

## **Networking by small-molecule hormones in plant immunity**

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**Abstract:** Plants live in complex environments in which they intimately interact with a broad range of microbial pathogens and insect herbivores with different lifestyles and infection or feeding strategies. The evolutionary arms race between plants and their attackers provided plants with a sophisticated defense system that, like the animal innate immune system, recognizes the attacker and responds by activating specific defenses that are specifically directed against the invader. Recent advances in plant immunity research provided exciting new insights into the underlying defense signaling network. Diverse small-molecule hormones play pivotal roles in the regulation of this network. Their signaling pathways cross-communicate in an antagonistic or synergistic manner, providing the plant with a powerful capacity to finely tailor its immune response to the attacker encountered. Pathogens and insects, on the other hand, can manipulate the plant's defense signaling network for their own benefit by affecting phytohormone homeostasis to antagonize the host immune response.

**Key words:** phytohormones, cross-talk, induced resistance, pathogens, insect herbivores

### **Introduction**

Phytohormones act as signal molecules and occur in low concentrations. Classic phytohormones are abscisic acid, auxins, cytokinins, ethylene (ET) and gibberellins, but small signaling molecules such as brassinosteroids, jasmonates (JAs) and salicylic acid (SA) are recognized as phytohormones as well. Changes in hormone concentration or sensitivity, which can be triggered under biotic and abiotic stress conditions, mediate a whole range of adaptive plant responses. The importance of SA, JAs, and ET as primary signals in the regulation of the plant's immune response is well established (Pieterse and Dicke, 2007; Pieterse *et al.*, 2009). The involvement of so many plant growth regulators in plant immunity suggests that the control of plant growth, development and defense is interconnected in a complex network of cross-communicating hormone signaling pathways. The great regulatory potential of such a network may allow plants to quickly adapt to their biotic and abiotic environment and to utilize their resources in a cost-efficient manner. It is generally believed that hormone-regulated induced defense responses evolved to save energy under enemy-free conditions, since they only involve costs when defenses are activated upon pathogen or insect attack (Walters and Heil, 2007). These costs arise from the allocation of resources to defense and away from plant growth and development. Trade-offs between plant growth rate and disease resistance have been well documented and support the hypothesis that plant growth and defense are regulated by a network of interconnecting signaling pathways.

### ***Pathway crosstalk to fine-tune defense***

In nature, plants often deal with simultaneous or subsequent invasion by multiple aggressors and beneficials, which can influence the primary induced defense response of the host plant (Poelman *et al.*, 2008). Activation of plant defense mechanisms is associated with ecological

fitness costs (Walters and Heil, 2007). Hence, plants need regulatory mechanisms to effectively and efficiently adapt to changes in their complex environment. Crosstalk between hormonal signaling pathways provides the plant with such a powerful regulatory potential and may allow the plant to tailor its defense response to the invaders encountered (Pieterse *et al.*, 2009). Upon pathogen attack, the quantity, composition, and timing of the phytohormonal blend produced by the plant vary among plant species and depend greatly on the lifestyle and infection strategy of the invading attacker. This so-called 'signal signature' results in the activation of a specific set of defense-related genes that eventually determines the nature and effectiveness of the immune response that is triggered by the attacker (De Vos *et al.*, 2005). In recent years, molecular, genetic and genomic tools have been used to uncover the complexity of the hormone-regulated induced defense signaling network. Besides balancing the relative abundance of different hormones, intensive interplay between hormone signaling pathways emerged as an important regulatory mechanism by which the plant may be able to tailor its immune response to the type of invader encountered. On the other hand, evidence is accumulating that pathogens can manipulate hormone-regulated signaling pathways to evade host immune responses.

### ***Cross-talk between SA, JA and ET signaling***

In recent years, research on their biosynthesis pathways and the way they are perceived by other biomolecules significantly advanced our understanding of the signaling pathways that these hormones regulate. However, the way these signal molecules function in a complex network of interacting pathways is less well studied. Early work in tomato (*Solanum lycopersicum*) demonstrated that SA and its acetylated derivative aspirin are strong antagonists of the JA signaling pathway (Doherty *et al.*, 1988), and that JA and ET signaling can act synergistically (Penninckx *et al.*, 1998). The genomics era provided a wealth of new opportunities to investigate how the SA, JA, and ET signaling pathways are interconnected in the induced defense signaling network (Glazebrook *et al.*, 2003).

One of the best studied examples of defense-related signal crosstalk is the antagonistic interaction between the SA and the JA response pathway. Many cases of trade-offs between SA-dependent resistance against biotrophic pathogens and JA-dependent defense against necrotrophic pathogens and insect herbivory have been documented (Bostock, 2005; Stout *et al.*, 2006). For example, induction of the SA pathway in *Arabidopsis* by the biotrophic oomycete pathogen *Hyaloperonospora arabidopsidis* strongly suppressed JA-mediated defenses that were activated upon feeding by caterpillars of the small cabbage white *Pieris rapae* (Koornneef *et al.*, 2008). Activation of the SA pathway by the bacterial pathogen *Pseudomonas syringae* similarly suppressed JA signaling and rendered infected leaves more susceptible to the necrotrophic fungus *Alternaria brassicicola* (Spoel *et al.*, 2007). Also, exogenous application of SA rendered *Arabidopsis* plants more susceptible to the necrotroph *A. brassicicola* and the Western flower thrips *Frankliniella occidentalis* (Leon-Reyes *et al.*, 2009b).

### ***SA-mediated suppression of JA signaling***

Pharmacological experiments with *Arabidopsis* revealed that JA-responsive marker genes, such as *PDF1.2* and *VSP2*, are highly sensitive to suppression by exogenous application of SA, whereas the SA-responsive marker gene *PR-1* can be suppressed by JA signaling (Spoel *et al.*, 2003; Koornneef *et al.*, 2008; Leon-Reyes *et al.*, 2009a). SA-mediated suppression of JA-responsive gene expression was observed in a large number of *Arabidopsis* accessions collected from very different geographic origins, highlighting the potential significance of this phenomenon in the regulation of induced plant defenses in nature (Koornneef *et al.*, 2008).

Although many reports describe an antagonistic interaction between SA- and JA-dependent signaling, synergistic interactions have been described as well (Mur *et al.*, 2006). For example in *Arabidopsis*, treatment with low concentrations of JA and SA resulted in a synergistic effect on the JA- and SA-responsive genes *PDF1.2* and *PR-1*, respectively. However, at higher concentrations the effects were antagonistic, demonstrating that the outcome of the SA-JA interaction is dependent upon the relative concentration of each hormone (Mur *et al.*, 2006). Koornneef and co-workers (Koornneef *et al.*, 2008) demonstrated that timing and sequence of initiation of SA and JA signaling are also important for the outcome of the SA-JA signal interaction. Hence, the kinetics of phytohormone biosynthesis and signaling during the interaction of a plant with its attacker(s) could be highly decisive in the final outcome of the defense response to the attacker encountered.

### ***Signaling nodes in the SA-JA-ET network***

To date, several proteins with an important regulatory role in SA-JA crosstalk have been identified in *Arabidopsis*. Mutation or ectopic expression of the corresponding genes were shown to have contrasting effects on SA and JA signaling and on resistance against biotrophs and necrotrophs (reviewed in Koornneef and Pieterse, 2008; Pieterse *et al.*, 2009). Changes in the cellular redox state play a major role in the transduction of the SA signal (Mou *et al.*, 2003). In *Arabidopsis*, the ability of SA to suppress JA-responsive genes was shown to coincide with an increase in the level of glutathione, a major determinant of cellular redox homeostasis, suggesting that SA-mediated modulation of the cellular redox state is an important trigger for the attenuation of JA signaling (Koornneef *et al.*, 2008). The NPR1 protein is an important transducer of SA-induced redox changes. Besides functioning as a crucial transcriptional co-activator of SA-responsive *PR* genes, NPR1 appeared also to function as a key regulator in SA-mediated suppression of JA signaling (Spoel *et al.*, 2003).

### ***Concluding remarks***

To elucidate molecular mechanisms involved in plant immunity, *Arabidopsis* has been demonstrated to be an excellent model species. In many *Arabidopsis*-pathogen interactions, the roles of phytohormones in the regulation of plant immunity have been demonstrated and the underlying mechanisms uncovered. Currently, our research is focused on the mode of action of SA/JA cross-talk and the identification of potential targets in the JA signaling pathway through which SA can antagonize JA-dependent defenses. Furthermore, we are interested in how pathway cross-talk affects induced resistance against pathogen and insects.

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