Methyl jasmonate is transported to distal leaves via vascular process metabolizing itself into JA-Ile and triggering VOCs emission as defensive metabolites

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Introduction

Plants produce volatile organic compounds (VOCs) as defensive metabolites to protect themselves against biotic and abiotic factors such as damage, injury, insects and pathogens. VOCs emissions and their pattern are influenced by exogenous application of jasmonic acid (JA) and its metabolite methyl jasmonate (MeJA). JA is metabolized into its bioactive compounds, loosely called jasmolites. One important JA metabolite is its conjugate jasmonoyl isoleucine (JA-Ile). JA-Ile elicits defensive reactions and its level increases locally in damaged leaves. Recent studies have provided convincing evidence that JA-Ile is the active compound, not the free JA, responsible for triggering gene expression. Hence, JA-Ile plays vital roles in regulation of the JA- and MeJA-based signaling in plants.

It has been reported that plant JA and MeJA possess transportable property from leaves to roots or to tissues. Hence, JA and MeJA are considered long-distance signaling compounds. Signaling compounds can be transported to distal plant sites via air (airborne) and vascular process to perform its function as a long-distance signal. Previously, it has been demonstrated that airborne MeJA is converted into JA-Ile in the receiver leaves accompanying the defensive VOCs emission. Nonetheless, direct evidence is still lacking for transport of MeJA to distal plant sites by vascular process to elicit defense responses.

Keywords: methyl jasmonate, jasmonoyl isoleucine, deuterium labeling, plant signaling, long-distance signaling, VOC

Results and Discussion

MeJA is transported to distal leaves via vascular process leading to emission of VOCs as defensive metabolites. To investigate whether MeJA is transported and elicits defensive metabolites in distal leaves, the d2MeJA solution was supplied to the 4th node stem of Achyranthes (Fig. 1A; see inset). Stem absorbed 5 mL, at 24 h post-feeding, of the d2MeJA solution (10 mL). A combined GC-MS and GC-FID analysis of collected VOCs at 24 h post-feeding resulted in identification of multiple VOCs (Fig. 1B). Those VOCs were methyl 2-(E)-hexenoate, 3-(Z)-hexenyl acetate, 2-(E)-hexenyl acetate, (E)-β-ocimene, linalool, (3E)-4,8-dimethyl-1,3,7-nonatriene, linalool, (3E)-4,8-dimethyl-1,3,7-nonatriene,
Analysis of the reference leaves (i.e., control) gave a small peak of d\textsubscript{2}JA (12 ng/g.f.w), which is most likely derived from possible leakage of airborne d\textsubscript{2}MeJA in the glass container. The calculated d\textsubscript{2}JA amount accounted only 4% of the total d\textsubscript{2}JA (298 ng/g.f.w) obtained through the vascular process. Moreover, no detectable d\textsubscript{2}JA-Ile peak could be identified in the reference leaves (i.e., control). Hence, identified VOCs in Figure 1B are mainly due to d\textsubscript{2}MeJA supply and its conversion into an active d\textsubscript{2}JA-Ile metabolite.

Conversion to JA-Ile is perhaps one simple and efficient way to defensive responses. To understand plant’s systemic defensive reactions, it is important to consider the processes by which mobile signals are produced at the damaged site, transported and perceived by target cells.\textsuperscript{17} It has been suggested that factors qualifying as transportable signals have the properties to: (1) induce defensive response; (2) be produced or released at the site of attack; (3) be translocated from the site of attack to systemic tissue; and (4) accumulate in systemic tissue before conferring resistance reactions.\textsuperscript{14} To date, mounting evidence implies that MeJA are likely candidates to act as long-distance signaling compounds.\textsuperscript{6,7,12-14,17} But, the perception of MeJA at distal sites in plants remains to be demonstrated.

\begin{figure}
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\includegraphics[width=\textwidth]{figure1.png}
\caption{MeJA transport and metabolism in distal leaves. (A) The experimental set up includes Achyranthes plant and supply of the d\textsubscript{2}MeJA solution at the 4th node (number 4), as shown in an inset. Details are mentioned in the text. (B) Quantitative profiling of emitted VOCs. Numbers correspond to: 1, methyl 2-(E)-hexenoate; 2, 3-(Z)-hexenyl acetate; 3, 2-(E)-hexenyl acetate; 4, (E)-\textbeta-ocimene; 5, linalool; 6, (E)-4,8-dimethyl-1,3,7-nonatriene; 7, (E)-\textbeta-caryophyllene; 8, (E)-\textalpha-bergamotene; 9, sesquisabinene; 10, (E,\textbeta)-farnesene; 11, \textalpha-humulene; 12, (E,\textE)-\textalpha-farnesene; and 13, \textbeta-bisabolene. (C) Absolute quantification of exogenous/endogenous JA and JA-Ile in the upper developed leaves (Fig. 1A; leaf 1 and 2). Data shown in (B) and (C) are derived from eight independent biological experiments and were used to calculate means and SE. Error bars represent standard errors.}
\end{figure}
The dJA-Ile detection in the distal leaves shows that dMeJA is transportable via vascular process and is metabolized into an active signaling component dJA-Ile/JA-Ile. Present findings concur with the previous reported result suggesting that: (1) JA conversion/synthesis is required and (2) jasmonate action is involved in signal recognition in responding leaves by reciprocal grafting experiments. It appears that the JA-Ile-based signaling and perception is a simple and efficient defense strategy in Achyranthes, and perhaps in other plants. Inactive MeJA is transported to the distal sites and converted into active JA-Ile, which is proven to be recognized by the receptor COI1 leading to transcription of JA-responsive genes. Moreover, it has been shown that cis-JA-Ile binds more tightly to the receptor COI1/JAZ, suggesting that stereochemistry of a metabolite has an important role on its biological activity. To note, the upper leaves (Fig. 1A; leaf 1 and 2) contained both exogenous dJA-Ile and endogenous JA-Ile. As far as stereochemistry of JA-Ile is concerned, our previous report in Achyranthes has shown that cis-JA-Ile is a major component in endogenous JA-Ile, which will be essentially involved in the VOCs elicitation in distal leaves.

Materials and Methods

Chemicals and plant material. MeJA was purchased from Sigma-Aldrich. Standard compounds dMeJA and dJA-Ile were prepared as described previously. Other chemicals used in this study were of analytical grade. The dMeJA solution in water (distilled H2O) was prepared by dissolving 3 mg of dMeJA in 50 mL of H2O by vigorous stirring for 30 min. Achyranthes bidentata var tomentosa was used as an experimental plant as previously described. Achyranthes plants (ca. 25 cm tall) were cut at the 4th node from the top and used for all experiments.

Application of dMeJA and analysis of JA metabolites and VOCs. The stem with leaves cut at the 4th node (length of ca. 20 cm; fresh weight of the whole plant material was about 4.6 g) of Achyranthes plant was placed in a way that the stem was touching the bottom of a glass bottle (20 mL capacity, 5.5 cm length, 2.2 cm width) containing 10 mL of the dMeJA solution. The bottle neck was plugged with cotton and sealed with a flexible film (PARAFILM; American National Can) to prevent the d2-MeJA leakage (Fig. 1A). The entire experimental setup prepared was enclosed in a 2-L glass container (23.0 cm length, 10.5 cm width) for 24 h under light (intensity-50 μm/s/m²) which has resulted in the completion of this present study. VOCs emitted in the headspace were collected by solid phase micro extraction (SPME) fibers (Stable Flex PDMS/DVB, Supelco). Collected VOCs were analyzed by gas chromatography-mass spectrometry (GC-MS; Perkin-Elmer Turbo Mass).

After VOCs collection and their analysis, the plant material was taken out from the glass container. Upper two leaves [Figure 1A; leaf 1 and 2 (fresh weight of 1.13 g)] were detached and used together for extraction and analysis of JA/JA-Ile as described previously. In brief, detached leaves were extracted with acetone (60 mL) and concentrated to give a crude extract, followed by extraction twice with chloroform (20 mL x 2) under acidic condition (pH 3). The chloroform extract was concentrated and dissolved in 2 mL of 80% (v/v) aqueous methanol. Prepared aqueous methanol solution was passed through the C-18 short column (Waters Sep-pak C-18 light) twice. Of the eluant, 5 μL was applied to high performance liquid chromatography-tandem mass spectrometry [HPLC-MS/MS; TSQ Quantum Ultra MS/MS equipped with Accela 600 HPLC system (Thermo Fisher Scientific Inc. MA USA) for JA/JA-Ile analysis.

Evidence provided in this study suggests that MeJA is transportable via vascular process and metabolized into JA-Ile in the distal leaves, accompanying endogenous JA-Ile production and VOCs emission as defensive metabolites. Deuterium labeling approach and experimental design might be applied to better understand the systemic reactions and responses in plants. An idea of transportation with successive activation might be a key concept to understand the MeJA signaling. To strengthen the role of MeJA as a transportable signal, further study needs to investigate whether MeJA produced in the wounded local tissues is loaded onto the vascular system.

Concluding Remarks

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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