Review

Multifaceted role of geminivirus associated betasatellite in pathogenesis

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SUMMARY

Begomoviruses have emerged as a group of plant pathogens that cause devastating diseases in a wide range of crops in tropical and subtropical regions of the world. Betasatellites, the circular single-stranded DNA molecules with the size of almost half of that of the associated helper begomoviruses, are often essential for the production of typical disease symptoms in several virus-host systems. Association of betasatellites with begomoviruses results in more severe symptoms in the plants and affects the yield of numerous crops leading to huge agroeconomic losses. βC1, the only protein encoded by betasatellites, plays a multifaceted role in the successful establishment of infection. This protein counteracts the innate defence mechanisms of the host, like RNA silencing, ubiquitin-proteasome system and defence responsive hormones. In the last two decades, the molecular aspect of betasatellite pathogenesis has attracted much attention from the researchers worldwide, and reports have shown that βC1 protein aggravates the helper begomovirus disease complex by modulating specific host factors. This review discusses the molecular aspects of the pathogenesis of betasatellites, including various βC1-host factor interactions and their effects on the suppression of defence responses of the plants.

Keywords: βC1, betasatellites, chloroplast, defence, Geminivirus, interaction, pathogenesis.

INTRODUCTION

Specific interactions between the virus and the host proteins are prerequisites for both the pathogenicity determinant to execute its virulence function and the plant to activate its anti-virus surveillance mechanisms (Kong et al., 2014). But the complexities of such interactions impose challenges in understanding the detailed mechanisms of the defence responses of the plant generated against the invading viruses. The defence responses in plants are manifested through the pathways like R-gene-mediated defence, RNA silencing, ubiquitin-mediated proteasomal degradation etc. and involve several host factors that adversely regulate virus accumulation (Bhat et al., 2013; Bhattacharyya et al., 2015; Eini et al., 2009b; Li et al., 2014a; de Ronde et al., 2014; Shen et al., 2016).

The plant hormones such as salicylic acid (SA) and jasmonic acid (JA) contribute to the defence responses by eliciting the expressions of specific hormone-responsive genes. These gene products generate an antiviral state that restricts the invading pathogens (Spoel et al., 2007). During both compatible and non-compatible host-pathogen interactions, plants undergo autophagy (Alazem and Lin, 2015), which as an innate immunity response, degrades the viral protein(s) and reduces the viral infection (Haxim et al., 2017). In addition, necrotrophic pathogens, biotrophic pathogens and plant viruses induce the production of defence-related reactive oxygen species (ROS), which could also activate autophagy. Furthermore, chloroplasts, as the sites for production of defence hormones, play a central role in innate immunity of the plants and aid in restricting the viral spread and systemic infection (Bhattacharyya and Chakraborty, 2018). Furthermore, the photosystem-II dependent defence-related ROS production also could induce autophagy and programmed cell death (Doyle et al., 2010).

Geminiviruses severely interfere with the physiology of the host plants and are responsible for major crop losses in economically important dicots or monocots globally (Navas-Castillo et al., 2011). These viruses belong to a family of small, non-enveloped, single-stranded DNA viruses possessing a circular genome ranging in size from 2.5 kb to 3.2 kb (in case of monopartite viruses) and from 4.8 kb to 5.6 kb (in case of bipartite viruses), and are encapsidated in particles consisting of two joined incomplete icosahedra (Navas-Castillo et al., 2011; Zerbini et al., 2017). The International Committee on Taxonomy of Viruses classified the family Geminiviridae into nine different genera, namely,
Begomovirus, Bemisia tabaci, Alphasatellite, Deltasatellite (Kumar et al., 2015; Mansoor et al., 2016; Hassan et al., 2016).

Monopartite begomoviruses contain a single genome of size approximately 2.7 kb, named as DNA-A. The genomes of bipartite begomoviruses consist of two genomic components namely, DNA-A and DNA-B (Zerbini et al., 2017). DNA-A genome encodes for coat protein (CP, AV1/V1), pre-coat protein (AV2/V2; absent in the New World bipartite begomovirus), replication associated protein (Rep, AC1/C1), transcriptional activator protein (TrAP, AC2/C2), replication enhancer protein (REN, AC3/C3), and C4 protein (AC4/C4). DNA-B genome encodes for nuclear shuttle protein (NSP, BV1) and movement protein (MP, BC1) (Rojas et al., 2005; Zerbini et al., 2017). Studies in the last few decades revealed the association of begomoviruses with molecules like betasatellite, alphasatellite and deltasatellite (Kumar et al., 2015; Mansoor et al., 2006; Nawaz-ul-Rehman and Fauquet, 2009; Saunders et al., 2000). Betasatellites share little sequence similarity with the helper viruses, as many of them are trans-replicated by various helper begomoviruses have made them a serious threat to the agro-economy. Reports have suggested that βC1 protein, the only protein encoded by the betasatellite genome, elevates the disease manifestation by suppressing the plant defence machinery and by augmenting the accumulation of the helper begomovirus disease complex (Bhattacharyya et al., 2015; Li et al., 2014b; Mansoor et al., 1999; Zhong et al., 2017). Although the aspects like the evolution of betasatellites have been reviewed previously (Zhou, 2013; Nawaz-ul-Rehman and Fauquet, 2009), there is no recent reviews on the role of βC1 in disease development. The current review provides an exhaustive account of betasatellite pathogenicity with an emphasis on the molecular aspects of different host-virus interactions. It starts with a brief overview of the genomic organization and the genetic diversity of betasatellites followed by the discussion on the role of betasatellites as an important part of the begomovirus disease complex. Next, various interactions between βC1 and host factors as well as the implications of those interactions on the suppression of plant defence mechanisms and disease development are presented.

Genomic Organization and Replication of Betasatellites

The sequence analysis of different betasatellites associated with various begomoviruses revealed the presence of three common structural features: (i) an A-rich region, (ii) a 150–200 nucleotides long satellite conserved region (SCR) containing a potential hairpin loop structure with nonanucleotide TAATATTAC, (iii) a single ORF encoding multifunctional protein βC1, of size approximately 13 kDa–14 kDa, in its complementary sense-strand. In all known functional betasatellites, the location of this ORF is conserved with the start codon placed between 544 nt and 570 nt, and the stop codon placed between 195 nt and 209 nt from the origin of replication (Briddon et al., 2003; Mansoor et al., 2003a). The replication of a betasatellite is mediated by the replication associated protein (Zerbini et al., 2017) encoded by the helper begomovirus. Betasatellites undergo a rather relaxed interaction with the helper viruses, as many of them are trans-replicated by various helper viruses (Dry et al., 1997; Mansoor et al., 2003a; Ranjan et al., 2014; Saunders et al., 2002). Although betasatellites depend on the Rep proteins of the helper viruses for replication, they usually do not possess the conserved iterons in their genome (Rep binding sequences) like the helper viruses. Through deletion analysis, the origin of replication of betasatellites was identified to be encompassing the SCR, a part of the intergenic
region upstream of the SCR, and the ubiquitous nonanucleotide/stem-loop structure (Saunders et al., 2008). A study with Tomato yellow leaf curl China virus (TYLCCNV), Tobacco curly shoot virus (TbCSV), and their respective betasatellites namely, Tomato yellow leaf curl China betasatellite (TYLCCNB) and Tobacco curly shoot betasatellite (TbCSB) has shown the presence of a novel Rep binding motif (RBM) in the genome of betasatellites. The different binding affinities of RBM with cognate and non-cognate Rep proteins correlate with promiscuous selection and efficiency of trans-replication of various betasatellites (Zhang et al., 2015). The role of host-specific adaptability in the trans-replication and maintenance of betasatellites by various begomoviruses was also demonstrated (Ranjan et al., 2014).

GENETIC DIVERSITY OF BETASATELLITES

Symptom severity caused by the viruses is prompted by their evolutionary fitness, which in turn depends on the internal genetic modifications achieved primarily by mutation, recombination and pseudo-recombination (reassortment) (Seal et al., 2006). In addition, external factors such as climatic changes, synergistic/antagonistic effects of the associated helper viruses and the specific mediating vectors, play roles in the evolution of viruses (Acosta-Leal et al., 2011). Amongst others geminiviruses showed enormous genetic variations from their proposed prokaryotic plasmid origin (Krupovic et al., 2009). Betasatellites rapidly adapt to diverse geminiviral components leading to the generation of unique disease complexes and expanding their ecological niches by increasing the host range, disease severity and enhancement of the vector performance (Mansoor et al., 2006; Nawaz-ul-Rehman and Fauquet, 2009; Patil and Fauquet, 2010; Sattar et al., 2017; Zubair et al., 2017).

At least 66 distinct betasatellites, associated with diverse helper viruses and numerous disease complexes that infect a vast range of hosts, identified from around 20 countries in the Asian, African and European continents belonging to the “Old World” (Table. S1). The majority of these molecules (59/66) was identified from Asia, and amongst these molecules 32 distinct betasatellites were reported from the Indian subcontinent alone (Bangladesh, India, Nepal, Pakistan and Sri Lanka) (Fig. 1). In addition, high genetic diversity amongst betasatellites was found in China, too. Furthermore, the isolates of Cotton leaf curl Gezira betasatellite (CLCuGeB) and Ageratum leaf curl Cameroon betasatellite are the predominant betasatellite groups reported from the West and Central regions of Africa, while tomato leaf curl associated betasatellites are prevalent in Oman (Khan et al., 2014; Leke et al., 2015). Many of these satellites were isolated from

Fig. 1 Geographical distribution of distinct betasatellites across the ‘Old World’ countries. The presence of distinct betasatellites identified from different geographical locations is indicated in multiple colours. The full name of betasatellites mentioned here is provided in Table. S1.
the plants belonging to the families Solanaceae, Asteraceae or
Malvaceae (Table S1).

Betasatellites associated with chilli leaf curl disease in India, for
instance, *Tomato leaf curl Bangladesh betasatellite*, contain
high nucleotide variability and a high nucleotide substitution
rate in the βC1 coding regions (Kumar et al., 2015). The genetic
variation of begomovirus populations is mainly attributed to the
mutational dynamics involving point mutations generated by nu-
cleotide substitution (Lima et al., 2017). As begomoviruses use
the host DNA polymerase, the replication fidelity of these viruses
likely should be the same as that of the host. Therefore, the high
mutation rate might be due to a less stringent repair process of
the geminivirus genomes that lack the proper methylation pat-
terns for the host exonucleases (Sanz et al., 1999). In the case of
the correctly methylated begomovirus genomes, base-excision
repair might not take place as the genomes are only transiently
double-stranded during rolling-circle replication. Nevertheless,
the possibility of the viruses recruiting a more error-prone poly-
merase from the host’s nucleus for their own replication has not
been ruled out (Duffy and Holmes, 2008). Additionally, betasat-
ellites facilitate the conditions that favour the whitefly vectors
and thus, promote the propagation of the disease complexes.
These conditions include induction of the positive behavioural
responses through enhanced linalool emission in plants, suppres-
sion of the host’s anti-herbivory responses and increasing the
fecundity of the vectors (Jia et al., 2012; liu et al., 2007; Li et al.,
2014b; Salvau donation, 2013).

Mixed infection is a source of recombination and pseudo-re-
combination that contribute to begomovirus-betasatellite diver-
sity. Amongst chilli-infesting betasatellites, the A-rich region
and SCR have been reported as hot spots for recombination (Kumar
et al., 2015). Reassortment between CLCuGeB and Tomato yel-
low leaf curl Mali virus caused more severe growth stunting and
deforation of leaves than the usual leaf curling phenotypes in
tomato (Chen et al., 2009).

ASSOCIATION OF BETASATELLITE WITH
DISEASE COMPLEX INFLUENCES THE
BEGOMOVIRUS PATHOGENESIS

The earliest account of a plant virus disease, manifested by the
yellow vein symptom in eupatory plants, was found more than a
millennium ago in Japanese literature. The causative agent of
the disease was later identified as a begomovirus-betasatellite
disease complex (Saunders et al., 2003). Although initially attrib-
uted to *Ageratum yellow vein virus* (AYVV), a monopartite bego-
movirus (Tan et al., 1995), the typical yellow vein symptoms of
*Ageratum conyzoides* infected by the begomovirus (Swanson
et al., 1993) was eventually hypothesized to be associated with the
presence of additional factors (Saunders et al., 2000; Saunders
and stanley, 1999). Subsequently, a complete betasatellite mol-
ecule was isolated from the diseased Ageratum plant (Saunders
et al., 2000). Several studies reported the indispensable role of
betasatellite in the establishment and maintenance of diseases
in the host plants (Briddon et al., 2001; Jose and usha, 2003; Kuma
et al., 2010; Saunders et al., 2000; Singh et al., 2012).

Phylogenetic analyses of the viral genomes showed that beta-
satellites have undergone co-evolution with their helper viruses
(Zhou et al., 2003). Betasatellites are widespread amongst the
‘Old World’ begomoviruses that are mostly monopartite in na-
ture (Kumar et al., 2015; Saeed et al., 2007; Zubair et al., 2017).
In addition, betasatellites have also been reported to be associated
with a few bipartite begomoviruses such as *Sri Lankan cassava
mosaic virus, Tomato leaf curl Gujarat virus* and *Tomato leaf curl
New Delhi Virus* (ToLCNDV) (Jyothsna et al., 2013; Ranjan et al.,
2014; Sivalingam and Varma, 2012). This promiscuity is particu-
larly threatening for the agronomy as, even in the absence of any
helper begomovirus, a betasatellite can be maintained by a mas-
treivirus in the field-grown wheat plants elevating the accumula-
tion of the helper virus (Kumar et al., 2014). Such associations of
betasatellites with the viruses of different genera might generate
severe disease complexes that may invade new economically im-
portant crops. Furthermore, the synergistic interaction between
the betasatellite and multiple helper viruses enhanced the viral
DNA replication in resistant chilli cultivar and might result in
breakdown of the natural resistance (Singh et al., 2016).

THE CONTRIBUTION OF THE A-RICH REGION
AND SCR IN BETASATELLITE-MEDIATED
DISEASE DEVELOPMENT

As mentioned earlier, apart from the ORF of the pathogenicity
determinant βC1, the biologically active betasatellite molecules
contain two other conserved features—an A-rich region and an
SCR (Briddon et al., 2003). The conserved A-rich region, present
even in the naturally occurring but the truncated betasatellites,
is probably a ‘stuffer’ sequence essential in maintaining the size
of the betasatellite genome (Briddon et al., 2003; Mansoor et
al., 2003b). Although replication and encapsidation were not
affected in the betasatellites with deleted A-rich regions, such
mutant betasatellites induced milder symptoms on *Nicotiana
benthamiana*. This region includes the putative enhancer el-
ments for the βC1 promoter and hence might regulate the
symptom severity by affecting the protein expression (Tao et al.,
2004). The promoter of TYLCCNB is phloem-specific and capable
of inducing vein thickening in the host (Guan and Zhou, 2006),
while the promoter of TbCSB, which is constitutively expressed,
is unable to induce similar symptoms. The study of Ding et al.
(2009) involved different hybrid molecules containing the pro-
moters and βC1-ORFs of TYLCCNB and TbCSB. The result of this
study revealed that the promoter of βC1 indeed influences the
beta1-modulating the cellular niche through specifically interacting or C1 protein performs its pathogenicity function by plant cell, upon begomovirus-betasatellite infection (Xiao et al., 2014). Responsive miRNAs were found to be differentially regulated suppressor proteins to counter this mechanism (Pumplin and Host plants use the RNA silencing mechanism as an effective pathway of the host (Briddon et al., 2001). Nuclear localization mechanism of how different C1 proteins interfere with the silencing amongst them, a fact that suggests the plurality in the mechanism. Also, the study of Guan and Zhou (2006) suggested that the vein swelling and enation symptoms induced by TYLCCNV-DNA infection in the tobacco plants might be due to the phloem-specific expression of the βC1 gene. These reports indicate that although the pathogenicity factor βC1 protein is the symptom determinant, the promoter of the gene also influences symptom production, at least in the cases of some of the betasatellites. The regulatory element of the viral promoter might interact with distinct host factors (Yin and Beachy, 1995) causing tissue-specific expression of the gene. Also, the genetic elements in the viral promoter are exposed to the post-transcriptional gene silencing (PTGS) machinery and the methylation-mediated suppression of the plant (Dogar, 2006). The promoter of βC1 of the betasatellite associated with cotton leaf curl disease contains G-box motifs, which is important for the activity of the satellite molecule. This G-box element is capable of binding to the host factors and is important for the replication of the satellite, too (Eini et al., 2009a). Further studies are required to explore the possible role of the non-coding region, including A-rich region and SCR, of the betasatellites in interacting with the host factors and the effect of such interactions in pathogenesis.

THE ROLE OF THE βC1 PROTEIN, A PATHOGENICITY FACTOR, IN DISEASE DEVELOPMENT

Studies in the last two decades have established that βC1 acts as the pathogenicity determinant protein during begomovirus pathogenesis (Zhou, 2013). The multitasking βC1 protein suppresses the host defence responses mediated by PTGS, transcriptional gene slicing (TGS), ubiquitin-proteasome system and defence hormones of the plants (Bhattacharyya et al., 2015; Jia et al., 2016; Yang et al., 2008; Zhou, 2013) (Fig. 2). In an infected plant cell, βC1 protein performs its pathogenicity function by modulating the cellular niche through specifically interacting or targeting the host factors (Table 1). In addition, βC1 protein contributes to the disease and symptom development by assisting the intracellular and systemic transport of the virus and facilitating the virus-vector-host tripartite interactions. The next few sections discuss the prominent molecular aspects of βC1-mediated pathogenicity.

RNA silencing

Host plants use the RNA silencing mechanism as an effective antiviral defence strategy, whereas viruses encode silencing suppressor proteins to counter this mechanism (Pumplin and Voinnet, 2013). For instance, a diverse set of novel and defence responsive miRNAs were found to be differentially regulated upon begomovirus-betasatellite infection (Xiao et al., 2014). Various betasatellites were also reported to modulate the accumulation of virus-derived sRNAs, thereby targeting several stress-related defence proteins and transcription factors like myeloblastoses (MYBs), and subsequently promoting the virus infections (Wang et al., 2016; Yang et al., 2011a). This betasatellite-dependent differential expression of small RNAs could be due to the induction of RNA silencing components such as AGO1 and DCL1, and/or through the interaction of βC1 with AGO1 (Eini, 2017). Additionally, several βC1s have been demonstrated to suppress host-mediated transcriptional and PTGS processes.

Suppression of post-transcriptional gene silencing

The PTGS machinery, which involves sequence-specific degradation of double-stranded foreign RNA, serves as a robust and conserved mechanism employed by the plants to fight against pathogenic viruses, transgenes and transposons (Pumplin and Voinnet, 2013). In the process of plant-virus interaction, double-stranded replicative intermediates and overlapping segments of mRNA transcripts of RNA and DNA viruses, respectively, become the targets of the RNA silencing machinery. These targets are processed by DICER-like proteins (DCL) to dsRNAs of 21–24 nucleotides (Voinnet, 2001), known as siRNAs. These siRNAs are recruited by ARGONAUTE (AGO) proteins to form a nuclease cleaving complex called RNA-induced silencing complex (RISC) that guides binding and cleaving of homologous transcripts of viral pathogens (Hammond et al., 2000). Either viral RNAs or their cleavage products serve as templates for host RNA-dependent RNA-polymerases (RDRs) and yield dsRNAs that after being cleaved by DCLs produce secondary siRNAs.

To counter PTGS of the host, plant viruses encode proteins that suppress the gene silencing at various junctures of the silencing pathway (Pumplin and Voinnet, 2013; Roth et al., 2004). The viral pathogenicity determinant proteins usually act as the silencing suppressors. In addition to helper virus-encoded AC2/C2, AC4/C4, and AV2/V2 proteins, the βC1 proteins, encoded by betasatellites associated with different begomoviruses like TYLCCNV, Tomato leaf curl Java virus, Bhendi yellow vein mosaic virus (BYVMV), Cotton leaf curl Multan virus (CLCuMuV) etc. function as the extra silencing suppressor molecules (Cui et al., 2005; Gopal et al., 2007; Kon et al., 2007). Thus, βC1 proteins enhance the helper virus accumulation and the severity of infection. However, different βC1 proteins share limited sequence similarity amongst them, a fact that suggests the plurality in the mechanism of how different βC1 proteins interfere with the silencing pathway of the host (Bridon et al., 2001). Nuclear localization signal (NLS) of TYLCCNB-βC1 is essential for its nuclear localization and suppression of gene silencing activity (Cui et al., 2005). When expressed transgenically, TYLCCNB-βC1 overexpressed plants developed virus-like symptoms indicating the protein’s ability to cause developmental abnormalities in the plant. The developmental process of a plant is influenced by the microRNAs (miRNAs) that regulate the host gene expressions. Ranging

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in size from 20 to 24 nucleotides, the miRNAs are non-coding single-stranded small RNAs that target specific mRNAs for cleavage or translational inhibition and thus act as the master modulators of gene expression at the mRNA level (Bartel 2009). As both siRNA mediated gene silencing and miRNA biogenesis pathways require the nuclear localized DICER-like proteins, the point of interference of $\beta C1$ with the silencing pathway is likely present at the initial stage of maturation of miRNA (Xie et al., 2004). However, Cotton leaf curl Multan betasatellite encoded $\beta C1$ (CLCuMuB-$\beta C1$) possesses suppressor activity despite lacking an NLS (Amin et al., 2011; Tiwari et al., 2013).

Studies have revealed that a plant itself encodes the endogenous suppressors of RNA silencing (ESRs) for proper regulation of the gene silencing machinery (Anandalakshmi et al., 2000). $\beta C1$, as the pathogenicity factor, modulates the host ESRs to thwart host defence mediated by RNA silencing. TYLCCNB-$\beta C1$ acts as a viral suppressor by up-regulating the expression of N. benthamiana calmodulin-like protein, Nbrgs-CaM (Fig. 2), which in turn, represses the level of both RDR6 and secondary siRNAs causing suppression of gene silencing (Li et al., 2014b). Moreover, since $\beta C1$ expression suppresses viral siRNA production by an RDR6 independent pathway, it becomes clear that $\beta C1$ imparts a
pleiotropic effect on the host RNA silencing machinery (Li et al., 2014a).

Autophagy is reported to be an important antiviral defence mechanism. In plants, suppression of autophagy-related genes (ATGs) expression results in diminished vitality and disease resistance. Interestingly, reports suggest that viruses employ strategies to use autophagy for their own propagation with autophagy proteins acting as the proviral factors (Dreux and Chisari 2009).

### Table 1 βC1-host protein interactions.

| Associated betasatellite | βC1 targeting host factor | Implications in the scope of defence/counter-defence response | References |
|--------------------------|---------------------------|---------------------------------------------------------------|------------|
| Bhendi yellow vein mosaic betasatellite | Karyopherin-α | • BYVMV-movement protein interacts with BYVMB-βC1, which in turn interacts with karyopherin-α • Putatively facilitates viral movement through the nuclear membrane | Kumar et al., 2006 |
| Tomato yellow leaf curl China betasatellite | Asymmetric leaves 1 (AS1) | • Suppresses JA-biosynthesis and JA-responsive genes • Alters MIR165/166 and HHD2PIII transcripts | Yang et al., 2008 |
| Cotton leaf curl Multan betasatellite | Ubiquitin-conjugating enzyme (E2) | • βC1 modulates the host ubiquitin/26S proteasome pathway by inhibiting the ubiquitin conjugase E2 • Reduces polyubiquitination of protein and prevent degradation of the viral protein | Eini et al., 2009b |
| Tomato yellow leaf curl China betasatellite | Sucrose-nonfermenting1-related kinase (SnRK1) | • SnRK1 interacts with and phosphorylates βC1 through its kinase domain • Phosphorylation inhibits PTGS and TGS suppressor activity of βC1 • Attenuates disease symptom and lowers accumulation of viral DNA | Shen et al., 2011; Zhong et al., 2017 |
| Tomato yellow leaf curl China betasatellite | S-adenosyl homocysteine hydrolase (SAHH) | • βC1 inhibits the activity of SAHH required for the production of S-adenosyl methionine, active methyl group donor for methylation reaction • Hampers methyl cycle and reduces host and viral genome methylation level | Yang et al., 2011b |
| Tomato yellow leaf curl China betasatellite | Calmodulin-like protein (rgs-CaM) | • βC1 induces expression of rgs-CAM, an endogenous regulator of RNA gene silencing • Induced level of rgs-CAM suppresses the host RNA silencing by repressing the expression of RDR6 | Li et al., 2014a |
| Tomato yellow leaf curl China betasatellite | The basic helix-loop-helix transcription factor (MYC2) | • βC1 protein interacts with MYC2, interferes with its dimerization required for binding to the promoter • Suppress the terpene biosynthesis genes and establishes virus-insect vector mutualism | Li et al., 2014b |
| Radish leaf curl betasatellite | Oxygen-evolving complex (OEC) of PSII | • βC1 protein localizes into the chloroplast of the infected plant cell • Interferes with the electron transport in PSII probably affecting the OEC of PSII • βC1 protein inhibits the chloroplast defence response by affecting the structure and function of the chloroplast | Bhattacharyya et al., 2015 |
| Tomato yellow leaf curl China betasatellite | RING-finger protein (RFP1) | • Host ubiquitin-ligase E3, RFP1 interacts with and polyubiquitinitates βC1 and direct them to 26S proteasome-mediated degradation • Delays establishment of geminivirus infection | Shen et al., 2016 |
| Cotton leaf curl Multan betasatellite | S-phase kinase-associated protein (SKP1) | • βC1 protein interacts with SKP1 and interferes with SKP1-CUL1 interaction and thereby prevents SCF-E3 ligase complex formation • The βC1-SKP1 interaction impairs SCFco1 and subverts JA-mediated suppression of viral infection cycle | Jia et al., 2016 |
| Cotton leaf curl Multan betasatellite | Autophagy protein (ATG8) | • Plant ATG8 protein interacts with virulence protein βC1 protein and subsequently induces autophagy • Autophagy contributes to the plant innate immunity by degradation of viral protein and restricting its spread | Haxim et al., 2017 |
| Cotton leaf curl Multan betasatellite | Argonaute-1 (AGO1) | • βC1 protein physically interacts with AGO1 and possibly targets RNA silencing | Eini, 2017 |
In a recent study, the interaction between calmodulin-like protein (NbCaM) and suppressor of gene silencing 3 (SGS3) proteins has been shown to lead to phosphatidylinositol 3-kinase complex mediated degradation of SGS3. The class III phosphatidylinositol 3-kinase is involved in the initiation of autophagy, and degradation of SGS3 subsequently facilitates the infection by the geminiviruses TYLCCNV and TYLCCNB (Li et al., 2017a). As NbCaM promotes geminivirus infection via the autophagy pathway and jC1 induces up-regulation of NbCaM, the proviral function of autophagy in geminivirus infection might also be dependent on the presence of betasatellite (Li et al., 2017a).

**Suppression of transcriptional gene silencing**

In plants, TGS is an epigenetic phenomenon that involves repressive histone modifications and RNA-directed DNA methylation (RdDM). RdDM not only serves as a regulator of endogenous gene expression but also acts as an effective tool to repress the genes of the DNA viruses (Matzke et al., 2009). Geminiviruses do not encode polymerase and they depend on the host machinery for replication and transcription. Within the nucleus of an infected plant cell, geminivirus single-stranded genomic DNA is converted to double-stranded DNA, and associates with histone to form minichromosomes, which function as the template for replication and transcription (Kushwaha et al., 2017; Paprotka et al., 2015; Pilartz and Jeske, 2003). These minichromosomes become the target of the TGS machinery of the plant. TGS and PTGS processes complement each other to raise the antiviral defense by specifically inactivating viral RNAs resulting in reduced virus replication, hypermethylation of viral genomes and subsequent symptom disappearance (Raja et al., 2010). The pattern in which a viral genome becomes the subject to methylation is specific for a particular virus-host combination (Chellappan et al., 2004). As the plants target the regulatory elements of viral genome for TGS mechanism, the intergenic regions, in addition to the coding regions of Rep, Ren, TRAP and MP genes, seems to be the ‘hot spots’ for methylation (Rodriguez-Negrete et al., 2009; Yang et al., 2011b).

As a counter-defence mechanism, plant DNA viruses code for proteins that can serve as suppressors of TGS working at different stages of the process (Hohn and Vazquez, 2011). The protein jC1 has been demonstrated to suppress the methylation-mediated RNA silencing. TYLCCNB-jC1 acts as a suppressor of TGS by targeting the enzyme S-adenosyl homocysteine hydrolase (SAHH) and inhibiting the synthesis of S-adenosyl-methionine (Fig. 2), the methyl group donor for DNA methylation (Yang et al., 2011b). Further, in N. benthamiana, TYLCCNB changes the methylation pattern of both the helper virus promoter and the intergenic region, as well as the host genome. The level of methylation at both CG and non-CG sites were substantially reduced by jC1 expression (Yang et al., 2011b). In this case, too, disruption of the NLS of jC1 hampered its ability to interact with SAHH and the TGS reversal was suppressed. However, plant immunity has evolved to deploy host SUCROSE-NONFERMENTING1-related kinase (SnRK1) to overcome jC1 mediated suppression of TGS. SnRK1 mediated phosphorylation of jC1 protein reduces its TGS suppressor function without affecting its stability, self-interaction, subcellular localization and interaction with ASYMMETRIC LEAVES1 (AS1) transcription factor (Zhong et al., 2017).

**Implication of the interaction of jC1 and host ubiquitin-proteasome machinery on disease development**

The central role of the ubiquitin-proteasome system is to degrade the redundant/misfolded cellular proteins and the regulatory proteins with short half-lives (cell cycle regulators, transcription factors, signal transducer, etc.). Ubiquitination of protein substrate is mediated by the serial action of E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and E3 (ubiquitin-ligase) proteins (Stone et al., 2005). The proteins that are polyubiquitinated at their Lys-residues are recognized and degraded by the host ubiquitin-proteasome system. Plants defence mechanisms had evolved to deploy host proteasomal degradation machinery to degrade the viral and cellular proteins that contribute to the regulation of viral infections (Verchot, 2016). Targeting and exploiting the host ubiquitin system to invade the host cell machinery is a strategy used by different plant viruses (Alcaide-Loridan and Jupin, 2012). In recent studies, jC1 protein has been found to aid in the stabilization of viral proteins and the establishment of infection by modulating a component of the plant ubiquitin-proteasome system. A yeast two-hybrid screening revealed that Nicotiana tabacum RING-finger protein NTRFP1 interacts with TYLCCNV-jC1. NTRFP1, being a functional E3 ubiquitin-ligase, polyubiquitinates and degrades the jC1 protein by 26S proteasome-mediated degradation (Shen et al., 2016). During betasatellite infection, the up-regulation of NTRFP1 and subsequent degradation of the pathogenicity factor by the ubiquitin/26S proteasome system results in developing resistance against geminivirus infection.

The tomato SUCROSE-NONFERMENTING1-related kinase (SISnRK1) interacts with and phosphorylates the TYLCCNB-jC1 protein and this probably leads to its proteasomal degradation (Shen et al., 2011). The screening of a tomato yeast two-hybrid library with CLCuMuB-jC1 identified a host interacting factor namely Solanum lycopersicum ubiquitin-conjugating enzyme 3 (SIUBC3) (Fig. 2), a novel ubiquitin-conjugating enzyme (Eini et al., 2009b). The interaction of jC1 with SIUBC3-E2 protein correlates with the compromised polyubiquitination of host proteins in the betasatellite infected plants. Further, interaction studies confirmed that jC1 protein of ToLCV-associated
betasatellites also can interact with the SIUBC3-E2 protein (Eini et al., 2009b). Additionally, CLCuMuB-βC1 interacts with S-phase kinase-associated protein (SKP1) and interferes with SKP1-CUL1 interaction; this subsequently prevents the formation of plant SCF-E3 ubiquitin-ligase complex (SKP1/CUL1/FBX/RBX1) (Jia et al., 2016). The βC1 protein-mediated inhibition of the ubiquitin-conjugating system enhances the CLCuMV infection and accumulation of viral DNA in the infected plant. Therefore, the interaction of βC1 with components of the plant’s ubiquitin-proteasome system appears to be a crucial aspect of the betasatellite pathogenicity.

**βC1 MITIGATES THE ANTIVIRAL RESISTANCE ESTABLISHED BY CHLOROPLAST AND PLANT DEFENCE RESPONSIVE HORMONES**

Being obligate parasites, plant viruses exploit the host cellular machinery for their genome replication, protein synthesis, intracellular and systemic movement. The plant organelle chloroplast, itself being a chimera of bacterial, viral and plant components (Zhao et al., 2016), is a potential target for plant-virus interaction. Besides its role in photosynthesis, chloroplasts have crucial functions in the defence mechanisms of the plants against viruses and other biotrophic and necrotrophic pathogens (Haxim et al., 2017). Chloroplasts contribute to the defence response by providing the site for the production of SA, JA and ROS (Ascencio-Ibanez et al., 2008; Bowling et al., 1994; Nomura et al., 2012). Further, the antagonistic regulation of SA and JA biogenesis and signalling is accomplished by the components of the chloroplast (Fig. 3). The calcium spike signal, triggered during non-compatible plant-pathogen interactions, is perceived by *CALCIUM-SENSING RECEPTOR (CAS)* protein localized on the thylakoid membrane of the chloroplast. The SA accumulation, driven by the activation of CAS protein receptor, illustrates the association of chloroplast with nuclear and cytoplasmic immune responses (Nomura et al., 2012).

Geminivirus infection causes up-regulation of several genes associated with SA biosynthesis and signalling (Ascencio-Ibanez et al., 2008). Elevated transcript level of SA-responsive marker genes was found in the plants infected with Cabbage leaf curl virus (CaLCuV). In addition, the expression of transcription factors downstream of SA response pathways such as TGA1, TGA3, TGAS and WRKY70 was up-regulated during geminivirus infection (Ascencio-Ibanez et al., 2008). **CONSTITUTIVE EXPRESSION OF PR GENES (CPR1), an F-box protein, has been shown to negatively regulate SA production (Gou et al., 2009). The crp1-mutant plant showed constitutively elevated SA and its responsive gene and had elevated resistance to CaLCuV infection (Ascencio-Ibanez et al., 2008). The jasmonate signalling, as a part of the defence response of the plant, interrupts the geminivirus propagation and proliferation. Arabidopsis thaliana plants treated with exogenous jasmonate showed reduced susceptibility and DNA accumulation while challenged with Beet curly top virus (Lozano-Duran et al., 2011).**

*MITOGEN-ACTIVATED PROTEIN KINASE 3 (MAPK3)* also contributes to tolerance against *Tomato yellow leaf curl virus* infection through SA/JA signalling mediated defence response (Li et al., 2017b).

Extensive studies on the biological function of βC1 protein clarified its role in suppressing the plant defence response by disrupting the chloroplasts (Bhattacharyya et al., 2015). Interestingly, TYLCCNB-βC1 protein was detected in the nucleus as well as in the chloroplast of the infected *N. benthamiana* cells by immunoelectron microscopy (Cui et al., 2005). *Radish leaf curl betasatellite* (RaLCB) infection or mere transient expression of βC1 protein perturbed the chlorophyll pigment content, reduced the photosynthetic efficiency, resulting in inappropriate accumulation of the photoassimilates, and altered the expression of nuclear-encoded chloroplastic proteins. The RaLCB-βC1 protein was demonstrated to localize into the chloroplasts of the infected plant, affecting the ultrastructure and photosynthetic function of the organelle (Bhattacharyya et al., 2015). Inhibition of host photosynthesis might provide an optimal microenvironment for plant viruses (Bhattacharyya and Chakraborty, 2018). Also, considering the importance of chloroplasts in the defence response of the plants, the βC1-mediated disruption of chloroplast structure during betasatellite infection might be a part of the viral counter-defence strategy that hampers the elevation of plant defence hormones. TYLCCNB-βC1 protein functions as a pathogenicity factor by selective suppression of JA-responsive genes (Fig. 3). The expression of JA-biosynthesis genes (FAD3 and FAD7) and JA-responsive genes (PR4, PDF1.2, VSP1, COR13 and CYP79B2) were repressed in the betasatellite infected plants (Fig. 3) (Li et al., 2014b; Zhang et al., 2012). The suppression of JA response by βC1 is accomplished by its interaction with AS1 protein in the molecular disguise of *ASYMMETRIC LEAVES2* (AS2) protein (Yang et al., 2008). MYC2, a basic helix-loop-helix transcription factor is a key downstream component of JA signalling (Li et al., 2014b). TYLCCNB-βC1 interacts and interferes with the dimerization of MYC2, which is necessary for binding to G-box/G-box-like motif present in its promoter (Stone et al., 2005). Various studies showed that JA generates plant defence responses against geminivirus infection (Sun et al., 2017). JA exhibits its responses by binding to its receptor, SCF*Col1* complex. Recently, CLCuMuB-βC1 has been shown to hinder the JA signalling pathway in the plant through the interaction with SKP1 protein that impairs SCF*Col1*. As JA is likely involved in anti-virus defence, suppression of JA signalling resulted in enhanced viral accumulation and symptoms in plants (Jia et al., 2016).
βC1 PROTEIN AIDS MOVEMENT OF HELPER BEGOMOVIRUSES IN THEIR HOSTS

The DNA-B encoded NSP and MP mediate local and systemic movements of the bipartite viruses, respectively, inside the hosts (Gafni and Epel, 2002). According to the existing models describing the mechanism of systemic movement of the bipartite viral DNA in the host, functional complementarity between MP and NSP is a necessary condition. As proposed by Noueiry et al. (1994), NSP aids the intracellular movement of the viral genome from the nucleus to the cytoplasm, and MP transports the viral cargo from cytoplasm through plasmodesmata helping the cell-to-cell movement of the virus. In an alternative model of movement, MP mediates the NSP-DNA complex in both intra- and intercellular movement (Lazarowitz and Beachy, 1999). The ability of host histone H3 to specifically interact with NSP and MP implicates its role in the transport of geminiviral DNA complex from the nucleus to the cytoplasm as well as in cell-to-cell transport through plasmodesmata (Zhou et al., 2011). However, a monopartite geminivirus, in absence of DNA-B encoded MP and NSP, essentially needs an alternative strategy for the systemic and cell-to-cell movement. Interestingly, a number of betasatellites associated with diverse monopartite begomoviruses have been suggested to complement the movement function of DNA-B encoded proteins of different geminiviruses (Saeed et al., 2007; Saunders et al., 2000). Although ToLCNDV DNA-A alone induces local but not systemic infection, the presence of CLCuMuB facilitates the systemic infection of ToLCNDV DNA-A in tomato. CLCuMuB with disrupted βC1 failed to help ToLCNDV DNA-A in such systemic movements (Patil and Fauquet, 2010; Saeed et al., 2007).
Many of the βC1 proteins of different betasatellites have either an NLS or nuclear export signal (NES) (Kumar et al., 2006). The possibility of a correlation between symptom induction and intracellular movement of βC1 became stronger as the nuclear localization of TYLCCNB-βC1 was found to be crucial for symptom development in N. benthamiana (Cui et al., 2005). Further studies revealed that βC1 protein interacts with the CP of helper virus and also with the host nuclear importin like protein karyopherin α.βC1 encoded by Bhendi yellow vein mosaic betasatellite (BYVMV) possesses a strong NES and also physically interacts with BYVMV encoded CP, which lacks NES (Kumar et al., 2006). Such complementarity enables the interacting partners in nuclear export and import, and is suggested to be a process analogous to the interaction model of NSP and MP related to the nuclear transport and cell-to-cell movement of bipartite viruses (Gafni and Epel, 2002). Nevertheless, the βC1 deletion-mutant of TYLCCNB-Y10j) was capable of moving systemically in the plant and underwent encapsidation by the helper virus (Qian and Zhou, 2005). Thus, βC1 is considered to have a dispensable role in the systemic movement of a monopartite virus.

**ROLE OF BETASATELLITE IN THE TRIPARTITE INTERACTION OF HOST-VECTOR-VIRUS**

The complex tripartite interaction amongst host plant, insect vector and infecting virus produce the final outcome of the infection (Sun et al., 2017). Recent studies demonstrated that B biotype of Bemisia tabaci, the whitely responsible for begomovirus transmission participates in a synergistic relationship with the virus resulting in increased fecundity of the insect in TYLCCNV infected plants (Luan et al., 2013). Begomoviruses alter the host’s nutritional profile and defence responses to make the host more attractive to the vector (Luan et al., 2014). Wounding and herbivore attack on plants induce JA signalling, a major defence pathway against insects (Galis et al., 2009). Betasatellites manipulate the anti-herbivore response of the plant by interfering with JA signalling pathway through various strategies (Li et al., 2014b; Yang et al., 2008).

Firstly, βC1 down-regulates expression of JA-responsive genes by intervening with the AS1/AS2 complex formation (Fig. 2) (Yang et al., 2008). The other approach that betasatellite adopts in this context is to suppress the synthesis of organic volatile monoterpene, α-bergamotene and β-mycrene in the plant. Some of the volatile compounds derived from plants, like linalool, eugenol, myrcene, limonene and 1, 8-cineole, are involved in evoking positive behavioural responses from female B. tabaci of B biotype (Feng-Qin, 2008). βC1 protein of TYLCCNB interacts directly with MYC2 transcription factor of A. thaliana and N. benthamiana (Li et al., 2014b). The physical interaction of βC1 with MYC2 leads to inhibition of the dimerization and the DNA binding capability of the MYC2, which affects the induction of terpene synthase gene. Reduced synthesis and emission of α-bergamotene and β-mycrene attract more vectors to infect and lay eggs on the virus infected plants (Li et al., 2014b). Interference of βC1 with the function of a master regulator transcription factor-like MYC2 is highly significant for the physiology and cellular responses of the plant. Furthermore, genes related to indole and aliphatic glucosinolates, two components involved in the herbivore-induced response, were down-regulated in βC1-overexpressed plants (Li et al., 2014b).

**CONCLUSIONS AND FUTURE PERSPECTIVES**

The threats of geminivirus infection on economically important crops aggravates due to the association of betasatellites with the majority of monopartite begomoviruses. Plants generate defence responses against geminivirus infection by activating diverse mechanisms such as RNA silencing, ubiquitin/proteasome-related protein degradation system, autophagy, chloroplast machinery and innate immunity mediated by several host factors. The βC1 protein plays a multitasking role as it suppresses the gene silencing, attenuates plant defence responses, induces disease symptoms and possibly helps in the virus movement. The βC1 protein being a strong silencing suppressor facilitates the viral pathogenesis by interfering with host PTGS and TGS machinery. βC1 aggravates the symptoms by stabilizing the viral proteins. βC1 protein interacts with ubiquitin-conjugating enzyme, E2 and thereby exploits the ubiquitin-proteasome system of the plant leading to compromised polyubiquitination of proteins.

Chloroplast accomplishes the defence response against viruses by facilitating the autophagy-mediated viral protein degradation, production of defence-related ROS and contributing to the production of SA and JA. As a viral counter-defence strategy against chloroplast-mediated immunity response, βC1 localizes into chloroplasts and disrupts the structure and function of the chloroplasts. Additionally, betasatellite interferes with plant anti-herbivore response, attracts the insect vector for transmission and thereby supports the viral transmission into a new host. The βC1 protein adopts two independent strategies to interfere with JA response, either by βC1-AS1 interaction or by βC1-MYC2 interaction. In the future, investigating plant-geminivirus betasatellite interactions, in terms of the performance of photosystem, the fate of chloroplast, and the autophagy would help in deepening the understanding of the molecular mechanisms of βC1 mediated pathogenesis. Further, understanding of βC1 mediated regulation of the defence hormones of the plant would help to develop better strategies against geminiviral diseases.

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REFERENCES

Acosta-Leal, R., Duffy, S., Xiong, Z., Hammond, R.W. and Elena, S.F. (2011) Advances in plant virus evolution: translating evolutionary insights into better disease management. *Phytopathology*, 101, 1136–1148.

Alazem, M. and Lin, N.S. (2015) Roles of plant hormones in the regulation of host-virus interactions. *Mol. Plant Pathol.* 16, 529–540.

Alcaide-Loridán, C. and Jupin, I. (2012) Ubiquitin and plant viruses, let’s play together! *Plant Physiol.* 160, 72–82.

Amin, I., Hussain, K., Akbergenov, R., Yadav, J.S., Qazi, J., Mansoor, S., Hohn, T., Fauquet, C.M. and Biddon, R.W. (2011) Suppressors of RNA silencing encoded by the components of the cotton leaf curl begomovirus-betasatellite complex. *Mol. Plant-Microbe Interact.* 24, 973–983.

Anandalakshmi, R., Marathe, R., Ge, X., Herr, J.M., Jr, Mau, C., Mallory, A., Pruss, G., Bowman, L. and Vance, V.B. (2000) A calmodulin-related protein that suppresses posttranscriptional gene silencing in plants. *Science*, 290, 142–144.

Ascencio-Ibañez, J.T., Sozzani, R., Lee, T.J., Chu, T.M., Wolfinger, R.D., Anandalakshmi, R., Marathe, R., Ge, X., Herr, J.M., Jr, Mau, C., Mallory, A., Pruss, G., Bowman, L. and Vance, V.B. (2000) A calmodulin-related protein that suppresses posttranscriptional gene silencing in plants. *Science*, 290, 142–144.

Asencio-Ibañez, J.T., Sozzani, R., Lee, T.J., Chu, T.M., Wolfinger, R.D., Cella, R. and Hanley-Bowdoin, L. (2008) Global analysis of Arabidopsis gene expression uncovers a complex array of changes impacting pathogen response and cell cycle during geminivirus infection. *Plant Physiol.* 148, 436–454.

Bartel, D.P. (2009) MicroRNAs: Target recognition and regulatory functions. *Cell*, 136, 215–233.

Bhat, S., Folimonova, S.Y., Cole, A.B., Ballard, K.D., Lei, Z., Watson, B.S., Sumner, L.W. and Nelson, R.S. (2013) Influence of host chloroplast proteins on Tobacco mosaic virus accumulation and intercellular movement. *Plant Physiol.* 161, 134–147.

Bhattacharyya, D. and Chakraborty, S. (2018) Chloroplast: the Trojan horse in plant-virus interaction. *Mol. Plant Pathol.* 19, 504–518.

Bhattacharyya, D., Gnanasekaran, P., Kumar, R.K., Kushwaha, N.K., Sharma, V.K., Yusuf, M.A. and Chakraborty, S. (2015) A geminivirus betasatellite damages the structural and functional integrity of chloroplasts leading to symptom formation and inhibition of photosynthesis. *J. Exp. Bot.* 66, 5881–5895.

Bowling, S.A., Guo, A., Cao, H., Gordon, A.S., Klessig, D.F. and Dong, X. (1994) A mutation in Arabidopsis that leads to constitutive expression of systemic acquired resistance. *Plant Cell*, 6, 1845–1857.

Briddon, R.W., Mansoor, S., Bedford, I.D., Pinner, M.S., Saunders, K., Stanley, J., Zafar, Y., Malik, K.A. and Markham, P.G. (2001) Identification of DNA components required for induction of cotton leaf curl disease. *Virology*, 285, 234–243.

Briddon, R.W., Bull, S.E., Amin, I., Idris, A.M., Mansoor, S., Bedford, I.D., Dhawan, P., Rishi, N., Siwatch, S.S., Abdel-Salam, A.M., Brown, J.K., Zafar, Y. and Markham, P.G. (2003) Diversity of DNA beta, a satellite molecule associated with some monopartite begomoviruses. *Virology*, 312, 106–121.

Briddon, R.W., Brown, J.K., Moriones, E., Stanley, J., Zerbini, M., Zhou, X. and Fauquet, C.M. (2008) Recommendations for the classification and nomenclature of the DNA-beta satellites of begomoviruses. *Archiv. Virol.* 153, 763–781.

Ceniceros-Ojeda, E.A., Rodriguez-Negrete, E.A. and Rivera-Bustamante, R.F. (2016) Two populations of viral minichromosomes are present in a geminivirus-infected plant showing symptom remission (recovery). *J. Virol.* 90, 3828–3838.

Chellappan, P., Vanitharani, R., Pita, J. and Fauquet, C.M. (2004) Short interfering RNA accumulation correlates with host recovery in DNA virus-infected hosts, and gene silencing targets specific viral sequences. *J. Virol.* 78, 7465–7477.

Chen, L.F., Rojas, M., Kon, T., Gamby, K., Xoconostle-Cazares, B. and Gilbertson, R.L. (2009) A severe symptom phenotype in tomato in Mali is caused by a reassortant between a novel recombinant begomovirus (Tomato yellow leaf curl Mali virus) and a betasatellite. *Mol. Plant Pathol.* 10, 415–430.

Cui, X., Li, G., Wang, D., Hu, D. and Zhou, X. (2005) A Begomovirus DNAbeta-encoded protein binds DNA, functions as a suppressor of RNA silencing, and targets the cell nucleus. *J. Virol.* 79, 10764–10775.

Ding, C., Qing, L., Li, Z., Liu, Y., Qian, Y. and Zhou, X. (2009) Genetic determinants of symptoms on viral DNA satellites. *Appl. Environ. Microbiol.* 75, 5380–5389.

Dogar, A.M. (2006) RNAI dependent epigenetic marks on a geminivirus promoter. *Virol. J.* 3, 5.

Doyle, S.M., Diamond, M. and McCabe, P.F. (2010) Chloroplast and reactive oxygen species involvement in apoptotic-like programmed cell death in Arabisopsis suspension cultures. *J. Exp. Bot.* 61, 473–482.

Dreux, M. and Chisari, F.V. (2009) Autopahgy proteins promote hepatitis C virus replication. *Autophagy*, 5, 1224–1225.

Dry, I.B., Krate, L.R., Rigden, J.E. and Rezaian, M.A. (1997) A novel subviral agent associated with a geminivirus: the first report of a DNA satellite. *Proc. Natl. Acad. Sci. USA*, 94, 7088–7093.

Duffy, S. and Holmes, E.C. (2008) Phylogenetic evidence for rapid rates of molecular evolution in the single-stranded DNA begomovirus tomato yellow leaf curl virus. *J. Virol.* 82, 957–965.

Eini, O. (2017) A betasatellite-encoded protein regulates key components of gene silencing system in plants. *Mol. Bio*. 51, 656–663.

Eini, O., Behjatnia, S.A., Dogra, S., Dry, I.B., Randles, J.W. and Rezaian, M.A. (2009a) Identification of sequence elements regulating promoter activity and replication of a monopartite begomovirus-associated DNA beta satellite. *J. Gen. Virol.* 90, 253–260.

Eini, O., Dogra, S., Selth, L.A., Dry, I.B., Randles, J.W. and Rezaian, M.A. (2009b) Interaction with a host ubiquitin-conjugating enzyme is required for the pathogenicity of a geminiviral DNA beta satellite. *Mol. Plant-Microbe Interact.* 22, 737–746.

Feng-Qin, C. (2008) Behavioural responses of Bemisia tabaci B biotype to three host plants and their volatiles. *Acta Entomol. Sin.* 51, 830–838.

Gafni, Y. and Epel, B.L. (2002) The role of host and viral proteins in intra- and inter-cellular trafficking of geminiviruses. *Physiol. Mol. Plant Pathol.* 60, 231–241.

Galis, I., Gaquerel, E., Pandey, S.P. and Baldwin, I.T. (2009) Molecular mechanisms underlying plant memory in JA-mediated defence responses. *Plant Cell Environ.* 32, 617–627.

Gnanasekaran, P. and Chakraborty, S. (2018) Biology of viral satellites and their role in pathogenesis. *Curr. Opin. Virol.* 33, 96–105.

Gopal, P., Pravin Kumar, P., Sinilal, B., Jose, J., Kasin Yadunandam, A. and Usha, R. (2007) Differential roles of C4 and betaC1 in mediating suppression of post-transcriptional gene silencing: evidence for transactivation by the C2 of Bhendi yellow vein mosaic virus, a monopartite begomovirus. *Virus Res.* 123, 9–18.

Gou, M., Su, N., Zheng, J., Huai, J., Wu, G., Zhao, J., He, J., Tang, D., Yang, S. and Wang, G. (2009) An F-box gene, CPR30, functions as a negative regulator of the defense response in Arabidopsis. *Plant J.* 60, 757–770.

Guán, C. and Zhou, X. (2006) Phloem specific promoter from a satellite associated with a DNA virus. *Virus Res.* 115, 150–157.

Hammond, S.M., Bernstein, E., Beach, D. and Hannon, G.J. (2000) An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. *Nature*, 404, 293–296.

Hanley-Bowdoin, L., Bejarano, E.R., Robertson, D. and Mansoor, S. (2013) Geminiviruses: masters at redirecting and reprogramming plant processes. *Nat. Rev. Microbiol.* 11, 777–788.

Hassan, I., Orillo, A.F., Fiallo-Olivé, E., Briddon, R.W. and Navas-Castillo, J. (2016) Infectivity, effects on helper viruses and whitefly transmission of the
Arms race between betasatellite and plant defense

Leke, W.N., Mignouna, D.B., Brown, J.K. and Kvamhened, A. (2015) Geminivirus disease complex: emerging threat to vegetable production systems of West and Central Africa. *Agric. Food Sec. 4*, 1.

Li, F., Huang, C., Li, Z. and Zhou, X. (2014a) Suppression of RNA silencing by a plant DNA virus satellite requires a host calmodulin-like protein to repress RDR6 expression. *PloS Pathog. 10*, e1003921.

Li, R., Wedelgergis, B.T., Li, J., Jung, C., Qu, J., Sun, Y., Qian, H., Tee, C., van Loon, J.J., Dicke, M., Chua, N.H., Liu, S.S. and Ye, J. (2014b) Virulence factors of geminivirus interact with MYC2 to subvert plant resistance and promote vector performance. *Plant Cell, 26*, 4991–5008.

Li, F., Zhao, N., Li, Z., Xu, X., Wang, Y., Yang, X., Liu, S.S., Wang, A. and Zhou, X. (2017a) A calmodulin-like protein suppresses RNA silencing and promotes geminivirus infection by degrading SG3 via the autophagy pathway in Nicotiana benthamiana. *PloS Pathog. 13*, e0106213.

Li, Y., Qin, L., Zhao, J., Muhammad, T., Cao, H., Li, H., Zhang, Y. and Liang, Y. (2017b) SIMAPK3 enhances tolerance to tomato yellow leaf curl virus (TYLCV) by regulating salicylic acid and jasmonic acid signaling in tomato (Solanum lycopersicum). *PloS One, 12*, e0172466.

Lima, A.T.M., Silva, J.C.F., Silva, F.N., Castillo-Urquiza, G.P., Silva, F.F., Seay, Y.M., Mizutubi, E.S.G., Duffy, S. and Zerbini, F.M. (2017) The diversification of begomovirus populations is predominantly driven by mutational dynamics. *Virus Evol. 3*, vex005.

Lozano, G., Trenado, H.P., Fiallo-Olive, E., Chirinos, D., Geraud-Pouey, F., Briddon, R.W. and Navas-Castillo, J. (2016) Characterization of non-coding DNA satellites associated with Sweepoviruses (Genus Geminivirus, Geminiviridae) — definition of a distinct class of Geminivirus-associated satellites. *Front. Micro. 7*, 162.

Lozano-Duran, R., Rosas-Diaz, T., Gusmaroli, G., Luna, A.P., Taconnlat, L., Deng, X.W. and Bejarano, E.R. (2011) Geminiviruses subvert ubiquitination by altering CSN-mediated degradation of SCF E3 ligase complexes and inhibit jasmonate signaling in Arabidopsis thaliana. *Plant Cell, 23*, 1014–1032.

Luan, J.B., Wang, Y.L., Wang, J., Wang, X.W. and Liu, S.S. (2013) Detoxification activity and energy cost is attenuated in whiteflies feeding on tomato yellow leaf curl China virus-infected tobacco plants. *Insect Mol. Biol. 22*, 597–607.

Luan, J-B., Wang, X-W., Colvin, J. and Liu, S-S. (2014) Plant-mediated whiellyt-begomovirus interactions: research progress and future prospects. *Bull. Entomol. Res. 104*, 267–276.

Mansoor, S., Khan, S.H., Bashir, A., Saeed, M., Zafar, Y., Malik, K.A., Briddon, R., Stanley, J. and Markham, P.G. (1999) Identification of a novel circular single-stranded DNA associated with cotton leaf curl disease in Pakistan. *Virology, 259*, 190–199.

Mansoor, S., Briddon, R.W., Bull, S.E., Bedford, I.D., Bashir, A., Hussain, M., Saeed, M., Zafar, Y., Malik, K.A., Faquett, C. and Markham, P.G. (2003a) Cotton leaf curl disease is associated with multiple monopartite begomoviruses supported by single DNA beta. *Archiv. Virolog. 148*, 1969–1986.

Mansoor, S., Briddon, R.W., Zafar, Y. and Stanley, J. (2003b) Geminivirus disease complexes: an emerging threat. *Trends Plant Sci. 8*, 128–134.

Mansoor, S., Zafar, Y. and Briddon, R.W. (2006) Geminivirus disease complexes: the threat is spreading. *Trends Plant Sci. 11*, 209–212.

Matzke, M., Kanno, T., Deng, X.W., Daxinger, L., Huettel, B. and Matzke, A.J. (2009) RNA-mediated chlorosis-induced silencing in plants. *Curr. Opin. Cell Biol.*, 21, 367–376.

Navas-Castillo, J., Fiallo-Olive, E. and Sánchez-Campos, S. (2011) Emerging virus diseases transmitted by whiteflies. *Annu. Rev. Phytopathol. 49*, 219–248.

Nawaz-UL-Rehman, M.S., Misbah, N., Mansoor, S., Briddon, R.W. and Faquett, C.M. (2010) Post-transcriptional gene silencing suppressor...
activity of two non-pathogenic alphasatellites associated with a begomovirus. Virology, 405, 300–308.
Nawaz-ul-Rehman, M.S. and Fauquet, C.M. (2009) Evolution of geminiviruses and their satellites. FEBS Lett. 583, 1825–1832.
Nomura, H., Komori, T., Uemura, S., Kanda, Y., Shimotani, K., Nakai, K., Furuschi, T., Takebayashi, K., Sugimoto, T., Sano, S., Suwastika, I.N., Fukusaki, E., Yoshioha, H., Nakahira, Y. and Shiina, T. (2012) Chloroplast-mediated activation of plant immune signalling in Arabidopsis. Nat. Commun. 3, 926.
Noueiry, A.O., Lucas, W.J. and Gilbertson, R.L. (1999) Genetic variability of natural populations of cotton leaf curl geminivirus. J. Mol. Evol. 49, 77–89.
Paprotka, T., Deuschle, K., Pilartz, M. and Jeske, H. (2015) Form fol-

Saunders, K., Bedford, I.D., Briddon, R.W., Markham, P.G., Wong, S.M. and Stanley, J. (2000) A unique virus complex causes Ageratum yellow vein disease. Proc. Natl. Acad. Sci. USA, 97, 6890–6895.
Saunders, K., Bedford, I.D. and Stanley, J. (2002) Adaptation from whitefly to leafhopper transmission of an autonomously replicating nanovirus-like DNA component associated with ageratum yellow vein disease. J. Gen Virol. 83, 907–913.
Saunders, K., Bedford, I.D., Yahara, T. and Stanley, J. (2003) Aetiology: the earliest recorded plant virus disease. Nature, 422, 831.
Saunders, K., Briddon, R.W. and Stanley, J. (2008) Replication promiscuity of DNA-beta satellites associated with monopartite begomoviruses; deletion mutagenesis of the Ageratum yellow vein virus DNA-beta satellite localizes sequences involved in replication. J. Gen. Virol. 89, 3165–3172.
Seal, S., EvendenBosch, F. and Jeger, M.J. (2006) Factors Influencing Begomovirus Evolution and Their Increasing Global Significance: Implications for Sustainable Control. Crit. Rev. Plant Sci. 25, 23–46.
Shen, Q., Liu, Z., Song, F., Xie, Q., Hanley-Bowdoin, L. and Zhou, X. (2011) Tomato SiSnRK1 protein interacts with and phosphorylates betaC1, a pathogenesis protein encoded by a geminivirus beta-satellite. Plant Physiol. 157, 1394–1406.
Shen, Q., Hu, T., Bao, M., Cao, L., Zhang, H., Song, F., Xie, Q. and Zhou, X. (2016) Tobacco RING E3 ligase NLRFP1 mediates ubiquitination and proteasomal degradation of a Geminivirus-encoded betaC1. Mol. Plant, 9, 911–925.
Singh, A.K., Chattopadhyay, B. and Chakraborty, S. (2012) Biology and interactions of two distinct monopartite begomoviruses and betasatellites associated with radish leaf curl disease in India. Virol. J. 9, 43.
Singh, A.K., Kushwaha, N. and Chakraborty, S. (2016) Synergistic interaction among begomoviruses leads to the suppression of host defense-related gene expression and breakdown of resistance in chilli. Appl. Microbiol. Biotechnol. 100, 4035–4049.
Sivalingam, P.N. and Varma, A. (2012) Role of betasatellite in the pathogenesis of a bipartite geminivirus affecting tomato in India. Arch. Virol. 157, 1081–1092.
SpoeI, S.H., Johnson, J.S. and Dong, X. (2007) Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. Proc. Natl. Acad. Sci. USA, 104, 18842–18847.
Stone, S.L., Hauksdottir, H., Troy, A., Herschleb, J., Kraft, E. and Callis, J. (2005) Functional analysis of the RING-type ubiquitin ligase family of Arabidopsis. Plant Physiol. 137, 13–30.
Sun, Y.C., Pan, L.L., Ying, F.Z., Li, P., Wang, X.W. and Liu, S.S. (2017) Jasmonic acid-related resistance in tomato mediates interactions between whitefly and whitefly-transmitted virus. Sci. Rep. 7, 566.
Swanson, M.M., Harrison, B.D. and Wong, S.M. (1993) A geminivirus causing vein yellowing of Ageratum conyzoides in Singapore. Plant Pathol. 42, 137–139.
Tan, P.H., Wong, S.M., Wu, M., Bedford, I.D., Saunders, K. and Stanley, J. (1995) Genome organization of ageratum yellow vein virus, a monopartite whithfly-transmitted geminivirus isolated from a common weed. J. Gen. Virol. 76(Pt 12), 2915–2922.
Tao, X., Qin, L. and Zhou, X. (2004) Function of A-Rich region in DNA-beta satellite of Tomato yellow leaf curl China virus. Chin. Sci. Bull. 49, 1490–1493.
Tiwari, N., Sharma, P.K. and Malathi, V.G. (2013) Functional characterization of betaC1 gene of cotton leaf curl Multan betasatellite. Virus Genes. 46, 111–119.
Verchot, J. (2016) Plant virus infection and the ubiquitin proteasome machinery: arms race along the endoplasmic reticulum. Viruses. 8, E314.
Vinoth Kumar, R., Singh, D., Singh, A.K. and Chakraborty, S. (2017) Molecular diversity, recombination and population structure of
alphasatellites associated with begomovirus disease complexes. Infect. Genet. Evol. 49, 39–47.

Voinnet, O. (2001) RNA silencing as a plant immune system against viruses. Trends Genet. 17, 449–459.

Wang, J., Tang, Y., Yang, Y., Ma, N., Ling, X., Kan, J., He, Z. and Zhang, B. (2016) Cotton leaf curl Multan virus-derived viral small RNAs can target cotton genes to promote viral infection. Front. Plant Sci. 7, 1162.

Xiao, B., Yang, X., Ye, C-Y., Liu, Y., Yan, C., Wang, Y., Lu, X., Li, Y. and Fan, L. (2014) A diverse set of miRNAs responsive to begomovirus-associated betasatellite in Nicotiana benthamiana. BMC Plant Biol. 14, 60.

Xie, Z., Johansen, L.K., Gustafson, A.M., Kasschau, K.D., Lellis, A.D., Zilberman, D., Jacobsen, S.E. and Carrington, J.C. (2004) Genetic and functional diversification of small RNA pathways in plants. PLoS Biol. 2, 1E104.

Yang, J-Y., Iwasaki, M., Machida, C., Machida, Y., Zhou, X. and Chua, N-H. (2008) betaC1, the pathogenicity factor of TYLCCNV, interacts with AS1 to alter leaf development and suppress selective jasmonic acid responses. Genes Dev. 22, 2564–2577.

Yang, X., Wang, Y., Guo, W., Xie, Y., Xie, Q., Fan, L. and Zhou, X. (2011a) Characterization of small interfering RNAs derived from the geminivirus/betasatellite complex using deep sequencing. PLoS One, 6, e16928.

Yang, X., Xie, Y., Raja, P., Li, S., Wolf, J.N., Shen, Q., Bisaro, D.M. and Zhou, X. (2011b) Suppression of methylation-mediated transcriptional gene silencing by betaC1-SAHH protein interaction during geminivirus-betasatellite infection. PLoS Pathog. 7, e1002329.

Yin, Y. and Beachy, R.N. (1995) The regulatory regions of the rice tungro bacilliform virus promoter and interacting nuclear factors in rice (Oryza sativa L.). Plant J. 7, 969–980.

Zerbini, F. M., Briddon, R. W., Idris, A., Martin, D. P., Moriones, E., Navas-Castillo, J., Rivera-Bustamante, R., Roumagnac, P., Varsani, A. and ICTV Report Consortium (2017) ICTV virus taxonomy profile: Geminiviridae. J. Gen. Virol. 98, 131–133.

Zhang, T., Luan, J.B., Qi, J.F., Huang, C.J., Li, M., Zhou, X.P. and Liu, S.S. (2012) Begomovirus-whitefly mutualism is achieved through repression of plant defences by a virus pathogenicity factor. Mol. Ecol. 21, 1294–1304.

Zhang, T., Xu, X., Huang, C., Qian, Y., Li, Z. and Zhou, X. (2015) A novel DNA motif contributes to selective replication of a Geminivirus-associated betasatellite by a helper virus-encoded replication-related protein. J. Virol. 90, 2077–2089.

Zhao, J., Zhang, X., Hong, Y. and Liu, Y. (2016) Chloroplast in plant-virus interaction. Front, Microbiol. 7, 1565.

Zhong, X., Wang, Z.Q., Xiao, R., Cao, L., Wang, Y., Xie, Y. and Zhou, X. (2017) Mimic phosphorylation of a betaC1 encoded by TYLCCNB impairs its functions as a viral suppressor of RNA silencing and a symptom determinant. J. Virol. 91, e00300–17.

Zhou, X. (2013) Advances in understanding begomovirus satellites. Annu. Rev. Phytopathol. 51, 357–381.

Zhou, X., Xie, Y., Tao, X., Zhang, Z., Li, Z. and Fauquet, C.M. (2003) Characterization of DNAbeta associated with begomoviruses in China and evidence for co-evolution with their cognate viral DNA-A. J. Gen. Virol. 84, 237–247.

Zhou, Y., Rojas, M.R., Park, M.R., Seo, Y.S., Lucas, W. J. and Gilbertson, R. L. (2011) Histone H3 interacts and colocalizes with the nuclear shuttle protein and the movement protein of a geminivirus. J. Virol. 85, 11821–11832.

Zubair, M., Zaidi, S.S., Shakir, S., Amin, I. and Mansoor, S. (2017) An insight into cotton leaf curl Multan Betasatellite, the most important component of cotton leaf curl disease complex. Viruses, 9, pii, E280. https://doi.org/10.3390/v9100280.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher’s web site:

Table S1 Geographical distribution of helper virus betasatellite disease complexes across plant species.