



# Replication of DNA Satellites and Their Role in Viral Pathogenesis

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## Abstract

The white-fly borne begomoviruses (family *Geminiviridae*) have circular single-stranded (css) DNA genome, which is encapsidated as monopartite (DNA-A) or bipartite (DNA-A and DNA-B) in the twinned icosahedrons. During the course of their evolution and to escape host defense machinery, begomoviruses adopt small cssDNA satellites called alpha-, beta-, and deltasatellites. Alphasatellites are found to be associated with begomovirus–betasatellite complexes and encode their own replication-associated protein (Rep), thus capable of autonomous replication. These satellite-like molecules are not well known to serve any critical function for their helper begomovirus except for few reports about attenuation of helper-virus accumulation and/or occasionally suppression of the host defense. Most of the monopartite begomoviruses in the Old World (OW) are found to be associated with betasatellites; however, none of the New World (NW) begomoviruses are known to be associated with betasatellites. Begomoviruses replicate their genome through rolling circle replication (RCR), which requires the virus-encoded Rep to recognize and bind to the iterated sequences (iterons) in the origin of replication (*ori*) region. Betasatellites lack such iterated sequences; however, they can be transreplicated by a diverse range of begomoviruses, following a similar pattern for replication. Betasatellites play a significant role in viral pathogenesis by interacting with certain host factors, attenuation of disease symptoms, suppression of host defense, and sometimes

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inter- or intracellular shuttling of begomovirus genome. Likewise, the noncoding molecules deltasatellites depend upon their helper virus for their replication. However, their precise role in viral pathogenesis still needs to be explored.

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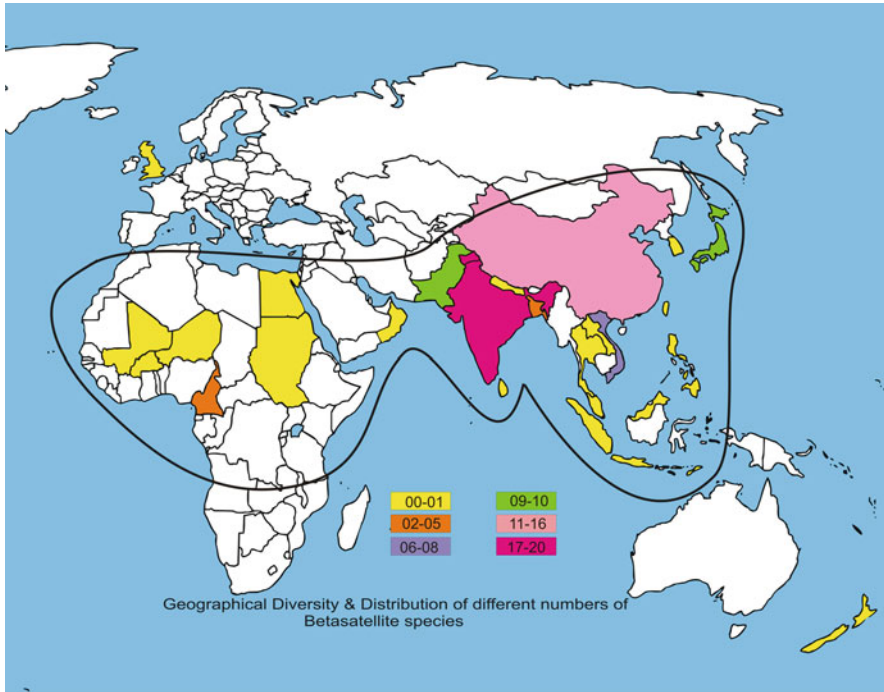
## 1 Introduction

Many plant viruses coexist with certain nucleic acid, either DNA or RNA molecules, termed as “satellites.” Satellites lack the ability for an independent existence and are entirely dependent on their helper virus for their replication, encapsidation, movement, and proliferation (Briddon and Stanley 2006). Satellite molecules have a very simple genomic organization and usually encode few or no genes and have little or no sequence homology to their helper virus. The word “satellite” coins two major classes of nucleic acid agents: satellite viruses, which are capable of self-encapsidation by producing capsid protein, and the virus-associated satellites, which lack their own capsid protein and therefore utilize helper-virus proteins for their encapsidation (Mayo et al. 2005). Satellite virus was described for the first time in 1962 when scientists discovered some exogenous nucleic acid agents being associated with few strains of *Tobacco necrosis virus* (TNV), a *Necrovirus*, laterally recognized as *Tobacco necrosis satellite virus* (TNSV) (Kassanis 1962). Until now, several satellite molecules have been found to be associated with different classes of plant viruses, particularly RNA viruses such as *Rice yellow mottle virus* satellite (RYMV-sat) and *Cucumber mosaic virus* satellites (CMV-sat) (Adams et al. 2017; Mayo et al. 2005). Majority of the satellites have single-stranded (ss) RNA genome; however, double-stranded (ds) RNA genome is also present in few members. RNA genome of few ssRNA satellites encodes some proteins that may or may not assist in replication process (Palukaitis et al. 2008). The first begomovirus-associated DNA satellite [Tomato leaf curl satellite (ToLCV-sat)] was reported in 1997 from tomato plants infected with *Tomato leaf curl virus* (ToLCV) (Dry et al. 1997). In most cases, these satellites overload the resources of helper virus for their own replication/survival and interfere with viral infectivity (Brown et al. 2012). However, few members of DNA satellites have been identified causing coinfections with helper viruses and result in severe disease symptoms as compared to single viral infection (Nawaz-ul-Rehman and Fauquet 2009).

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## 2 History and Current Status of ssDNA Satellites Associated with Begomoviruses

Most of the begomoviruses (family: *Geminiviridae*) in the Old World (OW) and few in the New World (NW) are being associated with circular ssDNA satellites (Fig. 1). Till now, DNA satellites associated with majority of the OW monopartite begomoviruses include more frequently found betasatellites and occasionally



**Fig. 1** Genome organization of cssDNA satellites. Schematic position and orientation of genes is shown with colored arrows. **(a)** Alphasatellites encode Rep protein and contain an adenine rich (A-rich) sequence. **(b)** Betasatellites encode single protein,  $\beta$ C1, have a satellite conserved region (SCR) and an A-rich sequence. The purple and green parts of deltasatellite SCR represent the predicted conserved stem-loop and secondary stem-loop structures, respectively. For all satellites, the intergenic region (IR) contains a predicted hairpin-loop structure, which contains the nonanucleotide sequence (TAA/GTATTAC) as part of the loop

alphasatellites, whereas newly characterized deltasatellites are most frequently reported in association with the NW begomoviruses (Adams et al. 2017) (Table 1). Recently, betasatellites and deltasatellites have been assigned new genera *Betasatellite* and *Deltasatellite* in the sub-viral family, *Toleucusatellitidae*, respectively (Adams et al. 2017).

## 2.1 Alphasatellites

After their first discovery in 1999 from *Ageratum conyzoides* (Saunders and Stanley 1999), approximately 200 complete alphasatellite (earlier described as DNA-1) sequences have been deposited in the GenBank database. Alphasatellites are circular and ssDNA (css) molecules associated with OW monopartite begomoviruses, begomovirus–betasatellite complexes, and/or NW bipartite begomoviruses

**Table 1** List of major DNA satellites prevailing in the Old World (OW) and the New World (NW)

Satellite	Acronym	Associated Begomovirus	Infected Crop	Origin	Accession #	Reference
<i>Betasatellites</i>						
Ageratum leaf curl betasatellite	ALCB	<i>Papaya leaf curl virus</i>	<i>Aster amellus</i>	India	JQ408217	Srivastava et al. (2013)
		<i>Ageratum enation virus</i>	<i>Amaranthus hypochondriacus</i>		IX512904	Srivastava et al. (2015)
		<i>Ageratum enation virus</i>	<i>Tagetes patula</i>		KC589700	Marwal et al. (2013b)
Ageratum leaf curl Cameroon betasatellite	ALCCMB	<i>Ageratum leaf curl Cameroon virus</i>	<i>Ageratum conyzoides</i>	Cameroon	FR717141	Leke et al. (2012)
Ageratum yellow vein betasatellite	AYVB	<i>Ageratum yellow vein virus</i>	<i>Ageratum conyzoides</i>	Malaysia	AJ542497	Bull et al. (2004)
Ageratum yellow leaf curl betasatellite	AYLCB	<i>Papaya leaf curl China virus</i>	<i>Nicotiana tabacum</i>	Vietnam	DQ641709	Ha et al. (2008a)
		<i>Chili leaf curl India virus</i>	<i>Mentha piperita</i>	India	KF364485	Saeed et al. (2014)
		<i>Wheat dwarf India virus</i>	<i>Triticum aestivum</i>	India	KC305092	Kumar et al. (2014)
		<i>Ageratum enation virus</i>	<i>Daucus carota</i>	India	JF728869	Kumar et al. (2013)
		<i>Tobacco curly shoot virus</i>	<i>Ageratum conyzoides</i>	Pakistan	NC_005046	Bridgdon et al. (2003)
Alternanthera yellow vein betasatellite	AIYVB	<i>Alternanthera yellow vein virus</i>	<i>Sonchus oleraceus</i>	Pakistan	AM412239	Mubin et al. (2010)
		<i>Ageratum enation virus</i>	<i>Ageratum conyzoides</i>	Pakistan	AM698010	Tahir et al. (2015)
		<i>Alternanthera yellow vein mosaic virus</i>	<i>Chrysogonum peruvianum</i>	Vietnam	DQ641716	Ha et al. (2008b)
Bean leaf curl China betasatellite	BLCCNB	<i>Tomato yellow leaf curl China virus</i>	<i>Phaseolus vulgaris</i>	China	DQ256459	Dong et al. (2007)

Bhendi yellow vein betasatellite	BYVB	<i>Bhendi yellow vein mosaic virus</i>	<i>Abelmoschus esculentus</i>	India	AJ308425	Jose and Usha (2003)
Chili leaf curl betasatellite	ChiLCB	<i>Chili leaf curl virus</i>	<i>Petunia hybrida</i>	India	KJ700655	Nehra and Gaur (2014)
Chili leaf curl Sri Lanka betasatellite	ChiLCSiB	<i>Pepper leaf curl Lahore virus</i>	<i>Capsicum sp.</i>	Pakistan	AM849549	Tahir et al. (2010)
Cotton leaf curl Gezira betasatellite	CLCuGB	<i>Chili leaf curl Sri Lanka virus</i>	<i>Capsicum sp.</i>	Sri Lanka	JN555600	Senanayake et al. (2013)
		<i>Cotton leaf curl Gezira virus</i>	<i>Gossypium sp.</i>	Sudan	AY669329	Fauquet et al. (2005)
		<i>Sweet potato leaf curl virus</i>	<i>Abelmoschus esculentus</i>	Niger	FJ469629	Shih et al. (2009)
Croton yellow vein mosaic betasatellite	CroYVMB	<i>Erechites yellow mosaic virus</i>	<i>Ipomoea purpurea</i>	India	IX050198	Geetanjali et al. (2013)
Erechites yellow mosaic betasatellite	ErYMB	<i>Honeysuckle yellow vein mosaic virus</i>	<i>Daucus carota</i>	Vietnam	DQ641713	Ha et al. (2008b)
Honeysuckle yellow vein betasatellite	HYVB	<i>Malvastrum leaf curl virus</i>	<i>Lonicera japonica</i>	Japan	NC_009571	Ogawa et al. (2008)
Malvastrum leaf curl betasatellite	MaLCB	<i>Okra leaf curl Oman virus</i>	<i>Ageratum malvastrum</i>	China	AJ971264	Huang and Zhou (2006)
Okra leaf curl betasatellite	OLCuB	<i>Papaya leaf curl China virus</i>	<i>Abelmoschus esculentus</i>	Oman	KF267444	Akhtar et al. (2014)
Papaya leaf curl betasatellite	PaLCuB	<i>Radish leaf curl virus</i>	<i>Carica papaya</i>	Vietnam	NC_009555	Ha et al. (2008b)
Radish leaf curl betasatellite	RaLCB	<i>Sida leaf curl virus</i>	<i>Daucus carota</i>	India	NC_010239	Singh et al. (2012)
Sida leaf curl betasatellite	SiLCuB	<i>Sida yellow mosaic China virus</i>	<i>Sida sp.</i>	China	AM050733	Guo and Zhou (2006)
Sida yellow mosaic China betasatellite	SiYMCNB	<i>Tobacco leaf curl virus</i>	<i>Sida sp.</i>	China	AJ810095	Xiong et al. (2005)
Tobacco curly shoot betasatellite	TbCSB		<i>Tobacco sp.</i>	China	AJ457822	Zhou et al. (2003)

(continued)

**Table 1** (continued)

Satellite	Acronym	Associated Begomovirus	Infected Crop	Origin	Accession #	Reference
Tomato leaf curl Bangalore betasatellite	ToLCBB	<i>Tomato leaf curl Bangalore virus</i>	<i>Solanum lycopersicum</i>	India	GU984046	Tiwari et al. (2010)
Tomato leaf curl Bangladesh betasatellite	ToLCBDB	<i>Tomato leaf curl Bangalore virus</i>	<i>Solanum lycopersicum</i>	Bangladesh	AJ542489	Bull et al. (2004)
Tomato leaf curl Gandhinagar betasatellite	ToLCGaB	<i>Tomato leaf curl Gandhinagar virus</i>	<i>Solanum lycopersicum</i>	India	NC_023038	Rathore et al. (2014)
Tomato leaf curl Philippines betasatellite	ToLCPHB	<i>Tomato leaf curl Philippines virus</i>	<i>Solanum lycopersicum</i>	Philippines	NC_009570	Sharma et al. (2011)
Tomato yellow leaf curl China betasatellite	TYLCCNB	<i>Tomato yellow leaf curl China virus</i>	<i>Solanum lycopersicum</i>	China	AJ781301	Tao and Zhou (2008)
Tomato leaf curl betasatellite	TLCB	<i>Tomato yellow leaf curl Vietnam virus</i>	<i>Solanum lycopersicum</i>	Nepal	AJ542492	Bull et al. (2004)
Vernonia yellow vein betasatellite	VeYVVB	<i>Vernonia yellow vein virus</i>	<i>Vernonia cinerea</i>	India	NC_013423	Packialakshmi et al. (2010)
Zinnia leaf curl betasatellite	ZLCuB	<i>Zinnia yellow leaf curl virus</i>	<i>Zinnia sp.</i>	Pakistan	AJ316028	Briddon et al. (2003)
Tomato yellow leaf curl Thailand betasatellite	TYLCThB	<i>Tomato leaf curl New Delhi virus</i>	<i>Solanum tuberosum</i>	Pakistan	LK933548	Hameed et al. (2017)
<i>Alphasatellites</i>						
Ageratum leaf curl Cameroon alphasatellite	ALCCMA	<i>Ageratum leaf curl Cameroon virus</i>	<i>Ageratum conyzoides</i>	Cameroon	NC_014744	Leke et al. (2012)
Ageratum yellow vein China alphasatellite	AYVCHA	<i>Ageratum yellow vein China virus</i>	<i>Syndrella nodiflora</i>	Philippines	KF785752	She et al. (2015)
Ageratum yellow vein alphasatellite	AYVA	<i>Ageratum yellow vein virus</i>	<i>Ageratum conyzoides</i>	Singapore	AJ416153	Saunders et al. (2002)
Ageratum yellow vein Pakistan alphasatellite	AYVPKA	<i>Ageratum yellow vein virus</i>	<i>Ageratum conyzoides</i>	Pakistan	AJ512949	Briddon et al. (2004)
Ageratum yellow vein Singapore alphasatellite	AYVSGA	<i>Tomato yellow leaf curl virus</i>	<i>Solanum lycopersicum</i>	Oman	FJ956707	Idris et al. (2011)

Cassava mosaic Madagascar alphasatellite	CMMAa	<i>Cassava mosaic virus</i>	<i>Manihot esculenta</i>	Madagascar	HE984148	Harimalala et al. (2013)
Chili leaf curl Multan alphasatellite	ChiLCMA	<i>Chili leaf curl virus</i>	<i>Solanum tuberosum</i>	Pakistan	NC_013103	Mubin et al. (2009)
Cotton leaf curl alphasatellite	CLCuA	<i>Cotton leaf curl virus</i>	<i>Gossypium sp.</i>	Pakistan	AJ132344	Mansoor et al. (1999)
Cotton leaf curl Gezira alphasatellite	CLCuGeA	<i>Cotton leaf curl Gezira virus</i>	<i>Solanum lycopersicum</i>	Sudan	KC763634	Fiallo-Olivé et al. (2013)
Cyamopsis tetragonoloba leaf curl alphasatellite	CyTLCA	<i>Gaur leaf curl virus</i>	<i>Guar sp.</i>	India	GU385877	Kumar et al. (2010)
Lantana yellow vein alphasatellite	LYVA	<i>Lantana yellow vein mosaic virus</i>	<i>Lantana sp.</i>	India	KC206075	Marwal et al. (2013a)
Malvastrum yellow mosaic Cameroon alphasatellit	MYMCA	<i>Tomato leaf curl Cameroon virus</i>	<i>Solanum lycopersicum</i>	Cameroon	FN675298	Leke et al. (2011)
Melon chlorotic mosaic alphasatellite	MeCMA	<i>Melon chlorotic mosaic virus</i>	<i>Melon</i>	Venezuela	KF670682	Romay et al. (2014)
Mimosa yellow leaf curl alphasatellite	MiYLCA	<i>Mimosa yellow leaf curl virus</i>	<i>Mimosa</i>	Vietnam	DQ641719	Ha et al. (2008a)
Okra leaf curl Oman alphasatellite	OLCOMA	<i>Okra leaf curl virus</i>	<i>Abelmoschus esculentus</i>	Oman	KF267445	Akhtar et al. (2014)
Okra yellow crinkle alphasatellite	OYCrCA	<i>Okra yellow crinkle virus</i>	<i>Abelmoschus esculentus</i>	Cameroon	FN675288	Leke et al. (2011)
Sida yellow vein China alphasatellite	SYVCA	<i>Tomato yellow leaf curl virus (TYLCV)</i>	<i>Solanum lycopersicum</i>	Pakistan	KC677736	Shahid et al. (2014)
Sida yellow vein Vietnam alphasatellite	SYVVA	<i>Sida yellow vein Vietnam virus</i>	<i>Sida rhombifolia</i>	Vietnam	DQ641718	Ha et al. (2008a)
Tobacco curly shoot alphasatellite	TCSA	<i>Tobacco curly shoot virus</i>	<i>Nicotiana benthamiana</i>	China	NC_005057	Xie et al. (2004)
Tobacco leaf curl PUSA alphasatellite	TLCPA	<i>Tobacco leaf curl Pusa virus</i>	<i>Nicotiana tabacum</i>	India	NC_014597	Singh et al. (2011)

(continued)

Table 1 (continued)

Satellite	Acronym	Associated Begomovirus	Infected Crop	Origin	Accession #	Reference
Tomato yellow leaf curl China aphasatellite	TYLCChA	<i>Tomato leaf curl China virus</i>	<i>Duranta sp.</i>	Pakistan	AM749494	Unpublished
Vernonia yellow vein Fujian aphasatellite	VYVFA	<i>Vernonia yellow vein Fujian virus</i>	<i>Vernonia cinerea</i>	China	JF265670	Zulfiqar et al. (2012)
<i>Deltasatellites</i>						
Croton yellow vein deltasatellite	CrYVD	<i>Croton yellow vein mosaic virus</i>	<i>Croton bonplandianus</i>	India	AJ968684	Unpublished
Malvastrum leaf curl deltasatellite	MaLCuD	<i>Malvastrum leaf curl virus</i>	<i>Malvastrum coromandelianum</i>	China	KF433066	Unpublished
Sida golden yellow vein deltasatellite 1	SIGYVD1	<i>Sida golden yellow vein virus</i>	<i>Malvastrum coromandelianum</i>	Cuba	JN986808	Fiallo-Olivé et al. (2012)
Sida golden yellow vein deltasatellite 2	SIGYVD2	<i>Sida golden yellow vein virus</i>	<i>Malvastrum coromandelianum</i>	Cuba	JN819490	Fiallo-Olivé et al. (2012)
Sida golden yellow vein deltasatellite 3	SIGYVD3	<i>Sida golden yellow vein virus</i>	<i>Malvastrum coromandelianum</i>	Cuba	JN819498	Fiallo-Olivé et al. (2012)
Sweet potato leaf curl deltasatellite 1	SPLCD1	<i>Sweet potato leaf curl virus</i>	<i>Sweet potato</i>	Spain	FJ914390	Unpublished
Sweet potato leaf curl deltasatellite 2	SPLCD2	<i>Sweet potato leaf curl virus</i>	<i>Merremia dissecta</i>	Venezuela	KF716173	Unpublished
Sweet potato leaf curl deltasatellite 3	SPLCD3	<i>Sweet potato leaf curl virus</i>	Unidentified host	Puerto Rico	KT099179	Rosario et al. (2016)
Tomato leaf curl deltasatellite	ToLCD	<i>Tomato leaf curl virus</i>	<i>Solanum lycopersicum</i>	Australia	U74627	Dry et al. (1997)
Tomato yellow leaf distortion deltasatellite 1	ToYLDD1	<i>Tomato yellow leaf distortion virus</i>	<i>Sidastrum micranthum</i>	Cuba	JN819495	Fiallo-Olivé et al. (2012)
Tomato yellow leaf distortion deltasatellite 2	ToYLDD2	<i>Tomato yellow leaf distortion virus</i>	<i>Sidastrum micranthum</i>	Cuba	KU232893	Fiallo-Olivé et al. (2012)



(Paprotka et al. 2010). Alphasatellites do not truly represent satellite molecules because of their self-encoded replication-associated protein (Rep) and autonomous replication ability (Briddon et al. 2004) and could survive in permissive hosts (Mansoor et al. 1999). However, for encapsidation, transmission by the insect vector, and *in planta* movement, they are reliant on helper viruses.

The genome of alphasatellites comprised of ~1380 nucleotides (nt) that encode a single open reading frame (ORF): *Rep* (36 kDa) subsiding in virion-sense strand (coding strand), a highly conserved A-rich genomic sequence (~200 nt), and an origin of replication (*Ori*) containing a conserved nonanucleotide sequence (TAGTATT/AC) present in a predicted hairpin structure (Fig. 1a) (Briddon et al. 2004). The nonanucleotide sequence and the Rep-encoding segments of alphasatellite genome resemble the nanoviruses (another family of ssDNA viruses) (Brown et al. 2012), which suggests their possible capture by a begomovirus during mixed infections (Briddon and Stanley 2006). It is presumed that the captured Rep-encoded component (~1000 nt) of nanoviruses was reorganized through embedding A-rich sequences to gain a ~1400 nt size (half the size of begomovirus, i.e., ~2800 nt) in order to encode a structurally stabilized Rep required for self-encapsidation (Briddon and Stanley 2006; Mansoor et al. 2003). Three different subclasses of alphasatellites, DNA-1-type, DNA-2-type, and DNA-3-type, are frequently reported. The most commonly occurring alphasatellites are DNA-1-type that predominantly occur in the Indian subcontinent (Paprotka et al. 2010). The DNA-2-type alphasatellites are far rare, found in Singapore and Oman having low nt sequence identity with DNA-1-type, while DNA-3-type are novel alphasatellites detected from Guatemala, Brazil, and Puerto Rico (Rosario et al. 2016). According to Rosario et al. (2016), the DNA-3-type alphasatellites share 51–55% nt sequence identity with the DNA-1-type. Moreover, they help to increase the symptom severity in the host plants and form a separate monophyletic group when analyzed through phylogenetic studies (Rosario et al. 2016).

Although predominantly alphasatellites are found to be associated with begomoviruses, quite recently, an alphasatellite has been found associated with a mastrevirus, *Wheat dwarf India virus* (WDIV), in a natural field infection, which shows that alphasatellites have fewer constraints for their helper virus, host plant, or the insect vector. Their enigmatic role in virus pathogenesis has not been clearly answered yet. However, in few studies, the Rep proteins of alphasatellites have been described as the post-transcriptional gene silencing (PTGS) suppressors (Nawaz-ul-Rehman et al. 2010). Alphasatellites still need extensive explorations in plant virology as there are no consolidated reports available that describe their precise function and association mechanism with begomoviruses.

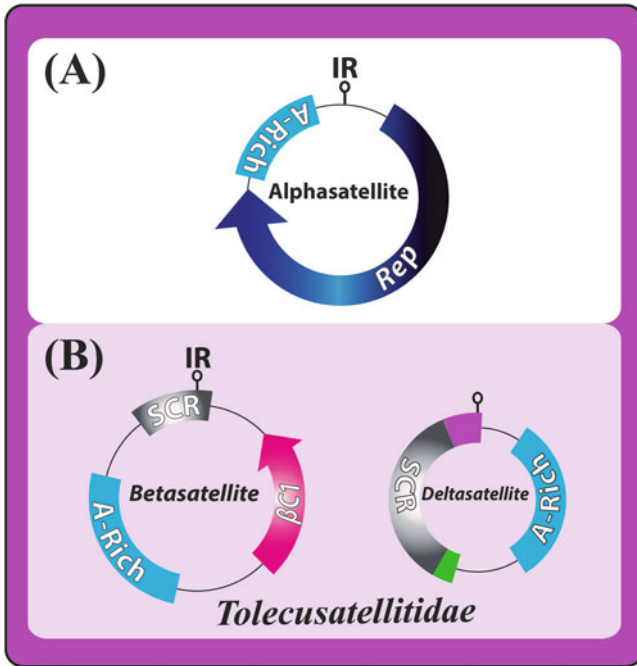
## 2.2 Betasatellites

Betasatellites (formerly described as DNA $\beta$ ) are cssDNA-satellite molecules, which have recently been classified into the sub-viral family *Tolecusatellitidae* genus *Betasatellite* (Adams et al. 2017) (Fig. 1b). Betasatellites are predominantly found

to be associated with monopartite begomoviruses in the OW; however, since the last few years, these molecules have also been found in association with bipartite begomoviruses (Hameed et al. 2017; Jyothsna et al. 2013) and recently with a mastrevirus (Kumar et al. 2014). Unlike alphasatellites, betasatellites are true satellite molecules as they entirely depend on the helper virus for their encapsidation, replication, and systemic dispersal (Briddon and Stanley 2006). Betasatellites variably interact with their helper-virus component and result in multiple types of coinfections. In some cases, the betasatellites synergistically infect the host plants by increasing their helper-virus accumulation and are essential for symptom induction (Chandel et al. 2016). For example, the interactions of *Cotton leaf curl Multan virus* (CLCuMuV) with *Cotton leaf curl Multan betasatellite* (CLCuMuB) and *Ageratum yellow vein virus* (AYVV) with *Ageratum yellow vein betasatellite* (AYVB) result in severe disease symptoms and enhanced virus titer as compared to their helper begomovirus alone in cotton and *A. conyzoides*, respectively (Briddon et al. 2001; Saunders et al. 2000). In other cases, a facultative interaction has also been observed in begomovirus:betasatellite complex, where the begomovirus component could infect alone and does not necessarily require betasatellite for symptom induction and/or enhanced viral titer (Chandel et al. 2016). For example, *Tobacco curly shoot virus* (TbCSV):(TbCSB) could make coinfections but TbCSV could infect alone; however, the presence of betasatellite induces more severe symptoms (Li et al. 2005). Another promising role played by betasatellite is the substitution of DNA-B component. Coinoculation of CLCuMuB and *Tomato leaf curl New Delhi virus* (ToLCNDV) DNA-A induced leaf curl disease phenotype in the model plant *Nicotiana benthamiana* in the absence of DNA-B component (Saeed et al. 2007). Since their first description in 2000, genome sequences of more than 1000 betasatellite isolates have been submitted to GenBank, depicting their ongoing diversity and evolution (Adams et al. 2017) (Fig. 2).

The betasatellite genome (~1350 nt) exhibits three conserved features: a complementary-sense single  $\beta C1$  gene (Briddon et al. 2003), a highly conserved A-rich region (~150-200 nt), and a ~100 nt satellite conserved region (SCR) (Nawaz-ul-Rehman and Fauquet 2009). The betasatellite genome shares no sequence homology with their cognate viruses except for a similar nonanucleotide sequence (TAATATTAC) present in the SCR (Briddon et al. 2003). Betasatellite-encoded  $\beta C1$  (13-14 kDa) is a multifunctional protein involved in pathogenesis, enhancing viral DNA accumulation in the nucleus and suppression of the host antiviral defense response (Saunders et al. 2004). The other important ability of  $\beta C1$  is self-interaction and localization at cell periphery, thus presumably having a role in viral movement (Cheng et al. 2011). Additionally,  $\beta C1$  interacts with numerous host factors like ubiquitin-conjugating enzymes (UBC) (Eini et al. 2009) and asymmetric leaves 1 (AS1) factor, etc. (Yang et al. 2008).

The studies on geographical occurrence and diversity of betasatellites showed that the major center of their diversity lies in the Indian subcontinent and Southeast Asia (Fig. 2). On the basis of phylogeny, betasatellites may be broadly categorized into two major groups: the first group constitutes all the betasatellites isolated from the plant family *Malvaceae* (hibiscus, cotton, okra, hollyhock, etc.) while the



**Fig. 2** Geographical genetic diversity of betasatellites. The map shows the diversification of betasatellites in colored parts, and the number of species identified in each country is labeled in respective colored boxes

betasatellites belonging to the second group were isolated from non-malvaceous plants (tomato, chillies, ageratum, zinnia, etc.). These findings are suggestive of the important role of host plants in the evolution of betasatellites.

### 2.3 Deltasatellites

Recently, another class of cssDNA satellites (approx. quarter the size of helper begomoviruses) has been classified as a new genus “*Deltasatellite*” (family *Toleucusatellitidae*) (Fiallo-Olivé et al. 2012; Lozano et al. 2016). Deltasatellites have been further categorized into three types of noncoding DNA satellites, i.e., *Tomato leaf curl virus*–satellite (ToLCV-sat) identified from Australia (Dry et al. 1997), DNA satellites associated with sweepoviruses in Venezuela and Spain (Lozano et al. 2016), and those isolated from malvaceous hosts in the Caribbean (Fiallo-Olivé et al. 2012). Besides their structural resemblance (Fig. 1b), phylogenetically deltasatellites are not closely related to each other; however, they entirely depend upon the helper virus for their vital functions. Their genome contains a stem-loop structure with a nonanucleotide (TAATATT/AC) sequence, an A-rich region, and does not encode any putative ORF. These satellites have a second

putative stem-loop structure situated close to the iteron-like sequences, and a short region that resembles the SCR of the betasatellites. Contrary to the betasatellites, the emergence of deltasatellites in the NW might be due to agricultural trades of infected plants, like sweet potato, from OW to the NW (Lozano et al. 2016).

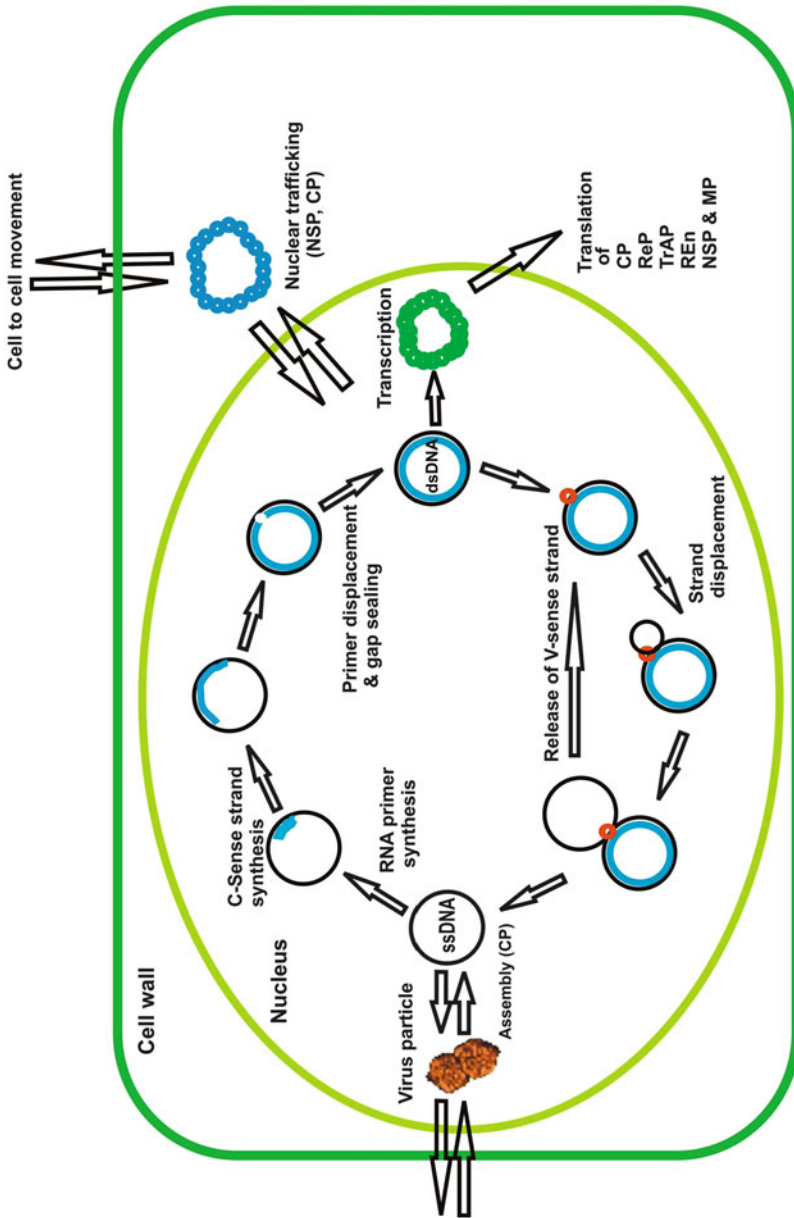
To date, the precise function of deltasatellites in context to begomovirus-deltasatellite complex is unclear, although some studies reported the influence of deltasatellite in lowering helper-virus accumulation in host plant that might facilitate the sequential movement of viruses to other plant parts (Fiallo-Olivé et al. 2016; Hassan et al. 2016).

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### 3 Replication Mechanism of DNA Satellites

In the course of successful infection after entering the host plant cell, begomoviral ssDNA genomes along with the genome of the associated DNA satellite(s) access the cell nuclei for replication. The replication of begomovirus genome is achieved through dsDNA intermediates either by a rolling circle replication (RCR) and/or recombination-dependent replication (RDR) mechanism (Hanley-Bowdoin et al. 2013) (Fig. 3). The dsDNA intermediates are transcribed by host RNA-polymerase II to translate the first viral protein Rep, which then initiates RCR of both the begomovirus and the satellite DNA (Hanley-Bowdoin et al. 2013). These circular dsDNAs are assembled into transcriptionally active viral mini-chromosomes with the help of host histone proteins (Pilartz and Jeske 2003). The viral Rep protein creates a conducive environment to commence the replication. The Rep protein of begomoviruses drives the initiation and termination of RCR by nicking the dsDNA intermediates and rejoining the circular DNA at the specific site in the nonanucleotide sequences (TAATATT/AC) (Hanley-Bowdoin et al. 2013; Laufs et al. 1995). The successful commencement of RCR specifically requires high-affinity interactions between the begomovirus Rep protein and *ori* in the intergenic region (IR). The synthesis of complementary strand is initiated with a nick by Rep protein in the nonanucleotide sequence of the ssDNA, and an RNA primer is synthesized by the host DNA primase to initiate this phenomenon. The host plant replisome machinery (DNA polymerase and associated factors) is hijacked and reprogrammed during the elongation step to accomplish the viral dsDNA synthesis (Bagewadi et al. 2004; Kaliappan et al. 2011). The newly synthesized strand is displaced and released by Rep as a circular ssDNA. The synthesized dsDNA is used as a template to start next replication cycle. At the end of optimum replication cycles, the Rep protein downregulates its own synthesis and ultimately activates the expression of TrAP, which leads to the production of CP to start the virus and DNA satellites assembly (Fig. 3).

The DNA satellites employ a similar mechanism of DNA replication as their helper begomovirus (Alberter et al. 2005). However, beta- and deltasatellites are devoid of Rep protein (unlike begomovirus and alphasatellites) and hence depend exclusively upon the helper virus to commence their replication (Zhou 2013). Alphasatellites are capable of autonomous replication mechanism through RCR



**Fig. 3** Pictorial model of replication mechanism of begomoviruses/satellites. Once the virion DNA is delivered to the nucleus, host machinery initiates the synthesis of the complementary strand. Host-derived polymerases convert the single-stranded virion DNA into a double-stranded intermediate, which performs

directed by their own protein, alpha-Rep. As betasatellites are devoid of self-replication, the replication strategy of the betasatellites is determined by the helper begomovirus (Alberter et al. 2005). The replication model for begomoviruses suggests the imperative binding of Rep protein to the iterative sequences upstream of the nonanucleotide sequences followed by the recognition of the *ori*. Apparently, betasatellites frequently lack the iteron sequences, which is suggestive of some other mechanism involved in the *ori* recognition of betasatellites (Leke et al. 2012). The nicking site for Rep in betasatellites is expected to present in the nonanucleotide stem-loop sequences adjacent to the SCR. The stem sequences and the adjacent hairpin structures of betasatellites are remarkably similar in all betasatellites, and thus, it reaffirms that they participate in the *ori* recognition by helper Rep (Zhou 2013). The position of the highly conserved SCR present immediately upstream of the stem-loop sequences is also analogous to the relative position of IR of the helper begomoviruses. Furthermore, the conservation of SCRs in the defective forms of betasatellites further supports that the SCR region is involved in the replication of the betasatellites (Zhang et al. 2016). However, the sequence between the downstream of SCR and  $\beta$ C1 is required for efficient replication of betasatellites (Eini and Behjatnia 2016). The betasatellites lack iteron-like Rep-binding motifs (RBM); thus, the presence of G-box motif (CACGTG) may serve the binding of Rep protein (Eini and Behjatnia 2016). Thus, the high-affinity binding of Rep has a critical role in betasatellite replication. However, the exact mechanism of Rep binding is needed to be explored yet.

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#### 4 Transreplication and Pseudo-Recombination of DNA Satellites

As discussed earlier, the only sequence homology between the betasatellites and their helper begomoviruses is the presence of stem-loop structure (Briddon and Stanley 2006). In contrast to DNA-B of bipartite begomoviruses, betasatellites do not necessarily require cognate DNA-A of a bipartite or monopartite begomovirus genome. Instead, they are transreplicated by a diverse range of non-cognate

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**Fig. 3** (continued) as a transcriptionally active mini-chromosome. This mini-chromosome mediates the cell-to-cell movement and nuclear trafficking. Virion derived replication-associated protein (Rep) then binds to the iterons, produces a site-specific nick in the origin of replication (*ori*), and becomes covalently linked to the 5' end of the nicked DNA via a tyrosine residue. The 3'OH end acts as a primer for synthesis of new virion-sense DNA by host-encoded factors, using the complementary-sense as a template. The nicking-joining activity of Rep releases unit length virion-sense ssDNA molecules. The newly synthesized ssDNA either continues the replication cycle (acting as a template for complementary-strand synthesis), is moved from cell-to-cell (possibly as virions), or is packaged by the coat protein (CP) for onward transmission by insect vectors. Image was reproduced from Briddon and Stanley (2006)

begomoviruses for their transreplication. For example, *Cotton leaf curl Gezira betasatellite* (CLCuGeB) is known to be a cognate associate of *Okra yellow crinkle virus* (OYCrV) and *Cotton leaf curl Gezira virus* (CLCuGeV) causing okra leaf curl disease (Leke et al. 2013). However, it can also be transreplicated with three other distinct begomoviruses *Tomato yellow leaf curl virus* (TYLCV), *Tomato leaf curl Mali virus* (ToLCMLV), and *Tomato yellow leaf crumple virus* (TYLCrV), each from a diverse geographic origin (Saunders 2008). Similarly, CLCuMuB is a cognate member of CLCuD-associated begomoviruses (CABs) in Asia (Iqbal et al. 2012; Sattar et al. 2017). The non-cognate associations between CLCuMuB with ToLCNDV and TbCSB with *Clerodendrum golden mosaic China virus* (CGMCV) are significant examples of its transreplication by a bipartite begomovirus (Li and Zhou 2010; Saeed 2010). Moreover, CLCuMuB can also be transreplicated by other non-cognate viruses such as TYLCV, *Tomato leaf curl Karnataka virus* (ToLCKnV), and *Tomato leaf curl virus* (ToLCV) (Kharazmi et al. 2012). Apart from begomoviruses, betasatellites are also known to be transreplicated by the members of other genera of the family *Geminiviridae*. *Beet curly top virus* (BCTV; genus *Becurtovirus*) successfully transreplicates *Ageratum yellow vein betasatellite* (AYVB) and *Tomato yellow leaf curl China betasatellite* (TYLCCNB) (Yang et al. 2011a). In another study, a *Curtovirus*, *Beet severe curly top virus* (BSCTV), successfully supported the transreplication of CLCuMuB (Kharazmi et al. 2012). Likewise, association of Cotton leaf curl Multan alphasatellite (CLCuMuA), Guar leaf curl alphasatellite (GLCuA), and *Ageratum yellow leaf curl betasatellite* (AYLCB) with the WDIV (genus *Mastrevirus*) highlights the natural transreplication of DNA satellites by the member of a different genus (Kumar et al. 2014). Such associations are quite surprising because the functional betasatellites are mostly known to be associated with monopartite begomoviruses during natural infections. Moreover, the DNA-B component of bipartite begomoviruses has specific interactions with the Rep of the cognate DNA-A only. Such indistinguishable replication of betasatellites depicts that these molecules are quite flexible for their transreplication as compared to the specificity of recognition between Rep protein and DNA-B component of a bipartite begomovirus.

Apart from the fact that betasatellites have quite a promiscuous mode of replication, two distinct betasatellite species rarely coexist with a single helper virus within the same host plant. Apparently, this is because betasatellites are adapted to their cognate helper virus for replication during the course of evolution (Zhou et al. 2003). Thus, the cognate betasatellites are shown to accumulate to higher levels than the non-cognate betasatellites within the same host (Qing and Zhou 2009). For example, the coinoculation of TYLCCNB and TbCSB with one helper virus creates a competition, which causes cognate betasatellite dominant over non-cognate betasatellite. However, switching their sequence elements also switched the preferential replication of the respective cognate helper virus (Zhang et al. 2016).

Under natural environmental conditions, although betasatellites may coexist with the alphasatellites, the binding of alpha-Rep with the Rep protein of the helper virus may obstruct betasatellite replication. The alphasatellites can ameliorate begomovirus symptoms and hinder high accumulation of betasatellites during coinfections (Idris et al. 2011).

The deltasatellites contain a stem-loop with nonanucleotide, TATA box, and a second predicted stem-loop with iteron-like sequences. Moreover, their A-rich region and a short region also share high homology with betasatellite SCRs (Lozano et al. 2016). However, further investigations are needed to decipher their mode of replication and roles in viral pathogenesis. Most probably, these molecules are also transreplicated by the helper begomovirus Rep due to the presence of begomovirus iteron-like sequences upstream of the second stem loop (Fiallo-Olivé et al. 2016). The deltasatellite, Tomato leaf curl virus-satellite (ToLCV-sat), has been shown to be transreplicated by ToLCV as well as geographically distinct geminiviruses like *Tomato yellow leaf curl Sardinia virus* (TYLCSV), *African cassava mosaic virus* (ACMV), and a becurtovirus BCTV. In another study, the deltasatellites sat-177 and sat-603 could only be transreplicated by the cognate begomoviruses, *Sida golden yellow vein virus* (SiGYVV), and a monopartite begomovirus *Tomato leaf deformation virus* (ToLDV) from the NW. However, the OW TYLCV, TYLCSV, and ACMV could not support their transreplication (Fiallo-Olivé et al. 2016).

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## 5 Deciphering the Role of DNA Satellites in the Begomovirus Pathogenesis

All the functions of betasatellites are accredited to their single gene product  $\beta$ C1. This protein, when expressed transiently through *Potato virus X* (PVX) or through a stable transformation in model host plants (*N. benthamiana* and *N. tabacum*) cells, induces typical begomovirus disease symptoms of leaf curling, vein thickening, and enations (Kon et al. 2007; Qazi et al. 2007). The  $\beta$ C1 protein regulates the expression of several different miRNAs involved in the developmental processes when expressed through PVX in *N. benthamiana* plants. The accumulation of miR159 and miR160 was significantly enhanced, while the accumulation of miR164, miR165/166, miR169, and miR170 was reduced when the  $\beta$ C1 gene was transiently expressed in the inoculated plants (Amin et al. 2011). The  $\beta$ C1 accumulates primarily in the nucleus, localizes at the periphery of the infected host cells, and colocalizes along the endoplasmic reticulum. These localization patterns and presence of both nuclear import and export signals point toward the putative role of  $\beta$ C1 in intracellular transport and movement (Cheng et al. 2011). Moreover,  $\beta$ C1 forms punctate bodies, both in vivo and in vitro, by self-interaction, which presumably has a role in symptom induction. A deletion mutagenesis study shows that amino acids spanning two  $\alpha$ -helices at C-terminal are important in self-interaction.

The  $\beta$ C1 interacts with a variety of host-encoded factors such as with AS1 and AS2 factors. Self-interaction of these two factors is required for leaf development;  $\beta$ C1 mimics the function of AS2 by interacting with AS1 and thus affects the leaf development (Yang et al. 2008). The CLCuMuB-encoded  $\beta$ C1 interacts with a UBC to induce betasatellite-specific symptoms in the host plant (Eini et al. 2009). It is speculated that  $\beta$ C1 interaction with UBC perturbs the ubiquitin-proteasome



pathway to enhance  $\beta$ C1 accumulation, which ultimately led to the development of viral symptoms.

Plants have developed a fine-tuned defense mechanism, which is operated through PTGS and TGS, against invading pathogens. To counter the host defense response,  $\beta$ C1 has the ability to suppress the PTGS-mediated host defense by interacting with one of the important host defense components, Argonaute-1 (AGO-1), which binds to the siRNAs and represses the target RNAs (Eini 2017). The  $\beta$ C1 protein can bind to ss- as well as dsDNA, dsRNA, and both long and short RNAs in a sequence-independent manner to suppress the host defense. This binding activity is mediated by the nuclear localization signals (NLS) present in the  $\beta$ C1. TYLCCNB-encoded  $\beta$ C1 has the ability to suppress PTGS by upregulating the *N. benthamiana* calmodulin-related protein (Nbrgs-CaM), which can repress the expression of *N. benthamiana* RNA-dependent RNA polymerase 6 (Zhou 2013). Besides PTGS, the  $\beta$ C1 also has the ability to reduce the TGS or can even reverse the established TGS (Yang et al. 2011b) in the host plants. This suppression of TGS is mediated by interacting with S-adenosyl homocysteine hydrolase (SAHH), an enzyme generally required for the generation of S-adenosyl methionine (SAM), through a NLS (49KKK51) present in  $\beta$ C1 (Yang et al. 2011b). The CLCuMuB- $\beta$ C1 can suppress the host defense by downregulating the jasmonic acid (JA)-responsive genes such as COR13, PR4, NbPHAN, and PDF1 (Yang et al. 2008) and can interact with certain host-encoded factors involved in metabolic and defense pathways (Tiwari et al. 2013). The expression of  $\beta$ C1 can differentially regulate the genes involved in electron carrier for photosynthesis, respiration, and ATP synthesis (Andleeb et al. 2010). The CLCuMuB- $\beta$ C1 also interacts with ATG8 protein, a ubiquitin-like protein having a role in the biogenesis of autophagosomes (Shelly et al. 2009). It is thus speculated that the interaction of  $\beta$ C1 with ATG8 may likely be an antiviral defense mechanism. Besides,  $\beta$ C1 can interact with tomato UBC, an enzyme required for ubiquitination and ultimately the degradation of the target protein (Eini et al. 2009). This interaction interferes with ubiquitin-proteasome pathway that could enhance the  $\beta$ C1 accumulation.

To counter the  $\beta$ C1 pathogenesis, host plants have developed a sophisticated counterattack mechanism. Tomato plants employed SucroSenonfermenting1-related kinase (SISnRK1)-mediated defense against the betasatellites. Hyperexpression of SISnRK1 leads to the reduction in betasatellite accumulation and delayed onset of the symptoms. It has been showed that SISnRK1 phosphorylates TYLCCNB- $\beta$ C1 at the amino acid positions 33 (serine residue) and 78 (threonine), thus negatively regulating the  $\beta$ C1 functions (Cui et al. 2004; Yang et al. 2008).

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## 6 Role of Rep-A of Alphasatellite in Viral Pathogenesis

To date, the interactions of the alphasatellite-encoded Rep protein with the host-encoded factors and its role in successful begomovirus infection have not been fully explored. Only a few studies are available, which reported that the Rep protein

encoded by few alphasatellites have PTGS suppressor activity (Nawaz-ul-Rehman et al. 2010), suggesting the role of alphasatellite in overcoming RNAi-mediated host defense. The type-2 alphasatellites are known to have a role in the symptom attenuation by reducing the accumulation of begomovirus (Nawaz-ul-Rehman et al. 2010) and/or betasatellites (Idris et al. 2011; Wu and Zhou 2005) in the begomovirus–betasatellite complexes. This attenuation in symptoms may likely increase the chance of host survival and virus transmission.

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## 7 Conclusion

Host–pathogen interactions are like arms race with typical zero-sum game, which ultimately leads to the disease development or the host recovery. In this subtle type of intimate relationship, both counterparts continuously deploy different strategies to take advantage over each other. The acquisition of DNA satellites by begomoviruses is the continuity of this process. DNA satellites have equipped their helper begomoviruses to suppress the host defense (both TGS and PTGS) and/or help in the symptom attenuation, which ultimately helps the virus to evade the host defense. During the acquisition process, begomoviruses resized these DNA satellites precisely in a mathematical way, alpha- and betasatellites are almost half, while deltasatellites are one-fourth of the helper-virus genome, to support their replication.

The maintenance of these DNA satellites by the helper-virus replication machinery is dependent upon dynamic, mainly undefined, interactions between begomovirus, DNA satellite, and host-encoded factors. However, *ori* is the only common feature between DNA satellites and the helper viruses, so interaction between the geminivirus Rep and DNA satellites is principally dependent on this region. Likewise, *ori* region (particularly nonanucleotide) determines the successful commencement of RCR. The importance of *ori* in replication has been proven experimentally where switching of the *ori* sequence has switched the preferential transreplication of betasatellite by helper begomovirus (Zhang et al. 2016). Although, no strong selection mechanism is present between DNA satellites and their helper virus, the interaction between DNA satellites and their helper virus is not merely a transreplication but stacking of a multilayer interaction (Iqbal et al. 2012, 2017).

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