The rapidly evolving associations among herbivore associated elicitor-induced phytohormones in Nicotiana

Shuqing Xu*, Wenwu Zhou, and Ian T Baldwin
Department of Molecular Ecology; Max Planck Institute for Chemical Ecology; Jena, Germany

Keywords: ABA, herbivore associated elicitors, JA, JA-Ile, SA, JA-SA correlation, JA - JA-Ile correlation, Nicotiana, phytohormone

Abbreviations: herbivore associated elicitors, HAE; jasmonic acid, JA; jasmonic acid isoleucine, JA-Ile; salicylic acid, SA; abscisic acid, ABA.

In response to herbivore attack, plants perceive herbivore associated elicitors (HAE) and rapidly accumulate jasmonic acid (JA) and other phytohormones, which interact in complex ways, such as the crosstalk between JA and salicylic acid (SA). Although recent studies have shown that HAE-induced individual phytohormones can be highly specific among closely related species, it remains unclear how conserved and specific the relationships among HAE-induced phytohormones are. Here we analyzed the correlations among 4 different phytohormones, JA, JA-isoleucine (JA-Ile), SA, and abscisic acid (ABA) in 6 closely related Nicotiana species that were induced by 3 different HAEs. Our results showed that while no clear association between ABA and other phytohormones were found, the positive association between JA and JA-Ile is mostly conserved among closely related Nicotiana species. Interestingly, the association between JA and SA are highly variable and can be regulated by different HAEs.

In response to herbivore attack, plants induce rapid phytohormonal changes through the perception of chemical cues (herbivore-associated elicitors: HAE) in insect oral secretion (OS). The phytohormonal responses include rapid accumulations of jasmonic acid (JA) and its derivatives, JA-Ile, which are the key plant hormones responsible for the activation of defense responses against most insect herbivores in plants. At same time, HAE can also induce accumulations of other phytohormones, such as SA, ABA and ethylene (ET). These different phytohormones can play either synergistic and/or antagonistic roles in defensive reactions. For example, the accumulation of SA, which plays a key role in defending against attack from piercing-sucking insects and biotrophic pathogens, can antagonize the accumulation of JA and JA induced anti-herbivore defensive functions. In addition to the antagonistic interaction of JA-SA, ABA was shown to have synergistic effects with JA on anti-herbivore defenses in Nicotiana attenuata. Therefore, the specificity of HAE-induced defense responses are thought to be depend on both induced individual phytohormones and their cross-talk. While HAE-induced individual phytohormonal responses were known to be highly variable among plant species and herbivore-dependent, how conserved and specific the relationships are among induced phytohormones remains unclear. Here, by analyzing our recently published phytohormone data that were collected from the leaves of 6 closely related Nicotiana species, we specifically investigated 2 questions: 1) how conserved are the relationships among HAE-induced phytohormones from closely related plant species? 2) can HAE elicitations regulate the relationships among phytohormones within a species?

We calculated the pair-wise correlations among the accumulations of 4 phytohormones (JA, JA-Ile, SA and ABA) within 2 hours of being induced by different HAEs in leaves of 6 closely related Nicotiana species. Since our aim was to investigate the HAE-induced phytohormonal associations, we excluded the un-induced samples (control samples at 0 h). We found that while ABA did not correlate with other phytohormones in most of species, JA-Ile and SA showed interesting patterns of correlations with JA. JA-Ile was positively correlated with JA among most of the Nicotiana species and among different HAE elicita- tions. The highly conserved positive correlation between HAE-induced endogenous JA and JA-Ile likely reflects their biosynthetic relationship in which JA-Ile is a conjugation product of JA and isoleucine (Ile). The only exception was N. pauciflora induced by C18:3-Glu (FAC) and Manduca sexta oral secretion (OS), which showed no or slight negative correlation between JA and JA-Ile. This is mainly due to the increased JA levels at later time points (2 h after elicitation), but no increase in JA-Ile levels, indicating that the genes related to the metabolisms of JA and JA-Ile, such as jasmonate-resistant (JAR) or
jasmonyl-$\alpha$-isoleucine hydrolase (JIH)\(^{15}\) are regulated by FAC or OS\(_{M}\) in \(N.\) pauciflora at later time points. However, further experiments to measure the HAE-induced regulations of these candidate genes are needed to falsify this hypothesis.

SA’s correlation with JA was found to be highly variable. When treated with only wounding and water, all species except \(N.\) miersii showed a strong antagonistic JA-SA relationship (Fig. 2, bottom row). Interestingly, HAE inductions regulated the correlation of JA-SA differently among the different species. After HAE elicitation, while the correlation between JA and SA remained negative in \(N.\) obtusifolia, \(N.\) acuminata and \(N.\) attenuata, results consistent with previous studies using transgenic approaches,\(^{7,16}\) this negative relationship was reversed in \(N.\) linearis and \(N.\) pauciflora (Fig. 2, second and fourth column). Interestingly, the regulations on JA-SA correlation within each species were largely consistent among different HAEs (Fig. 2).

The observed variations on JA-SA relationships have 3 implications. 1) Consistent with the patterns found among different \(A.\) spp.,\(^{12}\) HAE-induced JA and SA accumulations in \(N.\) attenuata can be regulated independently. Although at a qualitative level, the induction of JA and SA were mostly positively correlated - when JA is induced, SA is usually also induced\(^{11}\) - at quantitative level, however, no consistent relationships between JA and SA were found among species (Fig. 1). This is likely due to the fact that JA and SA were induced at different times. For example, after HAE elicitation, the highest JA accumulated was at 30 minutes in most of studied \(N.\) attenuata species, except \(N.\) pauciflora, however, from our data the highest SA accumulation was at 2 h in most of species.\(^{11}\) 2) The antagonistic associations between wound-induced JA and SA elicitation can be rewired when HAE are introduced into wounds. The fact that the negative correlations between JA and SA, representing the general JA-SA antagonism frequently reported in plants,\(^{8}\) can be either enhanced or reversed in different \(N.\) species after HAE elicitation (Fig. 1 and 2) indicates that other factors induced by HAE in \(N.\) attenuata, such as ethylene might contribute to the regulations of the antagonistic effects between JA and SA.\(^{17-19}\) Further investigations on HAE induced ethylene levels would provide insights into how did HAE regulate the association between JA and SA. 3) The HAE-induced JA and SA association can evolve rapidly among closely related plant species, since closely related \(N.\) species can have different induced JA-SA associations (Fig. 2). Although the antagonism between JA and SA are thought to have evolved early in the evolutionary
history of angiosperm, the breakdown of antagonisms was also reported in *Zea mays* and milkweed. Our study further suggests that such deconstructions of the antagonistic relationships between JA and SA might be more frequent than previously thought.

In summary, our data showed that among closely related *Nicotiana* species, while HAE-induced positive association between JA and JA-Ile is mostly conserved, the induced association between JA and SA can be regulated by HAE elicitation and can evolve rapidly among closely related species.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank T. Krügel for providing the seeds of different *Nicotiana* species, M. Kallenbach for providing the synthetic C18:3-Glu and an anonymous reviewer for constructive suggestions on the manuscript.

Funding

We are grateful for funding by Swiss National Science Foundation (Project number PEBZP3-142886 to SX), the Marie Curie Intra-European Fellowships (IEF) (Project Number 328935 to SX), the Max Planck Society and European Research Council advanced grant ClockworkGreen (Project number 293926 to ITB).

References

1. Bonaventure G, VanDoorn A, Baldwin IT. Herbivore-associated elicitors: FAC signaling and metabolism. Trends Plant Sci 2011; 16:294-9; PMID:21354852; http://dx.doi.org/10.1016/j.tplants.2011.01.006
2. Farmer EE, Ryan CA. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase-inhibitors. Plant Cell 1992; 4:129-34; PMID:12297644; http://dx.doi.org/10.1105/tpc.4.2.129
3. Wu J, Baldwin IT. New insights into plant responses to the attack from insect herbivores. Anna Rev Genet 2010; 44:1-24; PMID:20649414; http://dx.doi.org/10.1146/annurev-genet-102209-163500
4. Erb M, Meldau S, Howe GA. Role of phytohormones in insect-specific plant reactions. Trends Plant Sci 2012; 17:250-9; PMID:22305233; http://dx.doi.org/10.1016/j.tplants.2012.01.003
5. Gaffney T, Friedrich L, Vernooij B, Negrotto D, Nye G, Uknes S, Ward E, Kesmann H, Ryals J. Requirement of salicylic-acid for the induction of systemic acquired-resistance. Science 1993; 261:754-6; PMID:17757215; http://dx.doi.org/10.1126/science.261.5122.754
6. Delaney TP, Uknes S, Vernooij B, Friedrich L, Weymann K, Negrotto D, Gaffney T, Gut-Rella M, Kesmann H, Ward E, et al. A central role of salicylic-acid in plant-disease resistance. Science 1994; 266:1247-50; PMID:17810266; http://dx.doi.org/10.1126/science.266.5188.1247
7. Rayapuram C, Baldwin IT. Increased SA in NPR1-silenced plants antagonizes JA and JA-dependent direct and indirect defenses in herbivore-attacked *Nicotiana attenuata* in nature. Plant J 2007; 52:700-15; PMID:17850230; http://dx.doi.org/10.1111/j.1365-313X.2007.03267.x

8. Thaler JS, Humphrey PT, Whitman NK. Evolution of jasmonate and salicylate signal crosstalk. Trends Plant Sci 2012; 17:266-70; PMID:22498450; http://dx.doi.org/10.1016/j.tplants.2012.02.010

9. Dinh ST, Baldwin IT, Galis I. The HERBIVORE ELICITOR-REGULATED1 gene enhances abscisic acid levels and defenses against herbivores in *Nicotiana attenuata* plants. Plant Physiol 2013; 162:2106-24; PMID:23784463; http://dx.doi.org/10.1104/pp.113.221150

10. Schmelz EA, Engelberth J, Alborn HT, Tumlinson JH, Teal PE. Phytohormone-based activity mapping of insect herbivore-produced elicitors. Proc Natl Acad Sci U S A 2009; 106:653-7; PMID:19124770; http://dx.doi.org/10.1073/pnas.0811861106

11. Xu S, Zhou W, Portzinger S, Baldwin IT. Herbivore associated elicitor-induced defenses are highly specific among closely related *Nicotiana* species. BMC Plant Biol 2015; 15:2; PMID:25592329; http://dx.doi.org/10.1186/s12870-014-0406-0

12. Agrawal AA, Hastings AP, Patrick ET, Knight AC. Specificity of herbivore-induced hormonal signaling and defensive traits in five closely related milkweeds (*Asclepias* spp.). J Chem Ecol 2014; 40:717-29; PMID:24863490; http://dx.doi.org/10.1007/s10886-014-0449-6

13. Stawicki PE, Tiryaki I. The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis*. Plant Cell 2004; 16:2117-27; PMID:15258265; http://dx.doi.org/10.1105/tpc.104.023549

14. Kang JH, Wang L, Giri A, Baldwin IT. Silencing threonine deaminase and JAR1 in *Nicotiana attenuata* impairs jasmonic acid-isoleucine-mediated defenses against *Manduca sexta*. Plant Cell 2006; 18:3303-20; PMID:17085687; http://dx.doi.org/10.1105/tpc.106.041103

15. Woldernariam MG, Onkokesung N, Baldwin IT, Galis I. Jasmonoyl-l-isoleucine hydrolase 1 (JH1) regulates jasmonoyl-l-isoleucine levels and attenuates plant defenses against herbivores. Plant J 2012; 72:758-67; PMID:22866609; http://dx.doi.org/10.1111/j.1365-313X.2012.05117.x

16. Diezel C, von Dahl CC, Gaquerel E, Baldwin IT. Different lepidopteran elicitors account for cross-talk in herbivory-induced phytohormone signaling. Plant Physiol 2009; 150:1576-86; PMID:19498114; http://dx.doi.org/10.1104/pp.109.139550

17. Diezel C, Allmann S, Baldwin IT. Mechanisms of optimal defense patterns in *Nicotiana attenuata*: flowering attenuates herbivory-elicited ethylene and jasmonate signaling. J Integr Plant Biol 2011; 53:971-83; PMID:22054509; http://dx.doi.org/10.1111/j.1744-7909.2011.01086.x

18. Leon-Reyes A, Du YJ, Koornneef A, Proietti S, Korbes AP, Memelink J, Pieterse CM, Ritsema T. Ethylene signaling renders the jasmonate response of *Arabidopsis* insensitive to future suppression by salicylic acid. Mol Plant Microbe Interact 2010; 23:187-97; PMID:20064062; http://dx.doi.org/10.1094/MPMI-23-2-0187

19. Leon-Reyes A, Spoel SH, De Lange ES, Abe H, Kobayashi M, Tsuda S, Millenaar FF, Welschen RA, Ritsema T, Pieterse CM. Ethylene modulates the role of NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 in cross talk between salicylate and jasmonate signaling. Plant Physiol 2009; 149:1797-809; PMID:19176718; http://dx.doi.org/10.1104/pp.108.133926

20. Engelberth J, Viswanathan S, Engelberth MJ. Low concentrations of salicylic acid stimulate insect elicitor responses in *Zea mays* seedlings. J Chem Ecol 2011; 37:263-6; PMID:21360274; http://dx.doi.org/10.1007/s10886-011-9926-3