REVIEW ARTICLE

An Insight into the Approaches to Target Begomoviruses: RNAi Perspective

Saurabh Verma[1,2]*

DOI: 10.18811/ijpen.v5i03.7

Abstract

The destructive nature of viral diseases and its epidemic level of infection demand for a specific, effective and sustainable strategy. Begomovirus being the most destructive class of plant viruses, causes a widespread loss to crop production in the world. RNA silencing approaches have been implemented in past to raise virus-resistant transgenic crops. Some have found success while others failed terribly either in the laboratory or in field trials. In the present and future context, reviewing the potential of this technique is essential as Indian sub-continent region has an agriculture-based economy. This article aimed to review current research and future perspective in efficient targeting of begomovirus borne diseases in crops using RNA silencing as a molecular tool.

Keywords: Begomovirus, DNA methylation, Geminivirus, RNA interference, Short hairpin RNA, Small interfering RNA.

International Journal of Plant and Environment (2019)

Overview

The ever-increasing number of viral diseases in plants and the damage caused by them is hampering the global crop production. That is why there is a very high demand for the development of antiviral strategies to protect and also confer resistance towards viral infection in crop plants. The millions of hungry people being one major fraction of the hugely populated nation such as India; we need to develop indigenously developed virus-resistant crops to increase the crop productivity to feed millions of people. Begomoviruses are one of the major threats to Indian agriculture as evident from large scale losses to cotton, tomato, papaya and cereal crops in India (Meena and Chattopadhyay, 2016; Saxena *et al.*, 2016; Verma and Saxena, 2017a). Therefore, the problem related to begomovirus infection should be the focus of research in order to achieve the goal of attaining a sustainable food security in India. In this review, a glimpse of begomoviruses is presented along with various approaches used by researchers to address begomovirus infection in various crop plants.

Begomovirus: The complex genetic machinery

The Begomovirus is the largest genus belonging to the Geminiviridae family of plant-infecting viruses. The Begomovirus are whitefly-transmitted geminivirus, found frequently in both eastern and western hemispheres of Earth. Begomoviruses infect mostly dicotyledonous plants widespread in tropical, sub-tropical and other mild temperature regions (Zerbini et al, 2017). More than 16 different groups of Begomoviruses have been reported in India. The host range of these viruses comprises of horticulture, legumes and cash crops of India. Okra, chili, cotton, croton, papaya, cassava, legumes, and tomato are few commercial crops facing a tough begomovirus challenge in the fields (Borah and Dasgupta, 2012).

Begomoviruses mainly identified as geminate, twinned, icosahedral shaped particles containing circular single stranded DNA (ss-DNA) genome. The multifunctional begomoviral proteins are usually carried on ss-DNA known as helper DNA, also known as DNA-A. This DNA-A is associated with DNA-B and satellites DNA particles in case of bi- and monopartite begomovirus respectively (Zerbini *et al.*, 2017). The multifunctional proteins are encoded by overlapping regions called open reading frames (ORFs) present on virion and complementary strands. Both structural e.g. coat protein and those required for molecular functions i.e. replication, transcription, translation and host-resistance are encoded by ORFs whenever begomoviruses are transmitted into host cell. Apart from coding regions, the DNA-A, DNA-B and few satellites contain an intergenic regions spanning 200 nucleotides long that contains origin of replication (*ori*), transcription start site (TSS), bidirectional promoter and other elements required for efficient transcription and translation of viral peptides (Krupovic *et al.*, 2009; Fondong, 2013). In case of bipartite begomovirus, the other genomic component known as DNA-B, contains ORFs encoding for long range mobility of viral particles i.e. their movement from one cell to another. Thus, helping in systemic infection development in host plants (Noueiry *et al.*, 1994). The genomic component associated with monopartite begomovirus i.e. alpha- and betasatellite, may encode proteins which help in symptom elevation and suppression of host immune response (Zhou, 2013). Betasatellite encodes protein c1, reported to help in symptom development and host immune suppression through various mechanisms (Yang and Xhou, 2017). In DNA-A, the 7 ORFs in bipartite forms are named as AV1, AV2, AC1-AC5 whereas in monopartite they are named as V1, V2, C1-C5, respectively (Zerbini *et al.*, 2017). AC1 (C1) encodes replication-associated protein (Rep), a highly conserved protein in terms of sequence, position, and function; it is essential for viral replication and initiation of infection (Hanley-Bowdoin *et al.,* 1999; Fondong, 2013). AC2 (C2), a transcriptional activator protein (TrAP) is a multifunctional protein involved in gene activation (Shivaprasad *et al.*, 2005), suppression of gene silencing (Chowda-Reddy *et al.*, 2009) and virus pathogenicity (Hong *et al.*, 1996); resulting in activation of coat protein (CP) and

[1]Plant Science Division, Research School of Biology, Australian National University, Canberra, ACT-2601, AUSTRALIA

[2]DHR-MRU, King George's Medical University, Lucknow-226003, Uttar Pradesh, INDIA

Corresponding Author: Saurabh Verma, DHR-MRU, King George's Medical University, Lucknow-226003, INDIA; Mobile: +91-9565427565; +61-451155847; Email: saurabhv1982@gmail.com

How to cite this article: Verma, S. (2019). An Insight into the Approaches to Target Begomoviruses: RNAi Perspective. International Journal of Plant and Environment 5(3): 192-199.

Source of support: Nil

Conflict of interest: None

Submitted: 11.01.2019 **Accepted:** 01.07.2019 **Published:** 31.07.2019

viral movement protein (MP) in non-virus specific manner (Hanley-Bowdoin *et al*., 1999). AC3 (C3) encodes a replication enhancer protein which enhances viral DNA accumulation and symptom development in plants upon begomovirus infection (Sung and Coutts, 1995). AC4 (C4) ORF is contained within AC1 ORF but differ in frames and is least conserved of all begomoviral proteins with a different function in monopartite and bipartite viruses. Various studies have suggested its' role in symptom development i.e., development of leaf curl, chlorosis, vein swelling or enation due to abnormal cell division and virus movement (Mills-Lujan and Deom, 2010; Pooma *et al*., 1996). The AV1 or V1 ORF encodes the only structural protein CP which is involved in viral genome packaging, insect transmission, viral DNA shuttling in and out of nucleus and cell to cell movement in host plant (Boulton *et al*., 1989; Briddon *et al*., 1990; Liu *et al*., 1997; Pitakustheepong, 1999). AV2 or V2 ORF encodes a protein which has been putatively reported to be a viral suppressor of host silencing machinery (Chowda-Reddy *et al*., 2008).

The DNA-B genomic component is a characteristic of bipartite Begomoviruses and includes 2 ORFs namely BV1 on the complementary sense strand and BC2 on the virion sense strand. BV1 encodes nuclear shuttle protein (NSP) which is required for transport of the viral ss genome across nuclear membrane and cytoplasm (Noueiry *et al*., 1994). The MP is encoded by the BC2 ORF; required for the cell to cell and long-range movements through its cooperative interaction with the NSP-viral DNA complex (Lazarowitz and Beachy, 1999). Due to the similar redundant functions exhibited by AV1 and BV1, they are thought to be sharing common evolutionary origin (Zhou *et al*., 2007).

Betasatellite molecules are nearly half the size of DNA-A i.e. ~1.2-1.5kb. They utilize Rep protein produced by helper DNA i.e. DNA-A and initiate their replication and transcription of βC1 mRNA. Apart from being pathogenicity determinant, βC1 has also been shown to be helping in viral DNA accumulation, suppression of host RNAi machinery and helping in viral movement (Yang *et al*., 2012; Jia *et al*., 2016; Haxim *et al*., 2017). Because of these reasons, bipartite begomoviruses have been slowly taken over by monopartite begomoviruses populations in old world region. This phenomenon accounted for more than 250 betasatellite sequences being deposited in last 5-8 years. Such a large number of betasatellite components associated with DNA-A have given rise to several disease complexes in old world like Cotton leaf curl disease complex in Pakistan and Punjab area of India (Zubair, *et al*., 2017a,b). Such complexes have given rise to many epidemics in past few years leading to large scale economic losses (Pita *et al*., 2001; da Silva *et al*., 2011; Moriones *et al*., 2017; Sattar *et al*., 2017).

Plant immunity against virus invasion: RNA interference in plants

Post-transcriptional gene silencing or RNA interference involves suppression of gene expression by means of a complex population of small RNAs produced in response to the plant innate defence response called RNA silencing (Vance and Vaucheret, 2001). It is an evolutionary conserved, sequence-specific mechanism that regulates gene expression and defends organisms against abiotic stresses (Ghildiyal and Zamore, 2009; Kumar *et al*., 2017), invasive genetic material such as viral nucleic acids, transposons and other transgenes (Vaucheret, 2006; Ding *et al*., 2004; Matzke *et al*., 2015). Generally, 21-24-nt long small RNAs are called siRNAs whose genesis occurs from their dsRNA precursors or ssRNA molecules that form a self-complementary fold-back structure (Chapman and Carrington, 2007). The cloning of small RNA and Next Generation Sequencing (NGS) in Begomovirus infected plants has shown that the converging transcription of the nuclear transcripts is a major source of dsRNA precursors (Blevins *et al*., 2011; Seguin *et al*., 2014).

Transcriptional gene silencing: *De novo* and RdDM mediated cytosine methylation mechanism

DNA viruses need nuclei to replicate inside their plant hosts to increase their titer and thus cause infection in the host plant. The viral chromosomes are helped by host polymerases (host DNA Pol II in most of the cases) in generation of capped and polyadenylated viral RNAs (Sharma *et al*., 2013). The Begomovirus invasion is mainly countered by plants by means of DNA Methylation (DM) and by RNA dependent DNA Methylation (RdDM) which leaves a reversible epigenetic mark on the chromatin region (Fujimoto *et al*., 2012; He *et al*., 2014). The cytosine methylation (5meC) is catalyzed by de novo methyltransferases i.e. MET1, CMT3 AND CMT2 with the help of cofactors VIM and KYP. De novo establishment of methylation at both symmetric and asymmetric methylation sites is catalyzed by DRM2. DDM1 and RdDM proteins synergize to maintain all the cytosine methylation in the plant genome (Johnson *et al.,* 2007; Woo *et al.,* 2008; Zemach *et al.,* 2013). The DNA methylation occurs in the nucleosomal region involving both linker and core histones. The methylated DNA is repressed transcriptionally because it is packed into heterochromatin which is inaccessible to RNA polymerases. Thus, DNA methylation directed by 24-nt siRNAs plays a key role in the maintenance of chromatin states and regulation of gene expression in most eukaryotes (Xie and Yu, 2015). RNA directed DNA methylation (RdDM) is an important component of plant RNA silencing machinery as it defends it against transgenes, transposons, and viruses (Zvereva and Pooggin, 2012). It is mediated by Pol IV and Pol V, two plant-specific DNA dependent RNA polymerases functioning in the initiation of siRNA biogenesis and generation of scaffold transcript respectively. The scaffold transcripts produced by Pol V are *de novo* methylated and then targeted by AGO4 protein complex comprising of a 24-nt siRNA molecule interacting via the complementary pairing of siRNA and scaffold proteins and resulting into sequence-specific cleavage of their target RNAs (Ruiz-Ferrer and Voinnet, 2009; Bhatia *et al*., 2017).

How begomoviruses evade plant host silencing machinery?

The Geminiviruses mainly multiply inside host nucleus by Rep dependent DNA replication via rolling circle replication (RCR) and also partially by recombination-dependent replication (RDR) (Jeske *et al.,* 2001; Erdmann *et al.,* 2010). RDR generates a heterogeneous population of linear dsDNAs which become a target of cytosine methylation. RDR mediated generation of dsDNAs is the driving force for the Begomovirus evolution and their enhanced epidemiology all over the world through the formation of chimaeras in case of mixed infection and their vector-mediated transmission across the globe. The Begomoviruses have evolved various molecular mechanisms to evade plant RNA silencing pathways mainly bypassing the cytosine methylation/ DNA methylation, a primary defence response of plants against them. The Rep dependent RCR provides an efficient way for repression of methylation and thereby evasion of siRNA mediated transcriptional silencing (Stenger *et al*., 1991; Bian *et al*., 2006; Raja et al., 2010; Paprotka *et al*., 2011). RDR generated heterogeneous linear dsDNA is also incompatible with *de novo* and RdDM machinery in host plants. The above reasoning gets strength from the evidence that the geminiviral clones that were in vitro methylated gave rise

to unmethylated dsDNA strands in plant protoplasts, although there was some decrease in viral DNA titer as compared to the control protoplasts. This provides evidence of an initial role of cytosine methylation in repression of viral DNA replication in plants. This also is evidence that Geminiviruses demonstrate the ability to resurrect viral DNA from repressive cytosine methylation by bypassing the host maintenance methylation during its replication in the host nucleus (Brough *et al*., 1992; Ermak *et al*., 1993; Raja *et al*., 2008). Recruitment of begomovirus protein AC2, a transcription activation factor, AC4, a viral repressor protein (both DNA A) and betaC1 (β satellite) encoded by betastellite associated with begomoviruses also act as viral suppressors which activate the host genes initially repressed by methylation but later gets activated upon repression of maintenance methylation by viral dsDNA replication. The Old World Begomoviruses have shown to be up-regulating the WERNER-LIKE EXONUCLEASE 1 (WEL1) whose function is thought to be as a helper of viral DNA replication due to presence of a putative 3'-5' exonuclease activity similar to that of WERNER exonuclease involved in DNA replication and repair (Trinks *et al*., 2005). AC4, an open reading frame (ORF) lying within Rep encoding ORF but in a different reading frame is one of the least conserved of all begomovirus genes and its expression whether stable or transient resulted in no distinguishable phenotype in model plants. Yet, the action of AC4 is a major reason of the 'collateral damage' caused by binding single-stranded miRNAs and siRNAs; inhibiting the formation RNA-induced silencing complex (RISC) loading complex and leading to degradation of miRNAs and siRNAs thereby suppressing the RNA silencing mechanism of host plants (Chellappan *et al*., 2004, 2005; Vanitharani *et al*., 2005; Bisaro, 2006). Various begomoviral multifunctional proteins are thought to interact with ADENOSINE KINASE (ADK) and inactivate it thus, preventing the production of s-adenosylmethionine (SAM) thereby suppressing both TGS and PTGS (Wang *et al*., 2005; Buchmann *et al*., 2009). Thus, the viral minichromosome is armoured with various molecular shields and weapons which help them to modify cell cycle, initiate host DNA reduplication, suppress de novo and RdDM maintenance cytosine methylation and repress TGS and PTGS.

RNA interference as a tool against begomoviruses

The RNAi based approaches have an ability to ectopically reduce the expression of a target gene in a sequence-specific manner. RNAi has been effectively employed as an experimental tool for both functional characterizations of unidentified genes and for conferring virus resistance to host crop plants (Table 1). Apart from

Table 1: List of RNA interference based strategies used to develop virus-resistant transgenic plants.

S.N.	*Virus*	*Host*	*Approach*	*Target gene/region*	*Transgenic plant*	*Reference*
1	Cotton leaf curl burewala Virus (CLCuBuV)	Cotton	amiRNA	V2 gene sequence of CLCuBuV	*Nicotiana benthamiana*	Ali *et al*., 2013
2	Wheat streak mosaic virus (WSMV)	*Triticum aestivum*	amiRNA	5'UTR, polycistronic miRNA from P1, P3 and Hc-Pro genes	*T. aestivum*	Fahim *et al*., 2012
3	Cucumber mosaic virus (CMV)	*Cucumis sativus*	amiRNA	Gene silencing suppressor 2b of CMV	*N. benthamiana*	Qu *et al.,* 2007
4	Wheat dwarf virus (WDV)	*Triticum aestivum*	amiRNA	Different conservative sequence elements of WDV strains	*Hordeum vulgare*	Kis *et al*., 2016
5	Tomato yellow leaf curl virus (TYLCV)	*Solanum lycopersicum*	Antisense RNA	C1 encoded Rep protein	*N. benthamiana*	Bendahmane & Gronenborn, 1997
6	Cotton leaf curl virus (CLCuV)	cotton	Antisense RNA	Rep (AC1), TrAP(AC2) & REn (AC3)	*N.benthamiana*	Asad *et al.,* 2003
7	Vigna mungo yellow mosaic virus (VMYMV)	*Vigna mungo*	hpRNA construct	Promoter sequence of VMYMV 209bp long (2650-130nt) position	*V. mungo*	Pooggin *et al*., 2003
8	African cassava mosaic virus (ACMV)	cassava	Antisense RNA	Rep (AC1), TrAP(AC2) & REn (AC3)	cassava	Zhang *et al*., 2005
9	Citrus tristeza virus (CTV)	mexican lime	Antisense RNA	P23	mexican lime	Fagoaga *et al*., 2006
10	Tomato yellow leaf curl virus (TYLCV).	*S. lycopersicum*	siRNA construct	V1 gene encoding coat protein	*S. lycopersicum*	Zrachya *et al.,* 2007
11	Cassava brown streak virus (CBSV) & Cassava brown streak uganda virus (CBSUV)	Cassava	RNAi construct	Coat protein (CP)	*N. benthamiana*	Patil *et al*., 2011
12	Indian cassava mosaic virus (ICMV)	*Jatropha curcus*	Hair pin dsRNA	Fragment 1: 250 bp (CP/AV1 and AC5), Fragment 2: 250 bp (TrAP/AC2; Ren/AC3), Fragment 3: 609 bp (Rep/AC1 and AC4)	*J. curcus*	Ye *et al*., 2014

the above mentioned, the RNAi has been successfully employed to improve commercially useful traits and eliminate unwanted traits from plants (Mansoor *et al.,* 2006). The production of high stearic and oleic acid cotton seeds, thermally stable oil containing soybean seeds, tomato fruits with enhanced carotenoid and flavonoid content are few to mention here (Mansoor *et al.,* 2006). Early works in the production of virus resistant transgenic plants relied heavily on the pathogen-derived resistance approach, whereby a sequence or a part of the viral genome is introduced into the host plant in order to suppress viral invasion (Sanford and Johnston, 1985; Abel *et al.,* 1986).

But nowadays, with the development of advanced vector technologies based on Gateway® vectors technology i.e., pHELLSGATE, pIPK and PANDA and Golden Gate cloning technology; it has become quite easy to incorporate hairpin RNAs, sense RNA (co-suppression), anti-sense RNA, inverted-repeat (IR) sequences and artificial miRNA (amiRNAs) into the host plants for siRNA and miRNA induced gene silencing (Engler and Marillonet, 2011). The RNAi strategy was first reported in 1998 against Potato virus Y in potato plants where the vector containing sense and antisense transcripts of HC-Pro (a viral helper-component proteinase) conferred complete immunity in transgenic potato (Waterhouse *et al.,* 1998). Since then the RNAi has been successfully employed to confer resistance against Begomoviruses like Mungbean Yellow Mosaic Virus (MYMIV), African Cassava Mosaic Virus (ACMV), Tomato Yellow Leaf Curl Sardinia Virus, Indian Cassava Mosaic Virus (ICMV) and Ageratum Yellow Vein Virus (AYVV) using the above mentioned RNAi approaches (Pooggin *et al.,* 2003; Vanitharani *et al.,* 2003; Noris *et al.,* 2004; Yang *et al.,* 2014; Ye *et al.,* 2014).

Different RNAi based approaches against begomoviruses

RNA silencing, a homology-dependent siRNA-guided technique starts with the identification of conserved DNA sequence regions in a particular species of begomovirus. The conserved region can be submitted to any popular online siRNA target finder tools available to design siRNAs (Shukla *et al.* 2017). Following the identification of sense target sequences, the selected region is amplified using PCR (Verma and Saxena, 2017b), then an RNAi construct is designed incorporating the putative siRNA fragment as a hairpin forming precursor that favours constitutive expression of siRNAs upon transcription and processing by host siRNA machinery. The default length for a spacer hairpin sequence is 10-nt, however, longer spacers have been also reported in many cases; since it does not affect the potency and specificity of siRNAs. An additional purine must be added if not present at 5' end as the RNA pol III favours' C' 'G' at transcription initiation sites (Wang and Mu, 2004; Hirai and Kodama, 2008).

Various RNA silencing approaches for developing resistant crops against begomoviruses are:

Silencing mediated by sense RNA mediated 'co-suppression'

It is the earliest approach for RNA-mediated silencing in plants when accidentally the overexpression of chalcone synthase (*chs*) gene in Petunia resulted in silencing of 'Pink' colouration in flowers thus producing 'White' flowers (Napoli *et al.,* 1990). This pathway is also known as co-suppression as a result of 'over-abundance' of sense RNA resulting in suppression of both endogenous gene and the transgene as well. The 'over-abundance' of target transgene accumulation above a critical threshold signals the plant to eliminate these accumulated mRNA population from the nucleus irrespective of their origin and source. This mRNA degradation of PTGS signals initiation of RdRP mediated synthesis of templates for Dicer (DCL) mediated cleavage and chopping (Dougherty and Parks, 1995; Stam *et al.,* 1997).

Silencing by antisense RNA technology

The antisense RNA technology helps in gene silencing by providing a complementary strand that hybridizes against the transcribing endogenous mRNA in the nucleus; forming a dsRNA mediated inactivation or getting directed towards the silencing machinery of plants, thus, preventing the mRNA from translating a functional protein. This technique has not yielded efficient long-term silencing of Begomoviral DNA particles, however, combined targeting of ACMV replication genes AC1, AC2, and AC3 antisense RNA resulted in strong inhibition of ACMV in transgenic cassava. Short sense and antisense RNA specific to AC1 were identified in transgenic lines expressing AC1 antisense RNA, suggesting the involvement of PTGS mediated gene silencing (Zhang *et al.,* 2005).

Silencing by tandem Inverted Repeats (IR) containing hairpin RNA

It is the RNA silencing technique resulting in highest success rate in suppression of viral infection in crops which is approximately 90% as compared to meagre 10-20% shown by transgenic plants containing a single copy of sense and antisense RNA (Smith *et al.,* 2000; Waterhouse and Helliwell, 2003). This difference in efficiency could be attributed to the high level of dsRNA produced from single transcripts in case of IR without the requirement of host silencing machinery; these dsRNAs would thus act as a substrate for DCL4 and RISCs in case of DNA virus invasion in plants. This approach is suitable for RNA based viruses like Soybean Mosaic Virus (SMV), Bean Pod Mosaic Virus (BPMV), Sugarcane Mosaic Virus (SCMV), Maize Dwarf Mosaic Virus (MDMV) and Tobacco Mosaic Virus (TMV) to produce single or multiple virus resistant plants (Galvez *et al.,* 2014).

Artificial miRNA (amiRNA) mediated gene silencing:

MicroRNAs (miRNAs) have been considered the master regulators of gene expression in eukaryotes and they do so in a sequence-specific manner with evolutionarily conserved function as evident from studies based on functional characterization of miRNAs (Ruiz-Ferrer and Voinnet, 2009; Wang *et al.*, 2012). amiRNAs are synthetically designed 21-22 nt long dsRNA molecules which are incorporated into miRNA precursor molecules to modify the miRNA/miRNA* sequences in the duplex of a hairpin loop to generate mature miRNA with artificial sequences to control gene expression by PTGS (Ali *et al.*, 2013; Carbonell *et al.*, 2014; Chen *et al.*, 2016; Wagaba *et al.*, 2016). First report of amiRNA technology employed for conferring resistance towards viral silencing suppressor genes P69 and HC-Pro of Turnip Yellow Mosaic Virus (TYMV) and the Turnip Mosaic Virus (TuMV) respectively in Arabidopsis. They manipulated the 273 nt pre-miRNA159 backbone with amiR-P69 and amiR-HC-Pro into the hairpin loop to generate two independent amiRNA containing constructs. The resistance against TYMV and TuMV was astonishingly successful, even at low temperatures that inhibit PTGS (Niu *et al.,* 2006). The constructs containing multiple amiRNA directed against different viral nonstructural genes and repressors was generated to effectively delay infections or reduce the infection susceptibility in transgenic plants (Qu *et al.,* 2007; Carbonell *et al.,* 2014). The common limitation faced by this approach in conferring resistance to Begomoviral plant diseases is that the ssDNA viral genome replicates via rolling circle amplification as

well as by homologous recombination which helps in the genetic rearrangement of nucleotides in the viral genome. This enables the viral genome to evolve much faster than the plant miRNA (Lin *et al.,* 2009; Elena *et al.,* 2014), thus overcoming the conferred resistance.

Secondary siRNA mediated silencing

The secondary siRNAs are derived from ds- precursors, here the synthesis is stimulated by one or more upstream small RNAs requiring RDR6 and DCL4 as biogenesis factors. Slicing directed by 22nt miRNAs also initiates biogenesis of secondary RNAs from sliced target sequences (Axtell, 2013). The secondary siRNA mediated silencing signal acts through amplification pathway and traverses long distances from one cell to another as shown by grafting of silenced *Nicotiana tabacum* stock to a non-silenced scion expression the target gene (Voinnet *et al.,* 1998; Ryabov *et al.,* 2004). This implies that efficient silencing requires amplification steps in conjunction with the de novo synthesis of secondary siRNAs by host RDR in association with mobile RNA silencing. Thus, 20-30-nt duplexes with 3' overhang of 2-nt at each end (Ghildayal and Zamore, 2009) are diced and loaded onto RISC to target its complementary bases and direct PTGS by target mRNA cleavage. The amplification step multiplies the viral siRNA population by approximately 20-folds implicating the important role of RDR6 in the transitive spread of silencing signals against viral infection (Wang *et al.,* 2010). The concept of secondary siRNA has been reported in a unique approach where the silencing system allows induction of viral gene silencing in trans manner; this approach was named direct repeat-induced gene silencing (DRIGS) where a tandem array of single cistron incorporated into a silencing vector system confers resistance to multiple viruses (Mitra *et al.,* 2010). Therefore, this approach holds a great promise in the efforts to produce begomovirus resistance transgenic plants.

Conclusion and Future Perspective

The ss-DNA begomoviruses have an advantage of possessing viral suppressor genes which inhibit the adaptive response of plants by attenuation of their silencing machinery. They perform this silencing by partially inhibiting dicer activity and by interfering with the unwinding of duplex siRNA to prevent its incorporation into RISC; they also block the systemic silencing signals from traversing cellular boundaries (Bisaro, 2006). The successful siRNA targeting of begomoviral genome is difficult due to the presence of these viral suppressors which help the viral replication machinery to regain the invasive ability once the replicative DNA content crosses its relative inhibitory threshold limit. The unprecedented high rate of failure of RNA silencing has been observed in the cases where distinct begomoviral species are involved in co-infection. The synergism provided by the mixed infection enhances the fitness component of at least one of the members of this complex and thus the infection cannot be controlled by traditional anti-viral strategies (Noris *et al.,* 2004; Elena *et al.,* 2014).

In light of the serious begomovirus threat, we need to develop better RNA silencing approaches where the viral suppressors are targeted along with other structural and non-structural begomoviral genes to totally suppress the probability of begomovirus infection in important crops of the world. Therefore, a continuous effort is required to identify genomic adaptations acquired by the ever-evolving begomoviruses and design a suitable RNA silencing strategy to eliminate the risk of epidemiological invasion on crop plants globally.

Acknowledgment

SV acknowledges Division of Plant Sciences, Research School of Biology, Australian National University, Australia, Indian Council for Medical Research, Government of India, New Delhi and DHR-MRU, King George's Medical University for providing the necessary infrastructure and financial support in preparation of this manuscript.

References

Abel, P.P., Nelson, R.S., De, B., Hoffmann, N., Rogers, S.G., Fraley, R.T. and Beachy, R.N. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science* **232**: 738-743.

Ali, I., Amin, I., Briddon, R.W., and Mansoor, S. 2013. Artificial microRNA-mediated resistance against the monopartite begomovirus Cotton leaf curl Burewala virus. *Virology Journal* **10**: 231.

Asad, S., Haris, W.A.A., Bashir, A., Zafar, Y., Malik, K.A., Malik, N.N., and Lichtenstein, C.P. 2003. Transgenic tobacco expressing geminiviral RNAs are resistant to the serious viral pathogen causing cotton leaf curl disease. *Archives of Virology* **148**: 2341-2352.

Axtell, M.J. 2013. Classification and Comparison of small RNAs from plants. *Annual Reviews in Plant Biology* **64**: 5.1-5.23.

Bendahmane, M. and Gronenborn, B. 1997. Engineering resistance against tomato yellow leaf curl virus (TYLCV) using antisense RNA. *Plant Molecular Biology* **33**(2): 351-357.

Bhatia, G., Goyal, N., Sharma, S., Upadhyay, S. and Singh, K. 2017. Present Scenario of Long Non-Coding RNAs in Plants. *Non-Coding RNA* **3**: 16.

Bian, X.Y., Rasheed, M.S., Seemanpillai, M.J. and Rezaian, M.A. 2006. Analysis of Silencing escape of Tomato leaf curl virus: an evaluation of the role of DNA methylation. *Molecular Plant-Microbe Interactions* **19**: 614-624.

Bisaro, D.M. 2006. Silencing suppression by geminivirus proteins. *Virology* **344**: 158-168.

Blevins, T., Rajeswaran, R., Aregger, M., Borah, B. K., Schepetilnikov, M., Baerlocher, L., Farinelli, L., Meins, F.Jr., Hohn, T. and Pooggin, M.M. 2011. Massive production of small RNAs from a non-coding region of Cauliflower mosaic virus in plant defense and viral counter-defense. *Nucleic Acids Research* **39**: 5003-5014.

Borah, B.K., Dasgupta, I. 2012. Begomovirus research in India: A critical appraisal and the way ahead. *Journal of Biosciences* **37**: 791-806.

Boulton, M.I., Steinkellner, H., Donson, J., Markham, P.G., King, D.I. and Davies, J.W. 1989. Mutational analysis of the virion-sense genes of maize streak virus. *Journal of General Virology* **70**: 2309-2323.

Briddon, R.W., Pinner, M.S., Stanley, J. and Markham, P.G. 1990. Geminivirus coat protein replacement alters insect specificity. *Virology* **177**: 85-94.

Brough, C.L., Gardiner, W.E., Inamdar, N.M., Zhang, X.Y., Ehrlich, M. and Bisaro, D.M. 1992. DNA methylation inhibits propagation of tomato golden mosaic virus DNA in transfected protoplasts. *Plant Molecular Biology* **18**: 703-712.

Buchmann, R.C., Asad, S., Wolf, J.N., Mohannath, G. and Bisaro, D.M. 2009. Geminivirus AL2 and L2 proteins suppress transcriptional gene silencing and cause genome-wide reductions in cytosine methylation. *Journal of Virology* **83**: 5005-5013.

Carbonell, A., Takeda, A., Fahlgren, N., Johnson, S.C., Cuperus, J.T., and Carrington, J.C. 2014. New generation of artificial microrna and synthetic trans-acting small interfering RNA vectors for efficient gene silencing in *Arabidopsis*. *Plant Physiology* **165**: 15-29.

Chapman, E.J. and Carrington, J.C. 2007. Specialization and evolution of endogenous small RNA pathways. *Nature Reviews in Genetics* **8**: 884-896.

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. *Journal of Virology* **78**: 7465-7477.

Chellappan, P., Vanitharani, R., Pita, J. and Fauquet, C.M. 2005. MicroRNA-binding viral protein interferes with *Arabidopsis* development. *Proceedings of the National Academy of Science in* USA **102**: 10381-10386.

Chen, L., Cheng, X., Cai, J., Zhan, L., Wu, X., Liu, Q., Wu, X. 2016. Multiple virus resistance using artificial trans-acting siRNAs. *Journal of Virological Methods* **228**: 16-20.

Chowda-Reddy, R.V.C., Achenjang, F., Felton, C., Etarock, M.T., Anangfac, M.T., Nugent, P. and Fondong, V.N. 2008. Role of a geminivirus AV2 protein putative protein kinase C motif on subcellular localization and pathogenicity. *Virus Research* **135**: 115-124.

Chowda-Reddy, R.V.C., Dong, W., Felton, C., Ryman, D., Ballard, K. and Fondong, V.N. 2009. Characterization of the cassava geminivirus transcription activation protein putative nuclear localization signal. *Virus Research* **145**: 270-278.

da Silva, S.J.C., Castillo-Urquiza, G.P., Hora Júnior, B.T., Assunção, I.P., Lima, G.S., Pio-Ribeiro, G., Mizubuti, E.S. and Zerbini, F.M. 2011. High genetic variability and recombination in a begomovirus population infecting the ubiquitous weed *Cleome affinis* in northeastern Brazil. *Archives of Virology* **156**: 2205-2213.

Ding, S.W., Li, H., Lu, R., Li, F. and Li, W.X. 2004. RNA silencing: A conserved antiviral immunity of plants and animals. *Virus Research* **102**: 109-115.

Dougherty, W.G. and Parks, T.D. 1995. Transgenes and gene suppression: telling us something new? *Curent Opinion in Cell Biology* **7**: 399-405.

Elena, S.F., Bernet, G.P. and Carrasco, J.L. 2014. The games plant viruses play. *Current Opinion in Virology* **8**: 62-67.

Engler, C. and Marillonnet, S. (2011). Generation of Families of Construct Variants using Golden Gate Shuffling. In: Lu, C., Browse, J. and Wallis, J. (Eds.) cDNA Libraries. *Methods in Molecular Biology (Methods and Protocols),* Humana Press, pp. 729.

Erdmann, J.B., Shepherd, D.N., Martin, D.P., Varsani, A., Rybicki, E.P. and Jeske, H. 2010. Replicative intermediates of maize streak virus found during leaf development. *Journal of General Virology* **91**(4): 1077-1081.

Ermak, G., Paszkowski, U., Wohlmuth, M., Mittelsten Scheid, O. and Paszkowski, J. 1993. Cytosine methylation inhibits replication of African cassava mosaic virus by two distinct mechanisms. *Nucleic Acids Research* **21**: 3445-3450.

Fagoaga, C., López, C., de Mendoza, A.H., Moreno, P., Navarro, L., Flores, R. and Peña L. 2006. Post-transcriptional gene silencing of the p23 silencing suppressor of *Citrus tristeza* virus confers resistance to the virus in transgenic Mexican lime. *Plant Molecular Biology* **60**: 153-165.

Fahim, M., Millar, A.A., Wood, C.C. and Larkin, P.J. 2012. Resistance to Wheat streak mosaic virus generated by expression of an artificial polycistronic microRNA in wheat. *Plant Biotechnology Journal* **10**: 150-163.

Fondong, V.N. 2013. Geminivirus protein structure and function. *Molecular Plant Pathology* **14**: 635-649.

Fujimoto, R., Sasaki, T., Ishikawa, R., Osabe, K., Kawanabe, T. and Dennis, E.S. 2012. Molecular mechanisms of epigenetic variation in plants. *International Journal of Molecular Sciences* **13**: 9900-9922.

Galvez, L.C., Banerjee, B., Pinar, H. and Mitra, A. 2014. Engineered plant virus resistance. *Plant Science* **228**: 11-25.

Ghildiyal, M. and Zamore, P.D. 2009. Small silencing RNAs: an expanding universe. *Nature Review of Genetics* **10**: 94-108.

Hanley-Bowdoin, L., Settlage, S.B., Orozco, B.M., Nagar, S. and Robertson, D. 1999. Geminiviruses: models for plant DNA replication, transcription, and cell cycle regulation. *Critical Reviews in Plant Science* **18**: 71-106.

Haxim, Y., Ismayil, A., Jia, Q., Wang, Y., Zheng, X., Chen, T., Qian, L., Liu, N., Wang, Y., Han, S., Cheng, J., Qi, Y., Hong, Y. and Liu, Y. 2017. Autophagy functions as an antiviral mechanism against geminiviruses in plants. *eLife* **6**: 1-17.

He, X., Ma, Z., and Liu, Z. 2014. Non-coding RNA transcription and RNA-directed DNA methylation in *Arabidopsis*. *Molecular Plant* **7**: 1406-1414.

Hirai, S. and Kodama, H. 2008. RNAi vectors for manipulation of gene expression in higher plants. *The Open Plant Science Journal* **2**: 21-30.

Hong, Y., Saunders, K., Hartley, M.R. and Stanley, J. 1996. Resistance to Geminivirus infection by virus-induced expression of dianthin in transgenic plants. *Virology* **220**: 119-127.

Jeske, H., Lütgemeier, M. and Preiß, W. 2001. DNA forms indicate rolling circle and recombination-dependent replication of Abutilon mosaic virus. *EMBO Journal* **20**: 6158-6167.

Jia, Q., Liu, N., Xie, K., Dai, Y., Han, S., Zhao, X., Qian, L., Wang, Y., Zhao, J., Gorovits, R., Xie, D., Hong, Y. and Liu, Y. 2016. CLCuMuB βC1 Subverts ubiquitination by interacting with NbSKP1s to enhance geminivirus infection in *Nicotiana benthamiana*. *PLoS Pathogen* **12**: 1-30.

Johnson, L.M., Bostick, M., Zhang, X., Kraft, E., Henderson, I., Callis, J. and Jacobsen, S.E. 2007. The SRA methyl-cytosine-binding domain links DNA and histone methylation. *Current Biology* **17**: 379-384.

Kis, A., Tholt, G., Ivanics, M., Várallyay, É., Jenes, B. and Havelda, Z. 2016. amiRNA-mediated WDV resistance in barley. *Molecular Plant Pathology* **17**: 427-437.

Krupovic, M., Ravantti, J.J. and Bamford, D.H. 2009. Geminiviruses: a tale of a plasmid becoming a virus. *BMC Evolutionary Biology* **9**: 112.

Kumar, S., Verma, S. and Trivedi, P.K. 2017. Role of involvement of small RNAs in phosphorus and sulfur sensing, signaling and stress: Current update. *Frontiers in Plant Science* **10**: 285.

Lazarowitz, S.G. and Beachy, R.N. 1999. Viral movement proteins as probes for intracellular and intercellular trafficking in plants. *Plant Cell* **11**: 535-548.

Lin, S.S., Wu, H.W., Elena, S.F., Chen, K.C., Niu, Q.W., Yeh, S.D., Chen, C.C. and Chua, N.H. 2009. Molecular evolution of a viral non-coding sequence under the selective pressure of amiRNA-mediated silencing. *PLoS Pathogen* **5**: e1000312.

Liu, S., Bedford, I.D., Briddon, R.W., and Markham, P.G. 1997. Efficient whitefly transmission of African cassava mosaic geminivirus requires sequences from both genomic components. *Journal of General Virology* **78**: 1791-1794.

Mansoor, S., Amin, I., Hussain, M., Zafar, Y. and Briddon, R.W. 2006. Engineering novel traits in plants through RNA interference. *Trends in Plant Science* **11**: 559-565.

Matzke, M.A., Kanno, T. and Matzke, A.J.M. 2015. RNA-Directed DNA Methylation: The Evolution of a Complex Epigenetic Pathway in Flowering Plants. *Annual Reviews in Plant Biology* **66**: 243-267.

Meena, S.C. and Chattopadhyay, A. 2016. Begomovirus Disease Complex: Threat in India. Vaish, S.S. (Ed.) Plant Diseases and their Sustainable Management, Biotech Publishers, India.

Mills-Lujan, K. and Deom C.M. 2010. Geminivirus C4 protein alters *Arabidopsis* development. *Protoplasma* **239**: 95-110.

Mitra, A., Ma, C., Han, J. and Galvez, L. 2010. Gene silencing in plants. *Indian Journal of Agricultural Biochemistry* **23**: 69-82.

Moriones, E., Praveen, S. and Chakraborty, S. 2017. Tomato leaf curl New Delhi virus: An emerging virus complex threatening vegetable and fiber crops. *Viruses* **9**: 264.

Napoli, C., Lemieux, R. and Jorgensen, R. 1990. Introduction of a chimeric chalcone synthase gene into *Petunia* results in reversible co-suppression of homologous genes in trans. *Plant Cell* **2**: 279-289

Niu, Q.W., Lin, S.S., Reyes, J.L., Chen, K.C., Wu, H.W., Yeh, S.D. and Chua, N.H. 2006. Expression of artificial microRNAs in transgenic *Arabidopsis thaliana* confers virus resistance. *Nature Biotechnology* **24**: 1420-1428.

Noris, E., Lucioli, A., Tavazza, R., Caciagli, P., Accotto, G.P. and Tavazza, M. 2004. Tomato yellow leaf curl Sardinia virus can overcome transgene-mediated RNA silencing of two essential viral genes. *Journal of General Virology* **85**: 1754-1749.

Noueiry, A.O., Lucas, W.J. and Gilbertson, R.L. 1994. Two proteins of a plant DNA virus coordinate nuclear and plasmodesmal transport. *Cell* **76**: 925-932.

Paprotka, T., Deuschle, K., Metzler, V. and Jeske, H. 2011. Conformation-Selective Methylation of Geminivirus DNA. *Journal of Virology* **85**(22): 12001-12012.

Patil, B.L., Ogwok, E., Wagaba, H., Mohammed, I.U., Yadav, J.S., Bagewadi, B., Taylor, N.J., Kreuze, J.F., Maruthi, M.N., Alicai, T. and Fauquet, C.M. 2011. RNAi-mediated resistance to diverse isolates belonging to two virus species involved in Cassava brown streak disease. *Molecular Plant Pathology* **12**: 31-41.

Pita, J.S., Fondong, V.N., Sangaré, A., Otim-Nape, G.W., Ogwal, S. and Fauquet, C.M. 2001. Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. *Journal of General Virology* **82**: 655-665.

Pitaksutheepong, C. 1999. Biological and functional aspects of the movement proteins of Maize streak virus and Bean yellow dwarf virus in transgenic plants. PhD Thesis, University of East Anglia, Norwich, Norfolk.

Pooggin, M., Shivaprasad, P.V., Veluthambi, K. and Hohn, T. 2003. RNAi targeting of DNA virus in plants. *Nature Biotechnol*ogy **212**: 131-132.

Pooma, W., Gillette, W.K., Jeffrey, J.L. and Petty, I.T.D. 1996. Host and viral factors determine the dispensability of coat protein for bipartite geminivirus systemic movement. *Virology* **218**: 264-268.

Qu, J., Ye, J. and Fang, R. 2007. Artificial microRNA-mediated virus resistance in plants. *Journal of Virology* **81**: 6690-6699.

Raja, P., Sanville, B.C., Buchmann, R.C. and Bisaro, D.M. 2008. Viral genome methylation as an epigenetic defense against geminiviruses. *Journal of Virology* **82**: 8997-9007.

Raja, P., Wolf, J.N. and Bisaro, D.M. 2010. RNA silencing directed against geminiviruses: Post-transcriptional and epigenetic components. *Biochimica et Biophysica Acta-Gene Regulatory Mechanisms* **1799**: 337-351.

Ruiz-Ferrer, V and Voinnet, O. 2009. Roles of Plant small RNAs in biotic stress responses. *Annual Reviews in Plant Biology* **60**: 485-510.

Ryabov, E.V., van Wezel, R., Walsh, J. and Hong, Y. 2004. Cell-to-Cell, but not long-distance, spread of RNA silencing that is induced in individual epidermal cells. *Journal of Virology* **78**: 3149-3154.

Sanford, J.C and Johnston, S.A. 1985. The concept of parasite-derived resistance-deriving resistance genes from the parasite's own genome. *Journal of Theoretical Biology* **113**: 395-405.

Sattar, M.N., Iqbal, Z., Tahir, M.N., and Ullah, S. 2017. The prediction of a new CLCuD epidemic in the old world. *Frontiers in Microbiology* **8**: 1-9.

Saxena, S., Singh, V.K. and Verma, S. 2016. PCR mediated detection of sex and PaLCuV infection in papaya - A review. *Journal of Applied Horticulture* **18**: 80-84.

Seguin, J., Rajeswaran, R., Malpica-López, N., Martin, R.R., Kasschau, K., Dolja, Otten, P., Farinelli, L. and Pooggin, M. 2014. De novo reconstruction of consensus master genomes of plant RNA and DNA viruses from siRNAs. *PLoS One* **9**: 1-8.

Sharma, N., Sahu, P.P., Puranik, S. and Prasad, M. 2013. Recent advances in plant-virus interaction with emphasis on small interfering RNAs siRNAs. *Molecular Biotechnology* **55**: 63-77.

Shivaprasad, P.V., Akbergenov, R., Trinks, D., Rajeswaran, R., Veluthambi, K., Hohn, T and Pooggin, M.M. 2005. Promoters, transcripts, and regulatory proteins of Mungbean yellow mosaic geminivirus. *Journal of Virology* **79**: 8149-8163.

Shukla, N., Verma, S., Babu, G.S. and Saxena, S. 2017. Strategy for Generic Resistance Against Begomoviruses Through RNAi. In: Saxena, S. and Tiwari, A.K. (Eds.), *Begomoviruses: Occurrence and Management in Asia and Africa*. Springer Singapore, Singapore, pp. 137-155.

Smith, N.A., Singh, S.P., Wang, M.B., Stoutjesdijk, P.A., Green, A.G. and Waterhouse, P.M. 2000. Total silencing by intron-spliced hairpin RNAs. *Nature* **407**: 319-320.

Stam, M., Mol, J.N.M. and Kooter, J.M. 1997. The silence of genes in transgenic plants. *Annals of Botany* **79**: 3-12.

Stenger, D.C., Revington, G.N., Stevenson, M.C. and Bisaro, D.M. 1991. Replicational release of geminivirus genomes from tandemly repeated copies: evidence for rolling-circle replication of a plant viral DNA. *Proceedings of the National Academy of Science, USA* **88**: 8029-8033.

Sung, Y.K. and Coutts, R.H. 1995. Mutational analysis of potato yellow mosaic geminivirus. *Journal of General Virology* **76**: 1773-1780.

Trinks, D., Rajeswaran, R., Shivaprasad, P.V., Akbergenov, R., Oakeley, E.J., Veluthambi, K., Hohn, T. and Pooggin, M.M. 2005. Suppression of RNA silencing by a geminivirus nuclear protein, AC2, correlates with transactivation of host genes. *Journal of Virology* **79**: 2517-2527.

Vance, V. and Vaucheret, H. 2001. RNA silencing in plants - Defense and counterdefense. *Science* **80**: 2277-2280.

Vanitharani, R., Chellappan, P. and Fauquet, C.M. 2003. Short interfering RNA-mediated interference of gene expression and viral DNA accumulation in cultured plant cells. *Proceedings of the National Science Academy USA* **100**: 9632-9636.

Vanitharani, R., Chellappan, P. and Fauquet, C.M. 2005. Geminivirus and RNA silencing. *Trends in Plant Science* **10**: 144-151.

Vaucheret, H. 2006. Post-transcriptional small RNA pathways in plants: Mechanisms and regulations. *Genes and Development* **20**: 759-771.

Verma, S. and Saxena, S. 2017a. Dissecting Papaya Leaf Curl Disease PLCD complex and assessing its potential for siRNA based targeting. *International Journal of Plant and Environment* **3**: 17-29.

Verma, S. and Saxena, S. 2017b. Chapter 3: Recent Advancement in Diagnosis of Begomoviruses. In: Saxena, S. and Tiwari, A.K. (Eds.). Begomoviruses: Occurrence and Management in Asia and Africa, Springer Nature, Singapore.

Voinnet, O., Vain, P., Angell, S. and Baulcombe, D.C. 1998. Systemic spread of sequence-specific transgene RNA degradation in plants is initiated by localized introduction of ectopic promoter less DNA. *Cell* **95**: 177-187.

Wagaba, H., Patil, B.L., Mukasa, S., Alicai, T., Fauquet, C.M., and Taylor, N.J. 2016. Artificial microRNA-derived resistance to *Cassava* brown streak disease. *Journal of Virological Methods* **231**: 38-43.

Wang, H., Buckley, K.J., Yang, X., Buchmann, R.C. and Bisaro, D.M. 2005. Adenosine kinase inhibition and suppression of RNA silencing by geminivirus AL2 and L2 proteins. *Journal of Virology* **79**: 7410-7418.

Wang, L. and Mu F.Y. 2004. A web based design center for vector based siRNA and siRNA cassette. *Bioinformatics* **2011**: 1818-1820.

Wang, M.B., Masuta, C., Smith, N. A., and Shimura, H. 2012. RNA Silencing and Plant Viral Diseases. *Molecular Plant-Microbe Interactions* **25**: 1275-1285.

Wang, X.B., Wu, Q., Ito, T., Cillo, F., Li, W.X., Chen, X., Yu, J.L. and Ding, S.W. 2010. RNAi-mediated viral immunity requires amplification of virus-derived siRNAs in *Arabidopsis thaliana*, *Proceedings of the National Academy of Science USA* **107**: 484-489.

Waterhouse, P.M. and Heliwell, C.A. 2003. Exploring plant genomes by RNA-induced gene silencing. *Nature Reviews in Genetics* **4**: 29-38.

Waterhouse, P.M., Graham, M.W. and Wang, M.B. 1998. Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. *Proceedings of the National Academy of Science USA* **95**: 13959-13964.

Woo, H.R., Dittmer, T.A., Richards and E.J. 2008. Three SRA-domain methylcytosine-binding proteins cooperate to maintain global CpG methylation and epigenetic silencing in *Arabidopsis*. *PLoS Genetics* **4**(8): 1000156.

Xie, M. and Yu, B. 2015. siRNA-directed DNA Methylation in Plants. *Current Genomics* **16**: 23-31.

Yang, C.F., Chen, K.C., Cheng, Y.H., Raja J.A.J., Huang, Y.L., *et al*. 2014. Generation of Marker-free transgenic plants concurrently resistant to a DNA Geminivirus and a RNA Topsovirus. *Scientific Reports* **4**: 5717-5728.

Yang, X. and Zhou, X. 2017. Chapter 62 - Betasatellites of Begomoviruses. In: Hadidi, A., Flores, R., Randles, J.W. and Palukaitis, P. (Eds.) In *Viroids and Satellites*. Academic Press, 2017, pp. 671-678.

Yang, X., Xie, Y., Raja, P., Li, S.,Wolf, J.N., Shen,Q., Bisaro, D.M. and Zhou, X. 2012. Suppression of methylation-mediated transcriptional gene silencing by βC1-SAHH protein interaction during geminivirus-betasatellite infection. *PLoS Pathogen* **7**: e1002329.

Ye, J., Qu, J., Mao, H.Z., Ma, Z.G., Rahman, N.E.B., Chao, B., Chen, W., Jiang, S-Y., Ramachandran, S. and Chua, N-H. 2014. Engineering Geminivirus resistance in *Jatropha curcus*. *Biotechnology For Biofuels* **7**: 149-160.

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. *The Journal of General Virology* **982**: 131-133.

Zemach, A., Kim, M.Y., Hsieh, P.H., Coleman-Derr, D., Eshed-Williams,L., Thao, K., Harmer, S.L. and Zilberman, D. 2013 The *Arabidopsis* nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. *Cell* **153**: 193-205.

Zhang, P., Vanderschuren, H., Fütterer, J. and Gruissem, W. 2005. Resistance to cassava mosaic disease in transgenic cassava expressing antisense RNA targeting virus replication genes. *Plant Biotechnology Journal* **34**: 385-397.

Zhou, X. 2013. Advances in understanding Begomovirus satellites. *Annual Review of Phytopathology* **51**: 357-381.

Zhou, Y.C., Garrido-Ramirez, E.R., Sudarshana, M.R., Yendluri, S. and Gilbertson, R.L. 2007. The N-terminus of the Begomovirus nuclear shuttle protein BV1 determines virulence or avirulence in *Phaseolus vulgaris*. *Molecular Plant-Microbe Interaction* **20**: 1523-1534.

Zrachya, A., Kumar, P.P., Ramakrishnan, U., Levy, Y., Loyter, A., Arazi, T., Lapidot, M. and Gafni, Y. 2007. Production of siRNA targeted against TYLCV coat protein transcripts leads to silencing of its expression and resistance to the virus. *Transgenic Research* **16**: 385-398.

Zubair, M., Zaidi, S., Shakir, S., Amin, I., and Mansoor, S. 2017a. An insight into cotton leaf curl multan betasatellite, the most important component of cotton leaf curl disease complex. *Viruses* **9**: 280

Zubair, M., Zaidi, S. S. E. A., Shakir, S., Farooq, M., Amin, I., Scheffler, J. A., Scheffler, B.E. and Mansoor, S. 2017b. Multiple begomoviruses found associated with cotton leaf curl disease in Pakistan in early 1990 are back in cultivated cotton. *Scientific Reports* **7**: 1-11.

Zvereva, A.S. and Pooggin, M.M. 2012. Silencing and innate immunity in plant defense against viral and non-viral pathogens. *Viruses* **4**: 2578-2597.