**REVIEW ARTICLE** 



# Emerging threats of begomoviruses to the cultivation of medicinal and aromatic crops and their management strategies

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Abstract Begomoviruses (family *Geminiviridae*) are responsible for extreme yield reduction in a number of economically important crops including medicinal and aromatic plants (MAPs). Emergence of new variants of viruses due to recombination and mutations in the genomes, modern cropping systems, introduction of susceptible plant varieties, global trade in agricultural products, and changes in climatic conditions are responsible for aggravating the begomovirus problems during the last two decades. This review summaries the current research work on begomoviruses affecting MAPs and provides various traditional and advanced strategies for the management of begomoviruses and vector in MAPs.

**Keywords** *Bemisia tabaci* · Begomovirus · RNAi · Alphasatellite · Betasatellite · Medicinal and aromatic plants

# Introduction

Research and market on medicinal and aromatic plants (MAPs) emerges globally as one of the fast growing areas in the field of pharmaceutical and allied disciplines. In fact between 50,000 and 70,000 species of plants are recognized internationally for their medicinal and aromatic properties, and out of them 4000–10,000 species are endangered [30, 142]. More than 4,00,000 tons of MAPs of approximately 3000 species are traded internationally.

Abdul Samad samad\_cimap@yahoo.co.in China and India are the largest producers and exporters of MAPs [142]. Natural products from MAPs have lesser harmful effects on human systems rather than their synthetic analogues. An annual growth of 10–20%, the global market on MAPs is expected to touch \$5 trillion by 2050 [38]. With an increasing usage of herbal preparations internationally, the scientific fraternity is exploring new methods to meet out the growing demand without exhausting the existing resources, earning foreign exchange, generating employment opportunities and income, improving food security, alleviating poverty, and enhancing development in the region.

Principally agriculture practices in India are seasonal. However, with the release of more and more hybrid varieties, crop plants are cultivated throughout the year. A large number of crop plants are cultivated in India and some of them are unique in their own way. Many have medicinal properties also [95]. There are a large number of threats to the agricultural crops grown not in India but even the worldwide [13]. Quite large number of pest harms the crops and hinders the production yield [69]. Apart from these there are also other microscopic organisms that cause a great annual loss to the agriculture sector, which in turn harms the livelihood of the farmers and Indian economy. One of the microscopic organisms is viruses [34]. Several viruses affect the crop plants, but the major contribution is from the begomovirus.

The biomass and yield of these MAPs are vigorously reduced by devastating diseases caused by DNA viruses i.e. begomovirus. Begomovirus is the largest plant virus genus of family Geminiviridae that infect a broad variety of plants, monocots and dicots as well and causes significant loss. The yield loss contributed by Geminiviruses was estimated to be 18–100% depending on the host, environment and virus (es) strains [37]. During 1950s,

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begomoviruses have been reported to cause important losses in vegetables ranging from 20 to 100% [6, 28].

The family Geminiviridae includes seven genera Mastrevirus, Begomovirus, Topocuvirus, Curtovirus, Becurtovirus, Eragrovirus and Turncurtovirus. These genera have been established according to genome organization, transreplication of genomic component, vector range, host range and coat protein characteristics [44, 154]. Begomoviruses are divided into Old and New world where monopartite begomoviruses are mostly old world (OW) with few bipartite viruses while new world (NW) begomoviruses are all bipartites. Old world are more diverse in comparison to new world as AV2 gene is present in OW but absent in NW begomoviruses [123]. Genome of begomovirus is ss-circular DNA, either bipartite (DNA-A and DNA-B) or monopartite (DNA-A). In monopartite begomovirus all genes are present on the DNA-A molecule itself for replication while in bipartite begomoviruses Rep and CP gene are only present on DNA-A molecule and gene for movement MP and NSP are present on DNA-B [79, 93]. The monopartite genome often assisted by satellite molecules betasatellite or alphasatellite. These satellite molecules are of the half size of genome not related with the pathogenecity of the virus but depend on the helper virus [20] to enhance symptom/severity of the disease [2] and in some cases alphasatellites were reported to mask the symptoms [66]. The DNA-A segment encodes two genes V1 and V2 on the viral sense as coat protein (CP, 29.7 kDa) and movement protein as pre-coat protein (precoat ORF, 12.8 kDa). Some studies revealed that V2 or pre coat protein function as anti-defence protein to inhibit post transcriptional gene silencing (PTGS) [119]. On the other hand four genes; C1, C2, C3 and C4 are encoded as complimentary sense replication associated protein (Rep, 40.2 kDa), replication enhancer protein (REn, 15.6 kDa) and transcription activator protein (TrAP, 19.6 kDa) participates in the control of gene expression and replication. The C4 gene determines symptom expression (12.0 kDa). There is another protein encoded i.e. AC5 protein whose function is obscure but according to Li et al. [87], it plays a major role in post transcriptional silencing as a pathogencity determinant and RNA silencing suppressor in bipartite begomovirus. DNA-B genome encodes two genes one in viral sense nuclear shuttle protein (NSP) (BV1, 33.1 kDa) and another in complimentary sense movement protein (BC1, 29.6 kDa) responsible for the movement of virus. In monopartite begomoviruses, these BV1 and BC1 are not present and the function of NSP is played by CP (V1) revealed from studies done on Tomato yellow leaf curl virus (TYLCV) and Tomato leaf curl virus (TLCV) [111, 144]. Intergenic region in begomovirus contains hairpin/stem loop structure having a nanonucleotide sequence "TAATATTAC" for replication. A 200 bp region common to both DNA-A and DNA-B known as common region (CR) and is highly specific between DNA-A and DNA-B of same virus.

There is a form of rolling circle mechanism i.e. recombination-dependent replication is responsible for generating diversity in the viral genome [118]. Recombination is an evolutionary process that effects greatly on genomic evolution of geminiviruses [21, 47, 49, 105, 109, 134]. Apart from recombination, pseudo-recombination is also responsible for the genetic diversity in the genus begomovirus. Both the phenomenon are responsible for the diversification in begomovirus between variants of the same virus, between different species and between different genus leading to the ability to adapt in different environment and forming new variants in new hosts. Mutations and acquiring new components like satellite molecules in the viral genome resulted in the emergence of new viruses [23, 124, 135]. One of a key recombinant event in ToMoV DNA-A CR with that of BDMV DNA-B component has resulted in the emergence of new virulent fitter begomovirus. TPCTV has evolved due to intra-genera recombination [21]. Diversification has also resulted in satellite molecules i.e. betasatellite contributing more virulence and diverse hosts appearance [48]. These form of viruses have now becoming more powerful and overcoming the host resistance and leading to epidemic. The intergenic region is the hotspot for genetic recombination [146]. In begomoviruses, recombination breakpoints are conserved with hot spots in the Rep N-terminal portion and in the 5'-end of the intergenic region [82, 91, 117]. The begomoviruses are more diverse inhabiting new hosts and the reason is the recombination events. In Tomato leaf curl virus, different viral strains have emerged due to recombination [76]. Similarly, Cotton leaf curl Burewala virus, a recombinant CLCuMuV and CLCuKoV was evolved to break host plant resistance and develop infection process [9]. The regions/parts of recombination that are exchanged in begomoviruses can be identified using RDP [91], GENECONV [105], MAXIMUM CHI<sup>2</sup> [139], CHIMAERA [92], recscan [90], and SISTER SCAN [51] methods as implemented in rdp2 [92].

By the mid 1980's it was established that the whiteflies had become a serious pest and wide variety of crops including cotton, cucurbits, lettuce, pepper and tomato were adversely affected by the diseases caused by begomoviruses [46]. In recent years begomoviruses have been more interestingly researched as the diseases caused have reached epidemic status and poses a severe threat to global food security. Geminiviruses have been so long significant plant pathogens in Africa, Australia, Southeast Asia, Latin America and the Caribbean, Southern Europe and southwest United States with heavy loss in world economy and food security enormously [25, 26, 36, 43, 58, 89, 110, 111, 126, 148, 152, 153]. Latin America has been reported most affected region by begomovirus, with 5 million hectares of agricultural land in more than 20 countries being affected by more than 30 distinct begomoviruses [24, 25, 61, 89, 110, 148]. In India, yield loss was estimated to be US\$ 300 million due to begomoviral disease "vellow mosaic disease" affecting mungbean, blackgram and soybean [153]. "Tomato leaf curl and tomato yellow leaf curl" disease has caused tomato and other crop wipe out and 90% of fruit loss with spread to more than 20 countries [36, 130]. Another most destructive disease i.e., cotton leaf curl disease (CLCuD) of cotton crops in Pakistan and India [101]. Economic losses due to geminivirus infections are estimated to be US \$1300-2300 million in Africa [145], US \$5 billion in Pakistan between 1992 and 1997 [22], US \$300 million in India [153] and US \$140 million in Florida [98]. Despite intensive efforts to contain geminiviruses and their vectors, the menace caused by newly emerging or reemerging geminiviruses are becoming frequent and posing potential threats even in new regions with fresh crops, previously free from such diseases. Wider host range of vector and the genetic mutations/recombination in begomoviruses is responsible for threat to other crops. Viral infection profoundly affects the plant growth and development where virus interacts with the host defense mechanism to reduce crop yield and unmarketable fruits. The modern cropping practices has made these viruses more widespread [83, 131] and increased transmission efficiency and disease spread to new hosts. There were some of the known geminiviral diseases reported in the end of ninteenth century and early twentieth century: Cassava mosaic in Africa (1894), maize streak in South Africa (1901), curly top of sugar beet and other crops in United States of America and Mediterranean (1900), tobacco leaf curl in Indonesia (1912) and cotton leaf curl disease (1931) in Angola and Egyptian Sudan.

# Description of begomovirus infecting MAPs

Begomoviruses have been known to infect a wide range of crops including agriculture crops, vegetables, medicinal crops, ornamental plants, weeds etc. with a huge economic loss affecting common people. The rapid evolutionary potential of these ss-DNA viruses combined with the polyphagous feeding behavior of whiteflies (*B. tabaci*) can lead to the emergence of devastating viral strains. Hence, begomovirus drew more attention globally by researchers to establish host-pathogen interaction, vector-host relationship, characterisation of virus, virus reservoirs, phylogenetic relationships, role of the viral genes and management strategies. This review presents a current status of the research work on begomoviruses particularly infecting MAPs (Table 1) and their possible management strategies:

Andrographis paniculata Known as Kalmegh (King of Bitters), a medicinal herb known for its pharmaceutical properties anti-inflammatory, antibiotic, antimalarial, anticancerous, antidiabetic, antihepatotoxic, antioxidant, carminative, anti pyretic etc. Since last several years Kalmegh fields in Lucknow (2010-2014) and nearby areas showed typical symptoms yellow veins on younger leaves and later upward leaf curling, vein clearing, chlorosis, reduced leaf size, poor inflorescence, and stunted growth leading to drastic reduction in herb yield (Fig. 1a). The disease incidence was estimated to be 25-40%. Total nucleic acids were extracted from the leaves of symptomatic and asymptomatic plants and PCR amplified amplicon of  $\sim$ 771 bp fragment of DNA, with begomovirus CP gene-specific primers and subsequently full length genome was amplified using overlapping primers F1For/F1Rev and F2For/F2Rev [72]. On sequencing it showed maximum identity of 96% with Eclipta yellow vein virus (EYVV, GO478343). A betasatellite was also identified and showed 83-89% identity with sequences of other betasatellites, like Ageratum yellow vein betasatellite (AJ542498) hence named as Andrographis yellow vein leaf curl betasatellite. No DNA-B and alphasatellite was detected in any of the samples. Catharanthus yellow mosaic virus along with Andrographis yellow vein leaf curl betasatellite (KC967282) was identified from Barabanki district, Uttar Pradesh in Kalmegh [71]. The disease incidence was calculated to be about 15-20%.

*Capsicum annum* Chilli or pepper plant is known due to its active ingredient Capsacin. Peppers are an excellent source of calcium and vitamin C. While studies showed its anti-proliferative effect against cancer cells and used as a pain reliever in arthritis, in the treatment of peptic ulcers, sinusitis and bronchitis etc. Chilli leaf curl disease was reported earlier [40, 97]. A monopartite Chilli leaf curl virus (ChiLCV) was identified from Pakistan [73, 137]. Later, in 2008 complete sequence of ChiLCV-PK [PK:Mul:98] with 95% sequence identity was established [33]. In 2011, Palampur, Himachal Pradesh, a new monopartite begomovirus was found associated with betasatellite molecule from infected chilli plant that showed upward leaf curling symptom (Fig. 1b). It was characterized as Chilli leaf curl Palampur virus along with Chilli leaf curl betasatellite from Pakistan. The molecular infectivity of this virus has been established and hence proved the Koch's postulates in Chilli plants and tobacco plants. Chilli leaf curl Vellanad virus along with two betasatellites Radish leaf curl betasatellite (EF175734) and Tomato leaf curl Bangladesh betasatellite (AY438558) was reported from Velland, Kerala with 100% disease incidence [80]. A Chilli leaf curl virus (ChiLCV) JN555601 from Srilanka along with a betasatellite, Chilli leaf curl

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. [14]
					Baghel et al. [15]
Momordica charantia	Tomato leaf curl New Delhi virus and Pepper leaf curl Bangladesh virus	Monopartite	-	Yellow mosaic	Tiwari et al. [143]
					Raj et al. [120]
Andrographis paniculata	Eclipta yellow vein virus and Catharanthus yellow mosaic virus	Monopartite	Betasatellite	Vein yellowing and clearing, leaf curling.	Khan et al. [72] Khan et al. [71]
Solanum nigrum	Solanum leaf curl Lakshmangarh virus	Monopartite	-	Leaf curling, yellowing and stunting	Prajapat et al. [115]
Tagetus patula	Ageratum enation virus isolate	Monopartite	Betasatellite and Alphasatellite	Leaf curling, crinkling and stunting	Marwal et al. [94]
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. [132]
					Borah et al. [19]
					Saeed et al. [127]
Papaver somniferum	Tomato leaf curl New Delhi virus	Monopartite	-	Leaf curling and stunting	Srivastava et al [140]
Capsicum annum	Chilli leaf curl virus	Monopartite	Betasatellite	Leaf curling	Chattopadhyay et al. [33]
					Kumar et al. [80]
Catharanthus roseus	Catharanthus yellow mosaic virus and Papaya leaf crumple virus	Monopartite	-	Mosaic patches and curling	Ilyas et al. [67]
Capsicum frutescens grossum	Tomato yellow leaf curl virus	_	-	Leaf curling, leaf chlorosis and stunting	Khan et al. [70]
<i>Rosa</i> spp.	Rose leaf curl virus	Monopartite	Betasatellite	Dwarfing and leaf curling	Khatri et al. [74]
					Sahu et al. [128]
Ocimum spp.	Tomato leaf curl virus, Chili leaf curl virus and Tomato leaf curl Albatinah virus	Monopartite	Betasatellite	Leaf curling, crinkling and yellowing	Gaur [50] Ammara et al. [7, 8]
Mucuna prureins	Velvet bean severe mosaic virus	Bipartite	-	Mosaic and yellowing	Zaim et al. [160]
Clitoria ternatea	Rhynchosia yellow vein mosaic virus	Monopartite	Betasatellite	Yellowing, leaf curling	Unpublished data
Euphorbia heterophylla	Euphorbia mosaic Venezuela virus	Bipartite	-	Yellow mosaic and leaf curling	Zambrano et al [159]
Salvia hispanica	Sida mosaic Bolivia virus and Tomato yellow spot virus	Bipartite	-	Mosaic and yellowing	Celli et al. [31]
Sida cordifolia	Sida leaf curl virus	Monopartite	Betasatellite	Mild upward leaf- curling	Guo and Xhao [56]

Table 1 Begomovirus infection reported in medicinal and aromatic plants



Fig. 1 Begomovirus infected MAPs 1 a A. paniculata, b C. annum

betasatellite (ChLCB) JN638446 was found to be associated with 60–100% disease incidence [133].

*Capsicum frutescens grossum* It is commonly known as sweet pepper, and is enriched with beta carotenoids, vitamins A, C, E, P, B1, B2 and B3, proteins, fats, steroidal alkaloidal glycosides and scopoletin. These constituents help in treating arthritis, sore back muscles, rheumatism or sprains, improve digestion, colds and fevers, diabetes and regulate cholesterol level. In northern coastal region of Oman, every year during October-March, a new and unrecorded whitefly transmitted viral disease on cultivated sweet pepper (Capsicum frutescens grossum) and other vegetables as well [70]. Infected sweet pepper plants showed typical begomovirus symptoms as upward leaf curling, interveinal leaf chlorosis, and growth stunting. Begomovirus infecting sweet pepper in Oman was detected by polymerase chain reaction (PCR) using begomovirus specific degenerate primers (PAL1v1978/PAR1c496 and AV494/AC1048). The sequence analysis of sweet pepper virus from Oman showed 92.2, 96.5, 94.0, 93.8, and 96.5% with TomGV-Lebanon, TYLCV-Guadeloupe, TYLCV-Israel, TYLCV-Kuwait, and TYLCV-Mexico, respectively. Results revealed the causal virus associated with sweet pepper is TYLCV. Phylogenetic analysis discovered that sweet pepper Tomato yellow leaf curl virus clustered with its closest relatives from Middle East regions but formed a separate strain.

*Catharanthus roseus* It is also known as Madagascar periwinkle and famous for its medicinally important anticancerous, anti-diabetic and anti-viral property exhibiting alkaloids apart from its ornamental importance. The periwinkle plants exhibiting typical symptoms of irregular yellow mosaic symptom with severe curling and distortion were collected from University of the Punjab, Lahore, Pakistan. Genomic DNA was isolated and subjected to rolling circle amplification (RCA) and restricted with different enzymes and with enzyme XhoI, a band of 2.8 Kb was obtained. Two clones are obtained and sequenced (KN4 and KN6) [67]. Two clones are obtained and sequenced (KN4 and KN6) [67]. BLAST analysis showed that the sequence of clone KN4 has less than 89% identity to an unpublished virus, Chilli leaf curl India virus (ChiLCIV-[IN:08] FM877858) and the sequence of clone KN6 has 95 and 99% identity to a new reported virus Papaya leaf crumple virus (PaLCrV) HM140369. KN4 represents an isolate of a newly identified species in the genus Begomovirus, and thus named as "Catharanthus yellow mosaic virus" (CYMV) [67]. No DNA-B and betasatellite presence was found. Sequence analysis shows that KN4 and KN6 are recombinants of Pedilanthus leaf curl virus (PedLCV) and Croton yellow vein mosaic virus (CrYVMV) using Recombination Detection Program 3 [89]. Hence, recombination plays a major role in emergence of new virulent strains.

Clitoria ternatea It belongs to the family Fabaceae, commonly known as butterfly pea having high economical and pharmacological importance for secondary metabolites including steroids, glycosides, anthocynins, flavonol and triterpenoids. The extracts from plant species possess a wide range of pharmacological activities including antimicrobial, antipyretic, anti-inflammatory, analgesic, diuretic, local anesthetic, anti diabetic, insecticidal, blood platelet aggregation-inhibiting and for use as a vascular smooth muscle relaxing properties [99]. Recently, several plants ( $\sim 30\%$ ) have been found to exhibit the symptoms of yellowing, mosaic, distortion of leaf, and slight upward curling of leaf in the fields of CSIR-CIMAP, Lucknow during the month of January 2016. Full length genome was amplified using overlapping primers F1For/F1Rev and F2For/F2Rev [72]. On sequencing it showed maximum identity of 99% with Rhynchosia yellow vein mosaic virus (RYMV, JX258325). A betasatellite was also identified and showed 97% identity with croton yellow vein mosaic betasatellite (GQ183865). No DNA-B and alphasatellite was detected in any of the samples. Hence, the association of a monopartite begomovirus has been established. (Unpublished data).

*Euphorbia heterophylla* It is originally native to South and Central America, now widely naturalized most of the tropics and subtropics. This plant is widely used in traditional African medicine to treat stomach-ache, constipation, fungal diseases and gonorrhea [http://proseanet.org]. In 2003, *E. heterophylla* samples exhibited yellow mosaic and leaf curling symptoms were observed in the state of Aragua, Venezuela. Amplification of the complete viral genome was performed using rolling-circle amplification (RCA) methodology. Linearized full length fragments were obtained by digestion with *Eco*RI or *Bam*HI, cloned and sequenced. The BLASTn analysis showed 86% similarity with *Euphorbia mosaic virus* (*EuMV*) from Jamaica, hence it is named as *Euphorbia mosaic Venezuela virus* (EuMVV) and DNA-B clone showed 77% similarity with EuMV from Cuba [159]. The presence of bipartite begomovirus was confirmed. The description of a member of a new begomoviral species that is able to infect cultivated plants has implications for the development of strategies to control begomovirus diseases in Venezuela and elsewhere.

Mentha spp. Mentha or mint belongs to family Lamiaceae cultivated widely tropical and sub-tropical parts of the world. Mint is one of the important plants which are known as medicinal and aromatic crop since ancient period. It is well known for its essential oil i.e. mints oil, menthol and carvone. It is used as carminative, antiflatulence, treating diarrhea, as a pain reliever, relaxant and antibacterial action etc. In previous years, different varieties of mint in the major cultivating areas of our country, showed viral infestation that include mosaic, curling and deformation of leaves, yellowing and poor stunted growth (Fig. 2a) which in turn causes tremendous loss in biomass and consequently high economic loss to the farmers/growers. In 2007, typical begomoviral symptoms were observed with 50-60% disease incidence in Lucknow and presence of  $\sim 800$  bp CP gene amplified using CP specific primers confirms begomovirus presence. The sequence showed 93% identity with Tomato leaf curl Pakistan virus isolate [132]. Thereafter, a complete DNA-A monopartite begomovirus along with a betasatellite molecule in Mentha crop was established in 2008 in Punjab, Ludhiana. The DNA-A segment shared 94% identity with Tomato leaf curl Karnataka virus Bangalore along with Cotton leaf curl Multan betasatellite molecule. A 1.1 kb fragment amplified showed 98% match with DNA-II molecule. It contains no ORF and named as Mentha leaf deformity associated DNA-II (MLDA-DNA-II) [19]. Recently, In CIMAP,

mints crop showed yellow vein, leaf yellowing, mosaic, crinkling, and cupping with 40–50% disease incidence. To ascertain the presence of begomovirus, viral genome amplified using RCA and 2.7 kb and 1.4 kb band were seen with restriction enzyme *EcoRV*, *Hind*III, *EcoRI*, *SacI* and *Sal* I were cloned and sequenced. The sequence analysis showed maximum nucleotide identity (99%) with *Chilli leaf curl India virus* (FM877858) and  $\beta$ -satellite showed 93% identity with *Ageratum yellow vein virus* satellite (AJ252072.1). No presence of DNA-B was detected using the universal primer PBL1v2040/PCRc1 [127].

Momordica charantia Also known as Bitter guard and a highly cultivated crop of India, China and South East Asia for its medicinal values and use as a vegetable crop. It is a good source of Vitamins B1, B2, B3, C, Folic acid, iron, calcium and beta carotene. The bitter guards also possess antiviral activity [10, 64]. Bitter guards have been the host to many viruses which affected its cultivation and economic yield. Begomoviruses have been reported earlier Bitter gourd yellow mosaic virus [122], ToLCDV [141], Indian cassava mosaic virus [121], Squash vein yellowing virus and Cucurbit leaf crumple virus [1] and Pepper leaf curl Bangladesh virus [120] identified on bitter gourd. In the year, 2007 a severe yellow mosaic disease was observed on bitter guard in different location of Uttar Pradesh under field conditions with a considerable disease incidence (10-20%) [143]. The affected plants showed leaf curling and lesser number of fruits as well. The infected sample amplified a band of  $\sim 800$  bp cloned and sequenced. BLAST analysis shows highest identity with Tomato leaf curl New Delhi virus. Tomato leaf curl New Delhi virus (ToLCNDV) is an emerging problem for agricultural crops in India and neighboring countries.

*Mucuna prureins* It is commonly known as velvet bean mainly grow in tropical regions. It is used as herbal medicine for treating liver dysfunction and other ailments. The presence of high level of  $_{L}$ -Dopa as its main constituent is

Fig. 2 Begomovirus infected MAPs 2 a Mentha sp., b M. pruriens



7

the most important for the treatment of Parkinson disease. Genomic DNA was isolated from plants with typical symptoms of golden mosaic and yellowing (Fig. 2b) at CIMAP, Lucknow (2008), amplified using rolling circle mechanism (RCA) and digested with different enzymes. The 2.7 kb band obtained both of DNA-A and DNA-B cloned into *Hind*III site of pBluescriptII KS + vector. One representative sequence of DNA-A (FN543425) *Velvet bean severe mosaic virus* DNA A and one that of DNA-B (FN543426) *Velvet bean severe mosaic virus* DNA B is available in EMBL database [160]. Agroinfectious clones of DNA-A and DNA-B constructs were made and analysed to infer that the presence of DNA-A and B both needed to produce infection in *M. pureins*. No satellite component was found.

Ocimum spp. Ocimum is an important medicinal and aromatic plant of Family Lamiaceae. It is important for its essential oil methyl eugenol/methyl chavicol having bactericidal properties. It has been used as antimicrobial, antimalarial, anti-allergic, anti-stress, anti-diabetic and immunomodulator etc. Tomato leaf curl virus was identified for the first time in India to infect Ocimum sp. on the basis of CP gene [50]. During the field survey in 2014 at Oman, severe leaf curling, crinkling and yellowing was observed (Fig. 3a). A band of 2.8 and 1.4 kb was cloned and sequenced. Three clones of DNA-A fragment were obtained in restriction of rolling circle mechanism (RCA) that was 2790, 2765 and 2759 nts. These species are Tomato yellow leaf curl virus-Oman (TYLCV-OM; BZ-2), Chili leaf curl virus-Oman (ChLCV-OM; BZ-8,) and their recombinant Tomato leaf curl Albatinah virus and Tomato Leaf Curl Betasatellite isolate [7].

**Papaver somniferum** Opium (*Papaver somniferum*) is grown widely for its highly valued and medicinally important constituents such as morphine, papaverine, codeine, thebaine etc. During the year 2012 in the fields of CSIR-NBRI, Lucknow, typical begomoviral symptoms like leaf curling and stunting along with the presence of white flies (Bemisia tabaci) suspected the presence of begomovirus infection. Nucleic acid isolated and the full genome was amplified using rolling circle amplification (RCA, TempliPhikit, GE Healthcare, USA) and restricted digested with enzymes XbaI MspI, Pst I and Cla I. Only XbaI resulted to give 2.7 Kb products which was cloned in vector pCAMBIA 1300 vector and then sequenced (Gen-Bank Ac. No. KC513822). The sequence shows highest identity with the DNA-A genome of Tomato leaf curl New Delhi virus (ToLCNDV) isolates from bottle guard, muskmelon and tomato etc. reported in India [140]. No betasatellite, alphasatellite and DNA-B genome was found. Hence, the virus was monopartite. Here, the significance of finding is that tomato infecting begomovirus infection does 100% on opium poppy plants and imposes threat to other medicinal important crops.

Rosa spp. Rose is an ornamental and aromatic plant cultivated worldwide for its use in food, nutritional, flavouring and medicinal products [55]. Rosa chinensis plant exhibited leaf curling, stunted growth, and downward or upward curling symptoms in 2006, Pakistan. Association of DNA-A and a betasatellite molecule was found [74]. Sequence analysis showed that DNA-A fragment shared less than 89% nucleotide identity to other begomoviruses hence according ICTV guidelines named as Rose leaf curl virus isolate and betasatellite showed 96% identity with Digera arvensis yellow vein betasatellite (DiAYVB). The infectious clones for virus were prepared and inoculated through Agrobacterium tumefaciens to N. benthamiana plants. DNA-A alone was unable to show symptoms on N. benthamiana plants, but co-inoculation with cognate betasatellite produced infection symptoms. Rose (R. indica) is a woody perennial ornamental plant grown throughout India. In a survey 2012, plants exhibited dwarfing, leaf distortion and leaf curling typical of

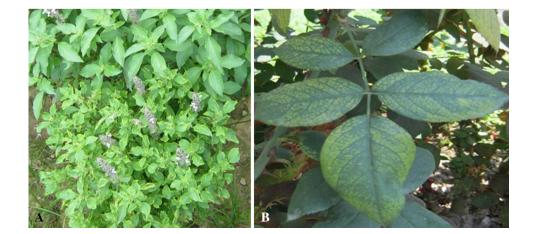


Fig. 3 Begomovirus infected MAPs 3 a O. basilicum, b R. indica

begomovirus symptoms with 50–80% disease incidence (Fig. 3b) [128]. In India for the very first time *Rose leaf curl virus* RoLCuV and *Digera leaf curl* betasatellite was found to be associated that was identified in *R. chinesis* in Pakistan.

*Sida cordifolia* Also known as country mallow or heartleaf, is a perennial shrub of the family Malvaceae native to India throughout the world. The roots and stems contain ephedrine, pseudoephedrine, hypaphorine, vasicinone, vasicinol, choline, and betaine constitute the major alkaloids. *S. cordifolia* is used in ayurvedic medicine for the treatment of inflammation of the oral mucosa, blenorrhea, asthmatic bronchitis, stomatitis, and nasal congestion [45].

In 2005, two virus isolates Hn57 and Hn60 were isolated from S. cordifolia which showed mild upward leaf-curling symptoms in Hainan province of China. Complete sequence comparison with other reported begomoviruses revealed that Hn57 DNA-A has the highest sequence identity (71.0%) with that of Tobacco leaf curl Yunnan virus. Consequently, Hn57 was considered to be a new begomovirus species, for which the name Sida leaf curl virus (SiLCV) is proposed [56]. In addition to DNA-A molecule, two additional circular single-stranded satellite DNA molecules corresponding to DNAB and DNA1 were also found to be associated with SiLCV isolates. Both DNA $\beta$  and DNA1 were approximately half the size of the viral DNA. Sequence analysis shows that DNAB of Hn57 and Hn60 share 93.8% nucleotide sequence identity, and they have the highest sequence identity (58.5%) with DNAB associated with Ageratum leaf curl disease (AJ316027).

**Solanum nigrum** A weed plant (Black night shade or Makoy) known for medicinal properties to treat inflammation, edema and hepatic cancer. *S. nigrum* in the year 2010 was surveyed in Rajasthan found to exhibit symptoms of leaf curling, yellow vein mosaic and stunting causing huge loss. The infected plants were checked for begomovirus infection using CP specific primers generating an amplicons of ~550 bp. The fragments were cloned and sequenced. On BLASTn analysis showed 96% identity with *Cucurbita pepo begomovirus* and hence named as *Solanum leaf curl Lakshmangarh virus* (JN009667). This is the first report of begomovirus infection on *S. nigrum* [116]. It plays a major role as alternate/reservoir host for the transmission of disease to other economically important crops.

*Salvia hispanica* It is also known as chia of family, Lamiaceae, native to Central and Southern Mexico and Guatemala, as well as Bolivia, Argentina, Ecuador, Nicaragua, and Australia [29]. It is grown commercially for its seed, rich in omega-3 fatty acids since the seeds yield

25–30% extractable oil, including  $\alpha$ -linolenic acid. Chia is one of the crops with the highest nutritional profile in Latin America and now marketed under various names. The chia oil proved to be as good antioxidant, anti-inflammatory, stabilize blood sugar levels, control high blood pressure and is best for cardiovascular health.

In Argentina for the first time infection of begomovirs is reported on chia (*S. hispanica*) and two viruses were identified. The comparison of the complete nucleotide sequences showed the presence of two viral species with the typical genome organization of bipartite New World begomovirus, identified as *Sida mosaic Bolivia virus* 2 and *Tomato yellow spot virus*, according to the ICTV taxonomic criteria for begomovirus classification [31]. DNA-A from *Sida mosaic Bolivia virus* 2 exhibited 96.1% nucleotide identity with a Bolivian isolate of *Sida micrantha*, and *Tomato yellow spot virus* showed 95.3% nucleotide identity with an Argentine bean isolate. This is the first report of begomoviruses infecting chia as well as of the occurrence of *Sida mosaic Bolivia virus* 2 in Argentina.

*Tagetus patula* It is also known as marigold widely cultivated as an ornamental plant in India. In the year 2012, diseased plants in Sikar province, India showed stunted plants with apical leaf curl and crinkled leaf (Fig. 4a). First report of complete nucleotide sequence of *Ageratum enation virus* isolate (KC589699) along with betasatellite and alpha satellite molecules was reported in 2013 [94]. These molecules have been identified as DNA- $\beta$  (*Ageratum leaf curl betasatellite*) and DNA- $\alpha$  (*Marigold leaf curl* alphasatellite). No DNA-B molecule was found. Moreover, to prove koch's postulates and to assess infectivity, virus clone constructs were prepared and tested in model plant *N. benthamiana* for disease development. Since the vector is likely to carry virus (DNA-A, DNA- $\beta$  and DNA- $\alpha$ ) poses a serious threat to other economical and ornamental plants.

*Withania somnifera* Also known as Ashwagandha. Its root and berry is used medicinal preparations for arthritis, insomnia, liver diseases, as an immuno modulator, anticancerous etc. In Ayurveda it is known as Indian Ginseng. The association of a begomovirus with yellow mosaic disease was identified but not at molecular level (Fig. 4b) [15]. During a survey at Aligarh, Lucknow and Rajasthan in the year 2008–2009, disease incidence was found between 15 and 20%. Total DNA isolated from infected *W. somnifera* leaf samples collected from three locations and begomovirus-specific primers degenerate primers PALIv 722/PALIc 1960 [125] resulted in the successfully amplification of 1.2 kb DNA fragments were cloned and sequenced. All three isolates shared a maximum of 91% identity with *Jatropha mosaic India virus* (JMIV) from

Lucknow (HM230683) [15]. This was the first report of JMIV in *W. somnifera* from India.

### **Management strategies**

# **Cultural practices**

Viral disease might be controlled effectively by timely use of cultural practices providing incomplete interaction among the virulent pathogen (virus), susceptible host and favorable environment. This management strategy is focused on elimination of primary inoculums and preventing the movement of inoculums from the infected to healthy plant. Regular weeding and general maintenance might reduce disease incidence. Host-free periods could be a successful way to control begomovirus infection [53, 147] but practically difficult since the knowledge about alternative hosts of begomoviruses is very limited. Other agricultural practices for control of begomoviral diseases include eradication of source plants, use of reflective mulches and physical barriers, intercropping or delayed sowing time to divert whiteflies and use of virusfree transplants [53, 102, 112]. The best-suited practices for control of begomovirus infections depend on the local conditions. In 2001, Hiljea et al. [62] presented a number of success stories with this approach on other crop pathosystem: okra in Mexico [39], tomato in Egypt [42], cotton in northern Mexico [59, 60], bean in Egypt [96] and tobacco in India [107].

## Vector management

Insecticides are commonly used to manage *B. tabaci* besides the harmful effects on human health and on environment with the frequent use that led to resistance in the

insect and also kills the beneficial insects that are natural enemies of whiteflies. The systematic use of pyrethroids, organophosphates and neonicotinoids has shown the reduction in susceptibility or resistance in populations of B. tabaci in Benin, Togo and Burkina Faso in Cotton [57]. However, neonicotinoids are applied to Bt cotton for controlling whiteflies, and this resulted in the selection of resistant Q-biotype of B. tabaci [54, 63]. A balanced approach for vector management would be the natural enemies of B. tabaci [103]. Although effective sources of resistance have been identified for non-Bemisia whitefly species in Latin America [17] and have suggested that these are less effective against African B. tabaci, [84, 85]. However, successes have been recorded with the identification of effective parasitoids of B. tabaci across Africa. Encarsia spp. and Eretmocerus spp. have been widely observed [35, 68] for management of B. tabaci of cassava in Africa.

## Virus reservoirs

Begomovirus infects weeds and wild plants in the surrounding of sowing host crops. To understand the complete epidemiology and establishment of proper management, identification of alternative hosts/reservoir is an important aspect for study. A recent study established that the common weed *A. conyzoides* in Cameroon is recorded a host to a complex consisting of *Ageratum leaf curl Cameroon virus* (ALCCMV), *Ageratum leaf curl betasatellite* (ALCCMB), and *Ageratum leaf curl Cameroon alphasatellite* (ALCCMA) [86]. The uncultivated/wild hosts serve as reservoirs for begomovirus has been demonstrated by the recent identification of *Lamium amplexicaule* as a host of *Tomato yellow leaf curl virus* (TYLCV) in Korea [77]. *Croton bonplandianum, Eclipta prostrate, Physalis minima* and *Solanum nigrum* are very



Fig. 4 Begomovirus infected MAPs 4 a *T. patula*, b *W. somnifera* 

common weeds and acted as potential source of begomovirus in India. These reports further proved that reservoirs remain largely unidentified and detailed investigation required to find out alternative hosts of begomoviruses and associated ssDNA satellites infecting commercially important crops including MAPs.

#### **Plant extracts**

Viruses are becoming major constraints to the economically and commercially important agricultural and MAPs. Like fungicide, weedicide, insecticide; viricides are lacking in the control of plant viruses. Botanical extracts have been known to combat bacterial and fungal diseases and thus gaining attraction in crop protection [32]. Endogenous viral inhibitors that are proteinaceous antiviral substances extracted from plants and used against plant viral infections [88, 152, 153]. Such antiviral substances have been reported in the extracts of Bougainvillea spectabilis, Azadirachta indica, Pongamia glabra, Clerodendron aculeatum, Phytolacca americana, Dianthus caryophyllous, Mirabilis jalapa, Boerrhea diffusa etc. against serious pathogenic viruses such as TMV, PVX, PVY, begomoviruses etc. [3, 18, 106]. These extracts reduce the infectivity of the viruses to 60-80% and thus reduce the number of local lesions. These proteins induce the resistance and highly active in reducing the infectivity of the virus, but their mode of action on virus and host is still needs more research.

## **Breeding for resistance**

Breeding for disease resistance and use of resistant planting materials serves the best management strategy; however, progress in incorporating durable resistance into cultivars with desirable agronomic and quality attributes has significant challenges to breeders. The use of resistant varieties has remained the most economical and ecologically sustainable control measure [12]. Additionally, most breeding programs have focused on yield and quality under intense management regimes and correspondingly low rates of disease, thus neglecting the impact of disease on yield [55]. A promising approach is to breed for disease escape means to shift cultivation time which is less favourable for disease spread. Breeders could select for short cropping cycle or for profuse flowering early or late in the season, depending on the climate of the area in question [157].

# Integrated pest and disease management

An integrated pest and disease management (IPDM) program is the integration of all available control methods for any disease into a single program. The vector, B. tabaci spread begomoviruses and causes serious damage thus limiting the production, hence it becomes necessary to combine a number of management strategies in a systematic and coordinated integrated pest and disease management plan. A number of possible control measures previously described can be combined. Management protocols should include biological control microorganisms, genetic and induced resistance, cultural practices, natural products, and use of approved chemicals. The application of IPDM to the selected crop enables farmers to opt management practices suited to their situations and needs. The use of an integrated management system reduces the levels of pests and diseases, reduces the inappropriate use of chemicals, provides alternatives for pest and disease management and improves the yield and quality of the end products, thereby increasing farmer income [78]. Frequent and complete harvesting, sanitation, and appropriate disposal of, infected plant parts, and husks will reduce the level of inoculums and flying vectors [11, 55]. The use of resistant planting materials, natural biopesticides and microorganisms can also be added to the IPDM programme and thus provides a promising and amenable to plant disease management under organic production conditions.

#### Virus-free planting material

Many vegetatively propagated plants like potato, rose, sugarcane, mints, cassava etc., are itself the main source of virus dispersal. One of the most successful forms of control is the development of virus-free clones of the particular virus [65]. It is possible to produce thousands of virus-free planting materials within a relatively short period of time through apical meristem and tip culture technology. It is helpful to eradicate the threatening viruses where the disease is causing serious losses to the farmers. Additionally, support from government in form of legislation and providing virus-free and resistant planting materials to farmers could motivate the farmers and will also help curb the pandemics.

#### **Cross-protection**

Cross protection, which involves protecting a susceptible plant from the infection of a virulent strain, has been used in the management of some plant viruses. A begomovirus mild strain infection was reported to save cassava plant from virulent begomoviral infection. To prove that mild strains of *East African cassava mosaic virus* (EACMV-UG) were providing a form of cross protection, plant grown from initially CMD-free parent and initially infected with mild strain of EACMV-UG were grown and subsequent pattern of infection, symptoms expression and tuberous root production were assessed by Owor et al. [104]. Plants grown from initially CMD-free parent developed more severe disease and yielded less than plants derived from mildly diseased parent [144]. There is also the risk of the viruses synergising to produce a more severe disease.

#### Training and extension education

Most of the farmers are illiterate so to educate them about the epidemiology and control of begomovirus diseases will be helpful for the disease management. Researchers constantly receive information through their participation in workshops and other scientific activities. So, farmers should be trained by giving tips on agro techniques and training, promoting interactions among farmers, orientation programs on the epidemiology, new products/varieties, low cost process and eco friendly control practices for the begomoviruses. Such type of training camps and extension services in the form of print media, radio and farmers field schools should also be organised as agricultural extension in order to disseminate the right management practices to the farmers, growers and industrial entrepreneurs.

# Molecular based approaches

The majority of approaches currently being used for begomovirus epidemiological and management studies rely entirely on molecular methods. Genetic engineering has been documented as most effective approach for controlling virus diseases in a wide range of crops grown worldwide. The coat protein mediated protection has been reported to be successful against numerous RNA viruses however; few results of engineered resistance against begomoviruses have also been reported. Kunik et al. [129] extended the idea of capsid protein mediated protection that could be applied successfully with many RNA viruses, to the DNA begomovirus. Begomoviral infections could be identified and established from plant leaves, stems, and roots/tubers with PCR using specific primers targeting CP gene or highly conserved gene mostly present in genome of begomoviruses and then the infected plant incinerated to avoid disease spread [130]. The resistant genes could be genetically engineered into other crops for protection. Although transcriptional gene silencing confers resistance in wild tomatoes and this could be genetically engineered in other plants. Ty resistance gene is responsible for this TGS phenomenon [27]. Combination of Ty (Ty-1, and Ty2, Ty3, Ty4 and Ty5) gene has been reported in wild tomato to provide high level of resistance against Tomato yellow leaf curl virus (TYLCV). Homologous Ds RNA engineered to express sequence of IR, CP gene, V2 gene and replication associated gene of Tomato yellow leaf curl virus-Oman prepared to reduce the symptom development against TYLCV-OM in N. benthamiana/Tomato [8]. Expression of a geminiviral promoter transgene could be silenced by infection with the homologous geminivirus [136]. This was conducted in blackgram (Vigna mungo) through bombardment with a hpRNA construct under 35 S promoter sequence of geminivirus Vigna mungo yellow mosaic virus (VMYMV) and plants completely recovered from the VMYMV infection [113]. Begomoviruses infection propels plant resistance mechanism to maintain its natural state of being healthy and one is RNAi resistance. In plants defense response certain DICER genes are present that are responsible to degrade the invading viral DNA/ RNA into pieces but as a coevolution viruses have also developed RNAi suppression activities that leads to disease in plant. These RNAi suppression factors were reported in begomoviruses [155]. The resistance could be induce by the expression of viral genes such as CP, Rep Ren gene, expression of non viral protein like dianthin against ACMV, expression of antibodies against RdRP/CP and expression of defective interfering (DI) reduces symptoms in plant [150]. To find a cure against these geminiviruses RNAi technology [81, 151] is a magnificent tool which employs a check on the devastating behavior of Geminiviruses infecting ornamental plants, weeds and MAP crops. Results of these techniques are very promising and effectively applied for the disease management and development of quarantine strategies.

## Resistance with CRISPR/Cas9 System

CRISPR/Cas9 technology could be a new ray of hope to engineer resistance against single and multiple geminivirus infections in plants [158]. Clustered regularly interspaced short palindromic repeats (CRISPRs)/CRISPR-associated 9 (Cas9) is a prokaryotic molecular immunity system against invading viruses and has been harnessed as a powerful tool for targeted genomic editing. During subsequent infections, spacers are transcribed as part of the CRISPR array; after transcription and maturation, CRISPR RNA guides the Cas9 endonuclease to scan invading DNA and cleave the target sequence [156] at a site preceding the protospacer associated motif (PAM), a trinucleotide sequence that is recognized by Cas9 and necessary for its binding to target DNA. Ali et al. [4, 5] showed that CRISPR/Cas9 technology could impart molecular immunity against three geminiviruses i.e., Tomato yellow leaf curl virus (TYLCV), Beet curly top virus (BCTV), and Merremia mosaic virus (MeMV) in N. benthamiana plants [4], and revealed that a sgRNA designed to target a conserved sequence (TAATATTAC) in the viral intergenic region could be used to target multiple geminiviruses simultaneously [4]. This sequence is conserved among geminiviruses and betasatellites of begomoviruses [57]. Thus, this approach could effectively impart resistance to multiple viruses under natural conditions, where mixed infections predominate. Although these three studies demonstrated the efficacy of the CRISPR/Cas9 system against geminiviruses in N. benthamiana, transformation in crop plants and field trials are needed to be conducted to evaluate CRISPR/Cas9-mediated resistance in natural conditions. The rate at which the virus evolves to evade the CRISPR/Cas9 machinery also remains to be determined. However, off target Cas9 activities in the plant genome have been detected only rarely [5]. Moreover, recent work has identified Cas9 enzymes that give fewer off-target effects, thus further reducing this concern in plants [138]. Harnessing the CRISPR/Cas9 machinery to engineer plant resistance to viral pathogens also opens the possibility of addressing basic questions in virus infection and plant host resistance. For example, the CRISPR/Cas9 platform could be used to investigate the evolution of the viral genome to counteract plant immunity, by examining the genomes of viruses that escape recognition by the CRISPR/ Cas9 system. The CRISPR/Cas9 platform could also be used for targeted mutagenesis to identify host factors that control plant resistance and susceptibility to viral infection. Thus, CRISPR/Cas9 technology offers a promising approach for understanding and engineering resistance to single and multiple viral infections in plants.

## Geminivirus Database (GVDB) in India

Geminivirus Database (GVDB) has also being developed at MITS (Mody Institute of Technology and Science (MITS), Lakshmangarh, Rajasthan (http://www.mitsuniversity.ac. in/). The objective of the Geminivirus Database (GVDB) is to design and provide tool for genetic and in silico analysis of geminivirus and its genus Begomovirus. It provides a platform for the current status and research on geminivirus especially with reference to begomovirus infection in different plants across India. The GVDB comprises of partial and complete nucleotide sequences of weed, ornamentals, vegetables, MAPs and commercially important crops etc. are infecting by begomoviruses. Homology modeling and docking results of different begomovirus proteins (cds) were also enclosed. Homology modeling is a bright field to understand and study the genetic diversity of begomoviruses. It involves the prediction of various proteins and helpful to biologist to develop resistance against various begomoviruses [114].

# **Concluding Remarks**

Excessive *B. tabaci*-induced losses have occurred worldwide in most of the agricultural, fruit, pulses, ornamental, medicinal and aromatic crops. *B. tabaci* transmitted plant viruses have increased tremendously, and total vield losses likewise increased. An increased number of begomovirus infections have been reported from India and other parts of the world. Potato vellow mosaic virus, a Begomovirus, first identified in the 1980 that infects tomato plant and till date 288 species are known of type Bean golden yellow mosaic virus (ICTV. "Virus Taxonomy" 2014). Due to the high survival and transmission rate of vector, whitefly and wide range of virus-host interaction, the disease day by day aggressively introgressing new crops. Weeds/wild hosts (Nicandra physaloides, Ageratum conyzoides, Lamium amplexicaule and Solanum nigrum) are commonly found infected and thus increase the chances of virus incidence [16, 77, 86, 116]. Recombination has played a major role in evolution and emergence of new one among species/strains [58]. Earlier it was believed that DNA viruses are less prone to recombination but certain studies found that begomoviruses are highly prone to recombination [49, 82, 105, 149]. This recombination leads to the formation of virulent strain than parent virus [149] and RNAi suppression of plant defence by begomoviruses is the key factor responsible for the disease development and transmission of virus [155]. In the early 1990s, geminiviruses caused up to 95% yield losses of tomatoes in the Dominican Republic, and inflicted over US\$2 billion/year yield losses in cassava in Africa [58]. In Brazil, during the last four years, more than 11,000 jobs have been lost in the tomato industry because of whitefly-transmitted geminiviruses and other factors. As whiteflies become more adapted to local climates and environments, geminiviruses will become one of the major constraints on agricultural production worldwide. The vast distribution and abundance of begomovirus isolates and continuously newly reporting species as well as high genetic diversity within species suggested that begomoviruses have high mutation rate and they produce highly diverse population in very short time [108].

In the endeavor to feed the ever-increasing population, intensive and extensive agricultural practices have been adopted and high yielding varieties are introduced. With the indiscriminate use of newer pesticides including synthetic pyrethroids resulted in enhancing the whitefly population, presence of whiteflies in newer areas and on additional hosts. As a result large number of begomovirus diseases emerged and some of them heavily damaged economically and pharmaceutical important crops [22, 36, 152]. Intense monitoring of emerging and reemerging begomovirus diseases on the regular basis should be faithfully followed so as to take timely measures to prevent the recurrence of the diseases. For the management of devastating begomovirus diseases, information on inheritance of resistance to most of the viruses is lacking. Moreover, epidemiological studies are required to address inter-relationship of vellow/golden mosaic viruses infecting legumes, leaf curl viruses infecting all the crop and weed plants. These studies coupled with developing resistant cultivars, could be as effective strategy for disease management [100]. Synthetic pesticides have largely failed to address the problem of whitefly population in field. Management of whiteflies and begomovirus diseases, IPM approach is needed [52]. Biological and ecofriendly management alternatives could be the answer as in the case of heavy population of biotype B of whitefly, which was successfully, controlled using its natural enemies [75]. Significant enhancement of chilli fruit yield was achieved in the case of chilli leaf curl disease using non-host barrier trap crop [41]. Crops with durable resistance, clean cultivation combined with eco-friendly management practices would help to combat against the diseases caused by begomoviruses. Enough numbers of begomoviruses in India infecting economically important food, horticultural, weeds, MAPs and ornamental crops which need to be fully characterized. Understanding the recombinants and phylogenetic relationship between them will help in establishing the evolutionary status. A rich diversity of begomoviruseses is of great concern, since this situation undoubtedly increases incidences of mixed infections and increases the possibility of yet more novel recombinant viruses arising within this species. Investigations revealed that geminiviruses which replicates in nucleus can induce PTGS and become the target for it. RNAi has a strong potential to reduce the infection of geminiviruses. Numerous studies are already performed on different genes of geminiviruses and that are used for developing virus resistance plants. The appearances of a new vector biotype and increases in vector populations have contributed to the emergence of geminivirus disease problems. Therefore, there is a need for a better understanding of the factors that have led to the increase in the vector populations in diverse cropping systems. The complexities of emerging geminivirus problems require an intensive effort by virologists as well as entomologists and plant breeders to contain fresh outbreaks and to reduce the damage caused by geminivirus diseases to allow sustainable crop production [152]. More research attention has to be given to combining disease resistance with vector resistance and commercialization of identified biological control agents that could be used for new diseases and pathosystems. Similarly, the efficacy of some other cultural methods-Weed/alternate host determinations, extension education/training of farmers, support from government in form of legislation and providing virus-free and resistant planting materials to farmers could motivate the farmers and will also help curb the pandemics. Recently, CRISPR/Cas9 platform could be used to investigate the evolution of the viral genome to counteract plant immunity, by examining the genomes of viruses that escape recognition by the CRISPR/Cas9 system. The CRISPR/Cas9 platform could also be used for targeted mutagenesis to identify host factors that control plant resistance and susceptibility to viral infection. Thus, CRISPR/Cas9 technology offers a promising approach for under-standing and engineering resistance to single and multiple viral infections in plants.

As begomoviruses are considered the worst virus in the world which causes 50–90% crop losses, current agricultural technology needs more and more molecular tools to reduce current crop loss and feed extra mouths, which according to a recent estimate by the FAO (Food and Agriculture Organization) will increase by two billion over the next 30 years. With this high diversity, recombination potential, limited knowledge of alternative hosts, and transmission by *B. tabaci*, begomoviruses and their associated ssDNA satellites possesses a serious threat to MAPs and agricultural crop production and food security in the world, thus needs deeper insights and intensive future research.

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