The Impact of Phytohormones on Virus Infection and Disease

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Abstract

Phytohormones play a critical role in nearly every aspect of plant biology, including development and pathogen defense. During virus infection disruption of the plant's normal developmental physiology has often been associated with alterations in phytohormone accumulation and signaling. Only recently has evidence emerged describing mechanistically how viruses modulate phytohormone levels and the impact these modulations have on plant

physiology and virus biology. From these studies there is an emerging theme of virus directed manipulation of plant hormone responses to disarm defense responses and reprogram the cellular environment to enhance replication and spread. In this review we examine the impact viruses have on plant hormone systems and the effects of this phytohormone manipulation on virus biology.

Introduction

Viruses utilize a variety of strategies to reprogram their host's cellular environment to one that is more conducive to replication and spread. As a consequence virus infections can directly or indirectly disrupt phytohormone accumulation and signaling pathways. Within plants there are an array of plant hormone pathways that contribute to nearly all aspects of plant physiology including growth, development and reproduction [1]. Salicylic acid (SA), jasmonic acid (JA) and ethylene (Et) are primarily involved in defense mechanisms [2]. Auxin (Aux), gibberellins (GA) cytokinins (CK), brassinosteroids (BR) and abscisic acid (ABA) also contribute to defense but play key roles in plant development and physiological processes [3,4]. In addition, there are extensive interactions or "cross-talk" between the different phytohormone pathways, providing a means for the plant to finely regulate responses to environmental cues or pathogen attack [3,5,6]. Here we discuss the role of phytohormones in the development of disease symptoms, the modulation of host defenses and enhancement of virus replication and movement. We focus primarily on specific virus-host interactions that have been linked to alterations in phytohormone synthesis and signaling and the role these interactions play in infection and disease.

Symptom development

Some of the most common symptoms produced by plant viruses include stunting, leaf curling and chlorosis. These types of symptoms have long been associated with disruptions in plant hormone production, accumulation and sensing [7,8]. Yet despite these associations our understanding of the viral components and interactions that affect phytohormone pathways and their role in symptom development is limited. Recently several interactions between viral and host components involved in phytohormone pathways have been identified and linked to symptom development. These interactions provide the first mechanistic explanation for how viruses modulate phytohormone regulatory systems within their hosts and how those modulations lead to symptom development. One phytohormone system that is directly disrupted by viral components is Aux. Disruption of Aux signaling has been linked to developmental phenotypes with Aux biosynthesis or signaling mutants resembling viral disease symptoms such as stunting, leaf curling, and loss of apical dominance [9]. The *Tobacco mosaic virus* (TMV) 126 kDa replication protein has been shown to disrupt Aux signaling via an interaction with select Aux/IAA family members [10,11]. These Aux/IAA proteins function as negative regulators of Aux responsive transcription factors (ARF) and control their ability to modulate genes involved in a range of plant processes [12]. Interaction with the TMV 126 kDa protein disrupts the nuclear localization of interacting Aux/IAA proteins and correlates with the development of leaf curling and developmental disease symptoms. In contrast, a mutant, TMV-V1087I or related virus, *Tobacco mild green mosaic virus*, do not interact with these Aux/IAA family members and produce attenuated disease symptoms even though these viruses replicate to wild-type TMV levels [11]. Thus the TMV – Aux/IAA interaction appears to be an important determinant in the development of disease.

1 The activity of viral silencing suppressors has also been linked to alterations in Aux
2 signaling and the development of disease symptoms. Specifically, transgenic *Arabidopsis* plants
3 constitutively expressing the *Turnip mosaic virus* (TuMV) silencing suppressor HC-Pro display
4 leaf developmental abnormalities similar to those that occur during virus infection[13].
5 Overexpression of HC-Pro was found to increase accumulations of several miRNAs, including
6 ones targeting Aux responsive transcription factors. Furthermore, increased levels of these
7 miRNAs corresponded with enhanced cleavage of their target mRNAs[13]. The authors
8 conclude that expression of viral suppressor proteins interferes with miRNA regulated pathways
9 including those under the control of Aux and that disruption of these pathways accounts for
10 many of the developmental symptoms induced during virus infection.

11 Disruption of the GA biosynthesis pathway has also been linked to viral disease
12 symptoms. GA is involved in cell division and elongation. *Rice dwarf virus* (RDV) induces
13 stunting and leaf darkening, symptoms that are characteristic of GA-deficient rice mutants. An
14 interaction between the RDV outer capsid P2 protein and the rice *ent*-kaurene oxidase has been
15 identified[14]. *Ent*-kaurene oxidases are key components in the synthesis of GA and have been
16 linked to dwarfing in rice[15,16]. Treatments of RDV infected plants with exogenous GA
17 restored the non-dwarf phenotype but not treatments with Aux [14]. It was speculated that the
18 virus directed disruption of *ent*-kaurene oxidase activity was responsible for symptom
19 development but could also potentially interfere with the synthesis of antimicrobial phytoalexins,
20 leaving the plant more susceptible to infection.

21 Another example of a viral component linked to symptom development is the P6 protein
22 of *Cauliflower mosaic virus* (CaMV). P6 is a multifunctional viral protein involved in virus
23 replication, movement and suppression of RNAi[17,18]. Transgene expression of P6 induces

1 stunting, chlorosis and vein banding [19]. P6 expression has been shown to interfere in the
2 ethylene response pathway as P6 transgenic Arabidopsis plants display an ethylene-insensitive
3 phenotype [20,21]. It was suggested that P6 may interfere with ethylene signaling leading to the
4 observed symptoms [21]. However, a direct interaction between P6 and a component of the
5 ethylene pathway has not been identified.

6 **Defense Responses**

7 SA is a key virus defense phytohormone involved in R gene mediated resistance,
8 systemic acquired resistance (SAR) and basal defense processes[22-25].Activation of SA
9 biosynthesis and signaling can lead to the accumulation of reactive oxygen species,
10 pathogenesis-related (PR) proteins, callose deposition and induction of the hypersensitive
11 response [22,23,26,27]. SA is also required for SARactivation in tissues distal from the site of
12 infection[24]. SA and JA/ ET mediated defense pathways are generally antagonistic, with SA
13 largely responsible for defense against biotrophic pathogens such as viruses and JA/ET largely
14 responsible for defense against necrotrophic pathogens and insects[28-30]. However, examples
15 of synergism between these pathways do exist [31-33].

16 In many studies depletion of endogenous SA or disruption of SA signaling leads to an
17 impairment of defense response and susceptibility to viral infections [34-38]. For example,
18 reducing the accumulation of SA through the use of a salicylate hydrolase (NahG) transgene
19 negates resistance conferred by the potato *Ny-1* R gene against *Potato virus Y*[39].Thus
20 inhibition of SA synthesis or SA dependent defenses is one strategy viruses may use to enhance
21 infection. Virus interactions that impact the SA pathway include the TMV replication protein,
22 which was found to target the proteasome degradation of a NAC domain transcription factor,

1 ATAF2, involved in the regulation of host basal defenses [40]. *ATAF2* knockout or repressor
2 lines displayed reduced levels of the SA marker gene *PR1* when treated with SA. In addition,
3 *PR1* is not induced upon SA treatment of systemically infected leaf tissues, indicating that host
4 defense responses become attenuated as TMV moves systemic. These findings suggest that
5 TMV targeted degradation of ATAF2 is involved in the suppression of SA mediated defenses.

6 In another example, the CaMV P6 protein has been shown to inhibit SA dependent
7 defenses by altering the expression and localization of the SA receptor NPR1[41,42]. Plants
8 expressing P6 display the miss-localization of an inactive form of NPR1 to the nucleus,
9 effectively disrupting SA signaling [42]. As a result plants expressing P6 are more susceptible to
10 SA sensitive pathogens but more resistant to JA sensitive pathogens [42]. Additionally, a recent
11 study with TMV-Cg, crucifer strain, has shown the virus coat protein (CgCP) can also suppress
12 SA signaling by stabilizing DELLA proteins without altering SA or JA levels[43]. DELLA
13 proteins are negative regulators of GA signaling and have been shown to repress SA defense
14 responses, possibly by modulating the antagonistic cross-talk between SA and JA pathways
15 [3,44]. In addition, CgCP expression also reduces plant growth and delays the timing of floral
16 transition, potentially linking this interaction to symptom development.

17 JA- SA antagonism appears to be a recurring factor in mediating virus defense processes.
18 In *N* gene resistant tobacco, exogenously applied methyl jasmonate reduced resistance to TMV
19 and conferred systemic viral movement [45]. Additionally, silencing of the JA receptor *COI1* or a
20 JA biosynthetic enzyme, allene oxide synthase, resulted in increased SA accumulation and
21 reduced TMV accumulations in *N* gene tobacco[45]. Antagonistic interactions between SA and
22 JA signaling have also been implicated in defense gene expression and the activation of *RCY1*

1 resistance to *Cucumber mosaic virus* where a mutant allele of *COII*, the JA receptor, restored
2 resistance in plants blocked in SA accumulation [35]. Thus, antagonism between JA and SA
3 pathways represents an important mechanism in regulating R gene mediated resistance to these
4 viruses. However, increased JA accumulation is not always favorable for viral infection.
5 Endogenous JA levels have been reported to increase in incompatible plant-virus interactions in
6 tobacco and potato [46,47]. Additionally, exogenous application of JA disrupts geminivirus
7 infection [48]. Furthermore, the geminivirus C2 protein has been found to interact with the
8 catalytic subunit of the COP9 signalosome, compromising SCF ubiquitin ligase activity and
9 altering its ability to regulate JA activity. C2 targeting of SCF ubiquitination thus provides a
10 mechanism for this virus to modulate host resistance.

11 Phytohormones also appear to modulate the general virus defense mechanism of RNA
12 silencing. Plants that contain mutations in RNA silencing pathway components including *HEN1*,
13 *DCL1* and *AGO1* are hypersensitive to ABA [49-51], while increased *AGO1* levels lead to ABA
14 hyposensitivity [50,51]. Additionally, miR168 which regulates *AGO1* contains ABA-responsive
15 elements in its promoter region and is up regulated by ABA [51]. There is also evidence for cross-
16 talk between SA and silencing defense pathways [52-55]. It was found that plants expressing
17 *NahG*, for reduced accumulations of SA, produce lower levels of siRNAs when infected with
18 *Plum pox virus* (PPV). Furthermore, overexpression of the potyvirus silencing suppressor protein
19 HC-Pro reduced SA-mediated defenses against PPV [53]. Combined these findings suggest a
20 strong connection between virus defense responses and phytohormone signaling.

21 **Virus Replication**

Only recently has evidence emerged linking specific phytohormone systems directly to virus replication. In one system SA was shown to inhibit the replication of *Tomato bushy stunt virus* (TBSV) by competitively binding cytosolic Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [56]. Cytosolic GAPDH binds the negative RNA strand of TBSV and is required for replication[57,58]. When SA accumulates it can directly bind to GAPDH, preventing its interaction with virus RNA and leading to suppression of TBSV replication[56]. In another system, a mutation in the ABA biosynthesis gene *ABA2* resulted in decreased *Bamboo mosaic virus* titers and a dramatic reduction of negative sense virus RNA[59]. Similarly, *Cucumber mosaic virus* (CMV) also failed to replicate in the *aba2-1* mutant. The authors propose the product of *ABA2* may be an essential component of the virus replication or translation complexes or that ABA2 negatively regulates host defense responses. Based on these findings it is clear phytohormones and their associated components can directly impact virus replication.

Movement

The movement of viruses from cell-to-cell as well as into the vascular phloem occurs via intercellular plasmodesmata (PD) connections. Mounting evidence indicates that several phytohormones play a role in regulating PD connections. For example, ABA can limit virus spread through inhibition of β -1,3-glucanase, which degrades callose[60-62]. Deposition of callose at PD is known to decrease viral cell-to-cell movement [61,63,64]. Furthermore, exogenous application of ABA has been shown to increase plant resistance to viruses such as TMV and *Tobacco necrosis virus* by limiting virus movement [65,66]. SA is another phytohormone linked to PD closure. Specifically, exogenous application of SA results in callose deposition within PD and reduced intercellular movement of a fluorescent marker dye [27]. This SA mediated PD closure requires the SA signaling pathway as well as a regulator of PD gating,

1 PDLP5[67].SA has also been tied to the inhibition of virus movement and replication via a
2 process linked to the mitochondrial alternative oxidase pathway (AOX) [38,68]. Specifically,
3 inhibition of AOX functions to counter SA induced resistance against TMV, PVX and CMV. It
4 would be interesting to determine if AOX can contribute to the regulation of PD. Recently, we
5 determined that TMV disrupts the nuclear localization of select Aux/IAA genes that are
6 specifically expressed in phloem (unpublished data). The disruption of these vascular specific
7 IAA regulatory proteins was shown to correlate with the ability of the virus to load into the
8 phloem and move systemically. Thus, TMV appears to target the Aux signaling pathway as a
9 means to enhance its ability to spread systemically and cause disease.

10 The plant to plant movement of viruses via their insect vectors represents another aspect
11 of virus biology impacted by phytohormones. In particular, JA, the primary hormone involved
12 in plant insect defenses appears to be a key target in vector transmission. For example, *Tomato*
13 *spotted wilt virus* (TSWV) is vectored by the western flower thrip, which prefers to feed on
14 infected tissues[69]. Plants infected with TSWV have increased SA levels and decreased levels
15 of JA[29,69]. However, it remains to be determined if TSWV directly targets these pathways
16 in order to enhance insect transmission. In another example, the 2b silencing suppressor from
17 CMV was found to interfere in the JA signaling pathway as a means to promote its own
18 transmission by its vector *Myzus persicae*. CMV infected plants or transgenic plants expressing 2b
19 display reduced levels of JA signaling[70]. In addition, aphid survival increases on tobacco
20 infected with CMV but decreases on tobacco plants infected with CMV strain lacking 2b
21 [71]. Finally, NIa-Pro (Nuclear Inclusion - Protease domain) from TuMV has been shown to
22 alter ethylene responses, suppressing aphid-induced callose defenses. NIa-Pro is highly conserved

among Potyviruses and the authors propose this interaction could represent a conserved mechanism for increasing aphid transmission of this important group of viruses[72].

Conclusion

From these studies it is clear that phytohormones play a significant role in many aspects of virus infection and disease. Alterations in phytohormone levels have been repeatedly linked to changes in virus accumulation. Furthermore, in a few systems we are beginning to understand the molecular mechanism whereby viruses target and modulate plant hormone synthesis and sensing systems to avoid host defenses and enhance their own infection and movement (summarized in Table 1). It is also becoming increasingly clear that cross-talk between phytohormone pathways is essential to the regulation of virus defense responses as well as a target for viruses to exploit during infection (summarized in Figure 1). However, despite these advances we still lack specific information on the phytohormone regulated genes and pathways that directly impact virus biology. What Aux regulated genes are involved in symptom development or what SA mediated processes directly impact virus accumulation are just a few of the questions that when answered will provide a greater understanding of the interactions between viruses and their plant hosts.

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1 This work shows that disruption of ethylene responses in plants is one function of the highly
2 conserved Potyvirus protein, NIa-Pro. Changes in ethylene responses may mediate vector-plant
3 interactions and be an important mechanism for increased insect transmission of this important
4 group of viruses.

5
6 Figure 1. Virus and phytohormone pathway interactions. Phytohormones shown in blue generally
7 have positive effects on plant defense against viruses, while phytohormones shown in red
8 generally have negative effects. SA and JA / ET are the phytohormones primarily involved in
9 defense responses, while Aux, GA, CK, BR and ABA also contribute to defense but play key
10 roles in plant development and physiological processes [2-4]. The ABA and JA pathways have
11 positive effects on defense against herbivorous insects that can act as viral vectors [29]. JA also
12 promotes defense against Geminiviruses[48]. Viruses boxed in red inhibit SA mediated defense
13 responses either through inhibition of the GA and SA pathways[14,40,42,43] or through
14 activation of the antagonistic phytohormone Aux [11,13]. Viruses boxed in blue inhibit JA and
15 ABA mediated defense responses against their insect vector either directly [71] or through
16 activation of the SA or ET pathways which can both be antagonistic to ABA [69,72].
17 Geminiviruses boxed in green inhibit JA signaling [48]. SA and ABA are also linked to the
18 general virus defense mechanism of RNA silencing [51,54]. SA, salicylic acid; JA, jasmonic
19 acid; ET, ethylene; Aux, auxin; GA, gibberellins; CK, cytokinins; BR, brassinosteroids; ABA,
20 abscisic acid; TMV, *Tobacco mosaic virus*; RDV, *Rice dwarf virus*; CaMV, *Cauliflower mosaic*
21 *virus*; TSWV, *Tomato spotted wilt virus*; TuMV, *Turnip mosaic virus*; CMV, *Cucumber mosaic*
22 *virus*.

Table 1.
Specific virus-host interactions that impact phytohormone pathways.

Phytohormone Pathway	Virus	Viral Protein	Host Component	Result	Reference
Increases Aux signaling	TMV	126 kDa	IAA26 IAA27 IAA18	Enhanced phloem loading and viral systemic movement, symptom development	[10,11] Unpublished Work
	TuMV	HC-Pro	miR167 (targets ARF8)	Symptom development	[13]
Alters ET responses	TuMV	NIA-Pro	unknown	Suppresses callose formation to promote transmission by insect vector	[72]
	CaMV	P6	unknown	Symptom development	[20, 21]
Inhibits GA synthesis	RDV	P2	ent-Kaurene oxidase	Enhanced susceptibility, symptom development	[14]
Inhibits GA signaling, inhibits SA mediated defense	TMV-Cg	CgCP	DELLA proteins	Enhanced viral accumulation	[43]
Reduces SA defense responses	TMV	126 kDa	ATAF2	Enhanced viral accumulation	[40]
Inhibits SA signaling, enhances JA signaling	CaMV	P6	NPR1	Enhanced susceptibility	[42]
Increases SA signaling, Inhibits JA signaling	TSWV	unknown	unknown	Promotes insect vector feeding	[69]
Inhibits JA signaling	Geminivirus	C2	CSN5	Enhanced viral replication	[48]
Decreases JA signaling	CMV	2b	unknown	Promotes insect vector feeding	[71]

Figure