Virus Research 318 (2022) 198844

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Plant epigenome alterations: an emergent player in viroid-host interactions

Gustavo Gómez [a,*], Joan Marquez-Molins [a], German Martinez [b], Vicente Pallas [c]

[a] Institute for Integrative Systems Biology (I2SysBio), Consejo Superior de Investigaciones Científicas (CSIC) - Universitat de València (UV), Parc Científic, Cat. Agustín Escardino 9, 46980 Paterna, Spain.
[b] Department of Plant Biology, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, SE-750 07 Uppsala, Sweden
[c] Instituto de Biología Molecular y Celular de Plantas (IBMCP), Consejo Superior de Investigaciones Científicas (CSIC) - Universitat Politècnica de València, CPI 8E, Av. de los Naranjos s/n, 46022 Valencia, Spain

ARTICLE INFO

Key words:
Viroid-host interaction
DNA methylation
Viroid-induced pathogenesis
Epigenetic plant-response to infections
RNA-dependent DNA-Methylation

ABSTRACT

It is well known that viroids promote significant alterations at diverse host regulatory levels. However the mechanisms by which these tiny RNAs subvert endogenous regulatory networks remain a to a large extent unsolved question. In the last years diverse studies have revealed the existence of a close interplay between viroid infection and host DNA methylation, suggesting that the modulation of the endogenous transcriptional activity by epigenetic alterations of the host genome may emerge as a novel player in plant-viroid interactions. Here, we summarize the more relevant findings related to alteration of the host DNA methylome in response to viroid infection and discus the potential strategies which may be exploited by these non-conventional pathogenic RNAs to hijack and redesign the plant epigenome.

1. ANTECEDENTS

Constrained by a naked and minimal genome (250 - 400 nt), viroids have evolved into versatile molecular entities capable of subvert the host-cell machinery at diverse functional levels to guarantee their infectious cycle (Gómez and Pallás, 2013; Gago-Zachert, 2016; Adkar--Purushothama and Perreault, 2020; Navarro et al., 2021a). In certain viroid-host interactions, this crosstalk can trigger phenotypic alterations recognized as symptoms (Navarro et al., 2021a). Although several studies have demonstrated that during the infectious process, viroids promote significant alterations at diverse plant regulatory levels, the mechanisms by which these tiny RNAs, with null or residual coding activity, hijack endogenous regulatory networks remain an unsolved question (Gómez and Pallás, 2013).

It is well established that a massive transcriptional reprograming constitutes the common characteristic response of plants to adverse environmental conditions, including pathogen infection (Waters et al., 2017; Wang et al., 2020b). Indeed, host transcriptional reprogramming is a central part of plant defense upon pathogen recognition (Tsuda and Somssich, 2015; Pesti et al., 2019). In agreement with this association between transcriptional reprogramming and defense against pathogens, significant changes at the transcriptomic level have been characterized in diverse viroid-host pathosystems including members of both *Pospiviroidae* and *Avsunviroidae* families (Owens et al., 2012; Rizza et al., 2012; Herranz et al., 2013; Katsarou et al., 2016a; Kappagantu et al., 2017; Xia et al., 2017; Zheng et al., 2017; Thibaut and Claude, 2018; Štajner et al., 2019; Takino et al., 2019; Wang et al., 2019b; Wiesyk et al., 2019)(Rizza et al., 2012; Herranz et al., 2013; Katsarou et al., 2016b; Kappagantu et al., 2017; Xia et al., 2017; Štajner et al., 2019; Wiesyk et al., 2019).

Transcriptional reprogramming is consequence of dynamic changes at a deeper level in the genome, and several regulatory layers (operating at both transcriptional and post-transcriptional levels) can modulate the complexity and/or intensity of the transcriptional response to infection (Tsuda and Somssich, 2015; Annacondia et al., 2018; Annacondia and Martinez, 2021). One of those regulatory layers is composed by epigenetic mechanisms, such as DNA methylation and histone modification, that modulate the gene accessibility to the transcriptional machinery and are a key component of the plant response to pathogens (Tsuda and Somssich, 2015; Annacondia et al., 2018; Hannan Parker et al., 2022).

DNA methylation is an epigenetic mark that modulates gene and transposable element (TE) expression and, by hence, regulates diverse processes such as genome stability and gene imprinting. In plants, DNA methylation is found in the CG, CHG and CHH sequence contexts (where H is A, C or T). This phenomenon plays a prominent role in the regulation of the gene expression through Transcriptional Gene Silencing

* Correspondence author.
E-mail address: gustavo.gomez@csic.es (G. Gómez).

https://doi.org/10.1016/j.virusres.2022.198844
Received 12 April 2022; Received in revised form 31 May 2022; Accepted 5 June 2022
Available online 6 June 2022

(TGS) (Zhang et al., 2018).

Diverse studies support the existence of a close interplay between infection and host DNA methylation in plants infected by virus (Raja et al., 2008; Yang et al., 2013; Wang et al., 2019a; Corrêa et al., 2020), bacteria (Pavet et al., 2006; Agorio and Vera, 2007; H. et al., 2012; Agnès et al., 2013; Deleris et al., 2016) and viroids (Martinez et al., 2014). All these studies suggest that the modulation of the endogenous transcriptional activity by epigenetic mechanisms of the host genome may constitute an emerging (and poorly described) player in plant-pathogen interactions (Zhu et al., 2016).

The first association between viroid infection and DNA methylation was reported at the end of the past century by researchers studying potato spindle tuber viroid (PSTVd) infection in tobacco plants transformed with a partial non-infectious cDNA of the PSTVd genome (Wassenegger et al., 1994). Although this analysis did not describe a direct relationship between viroid infection and epigenetic changes associated with the pathogen, it led to the discovery of the RNA-directed DNA-methylation (RdDM) phenomenon. In their analyses, Wassenegger et al (1994) showed that PSTVd transgenes became methylated at the DNA level when the transgenic plants where infected with the full viroid sequence. This was the first evidence that RdDM existed in any organism and it was crucial to the research on this topic (Wassenegger and Dalakouras, 2021). The current RdDM model propose that RNA polymerase IV (POL IV)-derived 24-nucleotide siRNAs (canonical RdDM) or, in exceptional cases, POL II-derived 21/22-nucleotide siRNAs (non-canonical RdDM), are loaded onto AGO4/6 or 9 and guide domains-rearranged methyltransferase 2 (DRM2) to methylate cognate DNA, most likely through a process wherein siRNAs directly interact with DNA or interact with the nascent transcripts produced by POL V (Erdmann and Picard, 2020; Gallego-Bartolomé, 2020).

In the last years, several pieces of evidence point to this mechanism as a previously ignored player in viroid-host interactions. Here, we briefly summarize the more relevant findings related to the alteration of the host DNA methylome in response to viroid infection (Table 1) and discus the potential strategies which may be exploited by these non-conventional pathogenic RNAs to subvert and redesign the plant epigenome.

2. VIROID INFECTION AND HOST EPIGENETIC CHANGES

As mentioned above, the initial experimental evidence supporting the link between viroid infection and DNA methylation was provided by the observation that PSTVd replication was associated to specific *de novo* methylation of a PSTVd-cDNA integrated as a transgene in tobacco plants (Wassenegger et al., 1994). However, it was not until the year 2014 when the direct interference of viroid infection with the methylation of host sequences was demonstrated. Employing hop stunt viroid (HSVd) as a pathogenic model (Marquez-Molins et al., 2021), it was established that viroid infection induces the hypo-methylation of the promoter region of the ribosomal genes in cucumber plants (Martinez et al., 2014). This alteration of the methylation pattern, predominantly affected cytosines in the CG context and was associated with an increased transcription and accumulation of ribosomal RNA (rRNA) precursors. Similar results were obtained analyzing transgenic *N. benthamiana* plants constitutively expressing dimeric transcripts of HSVd (Castellano et al., 2015). Additionally, the analysis of HSVd effects in the host male gametophyte of infected cucumber plants, showed that this viroid induced a dynamic demethylation of repetitive DNA regions (rRNA genes and TEs) in cucumber gametophytic cells (Castellano et al., 2016a). Altogether, these results support the notion that the plant epigenetic changes associated to HSVd infection constitute a regulatory phenomenon that is not restricted to a unique host-viroid interaction (cucumber or *N. benthamiana*) or plant tissue (vegetative or reproductive) and that those changes affect different type of repetitive sequences (rRNA and TEs).

Surprisingly, aiming to elucidate the molecular basis of the alterations in the host DNA methylation associated to HSVd infection, it was demonstrated, that in infected cucumber plants, mature forms of HSVd are able to interact *in vivo* with Histone Deacetylase 6 (HDA6) (Castellano et al., 2016b). Furthermore, HDA6 expression was increased in HSVd-infected plants and the transient overexpression of HDA6 reverted the hypomethylation status of rDNA in infected plants.

In *Arabidopsis,* HDA6 is recognized as a component of the RdDM pathway, involved in the maintenance and *de novo* DNA methylation of Transposable Elements (TEs), rRNA genes and transgenes (Aufsatz *et al.*, 2002; Probst *et al.*, 2004; May *et al.*, 2005; To *et al.*, 2011; Liu *et al.*, 2012 and 2012b; Hristova *et al.*, 2015) via interactions with Methyltransferase 1 (MET1). Based on these results, it was proposed that the host epigenetic alterations associated to the infection were related with the recruitment and functional inactivation of cucumber HDA6 by HSVd (Castellano et al., 2016b). Interestingly, the lack of HDA6 activity has been associated with spurious Pol II transcription of nonconventional rDNA templates (usually transcribed by RNA Pol I) (Earley et al., 2010). Similarly, in HSVd-infected plants, it was reported an increased accumulation of rRNAs precursors (pre-rRNAs) and sRNAs derived of ribosomal RNA (rb-sRNAs) indicating an unusual transcriptional environment (Martinez et al., 2014; Castellano et al., 2015; Castellano et al., 2016a; Castellano et al., 2016b). Therefore, it was proposed that the recruitment of HDA6 mediated by HSVd may promote a spurious POL II activity of rRNA repeats and in parallel favor the transcription of non-canonical templates such as viroid genomic RNA (Castellano et al., 2016b).

Further studies of the relationship between epigenetic regulation and viroid infection have shown different scenarios. For example, in the potato-PSTVd pathosystem, infection with PSTVd was associated with the hypermethylation of the promoter regions of certain potato genes and their consequent transcriptional repression in *N. benthamiana* (Lv et al., 2016). In this work, using transgenic *N. benthamiana* plants overexpressing GFP transcripts (line 16c) as experimental host, it was demonstrated that PSTVd replication enhanced GFP silencing by the hypermethylation of the promoter region of this transgene (Lv et al., 2016). The authors suggested that the interference of PSTVd in the host methylation pathway might be attributable to Virp1, a bromodomain-containing viroid-binding protein (Martínez de Alba et al., 2003) required for PSTVd replication (Kalantidis et al., 2007). Similarly, in the tomato-PSTVd pathosystem, a strong activation of host DNA methylation was observed (Torchetti et al., 2016). In particular,

Table 1
Detail of the the studies related to viroid-induced DNA methylation.

Year	Description	Host	Reference
1994	PSTVd replication is associated to specific *de novo* methylation of a PSTVd-cDNA integrated as a transgene	tobacco	*Wassenegger et al., 1994*
2014	HSVd infection induces the hypo-methylation of rDNA in vegetative tissues	cucumber	*Martinez et al., 2014*
2015	Constitutive expression of HSVd transcripts is associated to host epigenetic alterations	N. benthamiana	*Castellano et al., 2015*
2016	HSVd infection induced demethylation of repetitive DNA (rDNA and TEs) in gametophytic cells	cucumber	*Castellano et al., 2016a*
2016	Host epigenetic alterations associated to infection are related with the recruitment and functional inactivation of HDA6 by HSVd mature forms	cucumber	*Castellano et al., 2016b*
2016	PSTVd infection is associated with the hypermethylation and transcriptional repression of certain host genes	potato	*Lv et al., 2016*
2016	PSTVd infection up-regulated the expression of host genes involved in the DNA methylation pathway	tomato	*Torchetti et al., 2016*

viroid replication up-regulated the expression of key genes involved in the maintenance of DNA methylation (such as Methyltransferase 1 (MET1), Chromomethylase 3 (CMT3) and Domains Rearranged Methylase 2 (DRM2) and histone methylation (like the H3K9 histone methyltransferase KRYPTONITE/SUVH4, KYP). Altogether these results support the notion that (at least in the analyzed host) PSTVd infection may be associated to a general increase of the DNA methylation levels in the host genome. However, it remains unknown if the overexpression of methylation-related genes occurs indirectly as a host response or if the viroid might interfere by interacting with any host factor to promote these alterations. Future analysis of the global DNA-methylation and histone profile landscapes and their dynamism during PSTVd, HSVd or other viroid infection will help to shed light into the overall dynamism of viroid-induced epigenetic changes and their potential connection to transcriptional changes.

3. POTENTIAL WAYS OF PROMOTING VIROID-ASSOCIATED HOST EPIGENETIC CHANGES

A growing body of evidence link pathogen invasion with alteration of epigenetic modifications in the genome of animal and plant cells (Gómez-Díaz et al., 2012; Annacondia et al., 2018; Wang et al., 2019a; Tsai and Cullen, 2020). In the case of plants, the interaction of other molecular pathogens such as viruses with the epigenetic pathways of their hosts, has provided the field with good examples of how viroids might be mediating their induced epigenetic changes. For example, plant DNA viruses of the family *Geminiviridae* are known to interfere with the host DNA methylation machinery (Rodríguez-Negrete et al., 2013; Yang et al., 2013) to avoid the methylation of their genome, and the consequent transcriptional repression (Raja et al., 2008). This alteration is mediated by the activity of viral proteins (termed viral RNA silencing suppressors, VSRs) that suppress the host epigenetic silencing activity against the viral genomes. As a side-effect of this avoidance of epigenetic silencing in their own viral genomes, Geminivirus-VSRs induce the transcriptional reactivation of TEs in the host genome (Rodríguez-Negrete et al., 2013; Yang et al., 2013). Similarly, RNA viruses generally code for VSRs (Csorba et al., 2015) and, some of these,for example the 2b protein of cucumber mosaic virus, have been shown to interact with siRNAs from the RdDM pathway altering the methylation levels of its target genes (Hamera et al., 2012). Nevertheless, other viruses such as pelargonium line pattern virus (PLPV), which do not have any known interaction with the RdDM pathway through its silencing suppressor, was also able to induce methylation changes in its host genome (Pérez-Cañamás et al., 2020). Devoid of the ability to encode any protein homologous to VSRs, it is reasonable to assume that viroid might redesign the host epigenome during infection through alternative

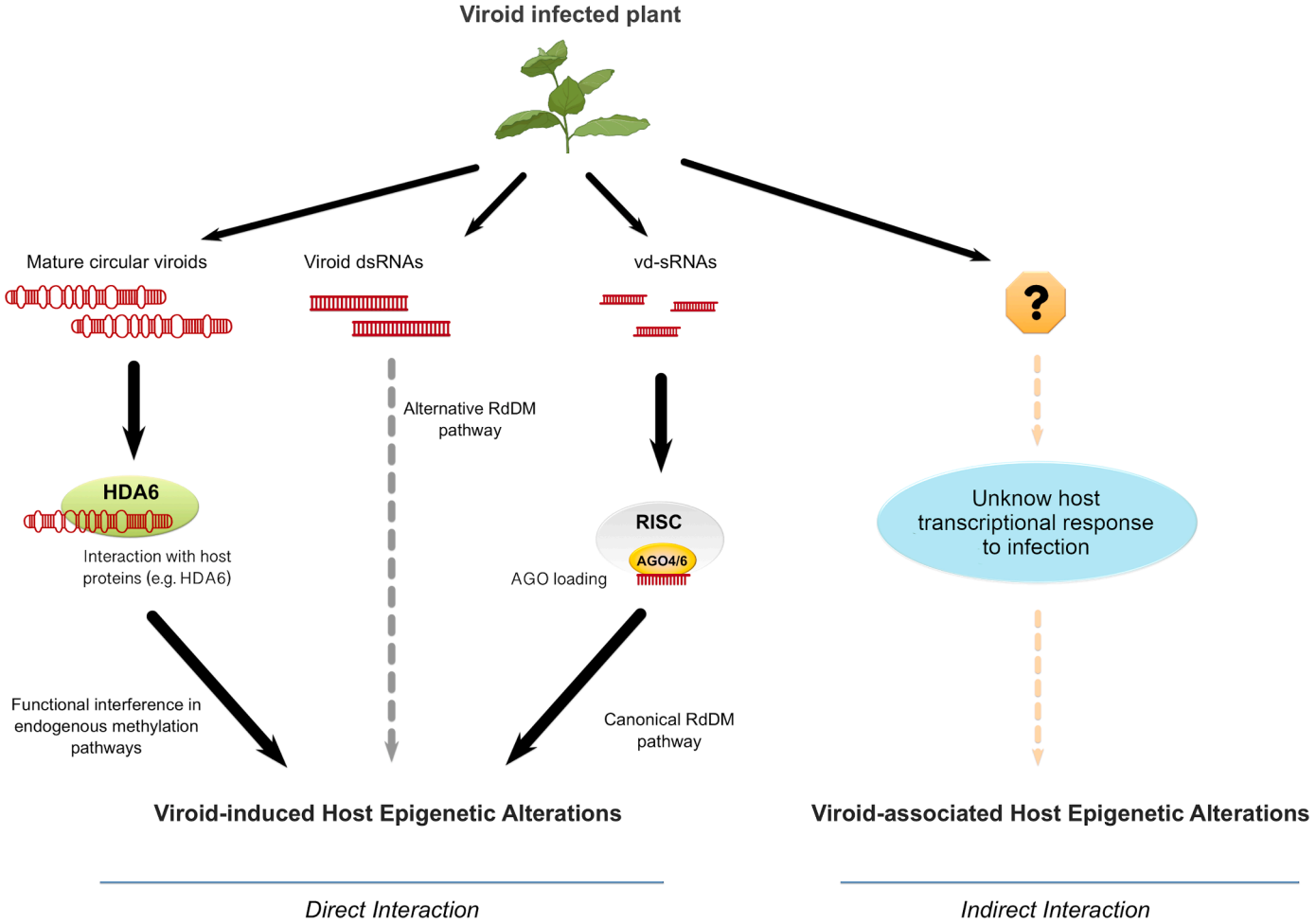

Figure 1. Potential mechanisms of interference with epigenetic routes in viroid infected plants. The HSVd-mediated recruitment of host-HDA6 is related to epigenetic changes observed in infected cucumber plants (Castellano, Pallas and Gomez, 2016b). The specific *de novo* methylation (mediated by vd-sRNAs) of PSTVd-cDNA sequences inserted in host-genome was originally described in PSTVd-infected plants (Wassenegger et al., 1994). Alternatively, has been recently proposed that *"de novo"* methylation step would be triggered by long dsRNAs rather than canonical siRNAs (Wassenegger and Dalakouras, 2021). The possibility that the epigenetic changes observed during viroid infection might be part of the transcriptional defense program of response to stress (Chang et al., 2020), cannot be discarded.

mechanisms. According to the experimental evidence obtained from diverse viroid-host interactions, we envision two alternative mechanisms to explain the interference of these pathogenic RNAs with the host epigenetic pathways, *i*) Direct Interaction: through the involvement of viroid RNAs (mature forms and/or vd-sRNAs) and *ii*) Indirect Interaction: by means of the unknown functional regulation of certain host factors (Figure 1).

3.1. Involvement of viroid RNAs (Direct interaction)

The early detection of vd-sRNAs recovered from plants infected by members of both *Pospiviroidae* (Itaya et al., 2001; Papaefthimiou et al., 2001) and *Avsunviroidae* (Martinez de Alba et al., 2002) families, supported the notion that the vd-sRNAs could guide the silencing of plant-endogenous transcripts (Papaefthimiou et al., 2001; Wang et al., 2004; Gómez et al., 2009). Although this hypothesis was validated in diverse viroid-host interactions (Wang et al., 2011; Navarro et al., 2012; Adkar-Purushothama et al., 2015; Avina-Padilla et al., 2015; Adkar--Purushothama et al., 2017; Adkar-Purushothama et al., 2018; Adkar--Purushothama and Perreault, 2018; Bao et al., 2019; Navarro et al., 2021b), it cannot be excluded the possibility that DCL4/2-synthesized 21-22 nt vd-sRNAs could be incorporated into non-canonical RdDM pathways or that spurious DCL3 activity could generate 24 nt length vd-sRNAs that could be incorporated into the endogenous RdDM pathway, and that both events could guide the specific methylation of complementary regions in the host genome (Figure 1).

This idea is supported by the first observation of the specific *de novo* methylation mediated by vd-sRNAs (generated during PSTVd replication) of full or partial length sequences of viroid cDNA inserted in the genome of tobacco plants (Wassenegger et al., 1994). Additionally, a plausible explanation for epigenetic changes taking place during viroid infection could be the colonization of the host AGO proteins, which are known to be influenced by anomalous presence of siRNAs (McCue et al., 2013; Minoia et al., 2014; Annacondia and Martinez, 2021). Hence, alteration of host RNA silencing pathways, including the RdDM pathway, might lead to altered DNA methylation levels and their associated histone marks.

Alternatively, diverse observations (Dalakouras and Wassenegger, 2013) have prompted the proposal of an extended model in which the *de novo* methylation step would be triggered by long (>50 bp) dsRNAs rather than canonical siRNAs (Wassenegger and Dalakouras, 2021). Although the involvement of longer viroid-derived sequences, might be containing specific structural motifs (Wüsthoff and Steger, 2022), significantly reduces the probability that homologous host DNA regions can exist, this last possibility cannot be completely ruled out (Figure 1). It must be noted that both DCL4/2- and DCL3-produced 21/22 and 24 nt TE-derived sRNAs are known to initiate silencing of “retrovirus-like” transcriptionally active TEs (Marí-Ordóñez et al., 2013; Nuthikattu et al., 2013).

Several studies performed in diverse viroid-hosts pathosystems have evidenced that viroid of both families are capable to subvert host factors in order to adapt the cell environment to their functional needs predominantly related to the intracellular compartmentalization (Martínez de Alba et al., 2003), replication (Mühlbach and Sänger, 1979; Navarro et al., 2000; Gas et al., 2007; Nohales et al., 2012a; Nohales et al., 2012b; Seo et al., 2021) and export to vascular tissue for long-distance trafficking (Gómez and Pallás, 2001; Owens et al., 2001; Gómez and Pallás, 2004; Morozov et al., 2014). Considering this experimental evidence, it can be envisioned a scenario in which viroids might modulate the host epigenetic landscape by interfering with the functional activity of certain host factors involved in the DNA methylation pathway (Figure 1). The mechanisms exploited by a viroid to functionally alter a host factor may be the direct physical interaction, as it was described for the interaction HSVd-HDA6 in cucumber plants (Castellano et al., 2016b), or by inducing the differential accumulation of host proteins directly (Torchetti et al., 2016) or indirectly (Lv et al., 2016) involved in the epigenetic pathways that maintain the stability of the plant genome.

3.2. Unknown regulation of host factors (Indirect interaction)

Another plausible scenario would be that the epigenetic changes observed during viroid infection were just part of the transcriptional defense program orchestrated during stress (Figure 1). Defense genes such as nucleotide binding site and leucine-rich repeat domains (NBS-LRR) proteins are localized in heterochromatic regions of plant genomes (Lee and Yeom, 2015) and are activated at the transcriptional level in epigenetic mutants (Stokes et al., 2002; López Sánchez et al., 2016). Additionally, DNA methylation changes are connected to the accessibility of transcriptional regulators such as transcription factors to genomic regions (O'Malley et al., 2016), which are well-known players in the transcriptional response to stresses (Fujita et al., 2006; Chang et al., 2020). It is interesting to note that actually several genes from diverse epigenetic pathways that respond to bacterial infection contain transcription factor binding domains in their promoter regions (Agnès et al., 2013). Future research will help to solve these unsolved questions that are basic to understand the interplay between epigenetic regulation and viroid biology.

4. REMARKS AND FUTURE PERSPECTIVES

The results obtained in the last years evidence a close interaction between the pathogenesis process induced by HSVd and PSTVd (two representative members of the *Pospiviroidae* family) and the DNA methylation level of the host genome. However, up to date, only viroid-induced changes affecting particular plant genes have been analyzed and the global impact on DNA methylation at a genomic scale is yet unknown. Consequently, deciphering the effects exerted by these plant-pathogenic RNAs on the plant epigenome (at both DNA methylation and histone modification levels) as well as identifying subverted host-endogenous mechanisms that might regulate these epigenetic changes emerge as relevant challenges for viroid research in the near future.

Another aspect to be elucidated is the specificity of the viroid-host interactions that produce epigenetic alterations, or in other words, whether some DNA methylation changes might be a common phenomenon triggered by nuclear-replicating viroids, or specific to certain viroid/host combinations. For example, the current results in the field (from a very limited number of studies) indicate that while HSVd induced the demethylation of cucumber (Martinez et al., 2014; Castellano et al., 2016a) and *N. benthamiana* (Castellano et al., 2015) genes, an opposite situation (strong hypermethylation of the analyzed genes) was observed in PSTVd infected tomato (Torchetti et al., 2016) and potato (Lv et al., 2016) plants. However, both effects may not be necessarily contradictory, since due to the absence of information about the temporal evolution of the host-epigenome during the infection and the intrinsically different target regions analyzed, it cannot be discarded that the different methylation effects associated to these two nuclear viroids might be consequence of a specific temporal stage of a dynamic host response. Alternatively, it should be considered the possibility that this functional inconsistence may be due, as mentioned above, to the limited number of host genes analized in both pathosystems. Additionally, it would be interesting to compare the potential methylation effects between severe and mild variants of a specific viroid to try to correlate these epigenomic alterations with their corresponding pathogenic processes. It is well known that induction of the viroid infection responses occurs earlier and is stronger in plants infected with a severe variant (HSVd-g54) than in those infected with a mild variant (HSVd-h) (Xia et al., 2017).

Another interesting issue to be considered is whether the infectious process triggered by viroids of the Avsunviroidae family could be associated to host epigenetic changes. Although these pathogenic RNAs replicate in the chloroplast, where no RdDM activity has been reported (Wang et al., 2020a), it is thought that they possess a nuclear processing

step before being targeted to the chloroplasts (Gómez and Pallás, 2012a; Gómez and Pallás, 2012b; Baek et al., 2017) and Avsunviroidae infection is associated to the recovery of vd-sRNAs (Martinez de Alba et al., 2002).

Finally, it is important to remark the that the first evidence linking viroid infection and epigenetic alterations in the endogenous host-DNA was only eight years ago (Martinez et al., 2014) and the road traveled so far is short. Future technical advances such as increased depth of sequencing technologies and availability of genome sequences from non-model species will help to understand these alterations to a broader extent. Consequently, much more work is needed to clarify the molecular basis and the functional impact (for both the pathogen and the plant) of the remodeling of the host epigenome in response to viroid infection.

FUNDING

This work was supported by the Agencia Estatal de Investigacion (AEI) (co-supported by FEDER) Grants PID2019-104126RB-I00 (GG) and PID2020-115571RB-I00 (VP). J.M.M. was recipient of a predoctoral contract ACIF-2017-114 from the Generalitat Valenciana. The funders had no role in the experiment design, data analysis, decision to publish, or preparation of the manuscript.

CRediT authorship contribution statement

Gustavo Gómez: Visualization, Conceptualization, Writing – review & editing. **Joan Marquez-Molins:** Visualization, Conceptualization, Writing – review & editing. **German Martinez:** Writing – review & editing. **Vicente Pallas:** Writing – review & editing.

Declaration of Competing Interest

All the authors declare no conflict interests.

REFERENCES

Adkar-Purushothama, CR, Brosseau, C, Giguère, T, Sano, T, Moffett, P, Perreault, J-P, 2015. Small RNA Derived from the Virulence Modulating Region of the Potato spindle tuber viroid Silences callose synthase Genes of Tomato Plants. Plant Cell 27, 2178–2194.

Adkar-Purushothama, CR, Iyer, PS, Perreault, J-P, 2017. Potato spindle tuber viroid infection triggers degradation of chloride channel protein CLC-b-like and Ribosomal protein S3a-like mRNAs in tomato plants. Sci Rep 7, 8341.

Adkar-Purushothama, CR, Perreault, J-P, 2020. Current overview on viroid–host interactions. WIREs RNA 11, e1570.

Adkar-Purushothama, CR, Perreault, JP, 2018. Alterations of the viroid regions that interact with the host defense genes attenuate viroid infection in host plant. RNA Biol. https://doi.org/10.1080/15476286.2018.1462653.

Agnès, Y, Gersende, L, Florence, J, Jingyu, W, Laure, B, Yu, W, Anne-Laure, A, Jon, P, FR, L., Olivier, V, et al., 2013. Dynamics and biological relevance of DNA demethylation in Arabidopsis antibacterial defense. Proc Natl Acad Sci 110, 2389–2394.

Agorio, A, Vera, P, 2007. ARGONAUTE4 Is Required for Resistance to Pseudomonas syringae in Arabidopsis. Plant Cell 19, 3778–3790.

Annacondia, ML, Magerøy, MH, Martinez, G, 2018. Stress response regulation by epigenetic mechanisms: changing of the guards. Physiol Plant 162, 239–250.

Annacondia, ML, Martinez, G, 2021. Reprogramming of RNA silencing triggered by cucumber mosaic virus infection in Arabidopsis. Genome Biol 22, 1–22.

Avina-Padilla, K, Martinez de la Vega, O, Rivera-Bustamante, R, Martinez-Soriano, JP, Owens, RA, Hammond, RW, Vielle-Calzada, J-P, 2015. In silico prediction and validation of potential gene targets for pospiviroid-derived small RNAs during tomato infection. Gene 564, 197–205.

Baek, E, Park, M, Yoon, J-Y, Palukaitis, P, 2017. Chrysanthemum Chlorotic Mottle Viroid-Mediated Trafficking of Foreign mRNA into Chloroplasts. Res Plant Dis 23, 288–293.

Bao, S, Owens, RA, Sun, Q, Song, H, Liu, Y, Eamens, AL, Feng, H, Tian, H, Wang, M-B, Zhang, R, 2019. Silencing of transcription factor encoding gene StTCP23 by small RNAs derived from the virulence modulating region of potato spindle tuber viroid is associated with symptom development in potato. PLOS Pathog 15, e1008110.

Castellano, M, Martinez, G, Marques, MC, Moreno-Romero, J, Köhler, C, Pallas, V, Gomez, G, 2016a. Changes in the DNA methylation pattern of the host male gametophyte of viroid-infected cucumber plants. J Exp Bot 67, 5857–5868.

Castellano, M, Martinez, G, Pallás, V, Gómez, G, 2015. Alterations in host DNA methylation in response to constitutive expression of Hop stunt viroid RNA in Nicotiana benthamiana plants. Plant Pathol. https://doi.org/10.1111/ppa.12358.

Castellano, M, Pallas, V, Gomez, G, 2016b. A pathogenic long noncoding RNA redesigns the epigenetic landscape of the infected cells by subverting host Histone Deacetylase 6 activity. New Phytol 211, 1311–1322.

Chang, Y-N, Zhu, C, Jiang, J, Zhang, H, Zhu, J-K, Duan, C-G, 2020. Epigenetic regulation in plant abiotic stress responses. J Integr Plant Biol 62, 563–580.

Corrêa, RL, Sanz-Carbonell, A, Kogej, Z, Müller, SY, Ambrós, S, López-Gomollón, S, Gómez, G, Baulcombe, DC, Elena, SF, 2020. Viral Fitness Determines the Magnitude of Transcriptomic and Epigenomic Reprogramming of Defense Responses in Plants. Mol Biol Evol. https://doi.org/10.1093/molbev/msaa091.

Csorba, T, Kontra, L, Burgyán, J, 2015. Viral silencing suppressors: Tools forged to fine-tune host-pathogen coexistence. Virology 479–480, 85–103.

Dalakouras, A, Wassenegger, M, 2013. Revisiting RNA-directed DNA methylation. RNA Biol 10, 453–455.

Deleris, A, Halter, T, Navarro, L, 2016. DNA Methylation and Demethylation in Plant Immunity. Annu Rev Phytopathol 54, 579–603.

Earley, et al., 2010. Mechanisms of HDA6-mediated rRNA gene silencing: suppression of intergenic Pol II transcription and differential effects on maintenance versus siRNA-directed cytosine methylation. Genes Development 24, 1119–1132.

Erdmann, RM, Picard, CL, 2020. RNA-directed DNA Methylation. PLOS Genet 16, e1009034.

Fujita, M, Fujita, Y, Noutoshi, Y, Takahashi, F, Narusaka, Y, Yamaguchi-Shinozaki, K, Shinozaki, K, 2006. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr Opin Plant Biol 9, 436–442.

Gago-Zachert, S, 2016. Viroids, infectious long non-coding RNAs with autonomous replication. Virus Res 212, 12–24.

Gallego-Bartolomé, J, 2020. DNA methylation in plants: mechanisms and tools for targeted manipulation. New Phytol 227, 38–44.

Gas, M-E, Hernández, C, Flores, R, Daròs, J-A, 2007. Processing of Nuclear Viroids In Vivo: An Interplay between RNA Conformations. PLOS Pathog 3, e182.

Gómez-Díaz, E, Jordà, M, Peinado, MA, Rivero, A, 2012. Epigenetics of Host–Pathogen Interactions: The Road Ahead and the Road Behind. PLoS Pathog 8, e1003007.

Gómez, G, Martínez, G, Pallás, V, 2009. Interplay between viroid-induced pathogenesis and RNA silencing pathways. Trends Plant Sci 14, 264–269.

Gómez, G, Pallás, V, 2013. Viroids: A light in the darkness of the lncRNA-directed regulatory networks in plants. New Phytol 198, 10–15.

Gómez, G, Pallás, V, 2001. Identification of an in vitro ribonucleoprotein complex between a viroid RNA and a phloem protein from cucumber plants. Mol Plant-Microbe Interact. https://doi.org/10.1094/MPMI.2001.14.7.910.

Gómez, G, Pallás, V, 2004. A long-distance translocatable phloem protein from cucumber forms a ribonucleoprotein complex in vivo with Hop stunt viroid RNA. J Virol. https://doi.org/10.1128/JVI.78.18.10104-10110.2004.

Gómez, G, Pallás, V, 2012a. A pathogenic non coding RNA that replicates and accumulates in chloroplasts traffics to this organelle through a nuclear-dependent step. Plant Signal. Behav. 7.

Gómez, G, Pallás, V, 2012b. Studies on subcellular compartmentalization of plant pathogenic noncoding RNAs give new insights into the intracellular RNA-traffic mechanisms. Plant Physiol 159, 558–564.

H, DR, Mattia, P, SR, J., Ryan, L, DJ, M., NJ, R., DJ, E., EJ, R., 2012. Widespread dynamic DNA methylation in response to biotic stress. Proc Natl Acad Sci 109, E2183–E2191.

Hamera, S, Song, X, Su, L, Chen, X, Fang, R, 2012. Cucumber mosaic virus suppressor 2b binds to AGO4-related small RNAs and impairs AGO4 activities. Plant J 69, 104–115.

Hannan Parker, A, Wilkinson, SW, Ton, J, 2022. Epigenetics: a catalyst of plant immunity against pathogens. New Phytol 233, 66–83.

Herranz, MC, Niehl, A, Rosales, M, Fiore, N, Zamorano, A, Granell, A, Pallas, V, 2013. A remarkable synergistic effect at the transcriptomic level in peach fruits doubly infected by prunus necrotic ringspot virus and peach latent mosaic viroid. Virol J 10, 164.

Itaya, A, Folimonov, A, Matsuda, Y, Nelson, RS, Ding, B, 2001. Potato spindle tuber viroid as inducer of RNA silencing in infected tomato. Mol Plant-Microbe Interact 14, 1332–1334.

Kalantidis, K, Denti, MA, Tzortzakaki, S, Marinou, E, Tabler, M, Tsagris, M, 2007. Virp1 is a host protein with a major role in Potato spindle tuber viroid infection in Nicotiana plants. J Virol 81, 12872–12880.

Kappagantu, M, Bullock, JM, Nelson, ME, Eastwell, KC, 2017. Hop stunt viroid: Effect on host (Humulus lupulus) transcriptome and its interactions with hop powdery mildew (Podospheara macularis). Mol Plant-Microbe Interact 30, 842–851.

Katsarou, K, Mavrothalassiti, E, Dermauw, W, Van Leeuwen, T, Kalantidis, K, 2016a. Combined Activity of DCL2 and DCL3 Is Crucial in the Defense against Potato Spindle Tuber Viroid. PLOS Pathog 12, e1005936.

Katsarou, K, Wu, Y, Zhang, R, Bonar, N, Morris, J, Hedley, PE, Bryan, GJ, Kalantidis, K, Hornyik, C, 2016b. Insight on Genes Affecting Tuber Development in Potato upon Potato spindle tuber viroid (PSTVd) Infection. PLoS One 11, e0150711. –e0150711.

Lee, H-A, Yeom, S-I, 2015. Plant NB-LRR proteins: tightly regulated sensors in a complex manner. Brief Funct Genomics 14, 233–242.

López Sánchez, A, Stassen, JHM, Furci, L, Smith, LM, Ton, J, 2016. The role of DNA (de) methylation in immune responsiveness of Arabidopsis. Plant J 88, 361–374.

Lv, DQ, Liu, SW, Zhao, JH, Zhou, BJ, Wang, SP, Guo, HS, Fang, YY, 2016. Replication of a pathogenic non-coding RNA increases DNA methylation in plants associated with a bromodomain-containing viroid-binding protein. Sci Rep 6, 35751.

Marí-Ordóñez, A, Marchais, A, Etcheverry, M, Martin, A, Colot, V, Voinnet, O, 2013. Reconstructing de novo silencing of an active plant retrotransposon. Nat Genet 45, 1029–1039.

Martinez de Alba, AE, Flores, R, Hernandez, C, 2002. Two Chloroplastic Viroids Induce the Accumulation of Small RNAs Associated with Posttranscriptional Gene Silencing. J Virol 76, 13094–13096.
Martínez de Alba, AE, Sägesser, R, Tabler, M, Tsagris, M, 2003. A bromodomain-containing protein from tomato specifically binds potato spindle tuber viroid RNA in vitro and in vivo. J Virol 77, 9685–9694.
Marquez-Molins, J, Gomez, G, Pallas, V, 2021. Hop stunt viroid: A polyphagous pathogenic RNA that has shed light on viroid–host interactions. Mol Plant Pathol 22 (2), 153–162. https://doi.org/10.1111/mpp.13022.
Martinez, G, Castellano, M, Tortosa, M, Pallas, V, Gomez, G, 2014. A pathogenic non-coding RNA induces changes in dynamic DNA methylation of ribosomal RNA genes in host plants. Nucleic Acids Res 42, 1553–1562.
McCue, AD, Nuthikattu, S, Slotkin, RK, 2013. Genome-wide identification of genes regulated in trans by transposable element small interfering RNAs. RNA Biol 10, 1379–1395.
Minoia, S, Carbonell, A, Di Serio, F, Gisel, A, Carrington, JC, Navarro, B, Flores, R, 2014. Specific Argonautes Selectively Bind Small RNAs Derived from Potato Spindle Tuber Viroid and Attenuate Viroid Accumulation In Vivo. J Virol 88, 11933–11945.
Morozov, S, Makarova, S, Erokhina, T, Kopertekh, L, Schiemann, J, Owens, R, Solovyev, A, 2014. Plant 4/1 protein: potential player in intracellular, cell-to-cell and long-distance signaling. Front. Plant Sci. 5.
Mühlbach, HP, Sänger, HL, 1979. Viroid replication is inhibited by α-amanitin. Nature 278, 185–188.
Navarro, B, Flores, R, Di Serio, F, 2021a. Advances in Viroid-Host Interactions. Annu Rev Virol 8, 305–325.
Navarro, B, Gisel, A, Rodio, ME, Delgado, S, Flores, R, Di Serio, F, 2012. Small RNAs containing the pathogenic determinant of a chloroplast-replicating viroid guide the degradation of a host mRNA as predicted by RNA silencing. Plant J 70, 991–1003.
Navarro, B, Gisel, A, Serra, P, Chiumenti, M, Di Serio, F, 2021b. Degradome Analysis of Tomato and Nicotiana benthamiana Plants Infected with Potato Spindle Tuber Viroid. Int J Mol Sci. https://doi.org/10.3390/ijms22073725.
Navarro, JA, Vera, A, Flores, R, 2000. A chloroplastic RNA polymerase resistant to tagetitoxin is involved in replication of Avocado sunblotch viroid. Virology 268, 218–225.
Nohales, M-A, Molina-Serrano, D, Flores, R, Daros, J-A, 2012a. Involvement of the Chloroplastic Isoform of tRNA Ligase in the Replication of Viroids Belonging to the Family Avsunviroidae. J Virol 86, 8269–8276.
Nohales, MÁ, Flores, R, Daròs, JA, 2012b. Viroid RNA redirects host DNA ligase 1 to act as an RNA ligase. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.1206187109.
Nuthikattu, S, McCue, AD, Panda, K, Fultz, D, DeFraia, C, Thomas, EN, Slotkin, RK, 2013. The Initiation of Epigenetic Silencing of Active Transposable Elements Is Triggered by RDR6 and 21-22 Nucleotide Small Interfering RNAs. Plant Physiol 162, 116–131.
O'Malley, RC, Huang, SC, Song, L, Lewsey, MG, Bartlett, A, Nery, JR, Galli, M, Gallavotti, A, Ecker, JR, 2016. Cistrome and Epicistrome Features Shape the Regulatory DNA Landscape. Cell 166, 1598.
Owens, RA, Blackburn, M, Ding, B, 2001. Possible involvement of the phloem lectin in long-distance viroid movement. Mol Plant-Microbe Interact 14, 905–909.
Owens, RA, Tech, KB, Shao, JY, Sano, T, Baker, CJ, 2012. Global analysis of tomato gene expression during potato spindle tuber viroid infection reveals a complex array of changes affecting hormone signaling. Mol Plant-Microbe Interact 25, 582–598.
Papaefthimiou, I, Hamilton, A, Denti, M, Baulcombe, D, Tsagris, M, Tabler, M, 2001. Replicating potato spindle tuber viroid RNA is accompanied by short RNA fragments that are characteristic of post-transcriptional gene silencing. Nucleic Acids Res 29, 2395–2400.
Pavet, V, Quintero, C, Cecchini, NM, Rosa, AL, Alvarez, ME, 2006. Arabidopsis Displays Centromeric DNA Hypomethylation and Cytological Alterations of Heterochromatin Upon Attack by Pseudomonas syringae. Mol Plant-Microbe Interact 19, 577–587.
Pérez-Cañamás, M, Hevia, E, Hernández, C, 2020. Epigenetic Changes in Host Ribosomal DNA Promoter Induced by an Asymptomatic Plant Virus Infection. Biol 2020 9, 91. Page9: 91.
Pesti, R, Kontra, L, Paul, K, Vass, I, Csorba, T, Havelda, Z, Várallyay, É, 2019. Differential gene expression and physiological changes during acute or persistent plant virus interactions may contribute to viral symptom differences. PLoS One 14, e0216618.
Raja, P, Sanville, BC, Buchmann, RC, Bisaro, DM, 2008. Viral Genome Methylation as an Epigenetic Defense against Geminiviruses. J Virol 82, 8997–9007.
Rizza, S, Conesa, A, Juarez, J, Catarra, A, Navarro, L, Duran-Vila, N, Ancillo, G, 2012. Microarray analysis of Etrog citron (Citrus medica L.) reveals changes in chloroplast, cell wall, peroxidase and symporter activities in response to viroid infection. Mol Plant Pathol 13, 852–864.
Rodríguez-Negrete, E, Lozano-Durán, R, Piedra-Aguilera, A, Cruzado, L, Bejarano, ER, Castillo, AG, 2013. Geminivirus Rep protein interferes with the plant DNA methylation machinery and suppresses transcriptional gene silencing. New Phytol 199, 464–475.
Seo, H, Kim, K, Park, WJ, 2021. Effect of VIRP1 Protein on Nuclear Import of Citrus Exocortis Viroid (CEVd). Biomolecules 11, 95.
Štajner, N, Radišek, S, Mishra, AK, Nath, VS, Matoušek, J, Jakše, J, 2019. Evaluation of Disease Severity and Global Transcriptome Response Induced by Citrus bark cracking viroid, Hop latent viroid, and Their Co-Infection in Hop (Humulus lupulus L.). Int J Mol Sci 20, 3154.
Stokes, TL, Kunkel, BN, Richards, EJ, 2002. Epigenetic variation in Arabidopsis disease resistance. Genes Dev 16, 171–182.
Takino, H, Kitajima, S, Hirano, S, Oka, M, Matsuura, T, Ikeda, Y, Kojima, M, Takebayashi, Y, Sakakibara, H, Mino, M, 2019. Global transcriptome analyses reveal that infection with chrysanthemum stunt viroid (CSVd) affects gene expression profile of chrysanthemum plants, but the genes involved in plant hormone metabolism and signaling may not be silencing target of CSVd-siRNAs. Plant Gene 18, 100181.
Thibaut, O, Claude, B, 2018. Innate Immunity Activation and RNAi Interplay in Citrus Exocortis Viroid—Tomato Pathosystem. Viruses 10, 587.
Torchetti, EM, Pegoraro, M, Navarro, B, Catoni, M, Di Serio, F, Noris, E, 2016. A nuclear-replicating viroid antagonizes infectivity and accumulation of a geminivirus by upregulating methylation-related genes and inducing hypermethylation of viral DNA. Sci Rep 6, 35101.
Tsai, K, Cullen, BR, 2020. Epigenetic and epitranscriptomic regulation of viral replication. Nat Rev Microbiol 18, 559–570.
Tsuda, K, Somssich, IE, 2015. Transcriptional networks in plant immunity. New Phytol 206, 932–947.
Wang, C, Wang, C, Zou, J, Yang, Y, Li, Z, Zhu, S, 2019a. Epigenetics in the plant–virus interaction. Plant Cell Rep 38, 1031–1038.
Wang, L, Leister, D, Kleine, T, 2020a. Chloroplast development and genomes uncoupled signaling are independent of the RNA-directed DNA methylation pathway. Sci Rep 10, 15412.
Wang, MB, Bian, XY, Wu, LM, Liu, LX, Smith, NA, Isenegger, D, Wu, RM, Masuta, C, Vance, VB, Watson, JM, et al., 2004. On the role of RNA silencing in the pathogenicity and evolution of viroids and viral satellites. Proc Natl Acad Sci U S A 101, 3275–3280.
Wang, X, Li, N, Li, W, Gao, X, Cha, M, Qin, L, Liu, L, 2020b. Advances in Transcriptomics in the Response to Stress in Plants. Glob Med Genet 7, 30–34.
Wang, Y, Shibuya, M, Taneda, A, Kurauchi, T, Senda, M, Owens, RA, Sano, T, 2011. Accumulation of Potato spindle tuber viroid-specific small RNAs is accompanied by specific changes in gene expression in two tomato cultivars. Virology 413, 72–83.
Wang, Y, Wu, J, Qiu, Y, Atta, S, Zhou, C, Cao, M, 2019b. Global Transcriptomic Analysis Reveals Insights into the Response of "Etrog" Citron (Citrus medica L.) to Citrus Exocortis Viroid Infection. Viruses. https://doi.org/10.3390/V11050453.
Wassenegger, M, Dalakouras, A, 2021. Viroids as a Tool to Study RNA-Directed DNA Methylation in Plants. Cells 10, 1187.
Wassenegger, M, Heimes, S, Riedel, L, Sänger, HL, 1994. RNA-directed de novo methylation of genomic sequences in plants. Cell 76, 567–576.
Waters, AJ, Makarevitch, I, Noshay, J, Burghardt, LT, Hirsch, CN, Hirsch, CD, Springer, NM, 2017. Natural variation for gene expression responses to abiotic stress in maize. Plant J 89, 706–717.
Wiesyk, A, Fogtman, A, Lirski, M, Zagorski-Ostoja, W, 2019. Root Transcriptomic Analysis Reveals Global Changes Induced by Systemic Infection of Solanum lycopersicum with Mild and Severe Variants of Potato Spindle Tuber Viroid. Viruses. https://doi.org/10.3390/v11110992.
Wüsthoff, K-P, Steger, G, 2022. Conserved Motifs and Domains in Members of Pospiviroidae. Cells 11, 230.
Xia, C, Li, S, Hou, W, Fan, Z, Xiao, H, Lu, M, Sano, T, Zhang, Z, 2017. Global transcriptomic changes induced by infection of cucumber (Cucumis sativus L.) with mild and severe variants of hop stunt viroid. Front Microbiol. https://doi.org/10.3389/fmicb.2017.02427.
Yang, L-P, Fang, Y-Y, An, C-P, Dong, L, Zhang, Z-H, Chen, H, Xie, Q, Guo, H-S, 2013. C2-mediated decrease in DNA methylation, accumulation of siRNAs, and increase in expression for genes involved in defense pathways in plants infected with beet severe curly top virus. Plant J 73, 910–917.
Zhang, H, Lang, Z, Zhu, JK, 2018. Dynamics and function of DNA methylation in plants. Nat Rev Mol Cell Biol 19, 489–506.
Zheng, Y, Wang, Y, Ding, B, Fei, Z, 2017. Comprehensive Transcriptome Analyses Reveal that Potato Spindle Tuber Viroid Triggers Genome-Wide Changes in Alternative Splicing, Inducible trans-Acting Activity of Phased Secondary Small Interfering RNAs, and Immune Responses. J Virol 91, e00247. -17.
Zhu, Q-H, Shan, W-X, Ayliffe, MA, Wang, M-B, 2016. Epigenetic Mechanisms: An Emerging Player in Plant-Microbe Interactions. Mol Plant-Microbe Interact 29, 187–196.