ARTICLE ADDENDUM

Multifaceted defense and counter-defense in co-evolutionary arms race between plants and viruses

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ABSTRACT
Plants have evolved sophisticated surveillance and defense mechanisms against constant attacks by different types of microbial pathogens including viruses. Nucleotide-based RNA silencing and receptor-based innate immunity constitute the primary plant defense to viral infection. To establish their infections, plant viruses have also evolved virulence strategies to suppress host defenses. This everlasting co-evolutionary arms race between the plant hosts and viruses has shaped today’s multifaceted defense and counter-defense layout. A better understanding of this process will certainly assist in the development of new plant varieties with enhanced durable resistance and novel approaches to control viral pathogens for sustainable agriculture. In this addendum, we briefly review the current knowledge of the plant immune response to viral infection and discuss defense and counter-defense strategies.

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Plants are constantly exposed to diverse microorganisms such as nematodes, fungi, oomycetes, bacteria, and viruses, and have evolved sophisticated and efficient mechanisms to protect themselves against pathogen attack.1,2 For non-viral pathogens, plants deploy a complex innate immune system that consists of 2 interconnected tiers of receptors including pattern recognition receptors (PRRs) and nucleotide-binding site leucine-rich repeats (NB-LRR) receptors to modulate plant defense.3,4,5,6 For viral pathogens, in addition to the receptor-based innate immunity system, RNA silencing is another major antiviral cellular pathway.7 To establish their infection, viruses have also evolved counter strategies to suppress or evade plant defense surveillance using the relatively few proteins they encode. In this addendum, we focus on summarizing current knowledge about innate immunity and RNA silencing antiviral defense in plants and viral counter-defense strategies. Due to space constraint, we do not discuss other antiviral strategies such as recessive resistance.8,9

RNA silencing in plants, suppression of RNA silencing by viruses and plant counter strategies

RNA silencing, also called RNA interference (RNAi), is an evolutionary conserved and sequence-specific mechanism that is triggered by double-stranded RNAs (dsRNAs) to induce RNA degradation or inhibit translation.7,10 In virus-infected cells, dsRNAs include replicative intermediates of viral replication.11 dsRNAs may also originate as a result of discrete intramolecular pairing within viral genomic RNA, or through de novo synthesis by the RNA-dependent RNA polymerases (RDRs).12 These dsRNAs are recognized and processed by plant Dice-Like (DCL) proteins, which are type III endoribonucleases, into small 20- to 24-nucleotide (nt) RNA duplexes termed virus-derived short-interfering small RNAs (vsiRNAs).13 The vsiRNAs are then incorporated into Argonaute (AGO) proteins, the core component of RNA-induced silencing complex (RISC) and RNA-induced transcriptional silencing complex (RITS) that directly catalyzes the cleavage of homologous RNAs and suppress the transcription of homologous DNAs via methylation, respectively.14,15 Since knockout of the viral suppressor of RNA silencing (VSR) usually results in the loss of infectivity of the virus, RNA silencing has been recognized as an essential antiviral defense mechanism. For instance, a Turnip mosaic virus (TuMV, a potyvirus) mutant with a defective HC-Pro, which is the major VSR of TuMV, fails to infect Arabidopsis, and the infectivity of this mutant can be restored when the plant RNA...
silencing pathway is compromised.\textsuperscript{16} Moreover, the vsiRNA duplexes are motile that enable the plant to establish RNA silencing-based resistance against homologous virus infection in the non-infected tissues.\textsuperscript{17}

In response to host RNA silencing, most plant viruses have evolved one or more VSRs to suppress host RNA silencing.\textsuperscript{18} For example, most of potyviruses such as TuMV encode 2 VSRs, e.g., HC-Pro and VPG. The VSRs encoded by either the same virus or different viruses share no obvious sequence or structure similarity, suggesting that they have evolved independently. VSRs can suppress RNA silencing virtually at all steps of RNA silencing.\textsuperscript{19} For example, many VSRs, such as p19 of tombusviruses, NS3 of tenuiviruses and HC-Pro of potyviruses, function by sequestering viral dsRNA or vsiRNA to block the silencing initiation step.\textsuperscript{16,20,21,22} A number of others, such as VPG of potyviruses, P0 of poleroviruses and P25 of potexviruses subvert the host RNA silencing pathway by destabilizing AGOs or SGS3/RDR6.\textsuperscript{11,23,24} A few VSRs such as the cucumoviral 2b protein can target multiple steps of the RNA silencing pathway.\textsuperscript{19}

To counteract RNA silencing suppression by VSRs, plants simultaneously activate the bulk expression of resistance (R) genes to increase host resistance to viral pathogens.\textsuperscript{25} It has been shown that these R genes are regulated post-transcriptionally by DCL-4-dependent small interfering RNAs (siRNAs).\textsuperscript{26,27,28,29,30,31,32} Suppression of these siRNAs by VSRs activates expression of the R genes.\textsuperscript{26} This has been suggested to be a counter-defense mechanism.\textsuperscript{25} Since these R proteins are indispensable in plant innate immunity, plant RNA silencing and viral suppression of RNA silencing are interlinked to plant innate immunity.

**Plant immunity to viral infection and suppression of the immune response by viruses**

Plant immunity is another essential defense antiviral mechanism. This concept is adapted from research on the plant immune response to non-viral pathogens. Jones and Dangl summarized research progress in molecular plant–pathogen interactions and proposed a zigzag model.\textsuperscript{3} The Jones/Dangl model shows that plants use a 2-branched innate immune system against attacks by non-viral pathogens. The extracellular PRRs recognize and respond to evolutionarily conserved pathogen- or microbial-associated molecular patterns (PAMPs or MAMPs) to trigger the first layer of resistance and establish PAMP-triggered immunity (PTI).\textsuperscript{33} PTI can restrict the majority of potential pathogens. To counteract this defense, some pathogens have evolved to deploy variable pathogen molecules, termed effectors, to interfere with PTI. As a result, these pathogens can establish a successful infection. In response, plants have developed the second branch of defense that is activated by dominant resistance (R) gene-encoded proteins. Most of these R gene-proteins belong to the extremely polymorphic superfamily of NB-LRRs. R proteins can directly or indirectly recognize the effectors of specific pathogens, which function as avirulence (Avr) factors, leading to effector-triggered immunity (ETI). ETI contains the invading pathogen at the infection site by activating much faster and stronger resistant responses than PTI and inducing the hypersensitive response (HR).\textsuperscript{3,33}

Although the relevance of the 2-tier immunity concepts to viral pathogens were not initially included in the Jones/Dangl model,\textsuperscript{3} several lines of recent evidence suggest that plants also deploy the innate immune system to combat viral pathogens.\textsuperscript{6,34,35,36,37} Viral infections induce PTI-based antiviral responses in plants.\textsuperscript{36,37,38} Knock-out of BRI1-ASSOCIATED KINASE 1 (BAK1) in Arabidopsis encoding a central regulator of PTI results in an increased susceptibility to several viruses, suggesting that PTI indeed suppresses viral infection.\textsuperscript{34} dsRNA, a replicative intermediate of viral replication, has been shown as a PTI elicitor to induce typical PTI responses such as activation of mitogen-activated protein kinases (MAPKs), ethylene synthesis and defense gene expression.\textsuperscript{38} The dsRNA-mediated PTI signaling requires the pattern recognition co-receptor kinase SERK1, but is independent on DCLs.\textsuperscript{38} To overcome dsRNA-activated PTI, viruses seem to have also evolved counter-PTI strategies. It has been reported that Plum pox virus (PPV) uses its capsid protein (CP) to suppress PTI.\textsuperscript{36} Therefore, potyviral CP can function as a virus-encoded innate immunity suppressor (VIIS).

Plants activate ETI against viral infection through direction or indirect interaction between a specific virus-encoded effector and a corresponding R protein, leading to HR.\textsuperscript{2,37} Like antibacterial and antifungal R proteins, proteins encoded by most of cloned dominant R genes to plant viruses are NB-LRR proteins too.\textsuperscript{2,6,37} Regardless of these NB-LRR proteins conferring resistance to viral or non-viral pathogens, they share structural similarity. Therefore, it is not surprising that plant antiviral R proteins can activate typical ETI responses.

To protect plants against pathogen attacks, PTI and ETI upregulate a large number of integrated defense responses not only in local leaves but also in non-infected tissues, a phenomenon called systemic acquired resistance (SAR).\textsuperscript{2,39} Non-expressor of pathogenesis related genes-1 (NPR1) is the master regulator of the plant immunity signaling pathway in local resistance and SAR.\textsuperscript{39,40,41} NPR1 may be regulated by sumoylation, and sumoylation of NPR1 by SMALL-UBIQUITIN LIKER MODIFIER 3 (SUMO3) activates the NPR1-mediated...
resistance pathway and SUMO3 is one of the 4 functional SUMOs encoded in Arabidopsis, and is required to maintain the basal expression level of PATHOGEN-RELATED (PR) genes in plants without pathogen challenging. Viral infection upregulates SUMO3 expression, leading to enhanced NPR1-mediated resistance. Potyviral Nib is the only viral RNA-dependent RNA polymerase (RdRp) that is absolutely required for viral genome replication. Similar to NPR1, Nib targets the nucleus and is also sumoylated wherein by SUMO3. Sumoylation of Nib by SUMO3 effectively suppresses the host immune response, possibly through inhibition of NPR1 sumoylation. Therefore, Nib is another VIIS that suppresses the cellular immune response to promoter viral infection. Interestingly, the Nib protein of several potyviruses, such as Potato virus Y (PVY), Pepper mottle virus (PepMoV), and Pepper severe mosaic virus (PepSMV), has also been shown as an Avr protein that triggers the HR response in plants carrying corresponding R genes. These data suggest that potyviral Nib catalyzes biosynthesis of the replicative intermediates as a generator of the RNA silencing inducer, promotes viral infection as a VIIS, and inhibits viral infection as an ETI elicitor. Thus, a single viral component can play contrasting roles in viral infection, reflecting the complex and specific virus-plant interactions as a result of the co-evolutionary arms race.

**Perspective**

In the past 20 years, significant progress has been made in understanding the mechanisms of defense and counter-defense between plants and viruses at the nucleic acid level. We are just beginning to touch plant receptor-based innate immunity to viral infection and viral counter-defense strategies. We summarize what we have discussed above in Fig. 1. However, on the one hand, it still remains unresolved how plants recognize infection by diverse viruses, what the signaling pathways immediately after acceptor recognition are that lead to the activation of antiviral innate immune responses and if the signaling defense pathways are shared in different plants against different viruses. On the other hand, it is yet largely unclear how viruses...
suppress PTI and ETI to establish their infection in plants and if similar defense suppression strategies are used by distinct viruses. These are certainly future research directions. It is worth mentioning that at the protein level, recently autophagy and unfolded protein response (UPR) pathways have been shown to be involved in viral infection. These pathways are likely interlinked with the receptor-based innate immunity. Rapid research in these areas may also contribute to elucidation of the molecular events of PTI and ETI pathways.

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No potential conflicts of interest were disclosed.

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