

48. Signal signature of *Arabidopsis* induced upon pathogen and insect attack

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Abstract

Three plant signaling molecules play a dominant role in the regulation of defenses in a number of plant-attacker model systems: salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). In this study, the roles of these signaling compounds were determined in the induced defense responses of *Arabidopsis thaliana* upon attack by a set of microbial pathogens and herbivorous insects. The production of SA, JA and ET was activated in different combinations depending on the attacker encountered resulting in a specific signal signature. Analysis of the expression of SA-, JA-, and ET- responsive marker genes showed that the signal signature nicely correlates with the expression of the marker genes in each plant-attacker interaction. We hypothesize that the specific signal signature is involved in the activation of an optimal mix of defenses to counteract the intruder.

Introduction

Plant defense against microbial pathogens on the one hand, and insects on the other hand, have been studied in great detail but to date phytopathological and entomological research approaches have remained separated. Recently, research on defense signaling pathways has revealed that induced defense against microbial pathogens and insects utilize common signaling pathways. The two pathways induced by pathogens and insects are considered to be mutually exclusive due to negative regulation mechanisms [2, 6]. In this study we combine knowledge on induced defense against microbial pathogens and against insects and follow a unique, integrated approach to elucidate how plants differentially activate specific defenses depending on the type of invader encountered. Using *Arabidopsis*-based model systems, we studied the relative expression of SA-, JA- and ET-dependent signaling pathways in response to different microbial and herbivorous inducers of defense. To this end, we examined the dynamics of these signaling compounds in the induced defense responses of *Arabidopsis* that are triggered upon attack by various microbial pathogens and herbivorous insects. This integrated approach allows us to study the flexibility of a single plant species in its defense against a wide range of invaders.

Materials and Methods

Five-week-old *Arabidopsis thaliana* Col-0 plants were infested/inoculated with five different organisms: aphids (*Myzus persicae*), thrips (*Frankliniella occidentalis*), caterpillars (*Pieris rapae*), the fungal leaf pathogen *Alternaria brassicicola* and the bacterial leaf pathogen *Pseudomonas*

nas syringae pv. *tomato* DC3000 (*avrRpt2*). Infestations with 40 nymphs and apterous adults of *M. persicae*, 20 larvae of *F. occidentalis* and five first-instar larvae of *P. rapae* were done as described for *P. rapae* larvae [13]. Microbial infections were performed as described [11]. Leaf tissues were harvested in time and used for quantification of SA (n=20), JA (n=20), and ET (n=10) production as described [7], or for RNA blot analysis performed as described previously [12].

Results

Inducer-specific disease and damage patterns

Arabidopsis plants were infested/inoculated with a set of attackers with highly different modes of action: phloem-feeding aphids, cell-content feeding thrips, tissue-chewing caterpillars, the fungal leaf pathogen *A. brassicicola* and the bacterial leaf pathogen *P. syringae* pv. *tomato* DC3000. The interaction of *Arabidopsis* with each of these organisms resulted in the development of a characteristic disease or damage pattern (Fig. 1).

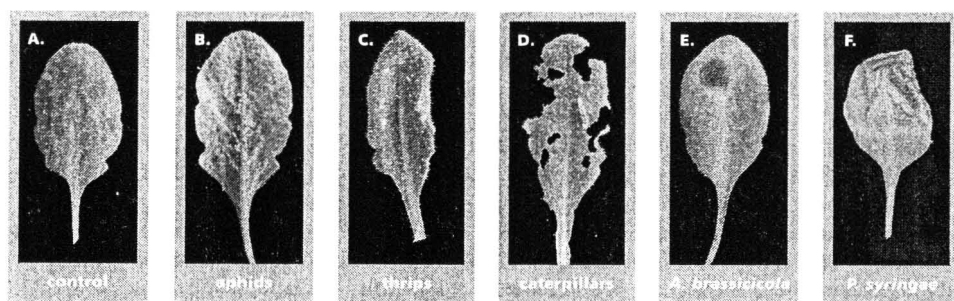


Fig. 1. Symptom development on *Arabidopsis* Col-0 plants infested/inoculated with five different organisms: (A) control; (B) aphids (*Myzus persicae*); (C) thrips (*Frankliniella occidentalis*); (D) caterpillars (*Pieris rapae*); (E) the fungal leaf pathogen *Alternaria brassicicola*; and (F) the bacterial leaf pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*avrRpt2*).

Inducer-specific signal signature

To identify the quantity, composition and timing of SA, JA and ET, the so-called signal signature, we monitored the production of these signal molecules in time. This revealed that the production of these signaling molecules by *Arabidopsis* was activated in different combinations depending on the attacker encountered (Fig. 2). *P. syringae* pv. *tomato* DC3000 infection stimulated the production of all three alarm molecules, whereas *A. brassicicola* infected resulted only in JA and ET accumulation. Of the herbivores, both *P. rapae* and *F. occidentalis* activated predominantly the production of JA, whereas no significant amounts of SA, JA, or ET were produced upon feeding by *M. persicae*. These results indicate that *Arabidopsis* responds to each inducer by producing a specific signal signature.

Marker gene expression

To verify whether the signal signatures induced in response to attack by the different invaders correlates with the expression of a set of well-characterized marker genes, we analyzed the expression of the SA-responsive *PR-1* gene, the JA/ET-responsive *PDF1.2* gene, the JA-responsive *VSP2* gene, and the ET-responsive *HEL* gene. Northern blot analysis of the expression of these genes in time showed that the gene expression profiles nicely matched the signal signatures for each plant-microbe and plant-herbivore interaction (Fig. 3).

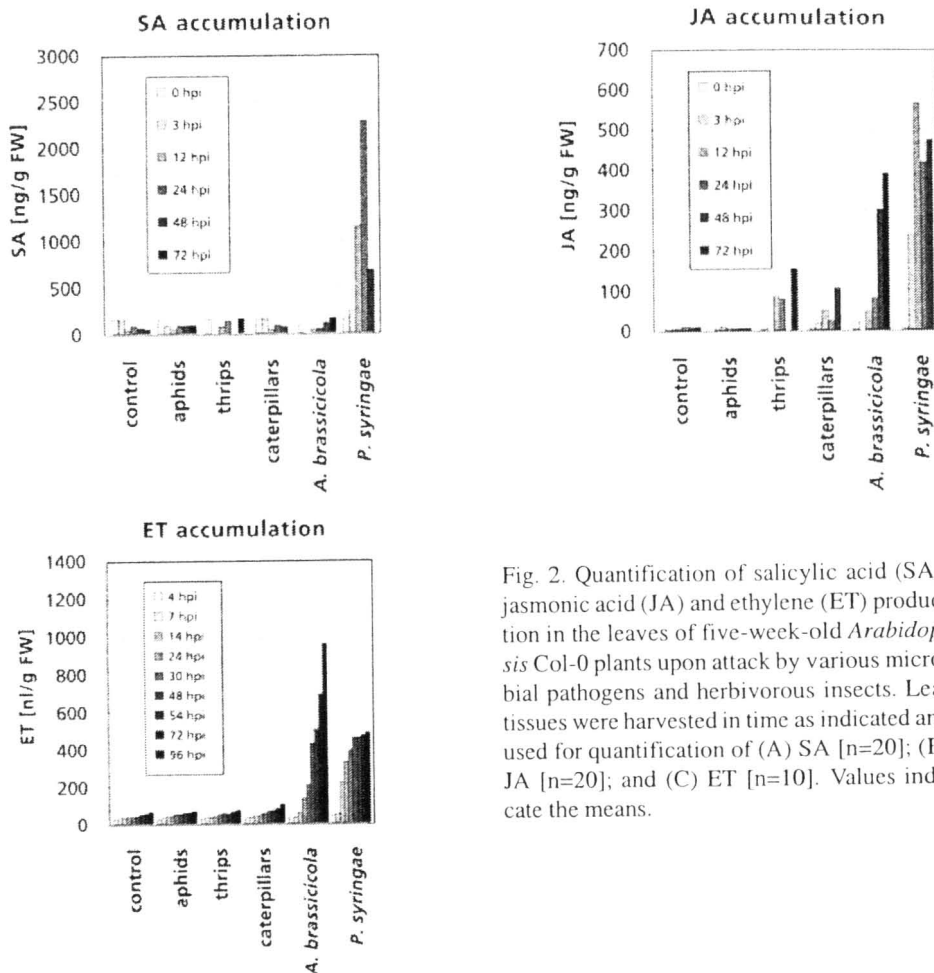


Fig. 2. Quantification of salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) production in the leaves of five-week-old *Arabidopsis* Col-0 plants upon attack by various microbial pathogens and herbivorous insects. Leaf tissues were harvested in time as indicated and used for quantification of (A) SA [n=20]; (B) JA [n=20]; and (C) ET [n=10]. Values indicate the means.

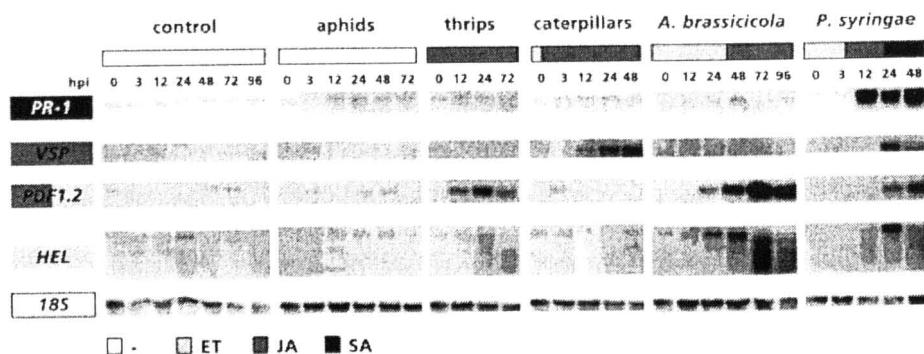


Fig. 3. RNA blot analysis of SA-, JA- and ET-inducible marker genes in the leaves of *Arabidopsis* Col-0 plants upon attack by various microbial pathogens and herbivorous insects. Leaf tissues were harvested in time as indicated. RNA blot analysis to study SA-responsive *PR-1*, JA/ET-responsive *PDF1.2*, JA-responsive *VSP2*, and ET-responsive *HEL* gene expression was performed as described previously [12]. The level of *18S* RNA served as a loading control.

Discussion

Recent advances in plant defense signalling research revealed that plants are capable of differentially activating inducible defense mechanisms depending on the type of invader encountered, leading to the activation of an optimal mix of defence responses to counteract the intruder [8]. SA, JA and ET play a dominant role in this signalling network: blocking the response to either of these signals can render plants more susceptible to insects [5] or microbial pathogens [1, 4, 10]. To date, little is known about how plants coordinate signals generated by different inducers of resistance into specific defenses. A well-accepted hypothesis is that this is accomplished by differences in the relative activation of the different pathways, where SA-dependent pathway is activated more in response to microbial pathogens and the JA- and ET-dependent pathways are more activated in response to herbivory [2, 3, 6, 8, 9].

Here we showed that the production of the defense signals SA, JA and ET in *Arabidopsis* upon attack by various microbial pathogens and herbivorous insects varies highly in quantity, composition and timing. These findings demonstrate that plants are highly dynamic in their response to a wide variety of harmful organisms. We hypothesize that the signal signature eventually determines the nature of the defense response that is triggered by the invader encountered.

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