

SHORT COMMUNICATION



Jasmonic Acid Modulates Xylem Development by Controlling Expression of *PIN-FORMED 7*

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ABSTRACT

Jasmonic acid (JA) modulates plant development, growth, and responses to stress. Previously, we showed that in *Arabidopsis thaliana*, JA promotes the formation of extra xylem in roots, and mutant plants unable to express *PIN-FORMED 3* (*PIN3*) and *PIN7* formed extra xylem in the absence of exogenous JA. Those results suggested that JA modulates root xylem development by controlling PIN-mediated polar auxin transport. Consistent with this, treatment with an auxin transport inhibitor induced extra xylem formation. Here, we characterized the expression of *PIN3* and *PIN7* in JA-treated *Arabidopsis* plants. *PIN3* expression was not altered in response to JA; by contrast, *PIN7* expression was reduced by JA, which suggested that *PIN7* is involved in JA-mediated xylem development. Indeed, overexpressing *PIN7* suppressed the formation of extra xylem in response to JA. Based on these results, we propose that JA mediates xylem development by controlling polar auxin transport with *PIN7* critically involved in this process.

ARTICLE HISTORY

Received 30 May 2019
Revised 19 June 2019
Accepted 20 June 2019

KEYWORDS

Jasmonic acid; xylem; cytokinin; auxin; polar auxin transport; PIN-formed proteins

Text

Auxin is a key phytohormone regulating various aspects of plant development including xylem development. The identification of the auxin carrier proteins including PINs and AUXs revealed that polar auxin transport provides crucial positional information for the specification of cells and tissues.^{1–3} Xylem cells in vascular tissues show high auxin responses compared with other cells, and the auxin signaling-defective mutant *axr3-1* produces a no-xylem phenotype, demonstrating the essential role of auxin in xylem development.^{4,5} This suggests that xylem-specific auxin accumulation produces the responses in xylem, consistent with the recent finding that inhibition of polar auxin transport affects xylem-specific auxin accumulation and xylem development.⁶

JA regulates plant defenses against biotic and abiotic stresses.⁷ JA is synthesized from linolenic acid via the octadecanoid pathway, after which it is metabolized into an isoleucine conjugate (JA-Ile).^{8,9} JA-signaling pathways are activated by the interaction between JA-Ile and the CORONATINE INSENSITIVE1 (COI1) receptor, which prompts proteolysis of transcriptional repressor JASMONATE ZIM-DOMAIN proteins.^{10,11} JA vitally underpins many plant responses to biotic and abiotic stresses and also modulates plant growth and development, strongly suggesting that JA is essential for coordinating development and stress responses in plants.^{12–14}

Our prior work revealed that JA promotes the formation of extra xylem in *Arabidopsis*^{15,16} (Figure 1). Wild-type plants form xylem cells in a single axis in the middle of the vasculature; by contrast, JA-treated wild-type plants produce extra xylem cells adjacent to the axis. Recently, we observed the extra-xylem phenotype in mutant plants that lacked expression

of *PIN3* and *PIN7*.⁶ *PIN3* and *PIN7* are responsible for polar auxin transport from the procambium to the xylem;⁵ this suggested that JA modulates xylem development by controlling auxin movement. Consistent with this, the *PIN3* and *PIN7* expression responsible for xylem patterning is activated by cytokinin (CK), yet JA antagonistically interacts with CK to modulate xylem development.^{5,6,15} Therefore, CK-responsive *PIN3* and *PIN7* expression could be involved in JA-mediated xylem development in plants.

To investigate the involvement of *PIN3* and *PIN7* in JA-mediated xylem development, we analyzed changes in CK responses and *PIN3* and *PIN7* expression in response to JA (Figure 2). When the CK response was visualized in *Arabidopsis* plants expressing the CK-responsive marker *ARR5::GFP*, JA reduced the CK response in the vasculature and columella (Figure 2a). To analyze changes in *PIN3* and *PIN7* expression in response to JA, we performed qRT-PCR using total RNA extracted from these plants. The *PIN7* expression level in JA-treated plants was approximately 3-fold lower than that in untreated plants (Figure 2b). In contrast to *PIN7*, expression levels of *PIN3* were similar between JA-untreated and -treated plants, indicating that JA has a negligible effect on *PIN3* expression. The JA effect on *PIN7* expression was also observed in wild-type plants (Figure 2c). JA-treated wild type exhibited reduced expression of *PIN7* compared to the JA-untreated wild type, suggesting that *PIN7* is involved in JA-mediated xylem development.

Based on the evidence that JA reduces expression of *PIN7*, we hypothesized that plants overexpressing *PIN7* would not show JA-induced development of extra xylem. To test this, we quantified formation of extra xylem in *PIN7*-overexpressing plants (*35S::PIN7*) grown in JA-untreated and -treated conditions (Figure 3).

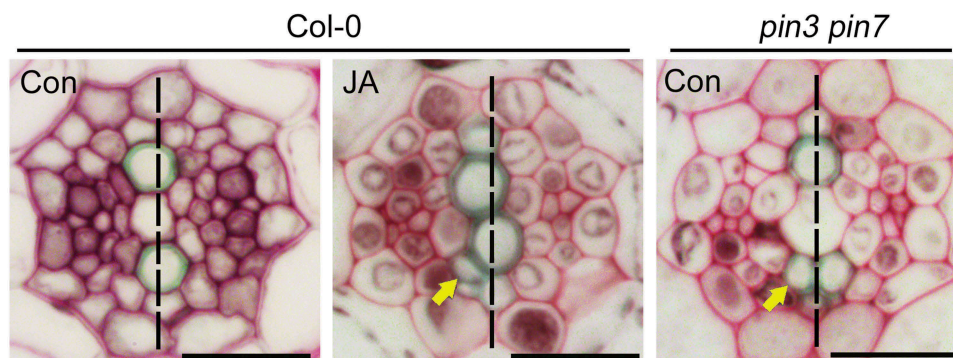


Figure 1. JA and knockout-out mutation of *PIN3* and *PIN7* promoted the formation of extra xylem cells.

Cross-sectional images showing the extra xylem formed in the roots of Col-0 plants grown in 10 μM of methyl jasmonate (JA) for 7 days unlike those of JA-untreated Col-0 plants (Con). The mutant plants lacking expression of *PIN3* and *PIN7* (*pin3 pin7*) formed extra xylem even under JA-untreated conditions. Arrows and dashed lines respectively indicate the formation of extra xylem and the xylem axis. Scale bar = 20 μm .

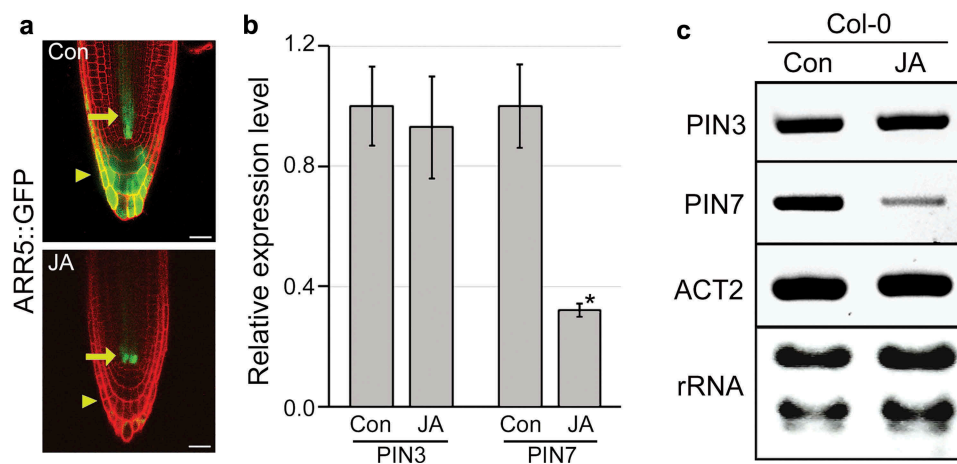


Figure 2. Suppression of *PIN7* expression by JA.

(a) Images showing the distribution and intensity of GFP signals in *ARR5::GFP* transgenic plants grown in JA-untreated (Con) and -treated conditions (JA) for 5 days (JA, 10 μM of methyl jasmonate). (b) Expression levels of *PIN3* and *PIN7* genes in the *ARR5::GFP* transgenic plants. Data represent mean values of three technical replicates, and error bars indicate SD. Asterisks show statistically significant differences between JA-untreated and -treated samples (p -value < 0.01, Student's t -test). *GAPDH* (*At1G13440*) was used as an internal control to normalize gene expression. (c) Semi-quantitative RT-PCR results showing that expression levels of *PIN3* and *PIN7* in wild-type plants (Col-0) grown in JA-untreated and -treated conditions (10 μM of methyl jasmonate) for 9 days. *ACT2* (*At3G18780*) was used as an internal control. Scale bar = 20 μm .

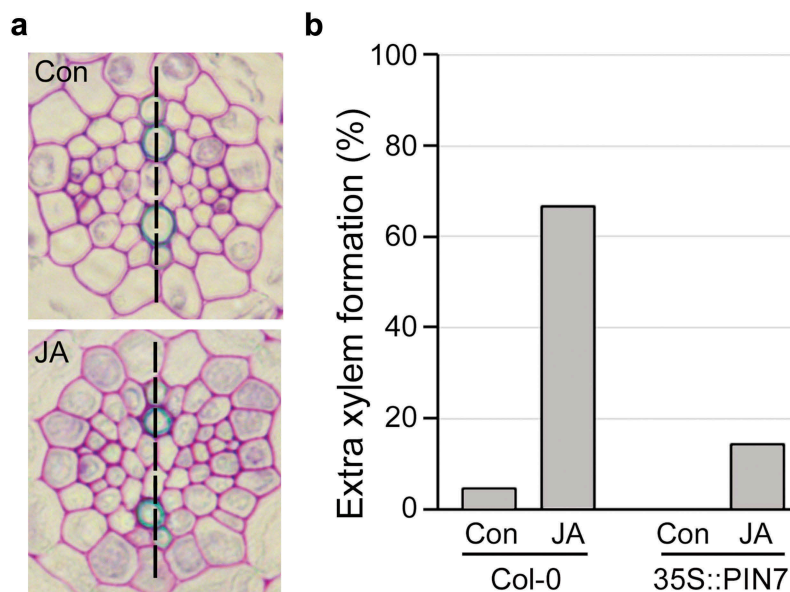


Figure 3. Overexpression of *PIN7* suppressed the JA-mediated extra-xylem phenotype.

Cross-sectional images of the roots of *35S::PIN7* grown in JA-untreated (Con) and -treated conditions (JA) for 7 days (JA, 10 μM methyl jasmonate). (b) Quantification of extra xylem formation in these plants. Percentages were calculated by dividing the number of plants with extra xylem by total number of plants observed ($n > 20$). Scale bar = 20 μm .

Approximately 15% of these plants we tested displayed the extra-xylem phenotype, a proportion approximately 4-fold lower than that of wild-type plants grown under the same conditions ($n > 20$). This indicates that JA regulates xylem development by controlling polar auxin transport, in a process mediated by PIN7. This crucial role of PIN7 in xylem development is consistent with the findings of Murano et al. (2014), whose computational study showed that PIN7 activity was capable of establishing the xylem-specific auxin accumulation responsible for xylem development, by directing polar auxin transport from the procambium to xylem cells.¹⁷ Taken together, these findings suggest that PIN7 mediates JA-dependent xylem development by controlling polar auxin transport to xylem structures.

Modulation of plant development under stress conditions largely occurs through the interaction between hormones that mediate plant developmental process and stress response. Growing numbers of studies have proposed that JA extensively interacts with other phytohormones to regulate plant development under stress conditions. For example, JA interacts with gibberellic acid in the coordination of plant development and stress responses.^{18–20} Based on our results presented here, we propose that JA interacts with auxin and CK to modulate xylem development. Further molecular and genetic studies will enhance our understanding of the molecular mechanisms underlying JA-mediated xylem development and the regulatory interactions between JA and other key phytohormones.

Acknowledgments

This work was carried out with the support of “Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01364301 and PJ01323901)” Rural Development Administration, Republic of Korea and the National Research Foundation of Korea grant funded by the Korean Government (MOE) [NRF-2019R1A2C1007103].

Abbreviations

JA	jasmonic acid
CK	cytokinin, PIN, pin-formed protein
GFP	green fluorescent protein

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by the Rural Development Administration (ROK) [PJ01364301]; National Research Foundation (ROK) [NRF-2019R1A2C1007103]; Rural Development Administration (ROK) [PJ01323901].

References

- Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, Hamann T, Offringa R, Jürgens G. Efflux-dependent auxin gradients establish the apical-basal axis of Arabidopsis. *Nature*. 2003;426:147–153. doi:10.1038/nature02085.
- Benková E, Michniewicz M, Sauer M, Teichmann T, Seifertová D, Jürgens G, Friml J. Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell*. 2003;115:591–602.
- Bllilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, Heidstra R, Aida M, Palme K, Scheres B. The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. *Nature*. 2005;433:39–44. doi:10.1038/nature03184.
- Scarpella E, Marcos D, Friml J, Berleth T. Control of leaf vascular patterning by polar auxin transport. *Genes Dev*. 2006;20:1015–1027. doi:10.1101/gad.1402406.
- Bishopp A, Help H, El-Showk S, Weijers D, Scheres B, Friml J, Benková E, Mähönen AP, Helariutta Y. A mutually inhibitory interaction between auxin and cytokinin specifies vascular pattern in roots. *Curr Biol*. 2011;21:917–926. doi:10.1016/j.cub.2011.04.017.
- Jang G, Lee S, Chang SH, Kim J-K, Do Choi Y. Jasmonic acid modulates xylem development by controlling polar auxin transport in vascular tissues. *Plant Biotechnol Rep*. 2018;12:265–271. doi:10.1007/s11816-018-0491-x.
- Creelman RA, Mullet JE. Jasmonic acid distribution and action in plants: regulation during development and response to biotic and abiotic stress. *Proc Natl Acad Sci USA*. 1995;92:4114–4119. doi:10.1073/pnas.92.10.4114.
- Staswick PE, Tiryaki I. The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in Arabidopsis. *Plant Cell*. 2004;16:2117–2127. doi:10.1105/tpc.104.023549.
- Schommer C, Palatnik JF, Aggarwal P, Chételat A, Cubas P, Farmer EE, Zitzmann N, Deane C, Ohkura H, Wakefield JG. Control of jasmonate biosynthesis and senescence by miR319 targets. *PLoS Biol*. 2008;6:e230. doi:10.1371/journal.pbio.0060098.
- Chini A, Fonseca S, Fernandez G, Adie B, Chico J, Lorenzo O, Gagliardini V, Page DR, Wolfe KH, Grossniklaus U. The JAZ family of repressors is the missing link in jasmonate signalling. *Nature*. 2007;448:666. doi:10.1038/nature05984.
- Yan J, Zhang C, Gu M, Bai Z, Zhang W, Qi T, Cheng Z, Peng W, Luo H, Nan F, et al. The Arabidopsis CORONATINE INSENSITIVE1 protein is a jasmonate receptor. *Plant Cell*. 2009;21:2220–2236. doi:10.1105/tpc.109.065730.
- Wasternack C. Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Ann Bot*. 2007;100:681–697. doi:10.1093/aob/mcm079.
- Chen Q, Sun J, Zhai Q, Zhou W, Qi L, Xu L, Wang B, Chen R, Jiang H, Qi J, et al. The basic helix-loop-helix transcription factor MYC2 directly represses PLETHORA expression during jasmonate-mediated modulation of the root stem cell niche in Arabidopsis. *Plant Cell*. 2011;23:3335–3352. doi:10.1105/tpc.111.089870.
- Yang D-L, Yao J, Mei C-S, Tong X-H, Zeng L-J, Li Q, Xiao L-T, Sun T-P, Li J, Deng X-W, et al. Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proc Natl Acad Sci USA*. 2012;109:E1192–E200. doi:10.1073/pnas.1201616109.
- Jang G, Chang SH, Um TY, Lee S, Kim J-K, Do Choi Y. Antagonistic interaction between jasmonic acid and cytokinin in xylem development. *Sci Rep*. 2017;7:10212. doi:10.1038/s41598-017-10634-1.
- Jang G, Choi YD. Drought stress promotes xylem differentiation by modulating the interaction between cytokinin and jasmonic acid. *Plant Signal Behav*. 2018;13:e1451707. doi:10.1080/15592324.2018.1451707.
- Muraro D, Mellor N, Pound MP, Lucas M, Chopard J, Byrne HM, Byrne HM, Godin C, Hodgman TC, King JR, et al. Integration of hormonal signaling networks and mobile microRNAs is required for vascular patterning in Arabidopsis roots. *Proc Natl Acad Sci USA*. 2014;111:857–862. doi:10.1073/pnas.1221766111.
- Cheng H, Song S, Xiao L, Soo HM, Cheng Z, Xie D, Peng J, Copenhaver GP. Gibberellin acts through jasmonate to control the expression of MYB21, MYB24, and MYB57 to promote

- stamen filament growth in Arabidopsis. *PLoS Genet.* 2009;5:e1000440. doi:[10.1371/journal.pgen.1000440](https://doi.org/10.1371/journal.pgen.1000440).
19. Hou X, Lee LYC, Xia K, Yan Y, Yu H. DELLAs modulate jasmonate signaling via competitive binding to JAZs. *Dev Cell.* 2010;19:884–894. doi:[10.1016/j.devcel.2010.10.024](https://doi.org/10.1016/j.devcel.2010.10.024).
20. Um TY, Lee HY, Lee S, Chang SH, Chung PJ, Oh K-B, Kim J-K, Jang G, Choi YD. JASMONATE ZIM-DOMAIN PROTEIN 9 interacts with SLENDER RICE 1 to mediate the antagonistic interaction between jasmonic and gibberellic acid signals in rice. *Front Plant Sci.* 2018;9:1866. doi:[10.3389/fpls.2018.01866](https://doi.org/10.3389/fpls.2018.01866).