Virus-encoded RNA silencing suppressor protein critical for disease development: Focus on its' multifunctionality and co-evolution in Solanaceous hosts

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Abstract

Viral suppressors of RNA silencing (VSRs) are proteins that interfere with anti-viral defense mechanisms and promote infection. For plant viruses, VSRs can be encoded in viral genomes and satellite molecules, and play a role in the virus life cycle and aid in overcoming host defenses. However, a comprehensive review of VSRs and their role in the spread of plant pathogens worldwide, has not been performed. Here we provide a comprehensive and updated synthesis of the role of VSRs in pathogenesis of Solanaceous plants, a family with many crop and medicinal plants. We focus on (1) VSR diversity and the mechanisms used to suppress anti-viral defense, (2) the role of VSRs in viral pathogenesis other than interfering with host RNA-silencing, and (3) co-evolution between VSRs and plant host proteins. Our review shows that VSRs promote disease development by altering multiple steps in the viral pathogenicity process, and documents various counter-defense mechanisms. Specially, a breadth of evidence suggests VSRs induce suppression of antiviral silencing, abrogation of phytohormone signaling, and R-gene mediated host defense. We also discuss how identifying and characterizing novel interactions between VSRs and Solanaceous host factors may be leveraged for developing sustainable pathogen and pest management strategies.

1. Introduction

Crop plants in the Solanaceae family are grown worldwide and include tomato (*Solanum lycopersicum*), potato (*S. tuberosum*), and pepper (*Capsicum annum*) (Olmstead et al., 2008; Gebhardt, 2016). However, productivity of crops in this family are threatened by over 40 viral genera (Haňcinský et al., 2020). Plant viruses infecting Solanaceous hosts include species of Begomovirus (e.g., *Tomato leaf curl virus*), Potyvirus (e.g., *Potato virus* Y), Tospovirus (e.g., *Tomato spotted wilt virus*), Nepovirus (e.g., *Tomato ring spot virus*), Tombusvirus (e.g., *Tomato bushy stunt virus*), Tobamovirus (e.g., *Tomato mosaic virus*), and Cucumoviruses (e.g., *Tomato aspermy virus*; *Cucumber mosaic virus*). In response to these pathogens, Solanaceous plants have evolved multi-layered defenses including RNA silencing, viral degradation, and phytohormone-mediated defense (Incarbone and Dunoyer, 2013). However, RNA silencing is the primary and critical antiviral strategy that has been shown to be conserved across nearly all crop species.

In addition to acting as defenses against viruses, RNA silencing plays a role in regulating gene expression related to plant growth and development. Host RNA silencing can be grouped in at least three partially overlapping pathways: (i) siRNA-mediated cytoplasmic gene silencing (post-transcriptional gene silencing), (ii) microRNA-mediated silencing that regulates messenger RNA expression, and (iii) DNA methylation-dependent gene silencing (transcriptional gene silencing) (Baulcombe, 2004) (Fig. 1). During post-transcriptional gene silencing, long dsRNAs are cleaved by Dicer like proteins into duplex siRNAs, which interact with Argonaut proteins and RNA-induced silencing complexes for homology dependent degradation of target molecules (Vaucheret et al., 2001; Khvorova et al., 2003; Zhang et al., 2016). miRNAs are generated by the same mechanism, but the process starts in the nucleus (Liu and Chen, 2016; Liu et al., 2017). These defenses have evolved throughout the plant kingdom to promote tolerance to pathogens.

To cause successful infections, viruses must counteract defensive plant responses. Viral genomes encode proteins that aid in overcoming host defense along with proteins required for replication and translation (Basu et al., 2014; Csorba et al., 2015; Cheng and Wang, 2017; Islam et al., 2019; Wu et al., 2019; Ziegler-Graff, 2020). Viral proteins that specifically interfere with RNA silencing based host defenses are called suppressors of RNA silencing (VSRs). Due to their small genome size, viruses use several strategies to maximize the coding capacity of the genome. For example, virus encoded proteins are often multi-functional, and this is true for VSR proteins, which often play key roles in controlling the virus life cycle while also breaking down host defensive responses. However, the diversity and multi-functionality of VSRs in Solanaceous plants have not been extensively reviewed.

Here we address knowledge gaps regarding VSRs by reviewing: (1) VSR diversity and the mechanisms they use to suppress anti-viral defense, (2) the role of VSRs in viral pathogenesis, and (3) co-evolutionary dynamics mediated by VSRs. We then highlight research needs related to VSRs in Solanaceous crops, and research that could aid in improving management of viral diseases by manipulating plant and virus signaling. We focus our review first on VSRs encoded by four genera that include pathogens with a broad host range in Solanaceae (Table 1): (1) Begomoviruses, (2) Potyviruses, (3) Cucomovirus, and (4) Orthotospovirus. We then compare these with VSRs encoded by three genera that have a narrow host range in Solanaceae and are often challenging to manage: (1) Nepovirus, (2) Tobamovirus, and (3) Tombusvirus (Kubota et al., 2003; Feng et al., 2011; Ghoshal and Sanfaçon, 2014; Ocampo Ocampo et al., 2016; Bera et al., 2017; Basu et al., 2018; Bera et al., 2018; Gnanasekaran et al., 2019) (Table 1). Overall, our review identifies key functions of VSRs and how these functions may aid in future management of plant disease.

2. Diversity of VSRs and the mechanisms used for RNA silencing

2.1. VSRs encoded by Begomoviruses

Begomoviruses are whitefly-transmitted ssDNA viruses in the family Geminiviridae that consist of circular single-stranded DNA genomes (2.5-3kb) with overlapping open reading frames. Begomoviruses have the smallest known genome that can replicate independently in the nucleus and have bi-directional transcription. Begomoviruses encode multiple structurally and functionally different VSR proteins that suppress host gene silencing (Rojas et al., 2001; Zrachya et al., 2007). For example, *Tomato yellow leaf curl virus* V2 inhibits gene silencing 3 protein (*Sl*SGS3), a key component of the host viral silencing machinery (Glick et al., 2008; Kumakura et al., 2009). In contrast, *Tomato yellow leaf curl China virus* V2 suppresses RNA silencing by sequestering siRNA molecules and inhibiting methylation-mediated gene silencing, a part of transcriptional gene silencing pathways (Zhang et al., 2012; Wang et al., 2014; Wang et al., 2018; Wang et al., 2019). VSRs also serve as transcriptional activators of viral and host genes to suppress transcriptional and post-transcriptional gene silencing (Dong et al., 2003; Wang et al., 2003, 2005; Luna et al., 2012; Jackel et al., 2015). For example, AC2 of *Tomato leaf curl virus* aids in silencing suppression by blocking methylation or by suppressing plant defense machinery (Ramesh et al., 2017; Basu et al., 2018), while AL2 encoded by *Tomato golden mosaic virus* induces calmodulin-like protein (rgsCaM), a regulator of RNA silencing (Chung et al., 2014).

VSRs such as AC4 and C4 also function as transcriptional and post-transcriptional gene silencing suppressors that interact with single stranded si-/miRNAs (Chellappan et al., 2005a, 2005b) or AGO4 (Vinutha et al., 2018). AC4, for example, functions up- and downstream of the unwinding of siRNA strands and inhibits siRNA incorporation into the RNA-induced

silencing complex (Amin et al., 2011; Hanley-Bowdoin et al., 2013; Ramesh et al., 2017). Transient expression of βC1 monopartite viruses also have strong post-transcriptional gene silencing suppressor activity, as they bind to both ssDNAs and dsDNAs irrespective of size or sequence specificity (Cui et al., 2005; Kon et al., 2007; Shukla et al., 2013; Li et al., 2014).

2.2. VSRs encoded by Potyviruses

Potyviruses (family *Potyviridae*) are aphid-transmitted positive sense RNA viruses (Revers and García, 2015) that cause epidemic outbreaks in several crops (Parizad et al., 2017, 2018, 2019; Moratalla-lópez et al., 2021; Movi et al., 2022). A non-structural protein, HCPro, was the first VSR identified encoded by a Potyvirus. HCPro targets RNA silencing pathways by binding to virus-derived siRNA (Kasschau and Carrington, 1998; Del Toro et al., 2014). HCPro also regulates AGO1 function by inducing miR168, a microRNA that targets AGO mRNA (Várallyay and Havelda, 2013). Aside from HCPro, VPg acts as a VSR for potyviruses. Like begomovirus protein V2, VPg also interacts with SGS3, the cofactor of RDR6, to initiate its degradation by the proteasome and autophagy pathway (Cheng and Wang, 2017); this interaction appears to be evolutionarily conserved across the *Potyviridae* (Rajamäki et al., 2014; Cheng and Wang, 2017).

2.3. VSRs encoded by Cucomovirus

Cucumoviruses from *Bromoviridae* (e.g., *Cucumber mosaic virus*) have segmented, tripartite linear, positive sense ssRNA genomes comprised of RNA1 (3.4 kb), RNA2 (3.1 kb), RNA3 (2.2 kb), each of which has a 3′ tRNA-like structure and a 5′ cap. The CMV 2b protein encoded by RNA2 binds strongly to host-derived siRNA duplexes (e.g., miR171) and efficiently suppresses RDR6-mediated post-transcriptional gene silencing (Diaz-Pendon et al., 2007; Ye et

al., 2009; Wang et al., 2011). CMV 2b also interacts with various protein components of RNA silencing machinery, such as AGO1 and AGO4 (Baumberger and Baulcombe, 2005; González et al., 2010; Harvey et al., 2011; Hamera et al., 2012) (Fig. 1). CMV 2b protein blocks AGO1 mediated cleavage associated with both miRNA and siRNA pathways (Zhang et al., 2006), and suppresses AGO4 mediated systemic silencing and DNA methylation (Ye et al., 2009). CMV 2b was further reported to decrease accumulation of 21-24 nt vsiRNAs generated by DCL4, DCL2, and DCL3 through RDR1-dependent non-cell-autonomous antiviral silencing (Diaz-Pendon et al., 2007). Besides CMV 2b, *Tomato aspermy virus* (TAV) encoded 2b protein suppress post-transcriptional gene silencing by directly binding to siRNA duplexes (Chen et al., 2008). TAV 2b was also found to suppress the accumulation of both 5′ secondary siRNAs and host RDR6-specific mRNAs but has no control over the regulation of 3′ secondary siRNAs (Zhang et al., 2008).

2.4. VSRs encoded by Tospoviruses

Tomato spotted wilt virus from *Tospoviridae* is a devastating tospovirus with a genome containing three negative-sense ssRNA (Margaria and Rosa, 2015). Non-structural proteins encoded by RNA (Parrella et al., 2003) blocks this antiviral silencing by binding with dsRNA in a size-independent manner or by interacting with SGS3 (Chen et al., 2022). Unlike other VSRs, non-structural proteins exhibit antiviral silencing in a dose dependent manner (Takeda et al., 2002; Bucher et al., 2003; Hedil et al., 2015; Ocampo Ocampo et al., 2016). *Tomato spotted wilt virus* non-structural proteins can inactivate RNA silencing by interacting with small and long dsRNAs (ds-miRNA and -siRNA precursors) through the dsRNA binding motif and inferring their cleavage by dicer-likes and uploading into RNA-induced silencing complexes (Schnettler et al., 2010). *Tomato spotted wilt virus* non-structural proteins also inhibits siRNA sequestration by

binding to an evolutionary conserved WG/GW motif of AGO1 (Giner et al., 2010; Hedil et al., 2015). Besides *Tomato spotted wilt virus*, non-structural proteins of another tospovirus, *Tomato yellow ring virus* also blocks local and systemic silencing and sequesters both long and short double stranded RNAs (Hedil et al., 2015). Unlike *Tomato spotted wilt virus*, non-structural proteins of *Tomato yellow ring virus* possess NTPase/phosphatase activity and higher systemic RNA silencing activity although expressed at a very low level (Hedil et al., 2015).

2.5. VSRs encoded by other viral genera

Tomato ring spot virus is transmitted by a nematode, *Xiphinema americanum* and belongs to the family *Secoviridae* (Genus: nepovirus) (Brown et al., 1993). The genome consists of bipartite ssRNAs that encode two polypeptides and are cleaved by proteases. *Tomato ring spot virus* coat protein exhibits VSR activity by interacting with AGO1 and destabilizing them by reducing the steady levels of AGO1 in the presence of *Tomato ring spot virus* coat protein (Karran and Sanfačon, 2014). *Tomato ring spot virus* is also associated with temperature-dependent recovery by decreasing translation of viral RNA genome and reducing levels of viral proteins (Ghoshal and Sanfaçon, 2014). *Tomato ring spot virus* X4 protein (encoded by RNA2) has a diverse sequence across nepovirus species and was reported to possess silencing suppressor activity in some *Tomato ring spot virus* species (Jafarpour and Sanfaçon, 2009).

Tomato bushy stunt virus from the tombusvirus genus (family: *Tombusviridae*) has a (+) ssRNA genome (4.8 kb) with 5 open reading frames and is passively transmitted by wind and by mites, aphids, and the fungus *Olpidium brassicae*. *Tomato bushy stunt virus* encodes P19 protein sequesters siRNA duplexes of specific size with high affinity, particularly 21 nt dssiRNA with 2 nt, 3′ overhangs (Hsieh et al., 2009; Danielson and Pezacki, 2013). Because of its unique small

RNA-ligand binding property, this protein prevents entry of the specific siRNA into the RNA-induced silencing complex by competing with AGO1, but fails to destabilize programmed RNA-induced silencing complexes (Silhavy et al., 2002; Lakatos et al., 2006) (Fig. 1).

P19 has strong affinity for DCL4, a major enzyme involved in plant defense through post-transcriptional gene silencing (Dunoyer et al., 2005; Deleris et al., 2006). In addition to siRNA duplexes, P19 has high affinity for miRNA duplexes of 23 nt (Chapman et al., 2004; Chen et al., 2008; Nasheri et al., 2011). P19 also adopt alternative strategies to suppress RNA silencing in hosts. For example, expression of P19 during infection induces host miRNA, miR168, which downregulates AGO1 (Várallyay et al., 2010) (Fig. 1 A, B). Because of the ability of P19 to sequester small RNA duplexes, it is a tool for capturing small RNAs in various heterologous systems with more complex RNA silencing pathway (Danielson and Pezacki, 2013).

Tomato mosaic virus from *Virgaviridae* encodes a replication-associated protein (Rep) that can suppress post-transcriptional gene silencing (Kubota et al., 2003). However, while *Tomato mosaic virus* Rep inhibited post-transcriptional gene silencing in inoculated leaves, Rep failed to suppress *Tomato mosaic virus*-specific post-transcriptional gene silencing in hosts that already had established infections (Kubota et al., 2003). Thus, *Tomato mosaic virus* Rep suppress the use of *Tomato mosaic virus*-specific small RNAs and make them unavailable for being used for the homology dependent cleavage of *Tomato mosaic virus* RNA (Fig. 1) (Tamai et al., 2010).

3. Role of VSRs in pathogenesis

Successful pathogenesis occurs when a virus overcomes host defenses, replicates, and spreads through the plant and to the next host (Mandadi and Scholthof, 2013; Garciá and Pallás, 2015). VSRs can also play an important role in multiple aspects of pathogenesis due to multi-

functionality (Garciá and Pallás, 2015). In this section (Table 1), we highlight various ways VSRs can promote pathogenesis in addition to suppressing RNA silencing, and highlight limits in research on the multifunctionality of VSR, which identifies critical research gaps.

3.1. VSR interacts with host proteins and hormones to alter plant immunity

3.1.1. Begomovirus C2, C4, V2, and βC1

The N-terminal of *Tomato leaf curl Java virus* V2 protein contains nuclear export signals that promote viral movement from the nucleus to the plasmodesmata (Sharma et al., 2011), while the C-terminal affects viral pathogenicity and hypersensitive response (Sharma and Ikegami, 2010). *Tomato yellow leaf curl virus* V2 aggregates also bind to viral DNA molecule for nucleo-cytoplasmic shuttling, which drives *Tomato yellow leaf curl virus* infection (Moshe et al., 2015). *Tomato yellow leaf curl virus* V2 protein interacts with papain-like cysteine proteases and interferes with their ability to induce host defenses (Bar-Ziv et al., 2012).

Tomato leaf curl New Delhi virus AC2 protein causes deregulation of host miRNAs involved in the regulation of transcriptions factors associated with development processes in tomato (Kumar and Naqvi, 2016). *Tomato leaf curl New Delhi virus* AC2 also suppresses hypersensitive response in both tomato and *N. benthamiana* (Hussain et al., 2007). AL2 protein encoded by *Tomato golden mosaic virus* interacts and inactivates host Sucrose Non-Fermenting1 (SNF1)-related kinase 1 (SnRK1) and Adenosine kinase (ADK), responsible for viral genome methylation, an epigenetic defense against *Tomato golden mosaic virus* (Wang et al., 2003; Wang et al., 2005; Raja et al., 2008). Both SnRK1 and ADK are important host factors that maintain host methylation cycles through regulation of host metabolism and S-adenosyl methionine (SAM) dependent methylation, respectively.

The sequences of various C4 genes vary across geminivirus family. C4 of *Tomato leaf curl virus* when expressed transgenically can induce symptom expression (Rigden et al., 1994; Krake et al., 1998). *Beet curly top virus* (BCTV) C4, which share no sequence homology with *Tomato leaf curl virus* C4, can function as pathogenicity determinant and contribute to enhanced phloem cell division and elongation (Pooma and Petty, 1996; Latham et al., 1997). The presence of conserved N-myristoylation domains in AC4 proteins determine its membrane binding, pathogenicity and disease symptom expression (Fondong et al., 2007; Rosas-Diaz et al., 2018).

Transgenic overexpression of *Tomato yellow leaf curl China virus* βC1 also induces developmental abnormality in leaves by decreasing miR165/166 levels and by enhancing the transcription factors that are responsible for maintaining abaxial and adaxial leaf polarity (Yang et al., 2008). βC1 also suppress methylation through interaction and inactivation of S-adenosyl homocysteine hydrolase, an essential enzyme involved in the methyl cycle (Yang et al., 2011).

Geminiviral suppressors obstruct phytohormone biosynthesis or signaling pathways that are necessary to regulate homeostatic balance between growth and virus induced stress in plants. For example, the C2 protein of *Tomato yellow leaf curl virus* interacts through an evolutionary conserved mechanism with the ubiquitination domain of RPS27A (a ribosomal protein) and inhibits the degradation of JAZ1 protein, which represses jasmonic acid signaling and terpene biosynthesis (Luan et al., 2013). Similarly, *Tomato yellow leaf curl virus* C2 protein interacts with the catalytic subunit of constitutive photomorphogenesis 9 signalosomemulti-subunit protein complex, affecting its ability to regulate E3 ubiquitin ligase and impair jasmonic acid signaling (Lozano-Durán et al., 2011; Rosas-Díaz et al., 2016). Thus, C2 of begomoviruses attenuate jasmonic acid pathway through transcriptional repression of jasmonic acid-responsive

genes or interacting with ubiquitin and subverting JAZ1-MYC mediated jasmonic acid response (Lozano-Durán et al., 2011; Rosas-Díaz et al., 2016; Li et al., 2019; Ziegler-Graff, 2020).

Another plant hormone, Brassinosteroid associated with other phytohormones to promote plant growth and defense (Belkhadir and Jaillais, 2015). However, AC4 from *Tomato leaf curl virus-Australia* interacts with a novel shaggy-like kinase in tomato (SlSK) through 12 amino acids present in the C-terminal to interfere with brassinosteroid (Piroux et al., 2007; Dogra et al., 2009); Fig. 2B). *Tomato yellow leaf curl China virus* βC1 also interacts with asymmetric leaves (AS1) to attenuate jasmonic acid defense (Fu et al., 2007; Yang et al., 2008) (Fig. 2A). *Tomato yellow leaf curl China virus* βC1 can also interact with helix-loop-helix transcription factors to reduce terpene and glucosinolate biosynthesis and phytohormones (Li et al., 2014) (Fig. 2A).

3.1.2. Potyvirus HCPro and VPg

HCPro uses *autoproteolytic* activity to cleave the viral polyprotein through its C-terminus and initiate viral infection (Carrington et al., 1989). Recently, AGO1 was shown to be recruited by HCPro, which causes the production of stable virus particles and results in systemic infection (Pollari et al., 2020). HCPro is also active in specific protein-protein and protein-RNA interactions that affect plant metabolism and virus multiplication (Whitham and Wang, 2004; Du et al., 2011). Evidence reveals HCPro interacts with the plant's cytoplasmic exoribonuclease 4 (Xrn4), another major cellular antiviral mechanism involved in RNA decay and VSR activity (Li and Wang, 2018). HCPro also causes viral symptoms by inducing the production of reactive oxygen species and by reducing antioxidant accumulation (Ivanov et al., 2016; De et al., 2018; Mäkinen and De, 2019). Recently, Yang et al., (2020) showed HCPro directly interacts with catalase 1 (CAT1) and catalase 3 (CAT3) in the cytoplasm of tobacco plants (Supplementary

Table 1). As a result, H_2O_2 was produced to help viral infection, and a reactive oxygen butst induced systemic cell death in infected plants. Similarly, VPg, apart from acting as a VSR, plays a role in viral translation and systemic movement (Eskelin et al., 2011). A recent article demonstrated that a multiprotein complex consisting of HCPro and VPg recruits a host protein, Varicose, which assists in initiating systemic infection in the plant (De et al., 2020).

HCPro of TuMV suppresses salicylic acid mediated defense signaling by interacting with a *Arabidopsis thaliana* homologue of Salicylic Acid-Binding Protein 3 (SABP3). By limiting the production of salicylic acid, HCPro weakens host defenses to facilitate viral infection (Poque et al., 2018). However, another study with HCPro of *Tobacco vein banding mosaic virus* showed the induction of salicylic acid and associated host defense response (Yang et al., 2016). From these studies, it can be concluded that the function of salicylic acid in potyvirus infection is dependent on specific plant-potyvirus interactions. A putative *Chenopodium quinoa* VSR found in *Chenopodium quinoa* (CqCA1) also interacts with HCPro (Poque et al., 2018), suggesting a conserved interaction. Further, TuMV HCPro affect jasmonic acid-regulated gene expression in plants (Endres et al., 2010). Apart from salicylic acid and jasmonic acid, HCPro was also found to induce auxin accumulation in plants leading to abnormal growth (Yang et al., 2020b).

3.1.3. Cucomovirus 2b protein

Cucumber mosaic virus (CMV) 2b protein plays a role in systemic and small viral movements (Ziebell et al., 2011; Zhou et al., 2014). Stabilization of C terminal of CMV 2b protein maintains the CMV2b-siRNA-ribonucleoprotein complex structure that is necessary for infectivity and viral spread (Gellért et al., 2012). Alanine scanning mutagenesis had identified conserved amino acid residues in CMV 2b protein that were responsible for cell-to-cell and long-

distance movement and silencing suppressor activity (Nemes et al., 2014). There are also reports of interaction between CMV 2b and *Arabidopsis* catalase 3 (CAT3) that causes necrotic spots in systemic leaves (Nakahara and Masuta, 2014).

Tomato aspermy virus 2b binds primarily to duplex siRNAs in a length specific manner and can also bind to miRNA duplexes and single stranded RNAs of various length (Rashid et al., 2008). The N-terminal (12 amino acid) of *Tomato aspermy virus* 2b protein play an essential role in recombination with 2b proteins of CMV 2b, and is key for the systemic infection of host plants (Shi and Palukaitis, 2011). The ability of 2b protein of CMV and TAV to interact with miRNAs were also revealed by spatial and temporal changes in various miRNAs and their target mRNA expressions in response to viral infection in tomato (Feng et al., 2011). The CMV 2b protein promotes cell to cell movement of pseudorecombinant viruses and plays a vital role in hypersensitive cell-death and virus resistance (Shi et al., 2003); these mechanisms were further demonstrated as both activities were abolished through mutation in the functional domain (Li et al., 1999).

During CMV infection, 2b protein binds to JAZ1 to inhibit degradation and induction of jasmonic acid (Ziegler-Graff, 2020) (Fig. 2A). Constitutive expression of Fny- CMV 2b protein downregulates 90% of jasmonic acid-regulated genes without affecting jasmonic acid biosynthesis (Lewsey et al., 2010). CMV 2b protein also interferes with salicylic acid signaling by interacting with rgs-CaM (Ji and Ding, 2001; Lewsey et al., 2010; Jeon et al., 2017) (Fig. 2B; Supplementary Table 1). Wu et al., (2017) investigated how CMV 2b protein can repress host JAZ1 protein, a repressor for MYC transcription factors. In non-stressed hosts, levels of jasmonic acid favor JAZ1 accumulation and suppress jasmonic acid signals. However, under the

influence of biotic stressors (herbivores), increased jasmonic acid levels facilitate the degradation of JAZ1 through 26S proteosomal machinery (Fig. 2A).

3.1.4. Tospovirus non-structural protein

VSR non-structural protein (NSs) plays an important role in viral infection and movement within the Tospoviridae family (Takeda et al., 2002). This VSR also maintains pathogenicity in other heterologous viruses that are deficient in functional suppressors (Ocampo Ocampo et al., 2016). Non-structural proteins also induce development of systemic infection and *Tomato spotted wilt virus* induced symptoms expression through inhibition of host plant antiviral silencing (Garcia-Ruiz et al., 2018). Similarly, *Tomato spotted wilt virus* non-structural proteins promote persistent infection and vector-borne transmission by western flower thrips (*Frankliniella occidentalis*) (Margaria et al., 2014). Moreover, non-structural proteins interact with transcription factors to subvert jasmonic acid-mediated defense against western flower thrip vectors.

3.1.5. Tombusvirus P19 protein

Various mutations in the siRNA binding site of P19 generates a multitude of symptoms in host plants that compromise systemic silencing, but mutations in other sites cause developmental defects (Hsieh et al., 2009). For example, silencing efficiency of siRNAs on target mRNA affect mismatches, where mutations in the central region produced stronger symptoms compared to mutations in the periphery. The generation of P19 mutants, and symptoms expressed by mutants, are also dependent on host physiology due to compromised host-dependent siRNA sequestration (Hsieh et al., 2009). P19 interferes with HEN-1 mediated methylation of miRNAs and decreases

endogenous miRNA stability (Lózsa et al., 2008). P19 also interacts with uncharacterized plant RNA binding ALY proteins (involved in nucleo-cytosolic mRNA transport and influence growth and development of plant) through its RNA binding domain and alter the localization of ALY from nucleus to cytoplasm (Uhrig et al., 2004; Canto et al., 2006). Moreover, ectopic expression of the VSR TBSV P19 in hosts results in mis-regulation of miR167 targeting Auxin response factor 8 (ARF 8) (Jay et al., 2011)(Fig. 2B; Supplementary Table 1) and causes abnormalities.

3.1.6. Replication protein of *Tomato mosaic virus* (ToMV)

Tomato mosaic virus Rep plays an important role in movement and encapsidation of the Tomato mosaic virus genome. In addition, *Tomato mosaic virus* Rep also interacts with host plant factors that drive symptom development (e.g., chloroplast ferredoxin I in tobacco, NAC domain transcription factors in *Arabidopsis*, various other cellular proteins from tomato) (Ishibashi et al., 2010; Sun et al., 2013). Membrane bound *Tomato mosaic virus* Rep also plays a critical role in guanylation of nascent RNAs to form 5′ cap. 5′ capping through guanylation is required for the stability of nascent RNA undergoing elongation and protein synthesis.

Tomato mosaic virus infection in tomato induced levels of trans-acting (ta)-siRNAs that regulate the Aux response factors (Yifhar et al., 2012) (Fig. 2B). *Tomato ringspot virus* CP in tobacco induces NahG expression and breaks down salicylic acid into catechol (Fig. 2B), which can result in increased lesion size, facilitating the spread of this virus efficiently and systemically (Jovel et al., 2011). Furthermore, salicylic acid has been reported to work upstream of siRNA pathway and amplify siRNA signaling in plants (Alazem and Lin, 2015). VSRs play a vital role in tuning these interactions to facilitate viral infection. Although, more studies need to be done to identify the protein/s that connects siRNA pathway to salicylic acid.

3.2. Role of VSRs in vector transmission

VSRs play a role in altering susceptibility of Solanaceous hosts to both vectors and non-vector herbivores by interfering with phytohormone signaling and volatiles emitted (Tungadi et al., 2017; Ziegler-Graff, 2020). In the following sections, we briefly discuss the role of VSRs in affecting vector fitness and behavior, and effects on pathogen transmission that build on a previous review (Ray and Casteel, 2022).

3.2.1. Begomovirus

βC1 interferes with feeding behavior of the vector whitefly by interfering with 3 different host factors: AS1, MYC2 and SKP1 (Yang et al., 2008; Li et al., 2014; Jia et al., 2016) (Fig. 2A). Conversely, accumulation of βC1 in the phloem of infected hosts and binding with transcription factor WRKY20 deter non-vector herbivores, but favor whitefly vectors (Zhao et al., 2019). βC1 favors herbivore insects by inhibiting glucosinolate mediated anti-herbivore defense (Hopkins et al., 2009). Another monopartite VSR, C2 was reported to improve the performance of whiteflies by inhibiting jasmonic acid-signaling and terpene biosynthesis and by subverting ubiquitination (Luan et al., 2014; Rosas-Díaz et al., 2016; Li et al., 2019) (Fig. 2A).

3.2.2. Potyvirus

HCPro acts as a bridge between virions and receptor proteins in aphid stylets (Blanc et al., 1997; Dombrovsky et al., 2007). From the insect vectors' aspect, there is little information on the receptors that bind to HCPro (Dombrovsky et al., 2007). However, HCPro manipulates aphid biology; *Potato virus Y* HCPro in transgenic *N. benthamiana* was reported to enhance the growth

of vector *M. persicae* (Westwood et al., 2014). In contrast, it has been revealed that transiently expressed *Turnip mosaic virus* HCPro decreased aphid fecundity on *N. benthamiana* leaves (Casteel et al., 2014). Moreover, Casteel et al. (2014) also showed a decrease in aphid fecundity in the presence of ectopically expressed VPg protein.

3.2.3. Cucomovirus

2b protein encoded by CMV releases volatiles that provide a favorable environment for both aphid vectors and parasitoids (Lewsey et al., 2010; Wu et al., 2017). CMV 2b protein also facilitates aphid invasion on plants by inhibiting the AGO1 mediated biosynthesis of an aphid repelling glucosinolate, 4-methoxy-indole-3-yl-methylglucosinolate by interfering with AGO1 (Westwood et al., 2013). CMV 2b protein affect attractiveness and fecundity of the vector green pea aphid (*Myzus persicae*) and indirectly affect virus transmission by suppressing jasmonic acid mediated defense signaling (Mauck et al., 2010; Ziebell et al., 2011).

3.2.4. Other virus genera

Non-structural proteins of *Tomato spotted wilt virus* alters preference of vector western flower thrips and increases their performance (Wu et al., 2019). *Tomato spotted wilt virus* non-structural proteins enhance plant attractiveness to thrips by interacting with various MYC transcription factors and inhibiting jasmonic acid-signaling (Wu et al., 2019) (Fig. 2A). Besides thrips, *Tomato spotted wilt virus* infected plants have enhanced performance and fecundity to two-spotted spider mite, *T. urticae* (Nachappa et al., 2013). Currently, it is not known if the coat protein (VSR) of Nepovirus affects the behavior of its vector.

4. Co-evolutionary dynamics between VSRs and host proteins

VSR is a critical pathogenesis factor and due to its role in suppressing host defense response can coevolve with the plant. To understand what is driving the evolution of VSRs, the gene-for-gene model can be implemented here, first described in the flax-flax rust system (Flor, 1955). With the gene-for-gene model, the interaction of specific plant and virus factors can cause an incompatible interaction and trigger a hypersensitive response which limits the infection (Fraile and García-Arenal, 2010). The hypersensitive response is often associated with an increase in salicylic acid leading to cell death and limiting the infection (Radojičić et al., 2018). Here, we point to studies where different factors from plants directly or indirectly detect VSR and trigger hypersensitive responses. We also highlight some known counter-defense strategies employed by viruses, allowing VSR to function without inducing a defense response.

4.1. Begomovirus

Begomoviral VSRs function as elicitors and pathogenicity determinants. For example, V2 protein of *Tomato leaf curl Java virus*, *Cotton leaf curl Kokhran virus*, and *Papaya leaf curl virus* elicit hypersensitive responses in *N. benthamina* and tomato (Mubin et al., 2010; Sharma and Ikegami, 2010). To counter the plant defense response, C2 protein neutralizes the effect of V2 protein and ensures efficient viral infection (Mubin et al., 2010).

Another VSR protein, AC4, from different geminivirus infect a range of hosts possess a N-myristoylation motif (conserved and consensus), responsible for membrane binding, elicitor of disease symptoms and pathogenicity determinant (Fondong et al., 2007). AV2 protein of *Tomato leaf curl Palampur virus* induces genes associated with salicylic acid-signaling in tomato (Roshan et al., 2020). Moreover, some recent findings suggest a role of C2 from *Tomato yellow*

leaf curl-Sardinia virus as a virulent factor that exhibits hypersensitive response in plants. The C2 protein of *Tomato yellow leaf curl Sardinia virus* acts as a pathogenicity determinant and a 16-amino acid domain is responsible for inducing a hypersensitive response in plants (Matić et al., 2016; Guerrero et al., 2020). Interestingly, the same study showed lack of hypersensitive response during *Tomato yellow leaf curl Sardinia virus* infection suggesting the presence of some other viral protein that counterattacks the host defensive response (Matić et al., 2016).

4.2. Potyvirus

HCPro can act as an elicitor of R gene-driven effector triggered immunity as per the gene-for-gene. This is the case for *Potato virus Y,* which induces hypersensitive responses that restrict the virus in necrotic local lesions in potato cultivars. These cultivars possess dominant resistance genes Nc_{tbr} and Ny_{tbr} (Moury et al., 2011), which may recognize similar structural determinants in the central region of HCPro of *Potato virus Y*^{0} (Ny_{tbr}) and *Potato virus Y*^{C} (Nc_{tbr}) strains (Tian and Valkonen, 2013; Tian and Valkonen, 2015). Nevertheless, resistance-breaking *Potato virus Y* isolates (*Potato virus Y*^{N}) can overcome Ny_{tbr} mediated resistance through some residues in the C-terminal part of the HCPro (K_{400} and E_{419}) causing induction of alternative defense response of vein necrosis in tobacco infected by *Potato virus Y* isolates (Tribodet et al., 2005; Faurez et al., 2012). Overall, the data suggest alterations in HCPro from mutations can overcome R gene-mediated resistance, affecting functional interactions with other host factors and inducing alternative defense responses (Tian and Valkonen, 2013; Tian and Valkonen, 2015).

VPg till now had not been reported to elicit a defense response against viruses in plants. However, VPg reduced aphid performance possibly mediated by the defense response which is in line with 6K1 protein that also reduced aphid performance, and later detailed investigation

showed phytohormones mediated defense response by 6K1 (Casteel et al., 2014; Bera et al., 2022). Due to the critical role of VPg in the viral genome translation, it has been targeted for breeding recessive resistance in plants (Moury and Verdin, 2012). Recessive resistance is defined as a lack of susceptibility in plants. In other words, the absence of host proteins are critical for virus infection (Fraile and García-Arenal, 2010). A recessive resistance gene, *pvr2*, was identified in pepper plants that code for translation initiation factor (eIF4e). VPg was found to interact directly with eIF4e that is vital for virus translation (Kang et al., 2005; Charron et al., 2008). Mutations in the *pvr2* gene encoding for the eIF4e protein interferes with VPg binding, resulting in resistance against potyviruses (Charron et al., 2008). However, VPg mutants restore the compatible interaction with eIF4e and break the host resistance conferred by the recessive gene (Gebre-Selassie et al., 1985). Moreover, recessive resistance was found to be highly durable for more than 50 years in pepper cultivars, suggesting most of the mutations in VPg was lethal and may have impaired its multi-functionality (Moury and Verdin, 2012).

4.3. Other viruses

Several resistance genes have been identified (e.g. *Tm-1 Tm-2*, *Tm-2²*, *Tm-2ᵅ*) in wild tomato species that confer resistance against tobamovirus species including *Tobacco mosaic virus*, *Tomato mosaic virus*, and *Tomato mild mottle virus* (Luria et al., 2017). *Tm-1* resistant gene from resistant tomato species encode proteins that interact with *Tobacco mosaic virus* Rep (Ishibashi et al., 2007) and prevent the formation of replication complex between *Tobacco mosaic virus* Rep and membrane bound host proteins (TOM1, TOM2A and ARF8) to inhibit *Tobacco mosaic virus* replication (Ishibashi et al., 2012; Ishibashi and Ishikawa, 2013; Ishibashi et al., 2014). Another resistance gene *Tm-2* in *S. peruvianum* confers a higher level of resistance

than *Tm-1*. The resistant gene *Tm-2²* was found more durable in conferring resistance than *Tm-2* (Lanfermeijer et al., 2005). The *Tm-2* and the *Tm-2²* resistance genes are considered allelic (Pelham, 1966; Young and Tanksley, 1989) and encode a coiled-coil/nucleotide binding-ARC/LRR protein class of plant resistance (R) genes (Lanfermeijer et al., 2003). Due to co-evolution, resistance-breaking *Tobacco mosaic virus* also show two nucleotide substitutions in the rep protein responsible for overcoming host plant resistance (Strasser and Pfitzner, 2007).

Interestingly, there are few studies that suggest the presence of avirulent factor associated with P19 (*Tomato bushy stunt virus*), 2b (*Tomato aspermy virus*), and non-structural proteins (*Tomato spotted wilt virus*). Depending on *Nicotiana* species, P19 upon agro-infiltration elicited defense responses, suggesting the presence of putative R-protein (Angel et al., 2011). Similarly, transient expression of TAV2b induced HR in *Nicotiana* species imply the presence of an R-protein. However, in the presence of virus infection no HR was detected (Li et al., 1999). In resistant pepper, non-structural proteins of *Tomato spotted wilt virus* were recognized by R-protein when ectopically expressed. The resistant breaking strains of *Tomato spotted wilt virus* were also reported that had mutations in non-structural proteins, impairing its RSS function however, resistance-breaking strains still suppressed RNA silencing indicating the presence of more than one VSR proteins in *Tomato spotted wilt virus* (de Ronde et al., 2013).

5. Conclusion and Future directions

Viral genomes are constantly evolving to optimize the number of coded proteins that can promote successful infection. Here we reviewed the multi-functionality associated with VSR proteins of some major viruses that affect Solanaceaous hosts. Apart from acting as a suppressor of RNA silencing, VSRs perform many critical functions in a viral life cycle and pathogenesis.

While synthesizing the available literature to understand the multi-functionality of VSR proteins in an ecological context, we found some knowledge gaps that suggest needed future research directions. This knowledge will help us to design and improve the strategies to manage plant viruses and their insect vectors efficiently (Wu and Ye, 2020) with the usage of fewer chemicals in agriculture leading to sustainable growth with less pollution (Parizad and Bera, 2021).

Our review also suggests a need to better understand the additional functions of VSRs and if any are conserved across VSRs. For example, a holistic overview is missing about how VSRs modulate phytohormones such as jasmonic acid and related defense responses. Jasmonic acid is a part of the oxylipin signaling pathway and mediates volatile production and vector attraction. Downstream of jasmonic acid signaling has a role in biosynthesis of anti-herbivore metabolites, such as terpenoids, sesquiterpenes, and monoterpenes, functions to repel herbivores or to attract natural enemies. Indeed, we have shown how VSRs manipulate upstream and downstream of jasmonic acid, but there are limited studies that investigated all the jasmonic acid dependent signaling pathways simultaneously with focus on vector attraction and repulsion during the virus life cycle. It would be also interesting to see if the function of VSRs change in the presence of healthy as compared to viruliferous herbivores. Exploring the possibility of dynamic multi-functionality of VSR to increase virus transmission will be an important direction in the future.

A clear role of HCPro in manipulating the ethylene hormone pathway is limited. HCPro interacts with exoribonuclease, Xrn4, to neutralizes host defense (Li and Wang, 2018; Supplementary Table 1). Xrn4 is also a component of the ethylene response pathway which is inhibited in the presence of ethylene (Olmedo et al., 2006; Potuschak et al., 2006). This suggests the ethylene hormone assists in potyvirus infection and allows HCPro to be available for other purposes as Xrn4 is suppressed by ethylene. Indeed, studies show potyvirus infection induces

ethylene which in turn mediates potyvirus spread by aphid vectors and HCPro is also key for vector transmission (Casteel et al., 2015; Bak et al., 2019). Thhe ethylene pathway thus likely plays a central and indirect role in HCPro's multifunctionality which needs to be investigated more to allow engineering of the ethylene pathway for sustainable virus and pest management in the coming years.

The multi-functionality of VSR proteins should also make them a good target to generate resistant plants. Moreover, the role of VSR proteins as an elicitor of defense response may lead to more durable strategies to control viruses. However, studies reviewed here often reported resistance-breaking viral strains often had fitness costs that may disrupt the multi-functionality of a viral protein (May et al., 2020; Liu et al., 2021). Assessing potential "trade-offs" among different virus life history traits has been conducted for various coat protein mutations of tobamoviruses (Moreno-Pérez et al., 2016; Bera et al., 2017; Moreno-Pérez et al., 2022) and the same rationale can be applied for the multifunctional VSRs. VSR affects the virus accumulation in systemic leaves due to its' role in systemic movement and vector transmission thus, trade-off between surviving in external environment and rate of multiplication.

Recently some approaches were designed to induce plant defense by using siRNA targeting VSR proteins. Begomovirus resistant tomato plants were developed by utilizing siRNA targeting both AC2 and AC4 ORFs. For example, Singh et al., (2015) used partial AC2 and AC4 sequences in RNAi vectors to silence AC2 and AC4 ORF of *Tomtao leaf curl New Dehli virus* and found relatively large amounts of *Tomtao leaf curl New Dehli virus* AC2 and AC4 specific transacting siRNAs. Artificial tasiRNAs also plays a key role in developing resistance against AC2 and AC4 suppressors of *Tomtao leaf curl New Dehli virus* (Singh et al., 2015). All these above studies suggest VSR proteins to be a good target to develop resistant crops. It can be

speculated that siRNA specific to VSR coding region creates a selection pressure on VSR proteins to mutate which can be non-functional, explaining the high efficiency of siRNA to produce virus-resistant crops. These kinds of research should be encouraged for other viruses that harbors VSR protein to produce virus-resistant crops.

While categorizing the different functions of diverse VSR proteins from different virus genera, we found consensus functions of VSR proteins related to silencing suppressor activity and in modulating phytohormones and related responses that affect vector behavior. Numerous studies also showed phytohormones mediate multi-trophic interactions consisting of herbivores, vectors, rhizobia *etc*, it is tempting to speculate that VSR proteins might indirectly affect other tropic levels by modulating the phytohormone pathways (Basu et al., 2021; Lee et al., 2021; Basu et al., 2022). Therefore, we would like to propose to focus more on unconventional interactions that might be mediated by VSR and are missed in control lab environment.

Figure Legends

Fig. 1. Antiviral RNA silencing pathways in tomato, depicting three unique silencing pathways: (1) Post-transcriptional gene silencing (PTGS) for degradation of viral mRNAs, (2) siRNA-directed methylation leading to transcriptional gene silencing (TGS) of the methylated DNA, and (3) Endogenous mRNA silencing by miRNAs. The figure also depicts the multiple mechanisms by which VSRs have evolved to suppress host-induced gene silencing.

Fig. 2: Schematic diagram showing roles of different VSRs in interfering with phytohormone signaling pathways.

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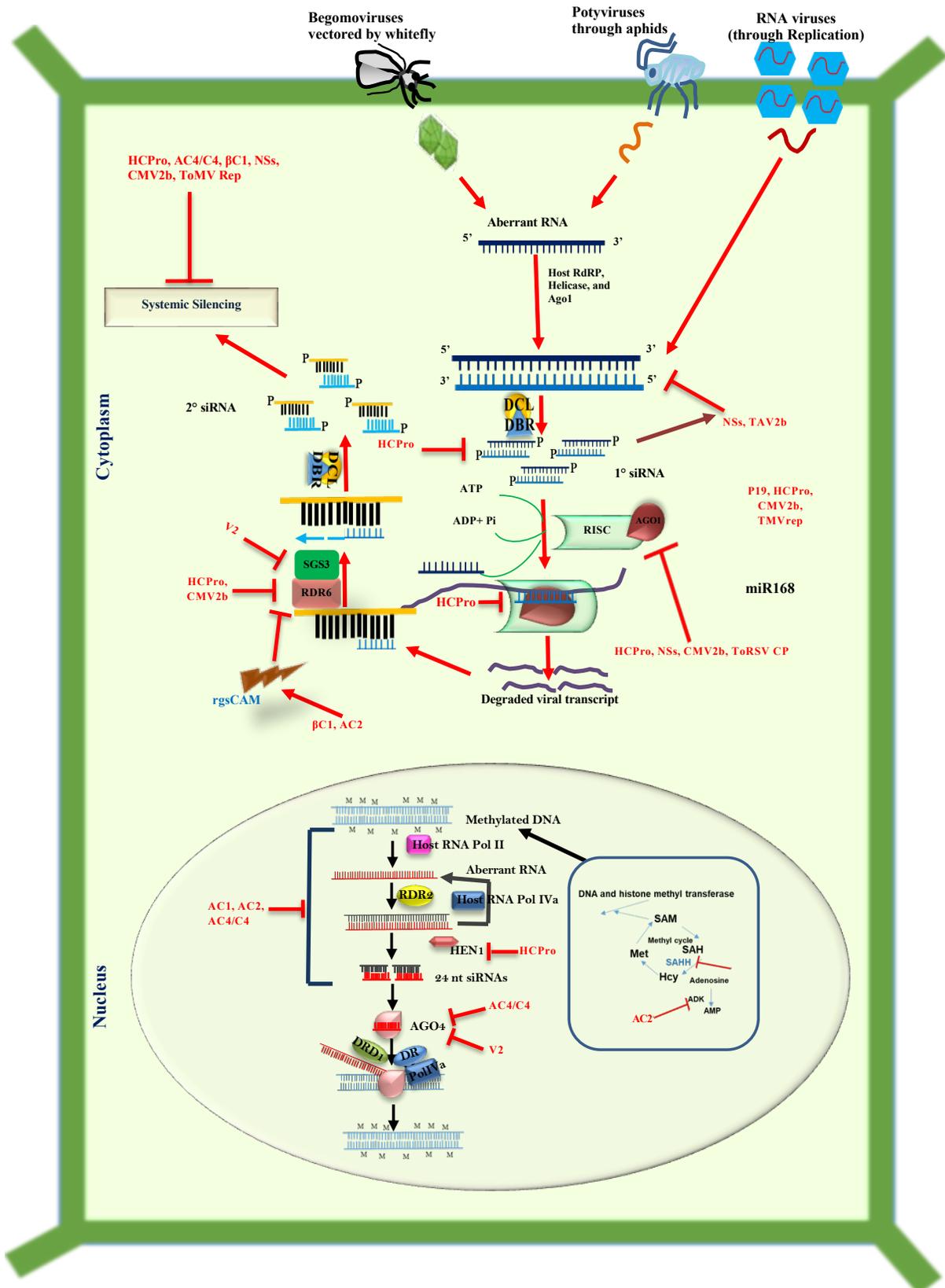
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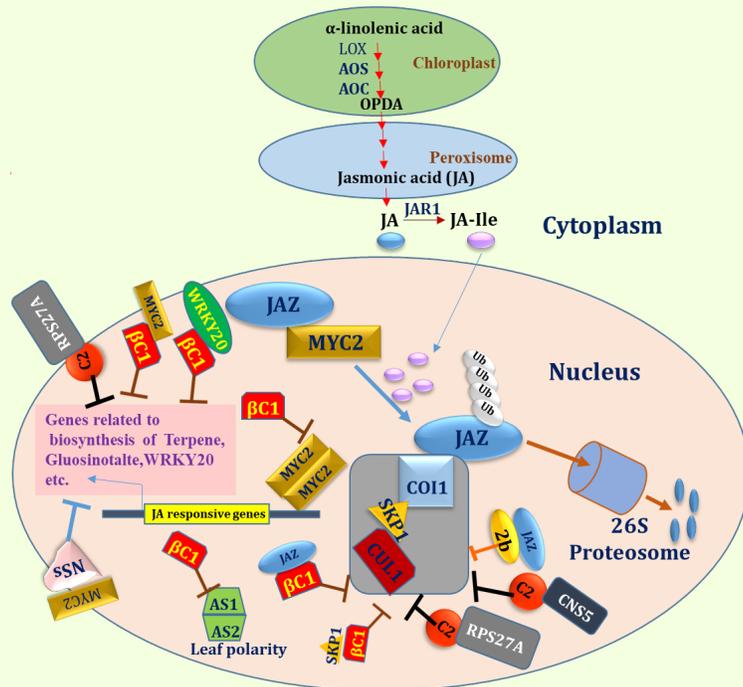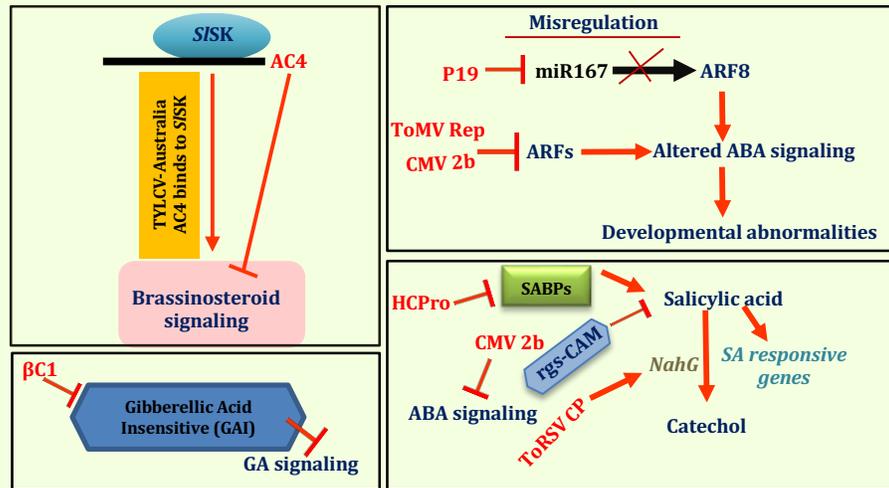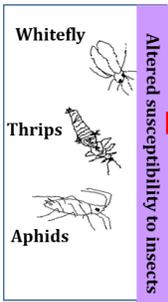
Table 1: Multifunctionality of different viral suppressors encoded by *Solanaceae* infecting viruses

Pathogenicity step	Viral silencing suppressor	Virus genus	References
Translation and protien functions	VPg	Potyvirus	Moury and Verdin, 2012; Charron et al., 2008
Replication	Rep	Tobamovirus	Ishibashi et al., 2010; Sun et al., 2013
Counter defense (JA-defense, SA-defense, RNA decay etc.)	V2/AV2	Begomovirus	Roshan et al., 2020;
	AC2/AL2/C2	Begomovirus	Guerrero et al., 2020
	βC1	Begomovirus	Gnanasekaran et al., 2019
	NSs	Tospovirus	Wu and Ye, 2020; Du et al., 2020
	Coat protein	Nepovirus	Jovel et al., 2007
	HCPro	Potyvirus	Endres et al., 2010; Yang et al., 2020b
	2b	Cucumovirus	Jeon et al., 2017; Ziegler-Graff, 2020
Cell-cell and Systemic movement	V2/AV2	Begomovirus	Zhao et al., 2020
	βC1	Begomovirus	Gnanasekaran et al., 2019
	HCPro	Potyvirus	Pollari et al., 2020
	VPg	Potyvirus	Eskelin et al., 2011; Du et al., 2020
	2b	Cucumovirus	Nemes et al., 2014; Shi et al., 2003
Symptoms	AC2/AL2/C2	Begomovirus	Matić et al., 2016; Guerrero et al., 2020
	C4/AC4	Begomovirus	Fondong et al., 2007; Rosas-Diaz et al., 2018
	Rep	Tobamovirus	Ishibashi et al., 2010; Sun et al., 2013
	HCPro	Potyvirus	De et al., 2018; Mäkinen and De, 2019
	P19	Tombusvirus	Hsieh et al., 2009
	NSs	Tospovirus	Garcia-Ruiz et al., 2018
Vector performance/transmission and host to host movement	βC1	Begomovirus	Zhao et al., 2019
	NSs	Tospovirus	Wu et al., 2019
	HCPro	Potyvirus	Dombrovsky et al., 2007
	2b	Cucumovirus	Mauck et al., 2010; Ziebell et al., 2011

Supplementary Table 1: Mode of action and multifunctionality of different viral suppressors encoded by *Solanaceae* infecting viruses

Virus genus	Susceptible crops from *Solanaceae*	VSR(s)	Molecular mechanism of silencing suppressor activity	Multifunctionality	References
Begomovirus	Tomato, pepper, Tobacco, eggplant	V2/AV2	Suppression of PTGS by inhibiting SGS3 activity, Sequestration of ds-siRNA (21 to 24 nt) and ss-siRNAs (24 nt), suppression of TGS through interaction with AGO4 and Histone deacetylase 6. Differential interaction with RDR1.	Pre-coat protein, viral pathogenicity determinant, induce HR, interact with papain-like cysteine protease to block host defense response Interaction with Catalase 2 enzyme for systemic spread, enhancement of SA-dependent defense signaling	Hancˇinský et al., 2020; Rojas et al., 2001; Zrachya et al., 2007; Glick et al., 2008; Sharma and Ikegami, 2009, Zhang et al., 2012, Bar-Ziv et al., 2012; Rojas et al., 2012; Wang et al., 2014, 2018, 2019 Roshan et al., 2018; Roshan et al., 2020 Basu et al., 2018
		AC2/AL2/C2	Suppression of silencing through interaction with AGO1 and inactivation of miRNAs Suppression of TGS mediated host defense through inactivation of SnRK1 and ADK and, interaction with host	Transcriptional activator, pathogenicity determinant, suppress HR induced by NSP, deregulation of host miRNAs (miR319 and miR172) responsible for various development processes, disrupts host methylation cycle, interaction between AC2 and	Praveen et al., 2007; Karjee et al., 2008; Basu et al., 2018; Kumar and Naqvi, 2016; Hussain et al., 2007; Wang et al., 2003, 2005; Raja et al., 2008; Chung et

			rgsCAM, H3K9me2 host methyl transferase and Su(var)3-9 homolog 4/Kryptonite (SUVH4/KYP).	methylation dependent host kinases inhibit transactivation of host genes through inactivating cellular transmethylation reaction.	al., 2014; Castillo-Gonzalez et al., 2015
			Suppress silencing machinery via sequestering viral DNA through DNA binding Zn-finger motif present in them	Interaction with CNS5 and inactivation of SCF-ubiquitin related cellular defense, suppress JA mediated defense through down regulation of JA genes	van Wezel et al., 2002; Dong et al., 2003; Trinks et al., 2005; Lozano-Duran et al., 2011; Rosas-Diaz et al., 2016
		C4/AC4	Suppress both TGS and PTGS through sequestration of miRNAs and siRNAs and, host AGO4 protein	Induces enhanced phloem cell division and elongation. Interact with shaggy-like kinase and interfere with brassinosteroid signaling, interact with BAM 1 and 2 and inactivation of cell to cell spread of silencing	Chellappan et al., 2005a, b; Vinutha et al., 2018; Dogra et al., 2009; Rosas-Dias et al., 2018
satellite DNA β		βC1	Suppression of both TGS and PTGS, non-specific binding with ssDNAs and dsDNAs and interference with host miRNA pathways, interaction and inactivation of SAHH (a methyl cycle maintenance enzyme), suppression of RDR6-SGS3	Virus movement, pathogenicity determination, induce development abnormality in leaves through interaction with AS1, interfere with JA mediated defense response and Gibberellic acid signaling by degrading a JA-receptor (SCF^{COI1}), abrogate host defense by interacting with Tm-1,	Cui et al., 2005; Yang et al., 2008; Shukla et al., 2013; Li et al., 2014, Zhao et al., 2019, Kon et al., 2007; Jia et al., 2016;

			mediated silencing through induction of host rgsCAM.	interacts with WRKY20 to activate SA signaling.	Voorburg et al., 2020
Tospovirus	Eggplant, Potato, Tobacco, Pepper, Tomato, Blackberry, Tomatillo,	NSs	NSs suppress both local and non-cell autonomous systemic silencing Sequestration of siRNAs and precursor dsRNAs of miRNAs and siRNAs; local silencing through AGO1 binding via WG/GW motif.	Pathogenicity determinant Alter vector thrips performance, inactivate JA signaling through interaction with JA-regulatory components (MYC2, 3 and 4)	Takeda et al., 2002;Ocampo et al., 2018; Wu et al., 2017; Margaria et al., 2015; Schnettler et al., 2010; de Ronde et al., 2014; Hedil et al., Hedil et al., 2015
Nepovirus	Tomato, Potato, Blackberry, Tamarillo,	Coat protein	RSS activity through interaction and destabilization of AGO1 through WG/GW motif (AGO hook), contribute to temperature dependent recovery by suppressing the production of viral proteins through reduced translation by AGO1 dependent silencing (without reducing viral siRNA titer)	Encapsidation and breakdown of salicylic acid to catechol through induction of *NahG* expression	Jovel et al., 2011; Karran and Sanfacon, 2014, Ghoshal and Sanfacon, 2014;
		X4 protein	PTGS suppression through unknown mechanism		Jafarpour and Sanfacon, 2009; Jafarpour 2010
Tombusvirus	Eggplant, Pepper, Tomato	P19	Very strong silencing suppressor for both local and systemic silencing.	Interact and inactivate nucleo-cytoplasmic protein ALY;	Qui et al., 2002; Dunoyer et al., 2004; Chen et al.,

			Molecular caliper; sequester siRNA (20-22 nt) and miRNA (23 nt) duplexes with very high affinity in size specific and sequence independent manner; P19 induces expression of miR168, which intern downregulate AGO1, interfere with HEN1 mediated methylation of miRNAs to decrease endogenous miRNA stability	Misregulation of miR167 targeting ARF8	2007, 2011; Chapman et al., 2004; Chen et al., 2008; Nasheri et al., 2011; Khan et al., 2011; Danielson and Pezaki, 2013; Varallayay et al., 2011; Lozsa et al., 2008; Catano et al.,2006; Uhrig et al., 2004; Lakatos et al., 2004
Tobamovirus	Eggplant, Potato, Pepper, Tomato, Tamarillo	Rep	Suppressor for both local and systemic silencing; PTGS suppression by working down stream of siRNA generation Suppress sequence specific RNA degradation by inhibiting 3'-terminal ToMV siRNA	ToMV replication, membrane binding and guanylation for 5' capping of nascent RNA, interfere with auxin signaling	Kubota et al., 2003; Tamai et al., 2013; Nishikiroi et al., 2012; Yifhar et al., 2012
Cucumovirus	Broad host range infecting almost all plants in Solanaceae family	2b	Sequestration of siRNA duplexes with strong affinity, suppress RDR6 mediated PTGS virus infected cells, interact with AGO1 through PAZ domain and PIWI box and with AGO4 to inhibit PTGS.	Pathogenicity determinant, induce HR, interfere with both SA, JA, ABA signaling and alter host susceptibility to herbivores	Zhang et al., 2006; Goto et al., 2007; Gonzalez et al., 2010; Hamera et al., 2012; Duan et al., 2012; Diaz-Pendon et al., 2007; Ye et al.,

					2007, 2009; Wang et al., 2011; Yifhar et al., 2012; Ziegler-Graff, 2020
Potyvirus	Pepper, Potato, Tomato, Tobacco, African eggplant, Cape gooseberry, Sweet pepino, tomatillo	HCPro	RNA silencing suppressor through different ways: Binding to vsiRNA and limiting RISC assembly by targeting multiple steps, Regulation of AGO1 function.	*Autoproteolytic* activity; AGO1 recruitment and systemic infection; Inhibition of the host RNA decay mechanism through interaction with the plant exoribonuclease 4 (Xrn4).	Carrington et al., 1989; Pollari et al., 2020; Li and Wang, 2018; Del Toro et al., 2017;
				Manifesting viral symptoms by inducing the reactive oxygen species (ROS) production; Helping viral infection by interaction with catalase 1 (CAT1) and catalase 3 (CAT3) and producing H_2O_2; HR induction	De et al., 2018; Ivanov et al., 2016; Makinen and De, 2019; Yang et al., 2020; Wen et al., 2013;
				Suppression of SA-mediated defense responses (probable related host factors are (SA)-binding proteins (SABPs); Affecting the JA-regulated gene expression in plants.	Endres et al., 2010; Poque et al., 2018;

				Mediates interaction between virus and aphid resulting in efficient virus transmission, manipulation of the aphids' biology.	Sigvald, 1985; Casteel et al., 2014;
		VPg	Mediates silencing suppressor activity through degradation of SGS3 along with RDR6	Viral translation and systemic movement; Interaction with eIF4E in CAP-dependent translation of viral genome. A multiprotein complex of HCPro, VPg and Varicose, assist in systemic infection.	Cheng et al., 2017; Eskelin et al., 2011; Coutinho de Oliveira et al., 2019; De et al., 2020; Gebre Selassie et al., 1985; Luis-Arteaga et al., 1993; Coutinho de Oliveira et al., 2019; Eskelin et al., 2011