Review Article

Viroid Pathogen Stress and Plant Immune Responses: Unraveling the Mechanisms of Defense and Resistance

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ABSTRACT

One-stranded circular noncoding RNAs called viroids infect plants. Research conducted over the past 50 years has revealed details on the genetic architecture of viruses, their replication processes, the host components engaged in infection, intracellular and extracellular trafficking patterns, and their connections to defense mechanisms. RNA silencing-based defenses and resistance (R) gene-mediated defenses are the two primary defense mechanisms that plants have evolved to fight against viroid infestations. Another consequence of mutations in important genes involved in viral infection is the development of recessive gene-mediated resistance in plants. These techniques have been applied to protect crops and have had a significant economic impact. A helpful paradigm for addressing basic virological and other issues is the unique class of subviral entities known as viroids. Plants and microbes have a long evolutionary history, are often dependent on one another, and are involved in a complex battle. The main topic of this essay review article is how plant immune responses are triggered by viroid pathogen stress.

Objective: The primary goal of this essay review is to present a comprehensive viewpoint in an easy-to-read format regarding the activation of the plant immune system upon coming into contact with viroids.

Methodology: A number of sources, including PUBMED, SCOPUS, Google Scholar, and several online sources, provided information on the immune responses triggered by viral and viroid stressors in plants. This information was then compiled and further assembled in a simplified way with the sole purpose of expanding our understanding of this topic.

Keywords: Immunity, Viroid, pattern triggered immunity, effector triggered immunity, PAMPs, RNA silencing.

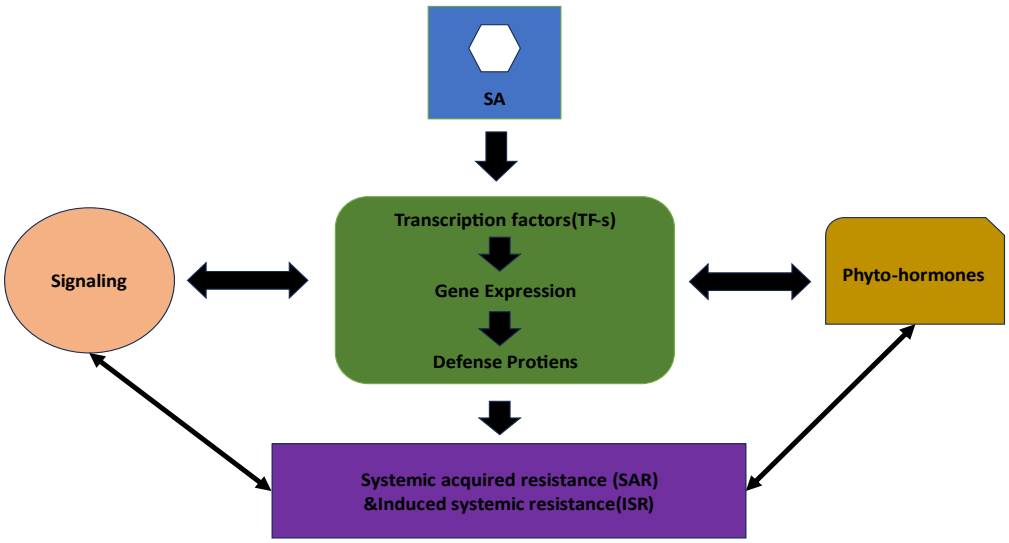

Figure 1: Viroid Pathogen Stress and Plant Immune Responses

INTRODUCTION

The first viroid was discovered in 1971 and was named PSTVd (Potato Spindle Tuber Viroid)[1, 2]. Approximately 40 viroids have now been identified and categorized as members of the Avsunviroidae and Pospiviroidae families [3,4]. The known viroids are circular noncoding RNAs that infect plants [5-7]. Members of the Pospiviroidae perform nuclear replication and have a rod-shaped genomic architecture. Their circular genome is known to enhance transcription into multimeric (-) intermediates by the utilization of DNA-dependent RNA polymerase II (Pol II) [8,9]. PSTVd, a model and example species of Pospiviroidae, requires an RNA-specific transcription variable (TFIIIA-7ZF) to instruct Pol II to use its whole RNA genome [10]. Hammerhead ribozymes are highly branched segments of the RNA genomes of all viruses in the

Avsunviroidae family that replicate in chloroplasts [5-7]. They depend on nuclear-encoded polymerase (NEP) for transcription based on RNA templates (11). After replicating in appropriate host plants, viroids can spread in a systematic manner. Through research on PSTVd structural patterns, several RNA 3-dimensional (3D) motifs have been discovered to regulate RNA mobility across various cellular borders [12,13]. It need an RNA-specific transcription component (TFIIIA-7ZF) to tell Pol II to use the RNA genome of PSTVd, a Pospiviroidae example species [14]. The idea that viroids move themselves within, between, and throughout the body using their RNA 3D patterns is supported by these studies. Despite the fact that many viroids are well-known illnesses that affect commercially important crops [15,16], some studies have reported that some hosts can contract viroid infection without any symptoms [17–19]. Viroid infection can trigger host immunological responses in addition to the well-known viroid interaction with the host RNA silencing machinery [20], according to recent extensive transcriptome analyses from multiple groups. The pathophysiology caused by foreign RNA alone can be better understood thanks to these findings. PSTVd, a common species of Pospiviroidae, has an RNAnas-specific transcription element (TFIIIA-7ZF) that directs Pol II to use its RNA genome. Through the vascular system, these viruses then enter the plant and spread throughout it. In the face of a resistant host, viral transit and/or multiplication may be hindered.

PLANT IMMUNOLOGY

Many different mutualistic attack and defense systems have evolved as a result of plants and microbes coexisting. A range of compounds are used by plant diseases to hinder the growth and reproduction of plants. Unlike mammals, plants rely on the innate immunity of particular cells and systemic signals from infection sites instead of having a somatic adaptive immune system or mobile defense cells. [1–23]. Plants have so developed sophisticated innate immune systems that are able to recognize and respond to infections by pathogens. Plants have two distinct immune systems: effector-triggered prompted immunity (ETI) and PAMP-triggered immunity (PTI).

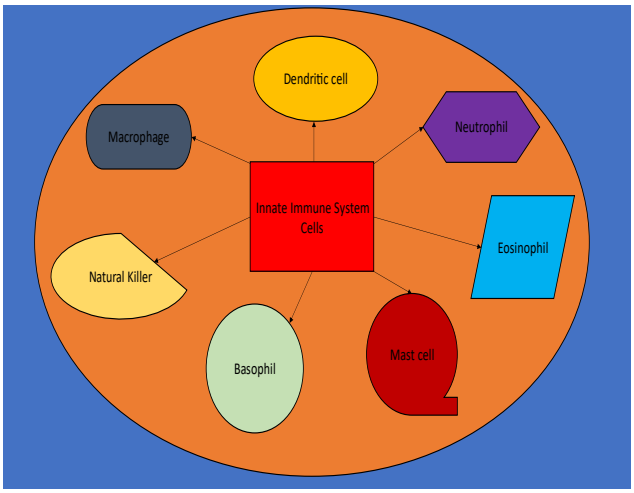

Figure 2: Innate Immune System Cells

Cell membrane-attached receptors serve as the initial line of defense. The ability to identify molecular patterns linked to damage or infections is possessed by transmembrane receptors. This type of immunity, known as PAMP-triggered immunity, is generated by receptors that recognize patterns that accurately recognize MAMP/DAMP. Receptor-like protein and receptor-like kinase are crucial regulators of plant growth and development as well as defense mechanisms against threats from abiotic stimuli.

Additionally, they are sensors that recognize patterns. Drugs that are especially susceptible to these receptors include carbohydrates and short peptides[24, 25]. PTI defensive methods include the creation of antimicrobial chemicals that activate signaling pathways, changes in the structure of plant cell walls, and reactive oxygen species (ROS) [26, 27]. Intracellular receptors, also known as R-genes, belong to triphosphate-binding site leucine-rich repeats (NBS-LRRs) and are more specialized than receptor-like proteins. These NLR proteins search for the effector molecules, or avirulence (Avr) factors, that are responsible for the infection. Pathogens have devised specific mechanisms to release Avr peptides into plant cells, thereby BLOCKING NLR protein responses. Effective Avr-factor recognition stops the infectious agent at the infection site and starts the hypersensitive reaction that results in programmed planned death of cells (PCD). Effector-triggered immunity (ETI) created mobile immunological signals, such as glycerol-3-phosphate (G3P), salicylic acid (SA), and azelaic acid. By transferring immunological signals from the infection site to uninfected areas, these molecules result in the significant accumulation of SA and transcriptional reprogramming. Finally, through a process called systemic acquired resistance (SAR), transcriptional reprogramming initiates the production of pathogenesis-related proteins (PR-proteins). Programed cell death effectively restrains the biotrophic pathogens. While receptor-like proteins and kinases are present in all three domains of life, eukaryotes have the largest abundance [28–31]. Receptor-like kinases are structurally composed of three domains: an internal cytoplasmic kinase for further signaling, a transmembrane domain, and an external ligand-binding domain that recognizes invasion patterns. The classification of cell surface immunological receptors into receptor-like kinases (RLK) and receptor-like proteins (RLP) is based on the kinase activity in their cytoplasmic tails. Cell surface receptor-mediated immunity involves the transmission of signals through the phosphorylation of MAP Kinases and calcium-dependent protein kinases.

MANAGEMENT OF CELL SURFACE IMMUNE RECEPTOR:

Cell surface immune receptors are regulated by the following mechanisms:

Transcriptional and metabolic post-translational regulation mechanisms subsequent to transcription. Ergosterol, squalene, and the symbiont of Trichoderma activate the transcriptional level of immune receptors. In solanaceous crop plants, including mungbean, hormone therapy, wounding, and pathogen infection all encourage the

synthesis of NLR genes and receptor-like kinase/receptor-like proteins. The use of alternative splicing allows for post-transcriptional regulation. Furthermore, post-translational regulation includes protein trafficking, stabilization, and degradation respectively. Predictable and controlled immune responses are established by the three regulatory systems.

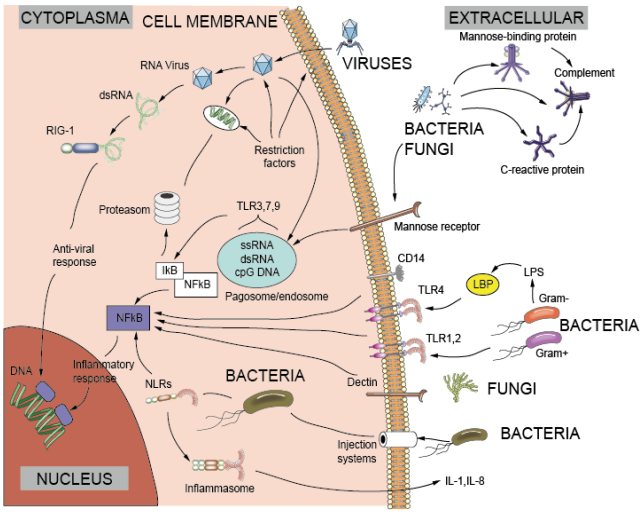

Figure 3: Receptors of the Innate Immune System(https://www.creative-diagnostics.com)

Immunological receptors that bind to effector molecules intracellularly are produced by PAMP-triggered immunity, hormone therapy, and certain stressors or traumas. Intracellular immunological receptors are divided into many groups according to their conserved domains. These immunological receptors have middle and C-terminal domains that contain the leucine-rich repeat (LRR) and nucleotide-binding adaptor (NB-ARC; shared by APAF-1, R proteins, and CED-4). Nucleotide-binding-leucine-rich repeat (TIR-NB-LRR) receptor subtypes include these NLRs, also referred to as NB-LRR receptors. together with the CNL subgroups (CC-NB-LRR; coiled trying to wind-nucleotide-binding-leucine-rich repeats). According to whether they possess a TIR or CC extra N-terminal domain [32]. These intracellular proteins identify effector molecules and hence cause gene-for-gene resistance. Through direct physical contact or indirectly through helper proteins, NB-LRR proteins are able to detect effector molecules. In an indirect way, the effector modifies an accessory protein that may be its target. As a plant defense, these two processes are triggered: rapid calcium ion inflow, rapid release of reactive oxygen species, stimulation of mitogen-induced protein kinases, control of gene expression, and hypersensitive reaction that results in cell death. PTI and ETI have similar gene expression profiles, indicating that they differ in magnitude but are similar overall [32-42]

INTEGRATING THE IMMUNE SYSTEM OF PLANTS:

The basis of plant defense is the immune system types known as pattern-triggered immunity (PTI) and effector-induced immunity (ETI). Efficient defense defense against host-adapted microbial infections is based on similar defense defense activation by PTI and ETI components. Numerous survival strategies are used by plant pathogens. The apoplast is an intercellular region where pathogenic bacteria can proliferate after entering through wounds, gas or water pores (stomata and hydathodes, respectively), or both. In order to feed, worms and aphids insert a stylet directly into a plant cell. Furthermore, fungi have the ability to directly infiltrate plant cells' epidermis and grow their hyphae on top of, inside, or through plant cells. Pathogenic and symbiotic fungi and oomycetes can embed feeding structures (haustoria) in host cell plasma membranes. The result of the encounter is determined by the close contact between the Haustoria plasma membranes, the host plasma membranes, and the extracellular matrix at this interface. Each of these different pathogen families inserts effector molecules (virulence factors) into the plant cell in order to increase microbial fitness. Plants do not have mobile defense cells or a somatic adaptive immune system like humans have. They rely on systemic signals from infection sites and the innate immunity of all cells instead [43,45]. The two parts of the two-tiered immune system that plants use to fight microbial invasion are, as previously mentioned, pattern-triggered immunity (PTI) and effector-induced immunity (ETI). ETI are commonly thought of as distinct branches of the plant immune system, and their mechanistic relationships are not well understood [44, 45]. Two recent investigations published in Nature dissected the PTI and ETI pathways using the Arabidopsis thaliana and Pseudomonas syringae system [45,46]. These publications made use of quite sophisticated methodological techniques. The two branches were found to have unexpected mutual dependencies by both teams. These included the shocking findings that ETI somewhat enhances PTI and that PTI components are required for ETI to function. PAMPs, or molecular patterns linked to pathogens, are recognized by pattern recognition receptors (PRRs) at the cell membrane, which mediates PTI. By increasing its brand-rich repeat (NLR) receptors secreted into the plant cell, ETI, on the other hand, detects polymorphic pathogen effectors. While PTI usually provides low-level, basal immunity that works against pathogens that have not evolved, ETI provides a more robust response to diseases that have adapted to the host. Until recently, microbial infections—with or without a variety of known effectors—were utilized in most ETI studies [47–51].

Despite the naming, most infections have many effectors, and PAMPs are also found in non-pathogens. It is therefore uncommon to find research on ETI that ignores PTI. Yuan et al. thoroughly investigated effector-mediated ETI using a strain of P. syringae that was free of the toxic coronatine and all of its agents except AvrRpt2. Although the lines lacking multiple PRRs or PRR co-receptors did not acquire resistance to this strain, A. thaliana of the wild type did [52]. According to Ngou et al., AvrRps4-induced resistance is likewise lost in a genotype that lacks PTI. According to these surprising results, effective ETI responses require PTI to be active. To investigate the effects of PTI and ETI, Ngou et al. and Yuan et al. used A. thaliana lines that encoded bacterial effectors and were inducible by estradiol. Conditional

expression of AvrRps4 enhances and prolongs the production of reactive oxygen species (ROS), which are produced by fungi, oomycetes, or microorganisms with structurally different immunogenic patterns.

Likewise, Yuan et al. show that AvrRpt2 expression significantly increases ROS sensitivity to bacterial flagellin (flg22) despite producing very little ROS [53,54]. Both pattern-induced phosphorylation of the cytoplasmic receptor kinase BIK1 and BIK1-dependent activation of the NADPH-Oxidase isoform RBOHD are necessary for the production of ROS in PTI [55,56]. These activities are reinforced by concurrent ETI activation, which clearly suggests that ETI enhances PTI signaling. Higher protein abundances of PRRs, PRR co-receptors, and downstream PTI signaling components were reported in both studies, which examined protein and transcript levels following ETI activation. They were better able to comprehend how ETI intensifies PTI as a result. Additionally, they found that transcriptional, post-transcriptional, and translational mechanisms controlled the rise in PTI protein abundance brought on by ETI. Additionally, a number of NLRs from the TOLL-INTERLEUCONIN RECEPTOR 1 and COILED-COIL NLR families add to the amplifying action of PTI. Together, these findings demonstrate that there is no correlation between the kinds of structures and effectors that ETI uses to stimulate PTI-induced physiological changes. The question of how PTI impacts effective disease resistance is brought up by the requirement for PTI to enable ETI-dependent immunity.

Microbes with host-adapted characteristics cannot infect plants because ETI often causes the hypersensitive response (HR), a type of regulated cell death. The conditional expression of bacterial AvrRps4 alone does not induce HR, according to Ngou et al. Similarly, Arabidopsis does not experience HR when the bacterial flagellin motif is treated; however, conditional AvrRps4 expression in conjunction with pattern treatment does. Similarly, Yuan et al. discussed how flg22 therapy speeds up AvrRpt2-controlled HR.In order to better understand how coactivation of ETI and PTI leads to HR, Ngou et al. investigated the function of RBOHD and found that ETI increases PTI's ability to activate this enzyme. In a rbohd rbohf mutant, they found that AvrRps4-induced HR was inhibited. Similarly, Yuan et al. discovered that a virus carrying AvrRpt2 was not well protected against a rbohd genotype[57,60].
These findings are exceptionally important because they demonstrate how ETI increases pattern-triggered activation of NADPH oxidase, which results in HR.

Important information is provided by these two studies:

(1) ETI raises PTI signaling element protein levels via as-yet-undiscovered molecular mechanisms;

(2) ETI needs PTI to provide complete resistance;

(3) PTI amplifies ETI outputs, like HR, to prevent pathogen reproduction. These findings support the theory that, upon infection, susceptible host plants develop PRR-induced defenses against pathogen transmission (PTI). Host susceptibility and disease development result from effector-mediated PTI suppression when there is no homologous effector recognized by an NLR receptor (no ETI).

PRR-mediated defenses, however, are strengthened when intracellular NLR receptors result in ETI. By restricting food, fortifying cell membranes, and producing antimicrobial compounds, the plant is able to stop germs from infiltrating. ETI activation in this situation counteracts the detrimental effects of PTI-suppressing effectors by increasing the quantity of PTI components, which may then enhance ETI outputs to prevent infection. The fact that these two studies describe plant immunity as a unified system with two interrelated, mutually reinforcing branches—in which internal NLRs and cell surface PRRs work together to impart resistance to microbiological infections—is their true contribution. Given their functional link, it is possible that the PTI and ETI branches developed simultaneously. These are not entirely consistent with the commonly accepted theory that ETI evolved as a consequence of PTI being rendered inoperable by microbial effectors. According to these findings, having enough NLRs to identify a range of microbial effectors may also lead to non-host resistance, which might stop a (non-pathogen) from avoiding its blockade.

THE DEFENSE OF THE HOST AND THE VIROID

The necessity of both innate immunity and RNA silencing for plant defense against viroid infection becomes clear. Virus infection results in the production of short RNAs (sRNAs) that range in size from? They range in length from 20 to 24 nt and are implicated in viral-derived sRNAs (vd-sRNAs). most likely prevent viroid replication. In addition to triggering host immune responses, viral infection also triggers ROS signaling, cell wall fortification, and hormone pathways linked to defense. In general, plant signaling and metabolism are often impacted by immune response activation, leading to cytopathic effects and morphological alterations [61-62].

INTERACTION OF THE VIRUS WITH THE HOST'S RNA SILENCING APPARATUS

According to the current understanding, viroids form double-stranded intermediates that can be cleaved by a variety of plant Dicer-like proteins (DCLs). Notably, plant-derived DCLs have the ability to target the PSTVd RNA genome without replication [63]. In Nicotiana benthamiana, DCL4 often almost constructively regulates PSTVd replication, whereas DCL2 and DCL3 collectively reduce PSTVd infection [64,65]. Function can be enabled by loading sRNAs generated from viroids into argonaute proteins (AGOs) [63,66]. Agrobacterium tumbefaciens transiently expressed Arabidopsis AGOs in RNA-immunoprecipitation assays demonstrated that vd-sRNAs can be attracted to plant AGOs AGO1, AGO2, AGO3, AGO4, AGO5, AGO8, and AGO9. AGO1, AGO2, and AGO3 prefer the binding of 21- and 22-nt vd-sRNAs, but AGO4, AGO5, and AGO9 concentrated a considerable amount of 24-nt vd-sRNAs.

According to Minoia et al. (2014), PSTVd levels in sick N were interestingly reduced by transgenic expression of the genes AGO1, AGO2, AGO4, or AGO5. benthamiana, suggesting their functions in interactions between plants and plants. An important part of the RNA silencing pathway, RNA-dependent RNA polymerase 6 (RDR6), prevents PSTVd from invading the shoot apical meristem in hosts including tomatoes and N. benthamiana [67]. RDR6 is important because it regulates plant pathogenicity caused by HSVd [68]. RDR6 regulates the tissue tropism and pathogenicity of PSTVd, however its exact mode of action is yet unclear. According to a recent study, PSTVd infectivity is also affected by changes in RDR1 expression [55]. However, new RNA-Seq (Thibaut) evidence indicates otherwise. RDR1 expression remains unchanged in plants infected with PSTVd and CEVd, raising doubts about its status as a true viroid defense gene.

INTERACTION BETWEEN VIROIDS AND PLANT INNATE IMMUNITY

In order to protect themselves from various pathogens, plants usually employ two forms of immunity: effector-triggered immunity (ETI) and molecular patterns associated with pathogens (PAMPs)-triggered immunity (PTI) [35]. The primary site of PTI's activity is the cell surface, whereas ETI mostly occurs inside cells. Plant survival depends on the precise planning of PTI and ETI operations. It has been demonstrated recently that the presence of viroids may result in host immunological reactions [69, 70]. How plant cells recognize the presence of foreign RNAs, or viroids, and initiate the innate immune response is unknown. PKV (protein kinase, viroid-induced), a protein kinase that binds to double-stranded RNA, was thought to be used by plants to identify viroid RNAs and start defense signaling [71]. However, comprehensive RNA-Seq analyses reveal that this PKV seems to be a pseudogene [70]. Viroids may not trigger the PTI response because they mostly enter host cells through wounds or, to a lesser extent, insect vectors. Within the infected plants, plasmodesmata allow viroids to move [70]. Therefore, it is rare to find viroids that trigger PTI on cell surfaces. It is unknown if viroids can activate ETI because no R gene is known to be able to detect viroids precisely. The immunological reactions that viroids elicit may be the consequence of damage-related molecular trend-triggered immunity, which was brought on by the release of as-yet-unidentified signal molecules associated with cell damage, if they do not elicit ETI responses. Notably, a recent study found that PSTVd replication can increase the production of reactive oxygen molecules, which is regulated by miR398 [72,73]. This discovery raises the possibility that innate immunity and RNA silencing activities are related in the fight against viroids. It will be essential to clarify the complex underlying mechanism for miR398 control and the several steps along such a regulatory chain within viroids-infected plants. Without a doubt, studying the immune reaction triggered by viroids will help us understand how plants sense the presence of foreign RNAs.

CONCLUSION

Plant-associated microbes frequently contain pathogens that impede plant development and reproduction. Two branches of an innate immune system aid plants in fending off infection. Both non-pathogens and a wide variety of microbes share molecules that the first branch can recognize and respond to. Through their impact on host targets, the second reacts either directly or indirectly to the pathogenicity of pathogen components. To address basic virological and other issues, the unique class of subviral entities known as viroids can be a helpful model. The immunological response of the plant to any viroids has been documented in this review. These defense mechanisms in plants, along with the harmful substances they react to, offer amazing insights into cell biology, molecular identification, and the evolution of several biological kingdoms. The development of crops for food, fiber, and biofuels will require a deep comprehension of the immune systems of plants.

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