cellular protein that may function as endogenous negative regulator in the host plants.

The intricacies of MYMIV-AC2 suppression of RNA silencing was investigated. Rahman et al. (2012) employed the AC2 of MYMIV for enhancing transgene expression, such as topoisomerase-II in the transgenic tobacco lines. They further elucidated the silencing suppression mechanism of MYMIV AC2 and showed that unlike several other suppressors, the AC2 of MYMIV does not bind to siRNA or dsRNA, but its suppression activity is mediated through interaction with the key components of the RNAi pathway, viz., RDR6 and AGO1 (Kumar et al. 2015). Interaction by AC2 inhibited the activity of RDR6, an essential component for biogenesis of siRNA and tasiRNA and also inhibited the function of AGO1, the major slicing factor of RISC (Kumar et al. 2015). They employed the AC2 of MYMIV to demonstrate the *in planta* activity of a hammerhead ribozyme designed

to target rep-mRNA of MYMIV as an antiviral agent (Mishra et al. 2014). Consequent upon identification of suppressors in begomoviruses, their application in achieving transgenic resistance has been looked into. Sunitha et al. (2013) and Shanmugapriya et al. (2015) demonstrated that tobacco plants transformed with MYMV TrAP gene accumulated siRNA and led to reduction in MYMV-DNA accumulation in tobacco.

5.6.19 Transcriptome Profiling

Upon geminivirus infection, plant remodels its cellular components involved in the diverse processes, such as transcription, hormone signaling, metabolic pathway and defense related processes. In order to get insights into the molecular events leading to compatible/incompatible interaction between the *Vigna mungo* and MYMIV, Kundu et al. (2013), performed proteome analysis. The analysis at 3, 7, 14 days post MYMV inoculation, revealed 109 differentially expressed proteins which were identified by mass spectrometry. Among all the proteins, photosynthesis related proteins were the most affected in susceptible genotypes. Photosystem II electron transport was influenced and many networks of defense related proteins expressions were altered.

Using substractive hybridization technique, transcripts altered in *V. mungo* under MYMV pathogenesis were studied by Kundu et al. (2015). Enhanced expression of genes involved in phenylpropanoid pathway, ubiquitin proteosomal pathways were seen which could contribute to resistance. Whereas in susceptible genotype repression of photosynthesis related genes affecting chlorophyll synthesis and functions resulting in yield penalty were observed.

Yadav et al. (2009) while studying differential response of soybean genotyes to MYMV discovered that a soybean variety resistant to MYMIV showed rapid degradation of viral RNA compared to a susceptible variety. Yadav et al. (2009) found that in resistant soybean lines the viral siRNA were generated complementary to noncoding intergenic region, whereas in susceptible variety siRNA generation was targeted to coding region. Most of the IR specifc siRNA generated were of 24 nt length and by bisulphite sequencing it was also discovered that the sequence of IR were highly methylated thereby suggesting that methylation of viral genome may be probable mode of resistance action.

Yadav and Chattopadhyay (2014) studied host gene response in soybean plant susceptible to MYMIV. A high-throughput microarray analysis of MYMIV infected soybean probed with 17,000 genes revealed the enhanced expression of various genes linked with systemic acquired resistance, programmed cell death and disease resistance response.

Kundu et al. (2017) investigated microRNA profile of blackgram plants inoculated with MYMIV. They found out that miRNA belonging to the family of miR156, miR159, miR160, miR162, miR398, miR1511, miR1514, miR2118 and novel vmu-miRn7 vmu-miRn, vmu-miRn13 and vmu-miRn14. These miRNA targetted transcripts like NB-LRR, NAC, MYB and several transcription factors. Thus miRNA are speculated to be involved in MYMIV induced stress repsonse.

5.6.20 Resistance to Yellow Mosaic Viruses

LYMVs of grain legumes continue to be a big challenge as the genetics of resistance is not understood clearly. There are contradictory reports on the genetics of resistance to YMV in blackgram. It is suggested to be controlled by a single recessive gene (Singh and Patel 1977; Singh et al. 1988; Thakur et al. 1997; Saleem et al. 1998; Reddy and Singh 1995; Sudha et al. 2013). There are also reports of single dominant gene (Sandhu et al. 1985), two recessive genes (Verma and Singh 1988; Pal et al. 1991; Ammavasai et al. 2004; Muraleedhar et al. 2015) and complementary recessive genes (Shukla et al. 1985). Absence of any clear cut lead in the genetic analysis was attributed to difference in strains of viruses, vector population, vector biotypes and environmental conditions. At present, with more clarity regarding viruses, and the facilities to separate the viruses and screening with the specific viruses, genetic analysis may give reliable information.

For example Karthikeyan et al. (2011) and Sudha et al. (2013) employed agroinoculation besides field level screening for resistance to MYMV. They inoculated MYMV DNA A separately with KA22 and KA 27 type of DNA B and found that only one genotype ML 818 showed resistance reaction. Screening of the blackgram accession in the field showed that four accessions were moderately resistant showing <20% incidence. They were challenged through agroinoculation with MYMV constructs and the resistant phenotype was further corroborated by less virus accumulation.

Efforts have been initiated to identify the resistance gene and develop marker near to the locus which can be employed in marker assisted selection breeding program. Kundu and Pal (2012) and Maiti et al. (2011) developed resistance linked molecular markers for resistance to MYMIV in blackgram using R gene analogues. Two MYMIV resistance markers, Yr4 and *CYR1* were identified, of these two, *CYR1* locus is completely linked to MYMIV resistance. The *CYR1* (R) gene which co segregates with resistance was isolated and characterized (Maiti et al. 2012). The transcript sequences and the 3D model revealed it to be a typical CC-NBS-LRR type of R gene protein. They also predicted the interaction between CYR1- LRR and MYMIV-CP and suggested that CYR1 protein may recognize the CP-effector of the virus and contribute to incompatible interaction.

Markers have been identified and validated in blackgram (Gupta et al. 2015). The whole genome resequencing of MYMV resistant soybean cultivar UPSM-534 and the susceptible cultivar JS335 was performed by Yadav et al. (2015) which should help in generating reliable and robust markers associated with resistance.

5.7 Begomoviruses Associated with Yellow Vein Mosaic Disease

Yellow vein mosaic disease (Fig. 5.5) was known in India since 1924 in bhendi (Kulkarni 1924) and pumpkin (Varma 1955) in Maharashtra. Pumpkin yellow vein mosaic virus (PYVMV) now known as squash leaf curl China virus was

Fig. 5.5 Yellow vein mosaic disease in different crops caused by begomoviruses. (**a**) bhendi yellow vein mosaic virus in okra; (**b**) mesta yellow vein mosaic virus causing yellow vein mosaic disease in mesta; (**c**) squash leaf curl China virus causing yellow vein mosaic disease in pumpkin

characterized as the first begomovirus infecting cucurbit in India (Muniyappa et al. 2003). Now, more than seventeen begomoviruses (Tables 5.4 and 5.5) are known to be associated with the yellow vein mosaic disease in crops and weeds. In many cases, especially in bhendi and other weed hosts, the yellow vein viruses may also cause enation and leaf curl symptoms. For example, both BYVMV and okra enation leaf curl virus (OELCuV) can cause YVM or leaf curl symptom depending on the genotype they infect. Likewise, in *Ageratum conyzoides* and *Croton bonplandianum*, both YVM and enation leaf curl symptoms are observed. An Old World begomovirus, Corchorus yellow vein virus has been identified in a weed plant, *Corchorus olitorius* showing yellow vein symptoms in Maharashtra (Malathi VG, unpublished result, GenBank KC196077, KC223600).

5.8 BYVMV and OELCuV

In India, begomoviruses associated with yellow vein mosaic and leaf curl diseases of bhendi are bhendi yellow vein mosaic virus (BYVMV, Jose and Usha 2003), bhendi yellow vein Bhubeneshwar virus (BYVBhV, Venkatarvanappa et al. 2013b), bhendi yellow vein Haryana virus (BYVMV-Har, Venkataravanappa et al. 2014b) and the bipartite tomato leaf curl new Delhi virus (ToLCNDV, Venkataravanappa 2008) and raddish leaf curl virus (RaLCuV, Kumar et al. 2012a, b). These are the only virus species approved by the ICTV. Other important yellow vein mosaic viruses are, mesta yellow vein mosaic virus (MeYVMV, Roy et al. 2009) and hollyhock yellow vein mosaic virus (HoYVMV).

5.8.1 Discovery and Distribution

Bhendi yellow vein mosaic disease constitutes the earliest record of whitefly transmitted virus disease in India. It was first reported by Kulkarni in Bombay district in 1924. The viral etiological nature and transmission by whitefly were subsequently studied by Uppal et al. (1940) who named the disease as bhendi yellow vein mosaic disease. Subsequently Capoor and Varma (1950) and Varma (1952, 1955) studied the whitefly transmission of the virus. Soon incidence of the disease in other states

was observed (Chelliah et al. 1975; Chelliah and Murugesan 1976; Khan and Mukhopadyay 1985; Bhugapati and Goswami 1992). Due to the earliest record, it can be reasonably assumed that the virus originated in India, (Nath et al. 1992). At present, the disease is widespread, occurring in all agroclimatic zones of India, in the absence of resistance sources, it is the most important constraint in productivity. A survey conducted on the incidence of yellow vein mosaic disease estimated the percentage disease incidence to range from 23 to 67 in Karnataka, 45–56 in Andhra Pradesh, 23–75 in Tamil Nadu, 42–75 in Kerala, 23–85 in Maharashtra, 24–65 in Haryana, 35–57in Uttar Pradesh, 45 in Delhi, 67 in Chandigarh and 45–66 in Rajasthan (Venkataravanappa 2008). Bhendi infected by BYVMV has also been recorded in Pakistan, Thailand, Sri Lanka and China (Tsai et al. 2013).

Contrasting to yellow vein mosaic disease, enation leaf curl disease was noticed only in 1980s (Singh and Dutta 1986) in Karnataka. The enation leaf curl was further detected in all the bhendi growing tracts of India (Venkataravanappa et al. 2016) and this disease has emerged as serious threat to cultivation. In some cultivars, yellow vein disease symptoms and enation leaf curl symptom appear separately, in some cultivars, both the symptoms appear together. The name of the virus as yellow vein mosaic and enation leaf curl virus does not imply that they cause only yellow vein mosaic or enation leaf curl. Thus the symptom expression is dependent on host genotype.

5.8.2 Economic Loss

If plants are infected at an early stage (20 days post germination) the fruits are severly malformed and yield loss is nearly 94–100%. If the infection occurs at later stage, the loss is reduced. There is 49–84% loss when infection occur at 50–60 days post germination (Sastry and Singh 1974; Pun and Doraiswamy 1999; Nath and Saikia 1992) recorded 84 and 49% yield loss when infection occur 50 and 65 days post germination. In the case of enation leaf curl disease the yield loss varies from 30 to 100%.

5.8.3 Symptomatology

The yellow vein mosaic disease has characteristics symptom of yellow veins surrounding green tissues. To begin with yellowing or vein clearing of small veinlets appear which intensify and the entire leaf becomes completely yellow or greenish in colour (Fig. 5.4a). Plants infected at early stage have small leaves and are extremely stunted. The fruits of infected plant are yellow in colour, deformed and are not marketable (Sastry and Singh 1974). Venkataravanappa et al. (2012a, b) differentiated three types symptoms, on the basis of developmental stage at which symptoms appear, Type I in which, plants get infected very early and develop symptoms, older leaves turn brown or wither, Type-II infection occurs after flowering, wherein only young leaves show symptom, older leaves are symptom free, fruits

produced are hard and yellow, Type III, where the plants appear normal, produce marketable yield but emerging axillary shoots produce symptomatic leaves.

The enation leaf curl disease has much more devastating phenotype. Initially, small pin head like enations appear on the leaves, followed by leaf curling. The undersurface of the leaves show prominent enations and leaves become leathery and brittle. There is severe twisting of stem, lateral branches and petioles. As the twisting becomes severe, the entire plant appears spreading on the ground. In some cases the veinal enations become brown and leaves have corky appearance. The severely affected plants do not produce any fruits, if formed the fruits are deformed and are not marketable.

5.8.4 Prevalence of Viruses in Field

There is a considerable variation in the incidence of the diseases in different states. The disease is severe in north India in *Kharif* season. The ideal location which are considered as hots spots for screening are Karnal, Tarai region of Uttarakhand, Nadia district of West Bengal and Varanasi area of UP. In central and south India, the disease is pronounced in summer season, the hot spot locations being Guntur in AP. Jalgaon in Maharashtra, Surat in Gujarat, and Coimbatore in Tamil Nadu.

Venkataravanappa et al. (2014b), summed up that of the four major cluster or virus species BYVMV, BYVMaV, OYVMV, and OELCuV are predominant. BYVMV is present in southern, central, western and eastern and north India, BYVMaV occurs only in some location in AP, TN and Maharahtra. OELCuV also seen distributed through out the country but distribution is less compared to BYVMV.

5.8.5 Transmission and Host Range

BYVMV is efficiently transmitted by whiteflies. The transmission studies have been accomplished by Varma (1952) who showed that 12–24 h of AAP and 30 min of IAP and fasting prior to acquisition enhanced the efficiency of transmission. Raychaudhuri and Nariani 1977, made an interesting observation that *B. tabaci* is capable of harbouring different viruses and exhibit differential transmission of individual virus, Pun and Doraiswamy (1999), described that the age of the bhendi seedling used in the tests is important; 100% transmission was obtained when inoculation was done on 1 week old seedlings, only 31.7% infection occurred with 7 week old seedling. Venkataravanappa et al. (2012a), demonstrated whitefly transmission with 24 of AAP and IAP for BYVMV. They achieved 100% transmission on the susceptible cultivar 1685. Venkataravanappa et al. (2013b), investigated the transmission characteristics of BYVBhV and observed that with 24 h of IAP and AAP, symptoms were observed in the cultivar 1685 within 15 DPI.

The vector transmission experiment conducted by Venkataravanappa et al. (2014a) with OELCuV revealed very significant results; it showed that a minimum of two whiteflies can bring10% infection. Transmission increased with more number of whiteflies. The minimum IAP and AAP required were 1 h and 30 min respectively, the maximum transmission rate was observed with 24 h. The adult female whiteflies were more efficient than male. They also observed that efficiency of transmission was highest when 7 days old seedling were inoculated and it decreased upto 50% when 25 days old plants were inoculated. The begomoviruses infecting bhendi are not sap or seed transmissible.

Capoor and Varma (1950) found that in addition to bhendi (*A. esculentus*) *A. moschatus*, *A. manihot* and *Althaea rosea* were susceptible to BYVMV. Handa and Gupta (1993) showed the yellow vein net symptoms were produced in *Croton bonplandianum* using BYVMV from bhendi as inoculum. For the OELCuV, Venkataravanappa and coworkers inoculated 50 plant species and found that only two species in the family Malvaceae, *A. esculentus* and *Althaea rosea* expressed symptoms; in the solanaceous hosts symptoms were observed in the *Datura stramonium, N. glutinosa, N. clevlandii, N. occidentalis, N. tabacum* and *N. benthamiana.*

5.8.6 Serology

The whitefly transmission of BYVMV though was well known, presence of geminate particles was first time shown only in 1991 using ACMV antibody by immune specific electron microscopy by Harrison et al. (1991).They also showed the positive reaction of BYVMV to polyclonal antibody to ACMV in DAS – ELISA and positive hybridization to ACMV DNA probe. Handa and Gupta (1993) found that BYVMV reacted specifically to two ACMV monoclonal antibodies SCR 17 and SCR 18. Harrison et al. (1997) further showed that the epitope profile of cotton leaf curl viruses from Pakistan generated by reaction to 31 MAb to ACMV, ICMV, Okra leaf curl geminviruses were not indistinguishable from YVMV affected okra samples from India.

5.8.7 Genome Comparison

The DNA A genome of BYVMV was characterized by using begomovirus specific primers and DNA B equivalent component could not be detected in the infected bhendi, however, a betasatellite associated with BYVMV was identified; subsequently, it was demonstrated that both DNA A and betasatellite were necessary to cause yellow vein mosaic disease in bhendi (Jose and Usha 2003). During last 5 years, several bhendi begomoviruses have been characterized.

The genome Organization is basically similar to Old World monopartitie begomoviruses with two virion (V1, V2) and five complementary sense ORFs. The iteron sequences were incomplete direct repeats of GGTGT. Venkataravanappa et al. (2012a, b, 2013a, b, c, 2014a, b, 2016) characterized begomoviruses infecting

bhendi throughout India, generated full length genome sequence of about 190 isolates and identified seven viruses, On the basis of 89% as the species threshold value, six species were identified, they were bhendi yellow vein Bhuvaneswar virus (BYVBhV) (Venkataravanappa et al. 2013a, b, c), bhendi yellow vein Delhi virus (Venkataravanappa et al. 2012b) bhendi yellow vein mosaic Maharastra virus (BYVMaV), bhendi yellow vein mosaic Harayana virus (BYVMV- Har) and bhendi yellow vein mosaic Karnal virus (BYVKaV), bhendi yellow vein India virus and ToLCNDV. However, the ICTV study group on geminiviruses reanalyzed the sequences and in the Xth report nomenclature of above mentioned viruses have been changed. As in the case of cotton leaf curl viruses, some of the new viruses were essentially recombinants, the committee perhaps decided to name them on the basis of the identity with the major parent, BYVMV.

The identity analysis performed by the authors (Venkataravanappa et al. 2016) by SDT, following the guidelines of the study group on taxonomy of geminivirus clearly justify separation of bhendi begomovirus isolates as distinct species on the basis of 91% identity. As the identity between some isolates is very low (<85%) in the future reports of ICTV, ambiguity may be cleared and the species status may be restored. In the present chapter the distinct status of species will be maintained as proposed by the authors when comparing the genome. Among the bhendi infecting begomoviruses 79–88% identity is observed between BYVMV, BYVIV, BYVMaV, BYVDV, BYVKnV, BYVHV viruses in DNA A component. However, bhendi yellow vein Bhuvaneswar virus is very distinct and shares only 66.9–76.6% identity with BYVMV. It is closely related to croton yellow vein virus (CYVMV) and 75–79% to mesta yellow vein mosaic virus (MeYVMV). In the case of OELCuV, highest percent identity of 82% is recorded with BYVMaV followed by cotton leaf curl Banglore virus (CLCuBaV) (81%) and MeYVMV (80%). Maximum identity was observed in the Rep region. The intergenic region of the genome shared very less identity (51–65%) between the viruses.

There is only one DNA B component sequence available, one DNA B of BYVDV (HQ 542082) which exhibits nearly 81–85% identity in the complete nucleotide sequence, 94% and 97% identity at amino acid level with ORF BV1 and BCI with DNA B component of ToLCNDV, respectively.

5.8.8 Phylogenetic Relationship

Venkataravanappa et al. (2014a, b) performed a comprehensive analysis involving 130 sequences of bhendi begomoviruses and observed four major clusters, consisting of BYVMV, BYVMaV, OELCuV, ToLCNDV. The BYVDV, BYVHV, BYVKnV belonged to the major cluster of BYVMV but occupied a separate group. The OELCuV and ToLCNDV clusters were distinct and well separated from BYVMV and BYVMaV. In all the analysis BYVBhV occupied a separate branch.

Venkataravanappa et al. (2014a, b, 2016) performed a neighbour net analysis of sequences using the split tree programme. The analysis involving bhendi infecting and related begomoviruses revealed extensive network structure rather than predominantly bifurcating tree like structures. The extremely networked tree is

indicative of recombination suggesting how different parts of genome have different origin due to recombinations. Neverthless the network analysis confirms the distinct nature of OELCuV and BVYBhV.

The population structure analysis of okra infecting begomoviruses (110 sequences of different begomoviruses) using model-based algorithm indicates existence of at least four genetically different populations (Prasanna et al. 2010; Venkataravanappa et al. 2014b). Most of the isolates that related to four previously characterized species namely BYVMaV, BYVMV, OELCuV and ToLCNDV segregated together and formed four genetically cohesive populations. Among these, two predominant populations observed included the group of viruses genetically similar to BYVMV (52 sequences) and BYVMaV (42). Further four major populations was analyzed separately to determine the optimum number of sub-populations that best represented within population structure using ARLEQUIN Ver. 3.0. The BYVMaV population contained five and BYVMV contained three minor sub-populations. There was evidence of potentially three and two minor sub-populations in OELCuV and ToLCNDV populations respectively. Majority of the member isolates assigned to these subpopulations showed more than 60% support. With regard to haplotype distribution, a total of 107 haplotypes were detected out of the 110 sequences analyzed. The highest number of haplotypes was observed in BYVMV-I with 44 haplotypes followed by BYVMaV-I with 27 haplotypes. All the sub-populations recorded high haplotype diversity (hd of >0.95) with an exception of BYVMaV-II. The number of polymorphic sites was highest in BYVMV-I with 988 polymorphic sites and nearly 181 average nucleotide differences. A high level of nucleotide diversity (0.26351) with the average number of nucleotide differences of 717 was noted in ToLCNDV-II sub-population (Venkataravanappa et al. 2014b).

5.8.9 Recombination

The recombinational events have led to emergence of important variants of the bhendi viruses, which may even be considered as distinct species is further proved by indepth analysis of recombinational events in the RDP programme.

Venkataravanappa et al. (2014b) revealed that the bipartite begomovirus BYVDV has sequences derived from ToLCNDV and BYVMV viruses which were confirmed in all six detection methods in RDP programme. They observed that the only DNA B component associated with bhendi begomovirus is derived through recombination between different DNA B components of ToLCNDV. In the case of BVYBhV, a very distinct bhendi virus, two recombinational events confirmed by six methods were detected in IAC3,AC5 region with CYVMV and CLCuMV-Rajasthan and RhYVMV as major and minor parents. Venkataravanappa et al. (2014a, b), suggested that BYVHV has entire coat protein sequences derived from one isolate of BYVMV (previously referred to as OYVMV) and the rest of the genome from ToLCNDV. The BYVKnV is a recombinant between BYVMaV and OELCuV the event detected was in the right half of the genome extending upto AC3. The analysis of OELCuV showed that most of its sequences originate from BYVMV, BYVBhV and MeYVMV. For all

the OELCuV isolates, the sequences containing the origin of replications originate from BYVMV, including the iteron sequences. For only one isolate of OELCuV (GU1119996) a small fragment is derived from ToLCNDV. Rishishwar et al. (2015) recorded presences of BYVMV in infected samples in Kalyani (west Bengal) and Aurangabad (Maharastra) but MeYVMV in samples from Varanasi (Uttar Pradesh) and Jalgaon (Maharastra).Interestingly they found that the Jalgaon isolate of MeVYMV showed a recombination event between MeYVMV as major parent and Malvastrum yellow vein Yunnan virus (MaYVYV, AJ786711) as a minor parent.The event located was between nt co-ordinate 2002 and 2020. The presence of sequence of (MaYVYV) is not reported earlier in India suggesting the possibility of its occurence in India potentially near to the location from where MeYVMV Jalgaon isolates were collected.

Vinoth-Kumar et al. (2017a) gave evidence for inter specific and inter strain recombination events and suggested that cotton infecting and bhendi infecting viruses may share a common ancestor. Different levels of recombination among BYVMV isolates have also been described. Serfraz et al. (2015) analyzed OELCuV isolates from Pakistan and found that OELCuV is a recombinant between BYVMV (isolates from south India) (at Rep region from 1522 to 1955) and OELCuV from northwest India as a minor parent. They suggest that cotton leaf curl Multan virus (CLCuMuV) and MeYVMV recombined to produce the entire region of rep present in OELCuV. They suggest that, BYVMV, MeYVMV and CLCuMuV are parents and as we move from north to north western regions CLCuMuV may dominate.

Vinoth-Kumar et al. (2016) found that distribution of SSR and recombinational events are linked, hence Vinoth-Kumar did an extensive analyzes of SSR and found that there is a good correlation between SSR and recombination events among BYVMV isolates. They found that 62 out of 67 begomovirus isolates had SSR sequences. Two types of SSR motifs (CG) 3-X1-T6–7 and $(AGA)_4$ –X1-A_6 were found frequently among begomoviruses.

5.8.10 Establishment of Koch's Postulates

For bhendi viruses, koch's postulates for the cloned components have been established for only one isolate of BYVMV-Madurai (Genbank accession no AF241479) by Jose and Usha (2003). Partial tandem repeat constructs of DNA A and betasatellite BYVB were agroinoculated to 125 plants in three different experiments of which 16 plants developed typical yellow vein symptoms 25 DPI. When DNA alone is inoculated, it resulted in mild leaf curl symptom.The functional role of betasatellite and C2 and C4 genes encoded in DNA A were further analyzed using the infectivity of these clones on to *Nicotiana benthamiana.*

For all other isolates belonging to different virus species, the whitefly transmission has been accomplished for every individual isolate before they have been subjected to molecular characterization. Therefore, it can be presumed that Koch's postulates have been established for these isolates also.

5.8.11 Molecular Basis of Pathogenicity

Various protein-protein interactions and the role of viral genes C2, C4 encoded by DNA A component and betasatellite C1 protein encoded by betasatellite were studied using the BYVMV/BYVMB combination. Kumar et al. (2006), studied subcellular localization, by fusing GFP to CP and βC1 coding sequences. They showed that CP was localized inside nucleus whereas βC1 is distributed in periphery. The nuclear localization signal in CP was predicted to be between amino acid residues 1 and 24, in βC1 nuclear export signal was located between aminoacids 105 and 115. They also demonstrated interaction between CP and βC1 in yeast two hybrid system. Transformation of *N. benthamiana* with βC1 ORF under the control of 35S promoter resulted in abnormal distorsion, twisting of stem and leaves and stunting of plants. Gopal et al. (2007) further extended their studies and showed strong suppression of gene silencing activities for C4 and βC1 but only a weak activity for C2. They showed that abnormal phenotypes were produced in *N. benthamiana* when transformed with C4 and βC1. They also analysed V sense and C sense promoters by both transient and stable expression in *N. benthamiana* that demonstrated efficiency of C sense promoters. Chandran et al. (2012), further looked into nuclear trafficking of C2 proteins. They found that BYVM2 C2 nuclear localization signal (NLS) was located in N terminus of the protein covering 17–31 amino acid. This NLS was recognized by the transport receptors, karyopherin α and βC2. Interaction through NLS with karopherin ensures its nuclear localization. The C2 protein was found both in cytoplasm and nucleus, which suggests that there is a NLS independent nuclear import of this protein. Interestingly, C2 was also demonstrated to play a role in symptom determination and virus replications by Chandran et al. (2014). When *N. benthaminana* plants were inoculated with BYVMV. DNA A engineered to have two stop codons in C2 ORF, plants did not develop any symptoms. The viral DNA level was drastically reduced. They concluded that C2 protein of BYVMV may have key role in symptom production and viral DNA replication (Chandran et al. 2014).

5.8.12 Resistance to BYVMV and OELCuV

Of all the virus diseases affecting vegetables, yellow vein mosaic disease of bhendi is the most devastating one as there is 100% infection even in elite varieties and hybrids and the yield losses range from 50 to 94%. The search for resistance and attempts to incorporate resistance genes have been many but with no impact. Sanwal et al. 2016 indicated contradiction between different research workers, which might also arise due to difference in virus species/strains, used as inoculum.

Screening of 941 indigenous and exotic germplasm of lines of Okra for YVMV field resistance under natural epiphytotic condition revealed that none of the accessions were immune or highly resistant, 43 were moderately resistant .The accessions IC 218887, IC 69286 and EC-305619 were resistant (Abdul et al. 2004). Out of eight wild species of bhendi, species having resistance to YVMV are *A. manihot, A. angulosus, A. crinitus, A. vitifolius, A. tuberculatus, A. panduraeformis, A.*

pungens and A. tetraphyllus (Dhankar et al. 1996). *A. manihot* ssp. *manihot* is widely used in developing resistant lines. Efforts are also being taken to induce mutation by gamma irradiation.

Venkataravanappa et al. (2012a, b), screened okra genotypes under both artificial and natural condition and found that the genotypes Nun 1145, Nun 1144, Nun 1142 and Nun 1140 showed resistance, genotypes M10, Nun 1142, Nun 1140 showed moderately resistance phenotype. In some selected genotypes symptoms free plants were found to contain virus by nucleic acid spot hybridization and PCR kits. Similarly Venkataravanappa et al. (2016), identified the genotypes Tulasi and Trisha also to have considerable resistance to BYVMaV.

About 36 betasatellites have been isolated from diseased okra which segregated into four groups (1) okra leaf curl betasatellite (OLCuB) (2) bhendi yellow vein betasatellite (BYVB) (3) bhendi yellow vein India betasatellite (BYVIB) and (4) croton yellow vein mosaic betasatellite (CroYVMB). Identification of CroYVMB, a "non-malvaceous betasatellite" in malvaceous okra might be the result of component reassortment between pathogens when they infect the hosts together (Venkataravanappa et al. 2011a, b).

5.9 Mesta Yellow Vein Mosaic Virus (MeYVMV) and Mesta Yellow Vein Mosaic Bahraich Virus (MeYVMBV)

MeYVMV and MeYVMBV are the two viruses causing yellow vein mosaic disease of mesta fibre crops, *Hibiscus cannabinus* (Kenaf) and *H. sabdariffa*. The disease in eastern India is caused by MeYVMV along with cotton leaf curl betasatellite, in northern India, the virus associated with the disease is mesta yellow vein Bahraich virus with Ludwigia leaf distortion betasatillite.

5.9.1 Discovery and Distribution

The yellow vein mosaic disease was first reported in mesta during 2005 from Eastern part of India (Chatterjee et al. 2005); similar symptoms were recorded in Mesta in U.P from Northern part of India in 2007 (Ghosh et al. 2007; Das et al. 2008a, b). Roy et al. (2009) surveyed for the disease incidence and observed that highest disease incidence occurred in southern West Bengal followed by eastern Uttar Pradesh and northern West Bengal; the lowest disease incidence was noted in northeastern Andhra Pradesh. Incidence of the disease at various locations ranged from 46 to 93% in eastern part of India, and 34–78% in Northern part.

5.9.2 Symptomatology

The symptoms of the disease are typical yellowing of veins, entire lamina turn yellow and form a yellow network (Fig. 5.5b). Entire lamina looks yellow, general

vigour and height of the plant are affected. The Mesta cultivar HC – 583 was the most susceptible one.

5.9.3 Transmission and Host Range

The virus MeYVMV was efficiently transmitted by whitefly upto 85% in the case of *H. sabdariffa,* 78% in case of *Hibiscus cannabinus* and they have a very narrow host range. The symptoms were produced 8–10 days post inoculation (Chatterjee et al. 2008; Das et al. 2008a, b).

5.9.4 Genome Comparison

The virus isolates from eastern and northern region of India belong to MeYVMV and shared 92% identity between them and showed 83% identity with isolates from northern India which belong to the species MeYVMBV. The isolates from northern India exhibited 98–99% identity between them. The betasatillite associated with MeYVMBV of northern India belonged to CLCuMB species, contrasting to eastern India which had Ludwigia leaf distortion betasatillite. In a phlyogenetic analysis the east and south Indian isolates comprising MeYVMV clustered separately from north Indian isolates comprising MeYVMBV. MeYVMV also produced leaf curl like symptoms in kenaf. Besides MeYVMV and MeYVMBV, also CLCuMuV and ToLCJV were found to be associated with leaf curl in Northern India. Papaya leaf curl virus infect another fiber crop *Crotalaria juncea* along with radish leaf curl betasatellite. Recently ageratum enation virus (AgEV) was also described from *H.cannabinus.*

5.10 Begomoviruses Associated with Leaf Curl Disease

In India, maximum numbers of begomoviruses are known to cause leaf curl disease in economically important crops (Fig. 5.6). The leaf curl disease includes various types of symptoms like leaf distortion, enation, twisting and stunting of plants.

5.11 Tomato leaf curl New Delhi virus (ToLCNDV) and Tomato Leaf Curl Bangalore Virus (ToLCBaV)

ToLCNDV and ToLCBaV constitute two divergent lineages of begomoviruses causing leaf curl disease in tomato in India. ToLCNDV is a bipartite begomovirus and was characterized from New Delhi and Lucknow (Padidam et al. 1995; Srivastava et al. 1995). ToLCBaV is a monopartite begomovirus, which was described from Bengaluru, southern India (Muniyappa et al. 2000; Kirthi et al. 2002).

Fig. 5.6 Leaf curl disease of different crops caused by begomoviruses. (**a**) tomato leaf curl New Delhi virus (ToLCNDV) in tomato; (**b**) cotton leaf curl Multan virus-Rajasthan strain in cotton; (**c**) chilli leaf curl virus in chilli; (**d**) papaya leaf curl virus in papaya; (**e**) tobacco leaf curl virus in tobacco; (**f**) ToLCNDV causing apical leaf curl disease of potato; (**g**) okra enation leaf curl virus in okra; (**h**), (**i**) croton yellow vein mosaic virus in rapeseed; (**j**) ToLCNDV in pumpkin; (**k**) kenaf leaf curl virus in kenaf

There are 19 begomoviruses recorded so far in tomato from India. In addition to ToLCNDV, there is one more bipartite begomovirus, tomato leaf curl Palampur virus (ToLCPalV) affecting tomato in northern and sub-Himalayan region. There are eight monopartite begomoviruses, tomato leaf curl Bangalore virus (ToLCBaV), tomato leaf curl Kerala virus (ToLCKeV), tomato leaf curl Patna virus (ToLCPtV), tomato leaf curl Gujarat virus (ToLCGuV), tomato leaf curl Karnataka virus (ToLCKaV), tomato leaf curl virus (ToLCV), tomato leaf curl Joydebpur virus (ToLCJV), pepper leaf curl Lahore virus (PeLCLaV). Two more begomoviruses, tomato leaf curl Pune virus (ToLCPuV) and tomato leaf curl Rajasthan virus (ToLCRaV) have been characterized from Maharashtra and Rajasthan, respectively, however, it is not clear whether they have monopartite or bipartite genome. Besides these viruses, chilli leaf curl virus (ChiLCV), chilli leaf curl India virus (ChiLCIV), AEV, PaLCuV, and tobacco curly shoot virus also infect tomato under natural field conditions. Recently, two begomoviruses were recorded, tomato enation leaf curl virus- KP195260, Venkataravanappa unpublished and tomato severe leaf curl virus (ToSLCV)-KP195267 (Venkataravanappa unpublished), however infectivity of the cloned DNA of these viruses in tomato have not yet been demonstrated.

5.11.1 Discovery and Distribution

Tomato leaf curl disease was first reported from northern India in 1948 (Vasudeva and Samraj 1948), from central India in 1950, subsequently the disease emerged as a problem in tomato growing region of southern India. Since then the disease has been recognized as threat to cultivation of the crop throughout the country. Table 5.7 shows the details on incidence, and year of record of the disease from which it is evident, that tomato leaf curl disease is the most devastating disease affecting productivity. In 1990, it struck as epidemic in Kolar region of Karnataka. The disease incidence has increased with introduction of high yielding hybrid varieties. On the basis of virus characterization it can be reasonably assumed that the predominant viruses in southern region are ToLCBaV and different isolates of ToLCV, eastern region ChiLCV. In Northern, Western and Central India ToLCNDV are predominant.

ToLCNDV is the only begomovirus which has no trans-boundary limitations in movement across different countries in Asia and Europe. In Asian continent, ToLCNDV has been recorded in Bangladesh, Iran, Sri Lanka, Malaysia, Taiwan, Thailand and Indonesia. In Europe, its occurrence was first noticed in 2013 in Southern Spain (Lopez et al. 2015). In Tunisia, ToLCNDV affect zucchini, cucumber and melon (Mnari-Hattab et al. 2015) and in several vegetable crops in Scily and Southern Italy (Panno et al. 2016). At present, it appears that ToLCNDV is well spread in all these Asian, North African and Southern European countries.

Table 5.7 Geographical distribution and incidence of leaf curl in tomato in India

S.No.	Reported from (state/place)	Cropping season	Disease incidence (%)	Reference
1	Andhra Pradesh	–	–	Reddy and Yaraguntaiah (1981)
2	Assam	Winter	11–21.4	Borah and Bordoloi (1998)
3	Bihar	–	35	Dubey et al. (1986)
4	Chattisgarh	–	70–80	Singh et al. (1999)
5	Delhi	Winter	83–90	Vasudeva and Samraj (1948)
		Summer	14	Tripathi and Varma (2003)
6	Gujarat	–	–	Shih et al. (2003)
				Chakraborty et al. (2003)
7	Haryana	Summer	95–100	Banerjee and Kalloo (1990)
8	Himachal Pradesh	–	–	Gupta et al. (2001)
9	Jammu	Summer	52.3	Sastry et al. (1978)
		Winter	16	
10	Karnataka	Summer	78–99	Sastry and Singh (1973)
		Summer	18.5–55	Sastry et al. (1978)
		Summer	52–100	Saikia and Muniyappa (1986)
		Summer	4.5–100	
		Winter	15–35	Sastry et al. (1978)
		Winter	6.4–52.2	Saikia and Muniyappa (1986)
11	Kerala	–	–	Reddy et al. (2005)
12	Madhya Pradesh	Summer	1.3–70.7	Singh et al. (1999)
		Winter	3.4–86.3	
13	Maharashtra	–	–	Pimpale and Summanwar (1986), Mote (1978)
14	Punjab	Summer Winter	–	Butter and Rataul (1981)
15	Rajasthan	Winter	–	Bhardwaj (1992)
16	Tamil Nadu	Summer	80	Jeyarajan et al. (1986, 1988)
17	Uttar Pradesh	Winter	35	Saklani and Mathai (1977)
	Pantnagar	Summer	69	Saklani and Mathai (1977)
	Lucknow	–	–	Verma et al. (1975)
	Varanasi	Summer	100	Srivastava et al. (1995)
				Kalloo (1996)
	Kanpur	Winter	25	Singh and Lal (1964)
18	West Bengal (Kalyani)	–	30	Mukhopadhyay et al. (1994)
		Winter	48–54	Verma et al. (1989)

5.11.2 Economic Loss

Depending on the time of infection, disease incidence and the severity, the yield loss ranges from 17.6 to 99.7% (Butter and Rataul 1981; Kalloo 1996). Sasthri and Singh (1973) reported 92.3% loss when infection occurs at 30 days after transplanting. The yield reductions were 94.9, 90.0, 78.0 and 10.8% when plants get infected

at 2, 4, 6, 10 weeks after planting (Sastry and Singh 1973). The tomato plants get infected at all developmental stages and Saikia and Muniyappa (1989) reported less yield loss in the summer planted crops (6.4–52.2%) compared to winter planted crops (52.5–100%).

5.11.3 Symptomatology

Typical symptoms of tomato leaf curl disease are curling, puckering of leaves, veinal yellowing, stunting, excessive branching, pale yellowing to deep yellowing of leaves (Vasudeva and Samraj 1948). In addition, the extreme distortion of leaves and stunting of plants are also observed. In severely stunted plants, flowers may drop off. In some genotypes, green vein banding, twisting, green enation are also seen on the under surface of the leaf. Sometimes upward rolling of margin and islands of golden colors scattered amidst the normal green tissue are also observed (Singh and Lal 1964). The type of symptoms produced is dependent on the genotype cultivated and the developmental stage at which infection occurred. The begomoviruses affecting tomato in Asian continent are referred by two generic names, tomato leaf curl viruses and tomato yellow leaf curl viruses; this gives impression as if yellow leaf curl symptoms are not caused by tomato leaf curl viruses, which is a misconception. In India, depending on the genotypes cultivated yellow leaf curl symptoms are caused by both mono and bipartite viruses in the field. Ultra structural changes like hypertrophy of nucleus and accumulation of dark granules and aggregate of virus like particles in the cytoplasm of matured sieve element was observed by Saikia and Muniyappa (1989).

5.11.4 Transmission and Host Range

A wealth of information is available regarding vector transmission of tomato leaf curl viruses. Since molecular characterization has been accomplished only in 1995s, it can be presumed that the details on transmission for the viruses from southern India may represent the data for monopartite begomoviruses mainly ToLCBaV; details from central and northern India may represent details for ToLCNDV. As early as 1948, Vasudeva and Samraj (1948) demonstrated whitefly transmission of leaf curl virus to several hosts. They observed that in winter, symptoms appeared 25 days post inoculation, while in summer it required only 15 days.

For ToLCBaV, Buttler and Rahul (1978) achieved 100% transmission with ten whiteflies/plant at optimum temperature of (33–39 °C). Muniyappa et al. (2000) reported minimum AAP of 10 min and IAP of 20 min for ToLCBaV-(Ban 4). They also described that geographically different isolates behaved in different manner. They reported that in one whitefly per plant inoculation tests, the females were more effective (95%) than males (25%) in transmitting the virus after 24 h of AAP, ToLCBaV persisted upto 12 days and not the entire life of vector (Muniappa et al. 2000). Similar results was reported for ToLCGuV by Chakraborty et al. (2003);

these results are contrasting to observations made by Reddy and Yaraguntaiah (1981) who found that the vector could retain the virus throughout the lifespan after 6 h of latent period.

In recent years, mechanical/sap transmission of ToLCNDV, ToLCGuV, ToLCKaV have been reported (Chatchawantcanphanich and Maxwell 2002; Chakraborty et al. 2003; Usharani et al. 2004a, b; Sohrab et al. 2004). The high virus titre in the host plants and their presence in mesophyll cells may explain the sap transmission.

The begomoviruses causing tomato leaf curl disease have a wide host range, affecting various dicotyledonous plants belonging to different families. Host range of the viruses has been determined by graft/whitefly transmission, agroinoculation/biolistic delivery of viral genome into tomato plants, or by detecting the viruses in naturally infected plants using specific primers or probes to virus species.

The tomato begomoviruses are known to infect economically important cultivated crops like chilli, papaya, sunnhemp, tobacco, *Physali*s sp., sesamum and potato (Vasudeva and Samraj 1948; Nariani 1968; Reddy and Yaraguntaiah 1981; Sastry et al. 1978; Rataul and Butter 1977; Saikia and Muniyappa 1986) and ornamental plants such as *Althaea rosea, Petunia hybrida, Phlox drumondii*, *Tithonia* sp., *Zinnia elegans, Vernonia cineria* (Rataul and Butter 1977; Sastry et al. 1978; Reddy and Yaraguntaiah 1979; Gupta et al. 2001). Other plant species infected are *Nicotiana sylvestris, N. glutinosa, N. rustica, Datura stramonium, Solanum nigrum, S. seaforthianum, Ageratum conyzoides, Acanthospermum hispidum, Centratherum anthelminticum, Cassia uniflora, Sida rhobombifolia, Euphoria hirta, E. geniculata, Scoparia dulcis, Shizanthus* sp., *Galinosoga parviflora, Nicandra physaloides, Flavaria australiasia, Sida mysoriensis, Xanthium strumarium* (Vasudeva and Samraj 1948; Nariani 1968; Singh and Lal 1964; Verma et al. 1975; Reddy and Yaraguntaiah 1981; Mariyappan and Narayanasamy 1986; Reddy and Ravi 1991; Sastry et al. 1978; Rataul and Butter 1977; Saikia and Muniyappa 1986; Gupta et al. 2001). Non host plants for these viruses were also identified. They are *Amaranthus caudatus, Achyranthus aspera. Gomphrena globosa, Catharanthus roseus, Chenopodium amaranticolor, Rhaphanus sativus, Brassica oleraceae, Cucumis sativus, Cucurbita pepo, Luffa acutangula, Momordica charantia, Acalypha indica, Phaseolus vulgaris, Dolichos lablab, Althaea rosea, Abelmoschus esculentus, Gossypium hirsutum, Solanum melongena, Solanum tuberosum, Datura metal, Withania sominifera, Vigna mungo, V. radiata, V. unguiculata, Lagenaria vulgaris, Clitoria sp. and Melilotus alba* (Reddy and Yaraguntaiah 1981; Verma et al. 1975).

The natural infection of begomoviruses has been identified in several plant species. Chilli (Hussain et al. 2004), potato (Usharani et al. 2004a, b), chayote (Mandal et al. 2004), *Luffa cylindrica* (Sohrab et al. 2004) and bitter gourd (Tahir and Haider 2005) are hosts for ToLCNDV; pepper is host for ToLCGuV (Chakraborty et al. 2003) and guar was identified as a host for TLCBaV (Khan et al. 2003).

5.11.5 Serology

Muniyappa et al. (1991a, b) purified the ToLCBaV virions from infected tomato sample and showed the association of geminate particles by immunosorbent electron microscopy. Using panels of monoclonal antibody to ICMV and ACMV Varma (1989) and Harrison and Robinson (1999) analyzed the epitope profile of tomato begomoviruses in TAS-ELISA and speculated that they are different from other Indian begemoviruses.

5.11.6 Genome Comparison

Full length genome characterization of ToLCNDV was initially accomplished by Padidam et al. (1995) and Srivastava et al. (1995). Subsequently more than 100 isolates of ToLCNDV have been characterized. Characterization of ToLCBaV was completed by Muniyappa et al. (2000) and Kirthi et al. (2002) and it was found that virus isolates which were labeled as tomato leaf curl viruses Ban1, Ban3, Ban4, Ban5 and Kolar belong to the species ToLCBaV. The genome organization of all the 16 begomoviruses (length varying from 2739 to 2759) resembles organization of OW begomoviruses, having two virion and sense and four complementary sense ORFs. In addition Padidam et al. (1999) predicted one small ORF, V3 on the viral strand, the function of which is not yet clear. In DNA B, there are two ORFs one each in viral and complementary strand.

From the details it is evident that leaf curl isolates from southern India have a monopartite genome, which is associated with betasatellite, while both mono (DNA-A with betasatellite) and bipartite begomoviruses have been found in leaf curl affected tomato in northern India. The characteristics feature of ToLCNDV, in Indian subcontinent is, it is invariably associated with either alpha or betasatellite, though it is bipartite.

The genomic component of the bipartite viruses, ToLCNDV and ToLCPalV when compared, around 80% and 89.4% identity was observed between DNA A and DNA B components respectively, the interesting point is 77–81% identity of the ToLCNDV is in the CR region.The sequence identity with the monopartite begomoviruses ranged from 69 to 73%. Interestingly with tomato leaf curl Rajasthan virus it was 86%. The ToLCBaV which is highly prevalent in the southern India shows 72–80% identity with other monopartite viruses, 70/71% with bipartite ToLCNDV and ToLCPalV.

The study group on taxonomy of geminivriuses has recommended 91% and 94% identity in the complete nucleotides sequence of DNA A component as the threshold values to demarcate the species and strains respectively. Consequently some of the tomato viruses have been regrouped as variants of existing species. Following are the isolates of ToLCNDV which show less than 90% identity; ToLCNDV, –1N-ND papaya, 2005- 89% (DQ989325), ToLCNDV- Bangladesh-cucumber-06- 89% (EF450316), ToLCNDV-India- Haryana, 2003-87% (FJ561298), ToLCNDV- 88% (FN645905), ToLCNDV Pakistan- Lahore- 89%

(HG316125), ToLCNDV- 90% (JX460805), ToLCNDV-90% (KC960492), ToLCNDV- 90% (KF002409), Parthenium -90% (JQ897969), ToLCNDV-2-71%(JQ897969), ToLCNDV-3- 85%(KC465466), ToLCNDV-484%(KF551592), ToLCNDV- spain - 90% (KT175406). All these isolates show less than 91% identity but have been included under ToLCNDV, which need to be rectified. Especially, the isolates ToLCNDV-2, ToLCNDV-3, ToLCNDV-4 which show very less identity need to be raised to species level.

ICTV in its ninth report grouped ToLCNDV isolates into three different strains; majority of ToLCNDV isolates as one strain, and other three isolates ToLCNDV-2,3,4 as three different strains, ICTV in its tenth report differentiates only two strains among ToLCNDV, one strain comprising all the isolates of ToLCNDV and another comprising ToLCNDV isolates from Spain (Zaidi et al. 2016). It appears that more stringent analysis need to be performed to group ToLCNDV isolates.

On the basis of infectivity Chatterji et al. (1999), identified severe and mild strains among ToLCNDV. They shared 94% nucleotide identity, but differed in the rep binding sites. The rep proteins of severe strain, the amino acid residue Asn10 was shown to specifically recognise the third base pair of the iteron sequences GGTGTCGGAGTC. However strainal differentiation based on symptom expression may not be conclusive as symptom expression is host genotype dependent. Therefore, categorization of ToLCNDV isolates into mild and severe strain is not being pursued presently.

5.11.7 Phylogenic Relationship

ToLCNDV represents unique begomovirus complex that comprises diverse isolates infecting hosts belonging to crop species, weeds and ornamentals distributed in diverse agroclimatic conditions. Whether, the grouping occurs in the context of geographical locations or on the basis of host species were examined by several workers.

Jyothsna et al. (2013a, b), proposed that ToLCNDV isolates can be categorised into three clusters, one major cluster comprising virus isolates from solanaceous hosts with exception of one isolate from ashgourd and pumpkin, a second cluster including isolates from cucurbitaceous hosts originating from South Asia and Indian subcontinent and a third cluster comprising isolates from okra, tomato and chilli pepper. Phylogenetic analysis clearly revealed that the three bipartite begomoviruses ToLCNDV, ToLCPalV and SLCNNV have originated together, in this group ToLCRaV is also present.

Zaidi et al. (2016), performed phylogenetic analysis including ToLCNDV isolates from Europe and from Southeast Asian countries. These isolates are evolutionarily distinct from ToLCNDV variants from India and they opined that, the ToLCNDV isolates from Europe and North African country had monophyletic origin.

An analysis performed including all the isolates (the present study) clearly showed that there is no association between host species and clustering of ToLCNDV isolates. The ToLCNDV isolates which showed less than 90% identity branched off independently akin to other monopartite viruses. The other major lineage consists of monopartite begomoviruses of which ToLCBaV represent the basal group. On the basis of 94% identity ToLCBaV isolates have been categorised into four strains A, B, C, D. Among the monopartite begomoviruses, ToLCKeV is distinct.

5.11.8 Recombination

Indian tomato begomoviruses share host range and are, often present in mixed infection (Kanakala et al. 2013), possibly co-exist with one another in an host, satisfying all the conditions required for recombination events. Prasanna and Rai (2007) analyzed the events by RDP and concluded very low or almost absence of recombination events for ToLCNDV isolates. Prasanna and Rai (2007) suggested that of the six isolates of ToLCNDV analyzed only three isolates showed recombination and there is a non-random distribution of events, the highest frequency being mapped in the N terminal portion of Rep. It is quite unexpected that despite wide host range and mixed infection only few recombination events are detected in ToLCNDV; that too only in some isolates. ToLCPalV was the major parent in the predicted events. On the contrary ToLCBaV was identified as the complex recombinant, arising from five to six events. Viruses from south India contained sequences closely related to isolates from Taiwan. In our analysis it was found that ToLCNDV was one of major parental sequences contributing to emergence of recombinants like ToLCRaV, radish leaf curl virus, ToLCPalV, papaya leaf crumple virus, ToLCPtV.

5.11.9 Component Complementation

Exchange of genetic components referred as pseudorecombination is common among bipartite viruses which may occur in mixed infection, for example, Kanakala et al. (2013) demonstrated pseudoreombination between ToLCNDV A and ToLCPalV DNA B, and *vice versa*. Interestingly, they also demonstrated whitefly transmission of pseudorecombinant progeny produced by inoculation of ToLCNDV-DNA A with ToLCPalV DNA B suggesting that hetero encapsidation of DNA B component of ToLCPalV by ToLCNDV DNA A. In an inoculation combining four components of both the viruses, the DNA B component of ToLCNDV dominates. Pseudorecombination between ToLCGuV and ToLCNDV has been shown by Chakraborty et al. (2008) and Jyothsna et al. (2013a, b) observed that there was an asymmetrical synergism that inoculation with ToLCGuV DNA A with ToLCNDV DNA B component led to expression of severe symptoms.

ToLCNDV DNA B is an interesting molecule which shares high identity with DNA B of atleast three viruses, ToLCGuV (Chakraborty et al. 2003) BYVMV (Venkataravanappa et al. 2015) and PepLCLaV (Shafiq et al. 2010). In all these cases identity between the viruses in DNA A is only 65% but DNA B shows 85–89% identity with ToLCNDV- DNA B. Infectivity and transreplication of these DNA B by ToLCNDV DNA A resulted in severe symptom expression. It is possible that these monopartite begomoviruses occurred in mixed infection with ToLCNDV and captured DNA B.

It is relevant to note here that ToLCGuV was found associated with tomato yellow leaf curl Thailand betesatellite (TYLCTHB) and produced very severe symptoms in *N. benthamiana* and tomato. Jyothsna et al. (2013a, b) suggested that occurrence of ToLCGuV in association with betasatellite is more frequent than its association with DNA B.

5.11.10 Virus Prevalence in the Field

The distribution of ToLCNDV and monopartite tomato begomoviruses were earlier thought to be geographical location specific (Chowdareddy et al. 2005). However, in recent years in northern India, the mixed infection of ToLCNDV and ToLCKaV or ToLCNDV and ToLCPalV are more frequent. In the southern India, ToLCNDV is recorded in almost all the cucurbitaceous hosts, okra and weeds. However ToLCNDV is not yet recorded from tomato in southern India. The tomato genotypes cultivated in southern India do not possess genetic resistance to ToLCNDV, as they succumb to infection when they are planted in north India. It is best speculated that, ToLCNDV in cucurbitaceous hosts in south India meet with some obstacles in getting transmitted to tomato, which need to be looked into.

The entry of ToLCNDV into cucurbits itself is of recent occurrence. ToLCNDV infection in okra has been identified in 2004, in majority of cucurbitaceous hosts between 2004 and 2007, later in ashgourd 2011, papaver 2012, cucumber 2012; in eggplant which was considered to be free of any WTG infection in India was found to be infected in 2009 (Pratap et al. 2011). Earlier researchers Vasudeva and Samraj (1948) and Singh and Lal (1964) considered cucurbitaceous hosts as non-host plants to ToLCNDV. Whether capturing betasatellite facilitates its expanding host range is one aspect which needs to be researched.

Whether the excessive spread of the virus in cucurbitaceous hosts could result due to its presence in seeds, need to be looked into in the context of seed borne nature and seed transmission of MYMV in India (Sathya et al. 2013).

5.11.11 Establishment of Koch's Postulate

Molecular characterization of nearly 100 isolates of ToLCNDV has been completed. However, Koch's postulates have been established through agroinoculation only for four isolates (U150115, AY428769, HQ264185, HQ141673). As a typical bipartite

virus, though DNA A alone is infectious, systemic spread and expression of symptoms occur only when both DNA A and DNA B are inoculated. Padidam et al. (1995) observed that, mutation in the coat protein region (amino acids 65 or 172) did not affect systemic movement and symptoms development but affected single stranded DNA accumulation. They also showed the mutation in AV2 region to affect generation of ds DNA.

Pratap et al. (2011) recorded for first time a ToLCNDV in eggplant causing severe yellowing diseases in Nagpur in central India and Koch's postulates were established by inoculating dimeric clones of DNA A and DNA B components.

Sivalingam and Varma (2012), found that, when ToLCNDV A alone is inoculated, limited accumulation of viral DNA occurred, which increased several folds in plants co-infected with DNA B or betasatellites. The increase in virus titre very much reflected in symptom severity and transmissibility by whitefly.

Since ToLCNDV was always associated with various types of betasatellite, the pathogenicity of ToLCNDV in association with different betasatellites was examined (Jyothsna et al. 2013a, b). Plants co-inoculated with betasatellites showed enhanced symptom severity in both *N. benthamiana* and tomato and increased in helper viral DNA A and DNA B levels.

For monopartite begomoviruses ToLCGuV (Chakraborty et al. 2003; Jyothsna et al. 2013a, b), ToLCKaV (Chatchawankanphanich and Maxwell 2002), ToLCJV (Tiwari et al. 2012), ToLCBaV (Tiwari et al. 2012), Koch's postulates have been established by inoculating DNA A with respective betasatellites. ToLCBaV was found to replicate well at 23 °C and produce symptom, than at 25 °C. Of all the viruses studied, ToLCJV was the most virulent, wherein DNA A alone could cause very severe symptoms in both *N. benthamiana* and tomato (Tiwari et al. 2012). The DNA A component of all these monopartite viruses *trans* replicated different betesatellite confirming the promiscuity between DNA A and betasatellite.

5.11.12 Molecular Basis of Pathogenicity

The functions of coat protein were examined by Kirthi et al. (2002) by expressing the coat protein of ToLCBaV. The purified recombinant CP bound preferentially to ssDNA in sequence nonspecific manner. They proved that the Zinc finger motif in CP (corresponding to 65–68 amino acid residue in ToLCBaV is involved in binding to Zinc and DNA.

5.11.13 RNAi and PTGS Suppressor

The viral suppressor AC2 was studied by Yadava et al. (2010) and ToLCNDV AC2 was demonstrated to inhibit the enzyme activities of RdR6 and AGO4 protein which are needed for initiation and effector activities of RNAi. Consequent upon identification of suppressor in begomoviruses, their application in achieving transgenic resistantce has been looked into. Thus Praveen et al. (2010) made four different RNAi

construct with varied length targeting AC4 gene of ToLCNDV, longer ds RNA constructs were more efficient in silencing of target gene. Using sense, antisense, self-complementary inverted repeats, non spliced hairpins and small hairpin constructs targeting Rep and AC4 segments they demonstrated suppression of viral symptoms.

5.11.14 Host Virus Interactions

A study by Sahu et al. (2010) highlighted the gene expression changes during incompatible interaction between tolerant tomato plant and ToLCNDV. A suppression substractive hybridization library (SSH) was prepared for a naturally tolerant cultivar of tomato, namely H-88-78-1. This study revealed that tolerant tomato plants have enhanced level of transcript related to cell cycle and DNA/RNA processing, signaling molecules, transporters, transcription factors along with the proteins of unknown functions. An interesting observation was that apart from these genes, classes of host ubiquitin proteasome pathway genes were also highly expressed in tolerant cultivar in comparison to a susceptible cultivar. Similar attempts were made to identify the differentially expressed genes during ToLCNDV-tomato interaction, (Naquvi et al. 2011a). The genes related to innate immunity, metabolism and ethylene signaling were implicated in the systemic infection during ToLCNDV infection in tomato (Naqvi et al. 2011a, b).

Kushwaha et al. (2015) inoculated ToLCNDV on to several solanaceous hosts, *Capsicum annuum*, *N. benthamiana*, and *Solanum lycopersicum,* and observed that *S. lycopersicum* and *N.tabaccum* developed symptoms and led to viral DNA accumulation more than other hosts. They observed that there were differential expression levels of RNAi pathway genes (RDR6, AGO1, and SGS3), as well as host defense pathway. The NBS-LRR type of protein and lipid transfer protein were upregulated and they concluded that, the expression levels of host defense genes, determined the viral DNA accumulation and symptom development. Sahu et al. (2016), subsequently attempted to characterize 26S proteosomal subunit RPT4a (SIRPT4) gene under ToLCNDV pathogenesis in the tolerant cultivar H-88-78-1. They showed specific binding of SIRPT4 at the stem loop region of IR in both DNA A and DNA B. They suggested that this binding is secondary structure specific and binding at IR inhibited RNA pol II activity, thereby reducing bidirectional transcription. When they silenced SIRPPT4 gene, the tolerant phenotype of H 88-78-1 was converted to susceptible phenotype. Overexpression of SIRPT4 gene resulted in programmed cell death and hypersensitive reactions. They suggest that SIRPT4 interference of viral pathogenicity is more due to specific binding and not due to any proteolytic function.

Mandal et al. (2015) studied the transcript level of SlTRNI gene which is important for cell expansion and vein formation. Though SITRN1 has two start sites, under viral pathogenesis there is a preferential use of one start site only. They found that the promoter sequences of SITRN1 have multiple W boxes which mediate induction of SITRN1 under ToLCNDV infection. They postulate that during stress SA pathway gets activated which induces WRKY16, leading to transcription of SITRN1gene.

5.11.15 Micro RNA and Their in Role In ToLCNDV Pathogenecity

Geminivirus proteins are known to modify the host PTGS pathways, which in turn deregulates normal cellular activities leading to disease development/resistance (Sahu et al. 2014b). In this regards, efforts have been made to identify the ToLCNDV-responsive-miRNAs responsible for either disease development or in providing tolerance (Naqvi et al. 2010; Pradhan et al. 2015). A NGS platform was used to identify the ToLCNDV-responsive miRNAs, which resulted in detection of 53 novel miRNAs (Pradhan et al. 2015). These novel miRNAs were not only involved in targeting leaf architecture and plant development related host genes, but were also implicated in plant defense response. For example, novel miRNAs such as Tom 14, Tom 43 have been shown to target transcription factors such as AP2/ERF; and teosinte branched1/cycloidea/PCF (TCP) transcription factor, respectively, and might lead to leaf curl phenotype in plant. Apart from this, disease resistance gene such as CC-NBS-LRR type protein was also shown to be targeted by a novel miRNA Tom 17. Besides these, role of conserved miRNAs has also been examined by Naqvi et al. (2010) which revealed that the differential accumulation of miR159/319 and miR172 have the correlation with leaf curl symptoms development in tomato. More interestingly, the authors have also postulated role of miR168 and miR162 in the alteration of global miRNA flux by targeting DCL1 and AGO1.

Moreover, numbers of reports on computational prediction of virus genome-derived miRNAs are also available, but few of them are reported to be involved in the disease resistance (Naqvi et al. 2011a, b; Shweta and Khan 2014).

5.11.16 Alpha and Betasatellites Associated with ToLCNDV and ToLCBaV

Though ToLCNDV is a bipartite virus in northern India, it is found associated with CLCuMuB (Sivalingam and Varma 2012; Jyothsna et al. 2013a, b) and can *trans* replicate ChLCB (Akhter et al. 2014), CLCuMuB, TYLCTHB (Jyothsna et al. 2013a, b) PaLCuB, BYVB and LuLDB. In all the cases there is enhancement in symptom expression and viral replication. ToLCBaV is associated only with ToLCBaB, but can trans replicate other betasatellites too (Tiwari et al. 2012). ToLCNDV is also found associated with alphasatellites.

5.11.17 Resistance to ToLCNDV and ToLCBaV

Identification of the resistant sources and deployment of the specific gene to counter the challenges of the specific virus in a selected location is the only strategy that will give rise to sustainable disease management. In the case of tomato leaf curl disease, the problem is more complicated as we are not sure how many viruses are causing the disease in a specific geographic location. Besides, the begomoviruses constantly

evolve and either by component capturing or by recombination, they emerge as super virulent strain that it is difficult to develop a resistant line. Until now, six genes (*Ty* genes) derived from different tomato wild species have been identified. From the perusal of the data it is evident that *Ty-2* genes while confer resistance phenotype against monopartite begomovirus, it was not effective against bipartite ToLCNDV, Prasanna et al. (2015) attempted to combine *Ty-2* and *Ty-3* genes through marker assisted selection and screened the hybrid lines for resistance to viruses by challenging through agroinoculation of specific, monopartite and bipartite viruses. They also performed field screening to know the phenotype of genotypes response to unidentified viruses other than ToLCNDV, ToLCBaV, ToLCJV, ToLCGuV. They found that the lines and hybrids with *Ty-2* were susceptible to ToLCNDV. The *Ty-3* gene showed dosage effect with partial resistance of plants to ToLCNDV in heterozygotes stage. They also observed that *Ty-2* gene in either homozygous or heterozygous stage enhanced level of resistance of plants with heterozygous *Ty-3*. By pyramiding *Ty-2* and *Ty-3* genes considerable resistance toToLCNDV can be achieved. The resistance of some of the tomato genotype Vaibav, Nandhini, having *Ty-2* gene to ToLCBaV were lost, when these genotypes were individually agroinoculated with ToLCBaV (Tiwari et al. 2012) and the cognate betasatellite.In the presence of betasatellites, the *Ty-2* gene effect seems to be neutralized which need to be looked into.

5.12 Chilli Leaf Curl Virus

Chilli leaf curl virus (ChiLCV) is the most predominant begomovirus affecting chilli production in India. This virus is present throughout India infecting wide range of solanaceous and non-solanaceous host. The virus is a typical Old World monopartite begomovirus associated with different betasatellites. Until now, 11 distinct begomovirus species are reported to be associated with chilli leaf curl disease in India e.g., ChiLCV, ChiLCINV, chilli leaf curl Kanpur virus (ChiLCKaV), chilli leaf curl Vellanad virus (ChiLCVV), PaLCuV, pepper leaf curl Bangladesh virus (PepLCBV), RaLCuV, ToLCJV, ToLCV, and ToLCNDV. Recently, a new recombinant virus, chilli leaf curl Ahmedabad virus has been recorded by Bhatt et al. (2016). Of all the viruses infecting chilli in India, ChiLCV is the most prevalent begomovirus species.

5.12.1 Discovery and Distribution

In India, the occurrence of leaf curl disease in chilli was first noticed in 1930 (Husain 1932). Successful whitefly transmission of the disease established it as a viral disease (Mishra et al. 1963; Dhanraj and Seth 1968; Shukla and Ram 1977). The incidence and severity of the disease has increased since 2007 (Chattopadhay et al. 2008). Due to high vector population, the incidence and spread of the disease are more in summer season. In the recent years (2014, 2015), chilli leaf curl emerged in

epidemic proportion in the states of Madhya Pradesh and Maharashtra. Although, the first report of the disease was about half- a century ago, the association of begomovirus with this disease has been confirmed only in 2007 based on partial sequencing of viral genome (Senanayake et al. 2007).

5.12.2 Economic Loss

Leaf curl is a major constraint in chilli production in India. If the chilli plants get infected within 3–4 weeks after transplantation the yield loss reaches upto 90% and the quality of the fruits is also affected. In Jodhpur (Rajasthan), a major chilli an epidemic of leaf curl disease in chilli was recorded in 2004, where14–100% incidence of the disease was recorded (Senanayake et al. 2012). In recent years, epidemic of chilli leaf curl emerged in central India. Chilli is a major crop in Khargone, Dhar and Badwani districts of Madhya Pradesh, where leaf curl disease was not a major problem till 2013, however during 2014–2015, leaf curl emerged in epidemic proportions in most places resulting in widespread crop failure. In Yavatmal District, Maharashtra, the disease caused a serious yield loss in chilli during 2015, where leaf curl appeared within 3–4 weeks post transplanting and affected entire fields in most of the places by October–November. Many of the farmers were forced to abandon chilli crop midway and many uprooted or sown wheat in the chilli field.

5.12.3 Symptomatology

The infected chilli plant exhibits symptoms such as curling of leaves, leaf rolling and reduced leaf size. Puckering and blistering of inter veinal regions associated with thickening and swelling of veins (Dhanraj and Seth 1968; Mishra et al. 1963) may also occur. The infected plants may get stunted, bear very less fruits resulting in heavy loss.

5.12.4 Transmission and Host Range

The virus is not sap transmissible and so far seed transmission has not been reported. It is efficiently transmitted by whitefly. Senanayake et al. (2007) found that 60% transmission was brought out by a single whitefly and eight whiteflies per plant resulted in 100% transmission and the plants developed symptoms within 7-10dpi; the minimum AAP and IAP were determined as 180 and 60 min. In the serial inoculation experiment whitefly survived upto 5 days post AAP and became aviruliferous 1 day prior to death.

Chilli leaf curl virus has a wide host range and infects solanaceous and non solanaceous hosts in combinations with various betasatellites. Among the solanaceous hosts the most commonly infected species are chilli, tomato and rarely eggplant.

The malvaceous host recorded are kenaf, and hibiscus. Some of the other hosts in which ChiLCuV was recorded are petunia, mentha, amaranthus and *Phaseolus aureus, Solanum nigrum.*

5.12.5 Genome Comparison

Geminate particles of 18 × 30 mm were detected by Senanayaka et al. (2007) in chilli samples fom Rajasthan. This combined with whitefly transmission and hybridization of field infected samples with DNA A gave sure indication of involvement of a begomovirus. Subsequently genomic components have been cloned which showed nearly 96% identity with ChiLCuV from Pakistan and Varanasi. The genome organization was found to be typically that of OW begomoviruses.

5.12.6 Recombination

Majority of the begomovirus isolates infecting chilli have been identified to be recombinants and the recombination breakpoints have been mainly identified to be located around AC1 and AV1 regions (Kumar et al. 2015). The recombination fragments in these begomoviruses were mainly contributed by other chilli-infecting begomoviruses which confirms that intra-species recombination predominates among these viruses (Kumar et al. 2015). However, it is known that recombination also facilitates the transfer of genetic elements even between distantly related species. In this context, it is important to note that the emergence of ChiLCV-Salem isolate has been facilitated by other non-solanaeceous crop-infecting begomoviruses. Among ChiLCD associated begomoviruses, a single species, ChiLCV, has been reported to be non-recombinant in nature. Apart from causing leaf curl disease in chilli, these begomoviruses has been found to infect various economically important plants such as amaranthus, bitter gourd, mentha, papaya and petunia (Saeed et al. 2014; George et al. 2014; Nehra and Gaur 2014; Raj et al. 2010a, b; Senanayake et al. 2012). Recombination might have facilitated the genetic potential to cross host barriers. In addition to recombination, these begomoviruses and betasatellites possess higher genetic variability and high rate of nucleotide substitution (Kumar et al. 2015). Furthermore, adaptive selection has been reported to be acting on the coding regions of these begomoviruses and betasatellites causing ChiLCD (Kumar et al. 2015).

5.12.7 Establishment of Koch's Postulate

Chattopadhyay et al. (2008), agroinoculated dimeric constructs of ChiLCV and tomato leaf curl betasatellite and demonstrated pathogenicity in chilli and in *N. benthamiana,* Kumar et al. (2011) proved the expression of symptoms for a Palampur isolate. Further Kumar et al. (2015) inoculated different begomoviruses

with different betasatellites, and established how association of the betasatellite is indispensable for the leaf curl disease development in chilli.

5.12.8 Distribution of Betasatellites Associated with ChiLCD

Recently, a survey conducted on the identification of the begomoviruses associated with ChiLCD reported the association of betasatellites with all the samples collected from major chilli growing regions in the country. Based on the revised species demarcation threshold for betasatellites (Briddon et al. 2008), a total of six different betasatellite groups have been found to be associated with this disease in India. These are chilli leaf curl betasatellite (ChiLCB), CroYVMB, radish leaf curl betasatellite (RaLCB), tomato leaf curl Bangladesh betasatellite (ToLCBDB), tomato leaf curl Joydebpur betasatellite (ToLCJB) and tomato leaf curl Ranchi betasatellite (ToLCRnB). The most prevalent betasatellites associated with these begomoviruses is ToLCBDB followed by ToLCJB (Kumar et al. 2015). Similar to the helper viruses, mixed infection of betasatellites also have been detected from ChiLCD infected samples. All these groups of betasatellites have been experimentally demonstrated to be trans-replicated by these begomoviruses (Chattopadhyay et al. 2008; Kumar et al. 2011, 2015). Further, the association of these betasatellites is indispensable for leaf curl disease development in chilli (Kumar et al. 2011, 2015).

5.13 Cotton Leaf Curl Multan (CLCuMuV) and Cotton Leaf Curl Kokhran Virus (CLCuKoV)

The most devastating epidemic outbreak of begomovirus is exemplified by cotton leaf curl disease in north western India in the years 1993–1996. In north western India, two important begomoviruses causing the cotton leaf curl disease are cotton leaf curl Multan virus (CLCuMuV) (Radhakrishnan et al. 2004a; Chowdareddy et al. 2005; Rajagopalan et al. 2012) and cotton leaf curl Kokhran virus (CLCuKoV); Kirthi et al. 2004). CLCuMuV includes the recombinant strain described from Rajasthan which was earlier referred to as separate species cotton lef curl Rajasthan virus (CLCuRaV) (Radhakrishnan et al. 2004a; Kumar et al. 2010b). CLCuKoV comprises two recombinant strains Burewala strain named earlier as separate species as cotton leaf curl Burewala virus (CLCuBuV) (Kumar et al. 2010b; Zaffalon et al. 2012; Rajagopalan et al. 2012) and Shadadapur strain earlier designated as separate species cotton leaf curl Shadadpur virus. Besides these two species, cotton leaf curl Bangalore virus (CLCuBaV), tomato leaf curl Bangalore virus (ToLCuBaV), tomato leaf curl Patna virus (ToLCuPtV), ToLCNDV viruses have also been described from cotton. A new virus named as cotton leaf curl Barasat virus has been reported from *Malachra capitata* from West Bengal. It is not yet clear whether it will infect cotton.The cotton leaf curl Alabad virus which occurs in cotton in Pakistan, is recorded only in okra in India. The geminiviruses, PaLCuV, ACMV, OELCuV and

CpCDV reported in cotton in Pakistan (Saleem et al. 2016) have not yet been isolated from cotton in India.

5.13.1 Discovery and Distribution

In India CLCuD was first reported at Indian Agricultural Research Institute (IARI), New Delhi in few isolated plants of *G. barbadense* in 1989 (Anonymous 1990); and in *G. hirsutum* plants near SriGanganagar, Rajasthan in 1993; Punjab in 1994 and Haryana in 1996 (Ajmera 1994, 1996). The disease spread further, in 1997 there was a severe epidemic, as per rough estimate an area about 2.19 Lakh/ha was affected by the disease (Narula et al. 1999). Within a short span of 4–5 years the disease spread to entire 15 lakh ha of the most potential irrigated cotton belts in north India. Elite cultivars like F846, Pakistani NIAB-72F505, LH1134, PL104, RST9, Somnath and Ganaganagar Ageti became susceptible and disease incidence in north west India ranged from 1 to 97% (Singh et al. 1994). During the past three decades cotton leaf curl struck as epidemics at least twice. In India the epidemic outbreak was between 1997 and 1998, when the recombinant strain of CLCuMuV, which was earlier referred to as CLCuRaV was the culprit. The recent epidemic outbreak in 2011 is attributed to a recombinant strain of CLCuKoV, which was earlier referred to as CLCuBuV.

Leaf curl disease was noticed in cotton grown in homestead garden in Bengaluru, Karnataka (Nateshan et al. 1996). In recent years CLCuMuV has been recorded in Okra, Cotton and *Hibiscus* plants in China. CLCuMuV also has been recorded in Hibuscus plants in Phillipines. It is possible that CLCuMuV has moved to other countries through virus borne Hibiscus plants.

5.13.2 Economic Loss

In India extensive field experiments have been conducted to estimate the yield loss due to CLCuD. In Rajasthan, at Sriganganager a reduction of 50.3% in seed cotton yield, 50.3% in number of opened bolls, 12.3% in boll weight and 16.1% in height per plant was estimated in the case of popular variety *G. hirsutum* F846. In the case of variety RST9, the reduction in seed cotton yield was 32.9%, in number of opened bolls 22.9%, and in height 3.5% but no reduction in boll weight was observed (Ajmera 1996). In Punjab, a reduction of 10.5–2.2% in seed yield/plant in variety F846 and 39.0–79.7% in Pakistani Narma (NIAB-72) were recorded (Singh et al. 1994).

Ten promising germplasm lines were evaluated to study the effect of CLCuD on yield and quality parameters. There was a drastic reduction in seed cotton yield in diseased plants (8.1–79.2%) of all the tested lines. Reduction in boll weight ranged from 1.6 to 37.1% whereas boll number reduction varied from 54.3 to 73.2%. Recently, the effect of CLCuD on seed cotton yield and fibre character of popular *Bt* cotton hybrids (RCH 134, MRC 6304) of Punjab have been studied; 46.1–52.7%

reduction in number of bolls, 43.4–54.2% in boll weight, 7.5% in fibre length 7.5% in fibre strength, 3.8% in micronaire value and 2.5% in span length were observed in all the cotton cultivars (Singh et al. 2013). During the period from 2008 to 2010, all the cotton varieties which were earlier resistant or tolerant succumbed to the disease and an average yield loss up to 53.6% were recorded in north western India.

5.13.3 Symptomatology

The initiation of disease is characterized by vein thickening or swelling on young upper leaves. Dark green bead like thickening of small veins can be clearly seen from the lower side against sun light. These irregular thickenings gradually extend and coalesce to form continuous reticulation of small veins. Veins of the leaves become thickened which are more pronounced on the underside. Affected leaves become thick, leathery brittle and greener than healthy leaves. Similar symptoms are also observed on bracts. The disease is further characterized by upward and downward curling of the leaves which occurs because of the uneven growth of veinal tissue on the abaxial side of the leaves. A single infected plant generally does not show both upward and downward curling in field as well as in green house condition (Godara et al. 2012). The disease also causes enation of the veins which frequently develop into cup shaped, leaf like outgrowths on underside of the leaves. In severe cases, spiral twisting of petiole, peduncle in the youngest part of the stem is also common. In severe cases buds are either totally supressed or die shortly after formation, causing partial or complete sterility of the plant. In plants affected at an earlier age, reduction of internodal length leading to stunting and reduced flowering- fruiting are observed (Narula et al. 1999).

5.13.4 Transmission and Host Range

The cotton leaf curl causal agent is transmissible naturally only by its whitefly vector (*Bemisia tabaci Genn*), but not by seed or by mechanical inoculation with sap or by soil (Singh et al. 1994).

In experimental studies, Nateshan et al. (1996) reported that a minimum of 1 h acquisition access period (AAP) and a minimum of 5 min inoculation access period (IAP) were required for transmission and the transmission threshold was 8 h for CLCuBaV isolate. The whole process of acquiring the virus and infecting a healthy plant was accomplished in 6 h 30 min to 8 h, suggesting that the virus is circulative and requires a relatively short latent period in the vector (Nateshan et al. 1996). If the AAP and IAP are increased up to 24 h each, efficiency of virus transmission increases which is shown by the increased proportion of *G. barbadense* seedlings that were infected from 20 to 87% (Nateshan et al. 1996).

Nateshan et al. (1996) reported that CLCuBaV was transmitted for at least 9 days after *B tabaci* left the virus source plants. Nateshan et al. (1996) also studied the transmission efficiency of male and female *B tabaci*. They reported that female

B. tabaci were efficient in transmitting CLCuBaV infecting 19/25 (76%), of the test plants than male *B. tabaci* in which 12/25 (48%) of the test plants were infected.

CLCuMuV-Rajasthan was easily transmitted by whitefly to cotton cv LH900 producing symptoms of vein thickening, leaf curling and enation. The virus could be transmitted by whitefly given a minimum AAP of 10 min and IAP of 10 min. The efficiency of transmission, increased with increase in AAP or IAP. Best transmission was obtained when the whiteflies were given AAP and IAP of 48 h (Radhakrishnan et al. 2004b).

Nateshan et al. (1996) determined the host range of CLCuBaV by whiteflies (*B. tabaci*) fed on infected *G. barbadense* plants. CLCuBaV is transmitted to 24 species in 6 plant families, (i) *Asteraceae (Acanthospermum hispidum, Ageratum conyzoides, Cosmos bipinnata, Dahlia sp., Sonchus brachyotis, Synedrella nodiflora and Zinnia elegans),* (ii) *Euphorbiaceae (Croton bonplandianum and Phyllanthus niruri)*, (iii) *Fabaceae (Phaseolus vulgaris)*, (iv) *Malvaceae (Althaea rosea, Gossypium barbadense* and *G. hirsutum*), *Oxalidaceae* (*Oxalis latifolia*), (v) *Solanaceae* (*Capsicum annuum, Datura stramonium, Solanum lycopersicum, Nicandra physaloides, Nicotiana benthamiana, N. glutinosa, N. occidentalis, N. rustica, N. sylvestris and N. tabacum*). All infected species developed leaf curl, leaf cupping or leaf rolling and in some cases enation or vein thickening. CLCuBaV could infect all *G. barbadense* cultivars and a few *G. hirsutum* cultivars tested, but the virus could not be transmitted to *G. arboretum* or *G. herbaceum* (Nateshan et al. 1996).

Radhakrishnan et al. (2004a) reported that CLCuMuV-Rajasthan is readily transmitted by whitefly to plants belonging to families *Malvaceae, Solanaceae* and *Fabaceae*. The plant species, which developed typical leaf curl symptoms are *A. esculentus, Alcea rosea, G. barbadense, G. hirsutum, S.lycopersicum, N. benthamiana, N. tabacum, P. vulgaris* and *Physalis floridana.*

5.13.5 Genome Comparison

The begomovirus association with CLCD was established in 1989 by ELISA and ISEM using polyclonal antibody to ICMV (Varma et al. 1993) and nucleo based diagonostic study was initiated by Radhakrishnan et al. (2004a); further begomoviruses associated with the disease in southern and north western India were characterized (Kirthi et al. 2004; Chowdareddy et al. 2005; Kumar et al. 2010b; Rajagopalan et al. 2012).

The genome organization of cotton leaf curl begomoviruses is similar to other OW monopartite begomoviruses with MP and CP genes on viral strand, Rep, REn, TrAP and *sd* genes on the complementary strand. The characteristic variation in Burewala strain of CLCuKoV is discussed in detail below. In addition to four ORFs in the complementary strand, 21 isolates of CLCuKoV -Burewala strain have an additional ORF C5 spanning from nucleotide co-ordinate 283–80. The function of this predicted protein is not yet deciphered. A extended version of this protein is also predicted in one of the isolates of Rajasthan strain of CLCuMuV. Some

begomoviruses have been show to have this additional ORFs like, MYMIV in which Raghavan et al. (2004) identified AC5 protein contributing to replication function in yeast.

The striking deviation observed among isolates of Burewala strain of CLCuKoV is the absence of intact functional C2 gene. The C2 or AC2 gene encodes a transcription activator protein of 134 aa compostion which activates the V1/CP gene promoter and is very much required for gene expression regulation in all begomoviruses. It also has silencing suppressor activity contributing to viral pathogenesis. Amrao et al. (2010a, b) when analyzed the resistance breaking strains of cotton leaf curl viruses, identified the virus isolates to be recombinant ones between CLCuMuV and CLCuKoV and named it as separate species CLCuBuV which lacked the functional C2 gene. Since CLCuMuV and CLCuRaV are recombinants, despite less than 91% identity in the complete nucleotide sequence, they are considered as distinct strains of CLCuKoV, CLCuBuV respectively. The Burewala strain in Pakistan, mutation in C2 region were of three types; type one (Amrao et al. 2010a, b) with one inframe stop codon (1S) resulting in prematured terminated product of C2 having only 35aa residues, the second type of mutation comprises of two inframe stop codon (2S), one at the same position as the first site of mutation codon, the second inframe stop codon in three amino acid downstream of the first; the third type of mutation, a frame shift at nucleotide co-ordinate 1535, due to loss of Guanine, and a Guanine to Thymidine mutation which resuted in stop codon.

Interestingly in India, isolates of Burewala strain characterized from 2004 to 2010 contain only 2S type of mutation and lacked functional C2 protein. Rajagopalan et al. (2012) analyzed C2 region of more than 258 isolates and found out that the isolates from Fazilka and Bathinda region contained a mixture of isolates, majority of them are of 2S – inframe stop codon type and some isolates had intact C2. However in Sri Ganaganagar and Hanumangarh all isolates of Burewala strains were with 2S mutation. No isolate was found to have intact C2. Contrastingly in Hissar and Dabavali regions, isolates had intact C2 gene. In summary, isolates with defective C2 gene was more prevalent than with intact C2 gene. The 14 isolates which lacked functional C2 gene had an addition ORF C5, function of which is not yet determined.

Godara et al. (2016) characterized cotton leaf curl viruses at Delhi. They found out that three isolates belong to CLCuMuV-Rajasthan and two isolates to CLCuKoV. Association of one betasatellite, CLCuMB, and three alphasatellites related to cotton leaf curl Burewala alphasatellite and Gossypium darwini symptomless alphasatellites.

Interestingly, eight DNA- B molecules have been isolated from different wild species of cotton in Pakistan (Sattar et al. 2013).They shared 88–98% identity between them and exhibited very low identity (65–71%) with DNA B of other begomoviruses like SLCMV, ICMV. However, from India, until now DNA B molecule has not yet been detected. The two major begomoviruses CLCuMuV and CLCuKoV between them share only 72–84% identity in the complete nucleotide sequence. Both the viruses exhibit 77–83% identity with CLCuBaV from southern India. CLCuAlV which occurs only in okra in India, exihibits 66–68% identity with

CLCuKoV, 73–75% with CLCuBaV, and 76–83% with CLCuMuV. With other begomoviruses PaLCuV, ICMV 62–71% identity was observed.

5.13.6 Phylogenetic Relationship

Rajagopalan et al. (2012) performed the phylogenetic analyses of cotton begomoviruses from India and showed that the species CLCuKoV, CLCuMuV, CLCuAlV and CLCuBaV stand well separated from each other. The isolates of Burewala strains could be categorised into three types; Those isolates which lack C2 due to two inframe stop codons (2S) from Bathinda, Abohar Fazilka and Sri Ganganagar clustered in same clade along with 2S isolates of Burewala strain from Pakistan. The isolates of Burewala strain having uninterrupted intact C2 gene (four isolates) clustered with Burewala isolates of CLCuKoV from Pakistan having intact C2 gene. However four Indian isolates of Burewala strain with intact C2 gene occupied a separate clade.

Interesting results emerged from analysis of 191 full length of genomes of cotton and related begomoviruses (BYVMV) by Saleem et al. (2016). They observed that depending on the recombinational events occurring between CLCuMuV and CLCuKov, two major groups could be recognized among cotton leaf curl viruses. Group A represents recombinant viruses mainly derived from CLCuMuV and group B consists of isolates derived from CLCuKoV. Depending on the recombinational events, clade I to clade VII was differentiated.

5.13.7 Recombiantion

The contribution of recombination to emergence of newer super virulent strain – a theoretical prediction became a reality in the case of cotton leaf curl viruses. Between the two major viruses CLCuKoV and CLCuMuV, recombination occurred at different sites in genome and gave raise to Rajasthan, Burewala and Shadadpur strain. Kumar et al. (2010b) showed that Rajasthan strain of CLCuMuV they characterized had recombination in AV1 region; between CLCuMuV and CLCuKoV they also showed recombination in the Rep region with CLCuMuV and MeYVMBhV; another isolate of CLCuKoV they cloned, had recombination event in AC1 region with chilli leaf curl Pakistan virus. Interestingly when Rajagopalan et al. (2012) performed recombination analysis for isolates of Burewala strain from 2004 to 2010, no recombination events were detected in the genome from other begomoviruses. Kumar et al. (2015) identified recombination in an isolate of CLCuMuV at the 673–1981 region and the burewala strain of CLCuKoV exhibited recombination in the region between 1535 and 1817 nucleotides.

Saleem et al. (2016) observed two recombination events involving BYVMV; one in CLCuMuV – Sri Ganganagar and another in CLCuAlV viruses in CP region. All isolates of Rajasthan strains are recombinants between CLCuMuV and CLCuKoV with varying lengths of CLCuKoV sequences; in only one isolate of Rajasthan

strain (CLCuMuV-HM037920 contribution from CLCuKoV spans both virion and complementary strand, CLCuMuV shares only a short stretch in CP region.

Rajasthan strain is the only strain where CLCuKoV contributes to the complementary sense strand. Saleem et al. (2016) suggest that since it is prevalent in India, this strain might have originated in India. On the contrary, the Burewala strain is dominant both in India and Pakistan, probably this strain originated first in Pakistan and got introduced through viruliferous whiteflies to India. The successful establishment of Burewala strain in both the countries suggest that successful and fittest recombination is the one in which complementary strand gene is from CLCuMuV and virion sense gene from CLCuKoV.

5.13.8 Establishment of Koch's Postulate

Radhakrishnan et al. (2004a) made complete tandem repeat constracts of Rajasthan strain of CLCuMuV and agroinoculated cotton cv LH 900. The plants showed enation and veinal thickening 28 days post inoculation. Kumar et al. (2015) made partial dimeric construct of CLCuMuV, CLCuKoV and Burewala strain of CLCuKoV and inoculated tobacco plants along with CLCuMB and CLCuMA. Eighty-five percent of the inoculated tobacco plants showed severe symptoms when CLCuKoV were inoculated together with beta and alphasatillite. Inoculation with alphasatellite and helper virus did not produce severe symptoms. The Burewala isolate with intact C2 gene induced severe symptom in comparison to the C2 mutants. Whitefly transmission of the progeny virus to cotton was performed using agroinoculated tobacco plants as inoculum. The cotton plants showed curling of leaves with Burewala isolate and with one isolate of CLCuMuV. The cotton plants inoculated with Burewala isolate showed severe symptoms. Sequencing of the viruses from the whitefly inoculated cotton plants revealed that the C2 gene mutation is retained.

5.13.9 Resistance

The first outbreak cotton leaf curl disease epidemic in Sri Ganganagar in 1992 was found to be caused by Rajasthan strain of CLCuMuV (Radhakrishnan 2002). CLCuKoV were also identified in adjacent field of Haryana and Punjab. However until 2004, Rajasthan strains of CLCuMuV was predominant in north western region of India. By 2005–2006, Burewala strain emerged, by 2010 Burewala strain displaced CLCuMuV and only in isolated places Rajasthan strain was located. The emergence of Burewala strains became obvious as most of the resistant lines succumbed to the disease. The resistant varieties derived from the resistant source LRA5166, RST9, RS875, RS810, RS2013, S1861, LH2076, H117, H1126, LH14144, CSH98, CSH238 and CSHH243 were highly susceptible to Burewala strain (Monga et al. 2008). The susceptibility of cotton cultivars, coinciding with build-up of whitefly population in the last 2 years resulted in increase in the incidence of the disease.

In this context, understanding genetics of resistance to leaf curl disease and deploying durable resistance gene become important. Ali (1997) found that resistance to cotton leaf curl viruses is controlled by single dominant gene, which can be introgressed into elite cultivars. Some of the wild species of Gossypium like *G. thurberi, G. anomalum, G. raimondii, G. armourianum, G. tomentosum* posses resistance genes to pests like whitefly which may be exploited (Azhar et al. 2010).

5.13.10 Molecular Basis of Pathogenecity

Khan et al. (2015a) examined the bidirectional promoter present in the IR of Burewala strains of CLCuKoV by transient agroinfiltration and transgeneic assay. Rep promoter in transformed tobacco plants showed two to fourfold higher activity than CaMV 35S promoter. Higher fluorescent identity of GFP was demonstrated in both tobacco and cotton leaves agroinfiltrated with CLCuKoV Rep promoter compared with CaMV 35S promoter and CLCuKoV CP promoter. The Rep promoter consisted of many cis- acting regulatory elements and growth regulator responsive transcription factor binding sites. Shukla et al. (2013a, b), identified the promoter sequence of CLCuMuB and confirmed the suppressor activity of the beta C1 protein by reversal of GFP silencing assay in *N. benthaminana.*

The functional attributes of CP and V2 of CLCuKoV was analyzed by over expression of the CP and V2 protein in bacteria, Sf21 cells and in planta by transient assay. Priyadharsini et al. (2011) purified the V2 and CP protein and showed the interaction between two proteins in ELISA and by surface plasmon resonance confocal microscopy studies of SF21 cells revealed the localization of CP in the nucleus and V2 in the periphery, when NLS signal of CP is abolished, CP is distributed in cytoplasm. They made V2- GFP and CP-YFP construct, expressed in *N. benthamiana* leaves and confirmed the localization of V2 in periphery and CP in the nucleus. When both constructs are co-infiltrated CP was found in the nucleus and in cytoplasm along with V2. The interaction between V2 and CP thus, may facilitate the movement of viral genome.

The CLCuMuV and CLCuKoV are associated with one predominant betasatellite CLCuMuB. In Pakistan, the resistance breaking Burewala strain was found associated with a recombinant CLCuMuB. The recombinant CLCuMuB is different from original sequence of CLCuMuB in having approximately 100 nucleotide fragment of the SCR originating from a betasatellite associated with tomato leaf curl disease (Amrao et al. 2010a, b). Kumar et al. (2015) identified Burewala strain in north western India and the satellites CLCuMuB and CLCuMA. CLCuBaV was found associated with Kenaf leaf curl betasatilitte .

Radhakrishnan (2002) identified the association of CLCuMuB with Rajasthan strains of CLCuMuV in Sri Ganganagar, Hissar, Delhi samples. Rajagopalan et al. (2012) analysed the samples from Abohar, Bathinda, Fasilika region and found that there was only one betasatellite CLCuMuB associated with burewala strains of CLCuKoV and Rajasthan strain of CLCuMuV. They could not isolate any alphasatellies. About 69 CLCUD associated betasatellites have been characterized which all

belong to only one betasatellite species CLCuMuB. Zaffalon et al. (2012) detected CLCuMuB in many host like cotton, papaya, *Tribulus terrestris*; about seven alphasatellite were detected by Zaffalon et al. (2012) in leaf curl affected cotton samples. They belong to three different clades; cotton leaf curl Dabwali alphasatellite; cotton leaf curl Lucknow alphasatellite; gossypium Davidsoni symptomless alphasatellite (GDarSLCV) have been found associated with the disease. Their contribution to viral pathogenesis is not yet resolved.

5.14 Papaya Leaf Curl Virus

Papaya leaf curl virus (PaLCuV) has been recorded in minimum fifteen hosts belonging to diverse families of Apocyanaceae, Caricaceae, Malvaceae, Euphorbiaceae and Asteraceae (Table 5.3). It is also relevant to note here that, the type of symptoms PaLCuV produce ranges from yellow vein mosaic symptom to leaf crumpling depending on the host it infects. Other than PaLCuV, papaya is infected by AEV, ChiLCV, papaya crumple virus and ToLCNDV.

5.14.1 Discovery and Distribution

The leaf curl disease of papaya was first reported in Tamil Nadu by Thomas and Krishnaswamy (1939). On the basis of symptoms produced they called it as papaya leaf crumple disease. Subsequently leaf curl disease was observed in North India (Nariani 1956), eastern India (Sen et al. 1946). At present the disease is widespread in Haryana, UP (Singh 2006), Maharashtra, Andhra Pradesh, Karnataka (Govindu 1964) and Tamil Nadu (Surekha et al. 1977; Pandey and Marathe 1986; Verma 1996; Raj et al. 2008; Krishnareddy et al. 2010).

5.14.2 Symptomatology

The infected papaya plants show downward curling of leaves, vein thickening and extremely twisted petioles. The leaves appear leathery, deformed and plants are stunted. The affected plants do not bear any fruit, if any fruits are produced, they are small and distorted. Summanwar and Ram (1993) and Singh-Pant et al. (2012) observed upward rolling of lamina, enation mosaic and upward curling too. The plants irrespective of their age from 6 months to 3 years exhibited symptoms.

5.14.3 Transmission and Host Range

The causal agent of the disease is transmitted by grafting and by whitefly and not mechanically through sap. Though Sen et al. (1946) reported sap transmission of virus it was not confirmed by the other workers. Chandra and Samuel (1999)

reported that graft inoculated plants show symptoms 5–7 weeks post inoculation.

The vector transmission of the virus has not been proved convincingly until recent years. Srivastava et al. (1977) showed more than 80% mortality of the whitefly, when allowed to feed on healthy papaya plants. Whitefly vector was thought unable to feed on papaya continuously. However Raichaudari (1977) reported that the virus can infect tomato, tobacco, sunnhemp, petunia and *Zinnia*. Summanwar and Ram (1993) recorded additional hosts of the viruses as chilli, *Datura* and hollyhock. Dubey et al. (2015), reported whitefly transmission of the virus, wherein symptoms appeared 4 weeks post inoculation. They showed that a minimum AAP of 30 min and IAP of 10 min are required for the transmission. The best transmission efficiency was obtained with IAP and AAP of 48 h. Raj et al. (2008) described successful transmission of PaLCuV from infected papaya to healthy papaya.

Of 12 test species tested by Dubey et al. (2015), 9 species, *Carica papaya, Carica annum, S. lycopersicum, N.tabacum, Crotolaria juncea, Petunia hybrida, Ageratum conyzoides, Datura stramonium* and *Zinnia elegans* plants expressed symptoms. These symptomatic plants were found positive in ELISA tests performed with polyclonal antiserum to ToLCNDV. On the basis of PCR detection and molecular characterization, PaLCuV has been detected in several hosts such as cotton (Mansoor et al. 2003), tomato, chilli, aster, tobacco (Kumar et al. 2009, 2012a, b), amaranthus (Srivastava et al. 2014a, b, c, d), radish, calotropis, cluster bean, Acalypha, soybean, and croton. In all above hosts, PaLCuV has been cloned and sequenced but none has been shown to be transmitted to papaya through whitefly transmission.

5.14.4 Genome Comparison

The association of begomovirus with papaya leaf curl symptom was established by Saxena et al. (1998) by Southern hybridization tests using DNA A probe to TGMV and ToLCNDV. They amplified DNA A fragments using Roja's primer and characterised the virus, which was designated as PaLCuV. Subsequently, Krishnareddy et al. (2010) characterised PaLCuV isolates from Andhra Pradesh, Karnataka, Tamil Nadu and Uttar Pradesh. Singh-Panth et al. (2012) further extended the studies to Haryana and Delhi and characterised more isolates.

Saxena et al. (1998) characterised the PaLCuV from Lucknow and until 2010, it was thought PaLCuV is the only virus infecting papaya. However, Krishnareddy et al. (2010) characterised the leaf curl etiological agents from four different states and came out with interesting results. The isolate from UP, exhibited 94.7% identity with Lucknow isolate and so was designated as variant of PaLCuV. He identified the viruses in New Delhi and Andra Pradesh samples to harbour ToLCNDV and CYVMV, respectively.

5.14.5 Phylogenic Relationship

Krishnareddy et al. (2010), in their phylogenetic analysis performed on a multiple alignment of DNA A sequences found that Papaya leaf curl Coimbatore virus clustered with BYVMV and CLCuMuV-Rajasthan viruses, CroYVMV, ToLCNDV and PaLCuV grouped along with the isolates of respective species.

Phylogenetic analysis performed by Sing-Panth et al. (2012), revealed the position of ChiLCuV clustering with one ChiLCuV isolate infecting papaya in Punjab and another one infecting tomato; The Papaya leaf crumple virus was positioned close to TOLCNDV and ToLCV isolates from Pakistan. Singh-Panth (2012), detected high degree of recombination in seven DNA A sequences out of ten characterized by them. Recombination events were described in PaLCrV in AV1, AC3, AC2 region. Some of the events were between ToLCNDV and ChiLCuV. The AC4 ORF was highly variable and exhibited highest numbers of non synonymous mutations. Sinha et al. (2016) identified recombination events in PaLCuV in AV2, AV1 region and from AC1 to IR region between PaLCuV and BYVBhV and between CrYVMV and ChilCuV.

5.14.6 Establishment of Koch's Postulate

The complete tandem repeat constructs of DNA A when inoculated onto *N. benthamiana* plants, a mosaic pattern and slight curling were observed after 24 days (Sinha et al. 2016). Infectivity of papaya by agro- or biolistic-delivery has not been established consistently which needs to be focused. Singh-Panth et al. (2012) identified four different betasatellite species, tomato leaf curl betasatellite, papaya leaf curl betasatellite and CroYVMB. It is interesting to note here that these betasatellites are found associated with ToLCNDV and chilli leaf curl virus.

5.15 Begomoviruses Infecting Weeds

Characterization of begomoviruses infecting important crop plants had received attention owing to the economic importance of diseases caused by the viruses. Though the begomovirus causing yellow vein mosiac disease infecting the weed plants were known and recorded much earlier, only in the recent years, the diversity of begomoviruses occurring in weeds was looked into. The begomoviruses belong to the group of viruses referred as WILPAD viruses by Harrison (1981), as these viruses are more adapted to wild plants and are persistently transmitted by vectors contrary to CULPAD viruses which are more adapted to cultivated plant species like tobamoviruses. The begomoviruses in weed may represent the unchallenged genetic entity which need to be studied in detail. Some of the viruses characterized are discussed below.

In any discussion on ecological aspects on the begomoviruses, often it is interpreted that the weeds serve as reservoir of the viruses and serve as inoculum source. However perusal of the data show that only few viruses from weeds infect cultivated crop plants and *vice versa*, such as ICMV having cassava and Jatropha; PaLCuV infecting papaya, soybean and radish also occurring on weeds like, Calotropis and Acalypha. Pramesh et al. (2013) proved the infectivity of CroYVMV isolate through biolistic delivery of DNA A, along with betasatellite. Typical yellow vein symptoms were produced 15 days post inoculation in croton; the constructs also induced severe leaf curl in tomato plants. Ageratum enation virus infecting *Ageratum* also causes disease in tomato, soyabean, carrot. In all these cases, viruses have been characterised from the crop plants and weeds but whether transmission between the weeds and crop plants occur in nature has not been proved categorically. Therefore, the role of weeds as inoculum source, contributing to the primary spread of the virus continues to be an enigmatic question.

5.15.1 Jatropha Viruses

Jatropha species occur in wild conditions and is also cultivated as bio-diesel resource plant and as ornamental plant. In both wild and cultivated crops, severe leaf curl, yellow mottling, enation and yellow vein mosaic symptoms are observed. Incidence of such infection are more than 40% through out India even endangering commercial cultivation of the crop for biodiesel purpose. Snehi et al. (2016) had observed that there are more than five viruses causing these symptoms. As per the recent recommendation of study group on geminiviruses of ICTV 2015, four virus species are identified to infect Jatropha, they are ICMV (Aswathanarayana et al. 2007), Jatropha leaf curl, Jatropha mosaic India virus, Jatropha yellow mosaic virus. Recently two more new viruses is Jatropha leaf crumple virus and Jatropha leaf crumple India virus have been identified by Snehi et al. (2016). In phylogenetic analysis performed. Jatropha leaf crumple India virus, Jatropha mosaic India virus are closely related to ICMV. Jatropha leaf curl virus is closer to ChiLCuV, however JMINV is very distinct and separate.

The congress weed which grows abundantly in all agricultural fields *Parthenium hysterophorus* was shown to be infected by ToLCKaV. Kumar et al. (2016) cloned ToLCKaV and tomato leaf curl batasatellite, tomato leaf curl alphasatellite component from parthenium plants showing severe leaf curl symptoms through agroinfilteration, they confirmed Koch's postulates. Leaf curl symptoms were expressed in *Parthenium hysterophorus* and tomato plants.

5.15.1.1 Ageratum Enation Virus (AEV)

AEV was first characterized from Nepal, Fauquet et al. (1990) recognized it as separate species distinct from other begomovirus isolates characterized from *Ageratum conizoides* from southeast Asia. Subsequently, AEV has been characterized from *Ageratum* sp. and several other hosts as indicated in the Table 5.3. The satellites associated with the virus has been identified as Ageratum yellow

leaf curl betasatellite and Ageratum enation alphasatellite. Besides AEV, Ageratum leaf curl virus and PaLCuV have been found infecting *Ageratum*. Interestingly Ageratum yellow vein virus, which is predominant in southern Asia is not yet detected in India.

5.15.2 Discovery and Distribution

The yellow vein, enation and leafcurl disease affected weed *Ageratum conzoides* and ornamental Ageratum are distributed throughout India. Though there is not yet an official record of the disease, the disease symptoms are observed thoughout India and the virus has been characterized in the year 2008.

5.15.3 Symptomatology

The disease plants show typical yellow vein symptom, accompanied by leaf curl, enation, leaf marginal rolling and reduction in leaf lamina and stunting. In *Cleome gynandra*, upward curling, crinkling, swelling of the petioles were observed.

5.15.4 Transmission and Host Range

The virus is readily transmitted by whitefly with AAP of 24 h and IAP of 24 h. The inoculated plants developed symptoms 25–30 DPI. The virus has been successfully transmitted from *Ageratum conzoides* to *Amaranthus, N. benthamiana* (Srivastava et al. 2013). The host range of the virus has been essentially revealed by the characterization of full length genome of DNA A sequences from plants showing yellow vein/ enation/ leaf curl symptoms. Thus besides Ageratum and ornamental species of Ageratum, AEV has been detected in pointed gourd (Raj et al. 2011), from grain *Amaranthus* (Srivastava et al. 2013), *Cleome gynandra* (Raj et al. 2010a, b), carrot (Kumar et al. 2013), and Zinnia (Kumar et al. 2010a, 2011). Presence of AEV in other hosts for which genome information is available in the database are tomato, soybean, *Papaver somniferum*, fenugreek, *Crassocephalum crepidioides* and papaya.

5.15.5 Genome Comparison

The genome organization of AEV is typically like that of Old World begomoviruses with intergenic region upto 281 nt. All the AEV isolates from India have iteron sequences GTACT and IRD sequences identified as FQIY. Between the AEV isolates from India, there is 92–99% identity at DNA-A nucleotide level, 97% with AEV from Nepal, 95% with AEV from Pakistan. The begomoviruses with which

AEV shares closest relationship (88.9%) is Tobacco curly shoot virus (Raj et al. 2011; Srivastava et al. 2013; Kumar et al. 2013).

On the basis of 94% identity for demarkation of strains within species, Tahir et al. (2015) categorised the AEV isolates into two strains, one Indian strain and one Nepal strain which characteristically differed in IRD and iteron sequences. The Koch's postulates with cloned components have been established for *Amaranthus* isolate (Srivastava et al. 2013) and for *Ageratum* and carrot isolates (Kumar et al. 2013).

5.15.5.1 Kenaf Leaf Curl

A disease causing leaf curl symptoms on kenaf has been observed in different parts of India. In eastern India, the begomovirus complex is composed of MeYVMV and CLCuMB while in southern India, ToLCJV and its associated betasatellite constitute the complex. In northern India, a new species of begomovirus, kenaf leaf curl virus, and an isolate of CLCuMuV were found associated with the disease (Paul et al. 2008).

5.16 Begomoviruses Infecting Ornamentals, Medicinal and Aromatic Plants

Efforts have also been made to characterize the begomoviruses infecting ornamental plants like hollyhock, (Srivastava et al. 2014a, b, c, d), *Calendula* sp. (Khan et al. 2007), *Dimorphothea* sp (Raj et al. 2007), jasmine (Srivastava et al. 2014a, b, c, d), *Duranta* (Jaidi et al. 2015a, b) and rose (Sahu et al. 2014a) and senna. However, further infectivity studies and cross inoculation studies are required to know their importance in the context of epidemic outbreak of crop diseases. In medicinal and aromatic plants several begomovirus associated diseases have been identified in India (Saeed and Samad 2017). *Withania somnifera* (ashwagandha), *Catharanthus roseus*, *Salvia hispanica* (chia) are known to be affected with yellow mosaic; *Andrographis paniculata* (Kalmegh) with yellow vein mosaic disease and *Mentha* spp, *Ocimum* spp, *Papaver somniferum* (Opium) and *Rosa* spp with leaf curl disease (Table 5.8).

5.17 Betasatellites Associated with Indian Begomoviruses and Their Role in Disease Development

The study group on geminiviruses submitted proposal in the year 2016 to ICTV on nomenclature and taxonomy of satellite DNA associated with begomo and mastreviruses. Accordingly the beta and deltasatellites have been included in the family designated as *Tolecusatellitidae,* comprising two genera *Betasatellite* and *Deltasatellite*. The decision regarding alphasatellites to be included as one of the genera under the family *Tolecusatellitidae* is deffered as they share lot of features

Table 5.8 Begomovirus reported in medicinal and aromatic plants in India

Name of the crop affected	Name of the virus/species	Nature of genome	Satellite/s molecule	Symptoms	References
Withania somnifera	Jatropha mosaic India virus	Monopartite	–	Yellow mosaic	Baghel et al. (2010)
Momordica charantia	Tomato leaf curl New Delhi virus and Pepper leaf curl Bangladesh virus	Monopartite	–	Yellow mosaic	Tiwari et al. (2012)
Andrographis paniculata	Eclipta yellow vein virus and Catharanthus yellow mosaic virus	Monopartite	Betasatellite	Vein yellowing and clearing, leaf curling.	Khan and Samad (2014), Khan et al. (2015b)
Solanum nigrum	Solanum leaf curl Lakshmangarh virus	Monopartite	–	Leaf curling, yellowing and stunting	Prajapat et al. (2013)
Tagetus patula	Ageratum enation virus isolate	Monopartite	Betasatellite and Alphasatellite	Leaf curling, crinkling and stunting	Marwal et al. (2013)
Mentha spp.	Tomato leaf curl Pakistan virus, Tomato leaf curl Karnataka virus and Chilli leaf curl India virus	Monopartite	Betasatellite	Leaf yellowing, mosaic, and crinkling	Samad et al. (2008)
Papaver somniferum	Tomato leaf curl New Delhi virus	Monopartite	–	Leaf curling and stunting	Srivastava et al. (2016)
Rosa spp.	Rose leaf curl virus	Monopartite	Betasatellite	Dwarfing and leaf curling	Sahu et al. (2014a)
Ocimum spp.	Tomato leaf curl virus, Chili leaf curl virus and Tomato leaf curl Albatinah virus	Monopartite	Betasatellite	Leaf curling, crinkling and yellowing	Gaur (2012)
Mucuna prureins	Velvet bean severe mosaic virus	Bipartite	–	Mosaic and yellowing	Zaim et al. (2011)

with nanoviruses. The family derives its name from the first satellite identified with Tomato leaf curl virus (Dry et al. 1997) which is a deltasatellite. The betasatellites and deltasatellites are believed to have common ancestor and so are placed together in one family. So far almost 1110 sequences of of full length betasatellites are available and they are differentiated into different species on the basis of nucleotide identity, <91% identity is recognized as threshold value to demarcate different species for both beta and deltasatellite. The deltasatellites are different from defective betasatellites in having more deletions or insertions in the SCR regions. While defective betasatellites occur with full length betasatellites, deltasatellites are not present along with betasatellites. So far only one deltasatellite, CroYVMB has been recorded from India, from *croton bonplandianum* (Dwakar et al. unpublished).

In India, the association of betasatellite in the begomovirus disease complex was first reported in bhendi yellow vein mosaic disease. In the absence of cognate betasatellite, BYVMV is unable to produce typical disease symptom in the host plant (Jose and Usha 2003). Extensive studies from several laboratories from India have confirmed association of betasatellites with begomoviruses infected crop plants of diverse types such as, *Abelmoschus esculentus* (okra), *Capsicum annuum* (chilli), *Citrullus lantus* (watermelon), *Cucumis sativus* (cucumber), *Gossypium hirsutum* (cotton), *Lagenaria siceraria* (long melon), *Luffa cylindrica* (sponge gourd), *Mentha arvensis* (mint), *Momordica charantia* (bitter gourd), *Raphanus sativus* (radish), *Solanum lycopersicum* (tomato), *Solanum tuberosum* (potato), *Phaseolus vulgaris* (French bean), *Vigna mungo* (blackgram) and *Vigna radiata* (mung bean) etc. (Varma and Malathi 2003; Borah et al. 2010; Singh et al. 2012; Jyosthna et al. 2013b; Kamaal et al. 2013; Satya et al. 2013). Betasatellite association has also been found in other plants like *Althaea rosea* (Hollyhock), *Kakimaeris indica* (Aster), *Hibiscus cannabinus* (Mesta), *Amaranthus hybridus* (Amaranth) which may act as reservoirs of infection (Chatterjee et al. 2007; George et al. 2014; Srivastava et al. 2013, 2014a, b, c, d). Totally about 545 betasatellites have been described from India.

Betasatellites are predominantly associated with the majority of the monopartite begomoviruses with notable exception of bipartite begomoviruses such as MYMIV (Rouhibakhsh and Malathi 2005) and ToLCNDV (Sivalingam et al. 2010; Jyothsna et al. 2013a, b). A monopartite begomovirus, ToLCBaV could transreplicate cognate betasatellite tomato leaf curl Bangalore betasatellite (ToLCBB) as well as non-cognate betasatellites namely, cotton leaf curl Multan betasatellite (CLCuMB) and luffa leaf distortion betasatellite (LuLDB). However, enhanced accumulation of viral DNA was found only in cognate betasatellite combination, suggesting a specific interaction between DNA A and its associated betasatellite (Tiwari et al. 2012). ToLCNDV DNA A and DNA B along with CLCuMuB or LuLDB betasatellite inoculated plants show 16-fold higher accumulation of DNA B and 60% reduced accumulation of betasatellite. In the case of association of betasatellite with bipartite begomovirus, DNA B and betasatellite act antagonistically (Jyothsna et al. 2013a, b).

Betasatellites, in contrast to its known association with begomoviruses, the association of betasatellite with mastrevirus was reported for the first time from wheat samples in the field infected by WDIV (Kumar et al. 2014). This betasatellite was identified as Ageratum yellow leaf curl betasatellite (AYLCB) and presence of this betasatellite induces more severe symptom as well enhances the accumulation of WDIV DNA (Kumar et al. 2014).

Ability of βC1 protein to suppress RNA silencing has been demonstrated for betasatellites such as ToLCBB, CLCuMB, LuLDB. The level of GFP siRNA was reduced in plants transiently expressing TOLCBB-βC1and not in CLCuMB-βC1, LuLDB-βC1 suggesting that βC1 encoded by different betasatellite interfere at different step of RNA silencing pathway (Shukla et al. 2013a, b). The interaction of βC1 with coat protein plays a collaborative role in inter- and intra-cellular dynamics of BYVMD complex (Kumar et al. 2006). BYVMV β-satellite based VIGS vector construction become possible by replacing the βC1 ORF with multiple cloning sites. The endogenous genes Su, PDS, PCNA and AGO1 could be successfully silenced using BYVMV β-satellite based VIGS vector (Jeyabharathy et al. 2015).

Radish, another important vegetable crop in India suffers from radish leaf curl disease (RaLCD) which has been found to be associated with radish leaf curl betasatellite. In subsequent study, this betasatellite was also found to be capable of substituting cognate DNA B's of ToLCNDV (Singh et al. 2012). The first evidences of chloroplast targeting by DNA virus encoded protein has been shown with the βC1 protein encoded by radish leaf curl betasatellite in *Nicotiana benthamiana*. The impediments caused by betasatellite at the different stages of chloroplast function causes photosynthetic inhibition and develops vein clearing symptom. In addition, the βC1 protein downregulates the expression of the genes involved in chlorophyll biosynthesis and chloroplast development (Bhattacharyya et al. 2015).

In 2009, Kumari et al. reported of a new tomato infecting virus from Patna in Northern India namely tomato leaf curl Patna Virus (ToLCPaV) and associated novel tomato leaf curl Patna betasatellite (ToLCPaB) which cause severe disease in tomato plants in Indo-Gangetic plain. Identification of ToLCV and associated tomato leaf curl Ranchi betsatellite further expands the list of tomato infecting begomoviruses and associated betasatellites in India (Kumari et al. 2011). Chilli leaf curl virus, causative agent of chilli leaf curl disease (ChLCD), has been shown to be associated with a betasatellite which is important to induce disease symptom in chilli (Chattopadhyay et al. 2008).

With increasing volume of systemic study involving begomovirus mediated disease complex, the importance of betasatellites in pathogenesis appears to be greater than ever. In India *Ipomoea purpurea* was a hitherto unknown natural host for sweet potato leaf curl virus (SPLCV). However, association of SPLCV with two different betasatellites i.e. croton yellow vein mosaic betasatellite (CroYVMB) and papaya leaf curl betasatellite (PaLCB) enables SPLCV to infect *Ipomoea purpurea* (Swapna Geetanjali et al. 2013). Similarly, a betasatellite associated with yellow vein mosaic disease of croton could infect radish and expand host range (Singh et al. 2012). Incidences of weed-infecting betasatellites infecting crop plants pose a serious threat for agro-economy in India as well as in world.

5.18 Alphasatellites Associated with Indian Begomoviruses and Their Role in Disease Development

The begomovirus/betasatellite complexes are often associated with a second type of circular ssDNA satellite, initially referred to as DNA-1 (Mansoor et al. 1999; Saunders and Stanley 1999; Briddon et al. 2004; Vinoth-Kumar et al. 2017b) and presently designated as alphasatellites. Alphasatellites components are about half the size (1350 nt) of begomovirus component and they have a highly conserved structure (Mubin et al. 2009). The alphasatellite Rep exhibits high levels of sequence identity to the Reps encoded by components of nanoviruses (Saunders et al. 2000; Saunders and Stanley 1999), a rolling-circle replication initiator protein encoded by viruses in the genus *Nanovirus*, family *Nanoviridae* that also have a genome of circular ssDNA (Gronenborn 2004). Consequently, alphasatellites are capable of autonomous replication, but require a helper begomovirus for spread in plants and for whitefly vector transmission. In addition to Rep, alphasatellites also have an A-rich region, ~200 nt long, down stream of the Rep-encoding region. In contrast to betasatellites, alphasatellites possess in their stem loop the nonanucleotide sequence, TAGTATTAC also found in the stem loop of viruses in the family *Nanoviridae*. Alphasatellites can affect both begomovirus titer and symptom development in host plants. Initially it was thought that the satellite molecules were limited to the OW, but recently, alphasatellites have been found associated with NW begomoviruses (Paprotka et al. 2010; Romay et al. 2010), thus expanding the geographical distribution of satellite molecules associated with begomoviruses. About 146 alphasatellites (Table-) belonging to 30 species have been described from India.

These molecules are not important in disease process and are not required for either infectivity or disease induction in host plants (Briddon et al. 2004). However these molecules reduce the accumulation of betasatellite DNA in plants and show little effect on accumulation of DNA A. Therefore they could have little minor modulating influence on the disease.

5.19 Concluding Remarks

In India, begomoviruses are distributed in all the agroclimatic zones from temperate sub-Himalayan foot hills to typical hot humid tropical climate zone. When the distribution of the virus (Table 5.3) is viewed, the maximum numbers of begomoviruses have been characterized from Uttar Pradesh (33) followed by Rajasthan (19) and Tamil Nadu (16). From this observation it is evident that some of the regions like north-eastern region, Gujarat and Odisha are not yet studied extensively. It is interesting to note that, there has been a gradual increase in the number of viruses described from 1990 to 2006; there is a spurt in the number of viruses recorded from 2006 to 2016, which may be due to discovery of RCA technique. The host plants from which viruses have been isolated, span from crop plants in 1990–2000 to weeds in 2005–2010.

Although as many as 80 begomviruses species have been indentified in India, the pan-Indian distribution is recorded only for BYVMV, ChiLCV and ToLCNDV. The wide distribution of ToLCNDV in all the states in southern India is in contrast to earlier understanding that ToLCNDV, a bipartite begomovirus is present only in northern India. However, it is to be noted that even now ToLCNDV in southern India is recorded only in cucurbitaceous hosts. MYMV and MYMIV occur both in northern and southern India; again the distribution pattern understood earlier has been negated. The most unexpected finding is that the leguminous host plants are infected by as many as 17 begomoviruses (Tables 5.3, 5.4, 5.6). Some of the viruses have been recorded only in the last 5 years. Whether, the viruses have moved to leguminous hosts in recent years or they have existed earlier but recorded now are not clear. The symptoms in these plants are not yellow mosaic but mosaic and leaf distortion. It is interesting that many begomoviruses are identified in soybean, cluster bean, French bean and *Phaseolus aureus*. Surprisingly, blackgram and mungbean are so far known to be affected only by MYMV and MYMIV. The vulnerability of leguminous plants especially soybean and French bean as revealed by infection by a large number of viruses is important in the context of possibility of recombination and emergence of new begomoviruses under the condition of mixed infection. Whether, these viruses will acquire ability to jump to blackgram, mungbean, pigeonpea and mothbean will have to be watched.

Solanaceous crops, tomato and chilli are infected by related viruses, those viruses which share more than 75% nucleotide identity. They are also infected by distantly related begomoviruses, RaLCuV, PaLCuv and AEV, which share less than 70% identity. The begomovirus, which infects a large number of hosts are PaLCuV (16 plant species), ToLCNDV (15 plant species) and AEV (14 plant species) (Fig. 5.7). Tobacco leaf curl is one of the earliest recorded diseases in India. The virus, tobacco leaf curl Pusa virus from Pusa, Bihar, may represent an isolate of the oldest virus reported. It is disheartening to see that though the disease is widespread in tobacco in different states, only one isolate has been characterized. Interestingly, tobacco curly shoot virus has been recorded from tomato, wild sunflower and French bean and not from tobacco. Some viruses like TbCSV, ToLCJV, ALYVV, ALCuV, HoLCV, MaYMV, SiLCuV have been reported earlier in other countries like China, Thailand and Pakistan. Whether, these viruses would have existed here in India or they gained entry in recent times needs to be investigated.

Phylogenetic relationship among Indian begomoviruses was inferred by analyzing the representative sequences of 80 virus species including the new species referred in the Table 5.2. The complete nucleotide sequences of DNA A component was analysed in CLUSTAL W programme in MEGA 6. It is evident, that Indian begomoviruses have polyphyletic origin and evolutionary pathway. The clustering of viruses is not dependent on geographical location; to a very limited extent grouping is governed by host species they infect. The begomoviruses analyzed fall into 14 major clusters; viruses clustered in IX and cluster XIII are of west Asian/African and American origin. The Indian begomoviruses could be grouped into 12 clusters, the most distinct one which do not share any common origin belong to cluster I comprising SLCMV, ICMV, JMINV, JLCrIV, cluster II, JYMV, cluster X- sweet

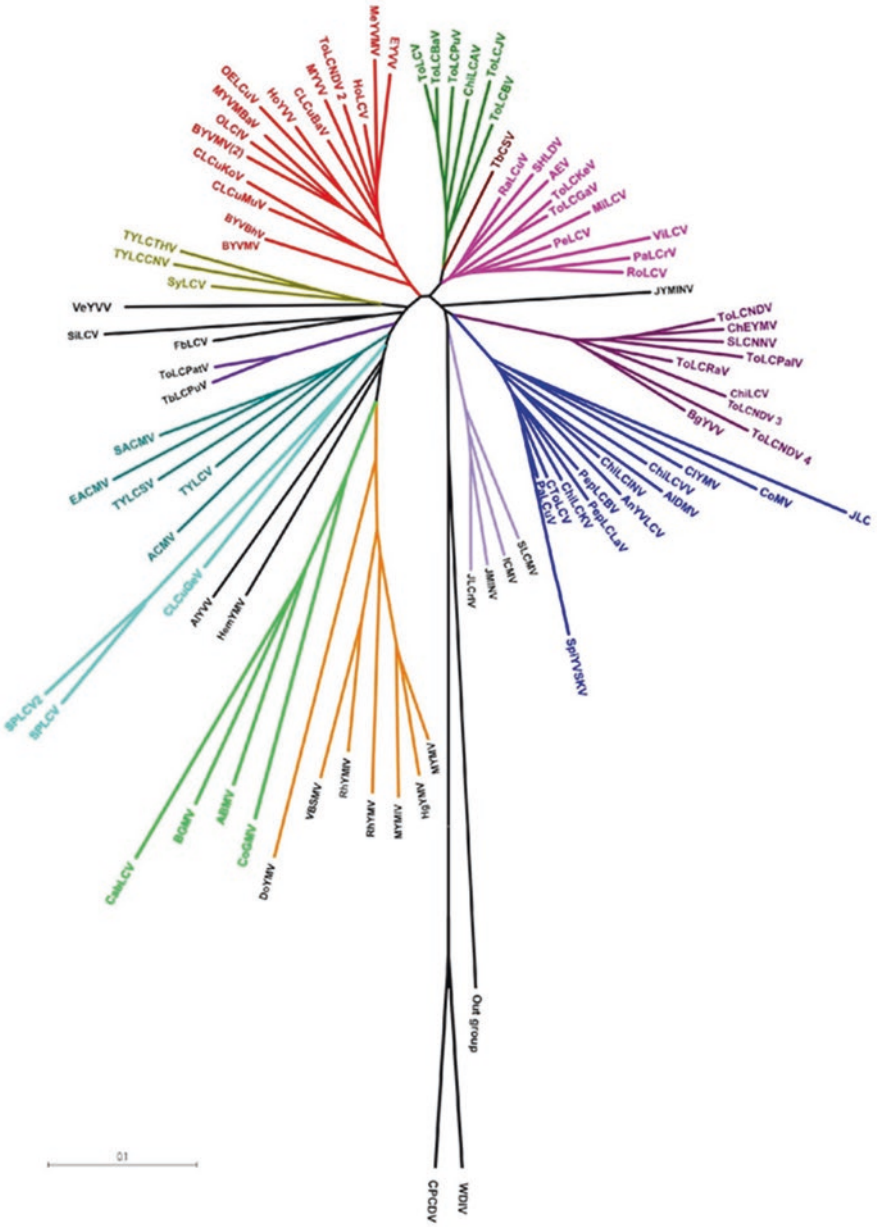

Fig. 5.7 Phylogenetic tree constructed on the basis of DNA A nucleotide sequence of Indian begomoviruses with other selected begomovirus sequences from NCBI database. The phylogenetic analyses were performed with MEGA 7.0 using neighbour joining method with 1000 bootstrap replications. Acronyms of viruses are as given in Table 5.1

potato leaf curl virus, cluster XIV- legume yellow mosaic virus. Of these, well separation of sweepoviruses and legomoviruses has been suggested by several workers (Qazi et al. 2007). The distinct nature of Jatropha viruses is an interesting finding, which further needs to be analyzed in detail as to which component of virus is divergent. Viruses in cluster II separate into two branches, one branch essentially having chilli viruses and second major branch having begomoviruses infecting Cucurbitaceae, ToLCNDV isolates and SLCCNV. It is relevant to note here that AEV the virus which infects cucurbits frequently is separated from cucurbitaceous cluster and belong to cluster IV. Of the four ToLCNDV isolates considered for analysis, ToLCNDV isolate infecting bhendi is grouped in cluster along with begomoviruses infecting Malvaceous and Teliaceous hosts (cotton, bhendi and mesta). Cluster IV contains array of leaf curl viruses in which, one branch is occupied by Solanaceous virus, other branch by AEV, RaLCuV, SHLDV and TbCSV. Positioning of some of the weed viruses are interesting. Synedrella leaf curl virus, which has been recorded from Andaman island, is grouped along with southeast Asian viruses (TYLCTHV, TYLCCNV). Vernonia yellowing virus is in the same cluster, but branches off independently. The weed infecting viruses seems to have independent origin and are well separated from crop infecting viruses.

International trade in agriculture, claimate change, resurgence of vector whitefly, deployment of new crop cultivars and intensive cultivations of crops throughout the seasons are important factors that aggravate begomoviral disease problems in the Indian subcontinent. The epidemic outbreak monitoring system is necessary to detect entry of an important begomovirus into new area or occurrence in new crop species, eg; whether ACMV occurs in cotton in India as in Pakistan, how extensive is ToLCNDV infection in cotton are some important questions which need to be answered. In this context, the cotton begomovirus movement from North-West India to South India needs to be monitored to avoid emergence of epidemic in a new production area.

Some of the begomoviruses like AEV occurring in weeds infect crop plants. Whether, the weed viruses contribute to crop disease development needs to be studied. The virus infecting ornamentals, especially *Hibiscus* sp., facilitate transboundary movement of begomovirus through cuttings, like cotton leaf curl viruses detected in *Hibiscus* in China. In the mixed infection situation, the transmission of individual virus by the vector, and its role either in eliminating the virus or making it more highly prevalent need to be studied. The genetics of resistance of begomoviruses is not addressed well, at present, there is ambiguity even in level of resistance as inferences have been drawn based on field infections. In most of the cases like bhendi there is no source of resistance available. Even, in some cases where resistance is observed, (e.g., *G. arboreum* to CLCuVs), the mechanism or the gene for gene interaction is not yet understood. The management involving exogenous application of dsRNA/siRNA will have to be strenghtened.

The classification of begomoviruses has become more challenging. The begomoviruses have been differentiated on the basis of 91% sequence identity in the DNA-A component. The current sequence based taxonomic method has resulted in a

complex situation in begomovirus nomenclature and classification. The rapid changes in the constitution of begomovirus genome sequences are expected to generate more confusion and challenges in the taxonomy of begomovirus in future. An alternative robust system needs to be debated and evolved based on the basis of their infectivity on diffential hosts and their genetics of resistance in addition to the sequence identity.

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