Visualization of wounding-induced root-to-shoot communication in Arabidopsis

Visualization of Wounding-induced Root-to-Shoot Communication in *Arabidopsis*.

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It is known that wounding systemically activates the expression of various defense-related genes in plants. However, most studies of wound-induced systemic response are concerned with a leaf-to-leaf response. We have recently reported that the long distance signaling was also observed in the shoots of *Arabidopsis* seedling with wounded roots. We identified early and late root-to-shoot responsive (*RtS*) genes that were up-regulated in the shoots of root-wounded seedlings at 30 min and 6 h post-injury, respectively. It is likely that the primary signals were rapidly transfered from injured roots to shoots, and then these signals were converted into chemical signals. In fact, increase of JA and OPDA content activated the expression of early and late *RtS* genes in shoots, respectively. In addition, we visualized wound-induced root-to-shoot response by using *RtS* promoter-luciferase (*Luc*) transgenic plants. Analysis of the *AtERF13* promoter*::Luc* transgenic plants clearly shows that the wound-induced root-to-shoot signaling was rapidly activated via the vascular systems.

In higher plants, "Root-to-Shoot" signaling plays an important role in their adaptation in response to various environmental stresses such as dehydration, flooding, larvae attack, and pathogen infection.[1, 2, 3, 4] However, little attention have been paid to root-to-shoot systemic gene expression in response to stresses.[5] To our knowledge, there have been no report of mechanical wounding-induced root-to-shoot systemic gene expression. In previous study, we found that the systemic induction of tobacco ethylene-responsive transcription factor (*ERF*) genes in shoots of seedlings with wounded roots (unpublished results). Therefore, we tried to establish a model system to monitor the gene expression during wound-induced root-to-shoot communication in *Arabidopsis* seedlings.[6] Then, we have performed the transcriptome analysis of wound-induced root-to-shoot response in *Arabidopsis.*[6] When the roots of *Arabidopsis* seedlings were wounded, the expression in the shoots of many genes was up-regulated more than 3-fold at 30 min and 6 h post-injury[6].

JA and OPDA regulate the wound-induced root-to-shoot response of *RtS* gene.

Early *RtS* genes encode the transcription factors, jasmonic acid (JA)

biosynthetic enzymes, mediators of calcium signaling, and so on.[6] It was reported that many of these genes were also rapidly induced by JA application.[7] Root-to-shoot response of all early *RtS* genes examined was significantly decreased in the JA-deficient mutant. In addition, ethylene (ET)-related genes such as 1-aminocyclopropane-1-carboxylic acid (ACC) synthase 6 (*ACS6*) were also induced in the shoots with wounded roots.[6,8] In fact, root-to-shoot response of some early *RtS* genes examined in the ET-insensitive *ein3* mutant was apparently low compared with wild type plants.[6] On the other hand, late *RtS* genes contain two genes encoding vegetative storage proteins (*VSP1*and *VSP2*), which are OPDA-inducible genes.[7] Correspondingly, JA and OPDA content were apparently increased in shoots of seedlings 30 min and 360 min, respectively (Fig. 1). Therefore, it is likely that the JA and OPDA signaling pathway is involved in the root-to-shoot systemic response of *RtS* gene expression. In, the ET signaling is at least partially involved in the regulation of early *RtS* gene expression.

Expression of most *RtS* genes examined was not induced in the injured roots[6]. Correspondingly, JA and OPDA content in roots were neither increased after 30 min or 6 h of root wounding.[6] This data implied that neither JA nor OPDA

directly moved from injured roots to shoots. It is likely that physical signals such as hydraulic signals were rapidly transfer from injured roots to shoots via vascular systems, and then these signals were converted into chemical signals such JA in shoots.[9, 10] Next, we tried to monitor the spatial expression pattern of *RtS* genes during wound-induced root-to-shoot response. For this purpose, we introduced some *RtS* promoter*::firefly Luc* fusion genes into *Arabidopsis* plants.

Wound-induced Root-to-Soot communication occurred via vascular systems.

As stated above, early *RtS* genes contained three *ERF* genes (*AtERF1, AtERF2*, and *AtERF#109*) in the B3 group of AP2/ERF superfamily.[11] Root-to-shoot response of the *AtERF13* gene, which also belonged to the B3 group, was statistically insignificant by microarray analysis, but its increase in expression was confirmed by qPCR analysis.[6] As shown in Figure 2A, the observed 5.1 -fold increases of *AtERF13* promoter activities in shoots of seedlings with wounded roots is quite consistent with the increase in expression of endogenous *AtERF13* mRNA by qRT-PCR (6.1-fold increase).[6] Results suggest that the root-to-shoot response of the *AtERF13* gene is mainly regulated at the transcriptional level.

Therefore, *AtERF13* promoter*::Luc* transgenic plants were subjected to further analysis.

As shown in Figure 2B, when roots of the *AtERF13* promoter*::Luc* transgenic plants were wounded, the promoter activity was found to be increased in vascular systems of whole shoots within 30 min. In contrast, such a systemic activation was not observed in mock-treated plants. This result suggests that primary signals generated in the roots are rapidly transmitted to shoot via vascular systems, and converted into chemical signals such as JA, ultimately leading to the transcriptional activation of *AtERF13* gene. As stated above, the promoter activity was not increased in injured roots. Thus, roots are capable of sensing environmental conditions in the subterranean area and generating signals that propagate signals to shoots through vascular systems. Root-to-shoot signaling communication plays an important role in environmental stress response especially in defense response.[3, 12]

References

1. Bailey-Serres J, Voesenek LA. Flooding stress: acclimations and genetic diversity. Annu Rev of Plant Biol 2008; 59:313-9.

2. Erb M, Ton J, Degenhardt J, Turlings TC. Interactions between arthropod-induced aboveground and belowground defenses in plants. Plant Physiol 2008; 146: 867–74.

3. Bakker PA, Pieterse CM, van Loon LC. Induced systemic resistance by fluorescent *Pseudomonas* spp. Phytopathol 2007; 97: 239–43.

4. Schachtman DP, Goodger JQ Chemical root to shoot signaling under drought. Trends in Plant Sci 2008; 13: 281-7.

5. Erb M, Flors V, Karlen D, de Lange E, Planchamp C, D'Alessandro M, Turlings TC, Ton J. Signal signature of aboveground-induced resistance upon belowground herbivory in maize. The Plant J 2009; 59: 292–302.

6. Hasegawa S, Sogabe Y, Asano T, Nakagawa T, Nakamura H, Kodama H, Ohta H, Yamaguchi K, Mueller MJ, Nishiuchi T. Gene expression analysis of wounding-induced root-to-shoot communication in *Arabidopsis thaliana*. Plant Cell Environ. in press.

7. Taki N, Sasaki-Sekimoto Y, Obayashi T, Kikuta A, Kobayashi K, Ainai T, Yagi K, Sakurai N, Suzuki H, Masuda T, Takamiya K, Shibata D, Kobayashi Y, Ohta H. 12-Oxo-phytodienoic acid triggers expression of a distinct set of genes and plays a role in wound-induced gene expression in *Arabidopsis*. Plant Physiol 2005; 139: 1268-83.

8. Lin Z, Zhong S, Grierson D. Recent advances in ethylene research. J Exp Bot. 2009; 60: 3311-36.

9. Hlavácková V, Naus J. Chemical signal as a rapid longdistance information messenger after local wounding of a plant? Plant Signal & Behavior 2007; 2: 103–5.

10. Hlavácˇková V, Krchnák P, Naus J, Novák O, Spundová M, Strnad M. Electrical and chemical signals involved in short-term systemic photosynthetic responses of tobacco plants to local burning. Planta 2006; 225: 235–44.

11. Sakuma Y, Liu Q, Dubouzet JG, Abe H, Shinozaki K, Yamaguchi-Shinozaki K. DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. Biochem Biophys Res Comm 2002; 290: 998-1009.

12. Verhagen BW, Glazebrook J, Zhu T, Chang HS, van Loon LC, Pieterse CM. The transcriptome of rhizobacteriainduced systemic resistance in Arabidopsis. Mol Plant-Microbe Interact 2004; 17: 895–908.

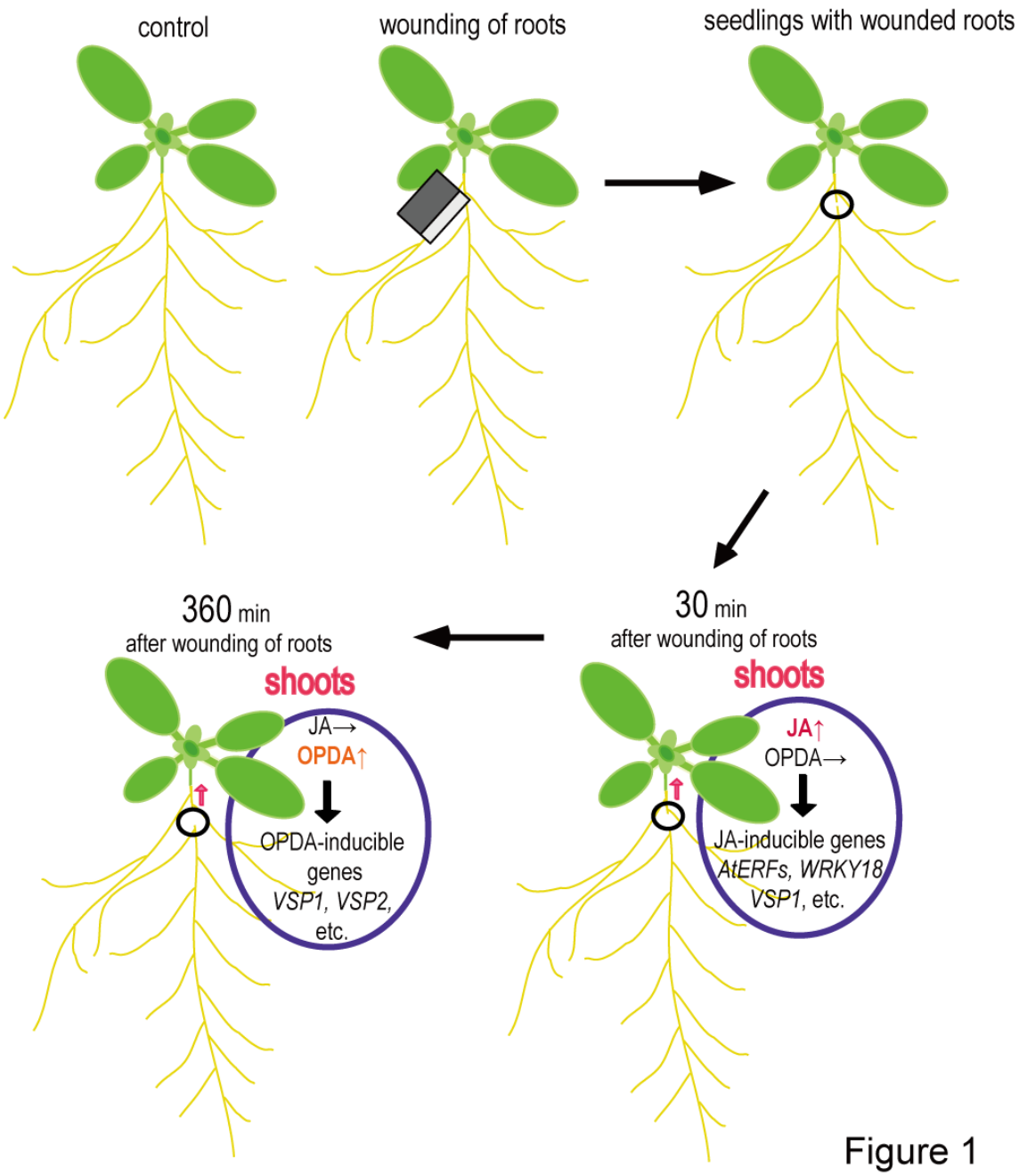
Wounding-Induced Root-to-Shoot Communication
control
wounding of roots
seedlings with wounded roots
30 min
after wounding of roots
shoots
JA↑
OPDA→
JA-inducible genes
AtERFs, WRKY18
VSP1, etc.
360 min
after wounding of roots
shoots
JA→
OPDA↑
OPDA-inducible
genes
VSP1, VSP2,
etc.

Figure 1

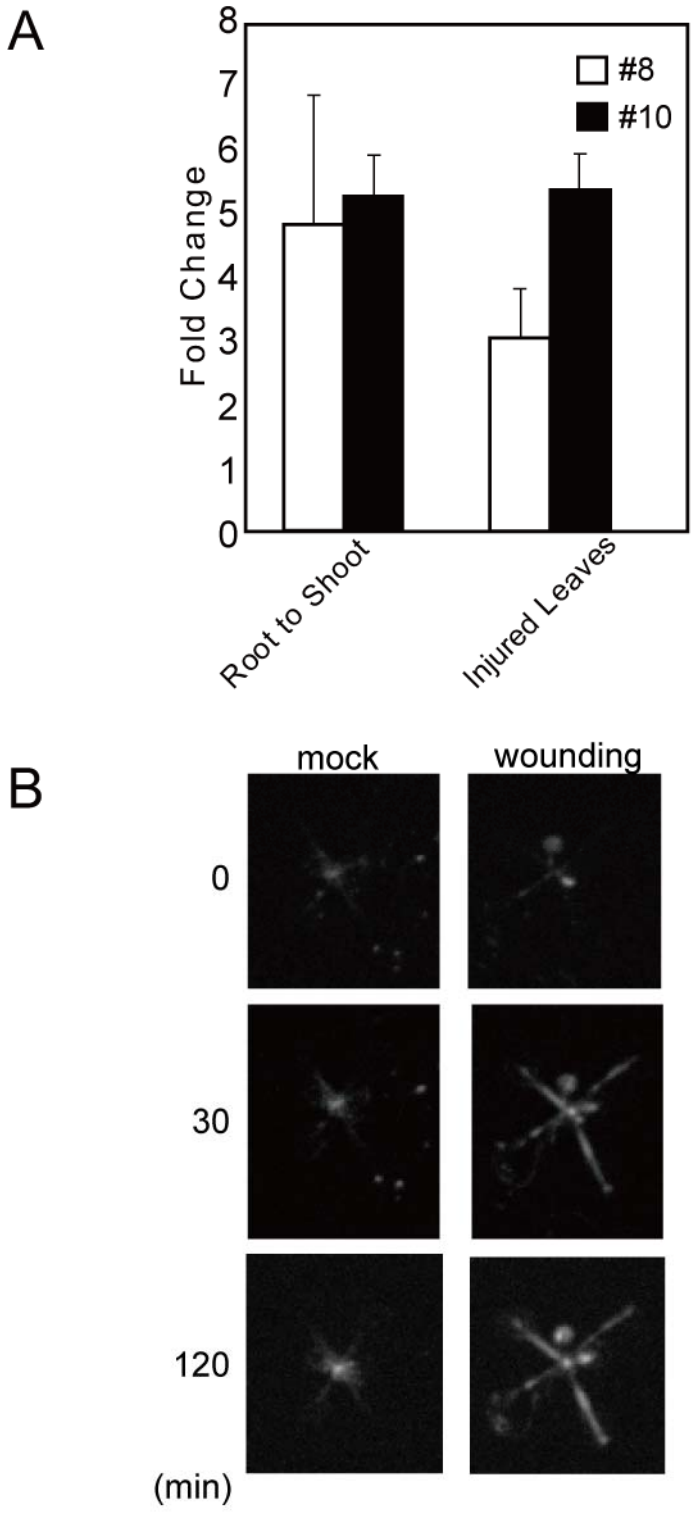
A
Fold Change
0
1
2
3
4
5
6
7
8
#8
#10
Root to Shoot
Injured Leaves
B
mock
wounding
0
30
120
(min)

Figure 2

Figure Legends

Figure 1. JA and OPDA were involved in the regulation of *RtS* gene expression during wound-induced root-to-shoot communication.

When root of seedlings were wounded with a razor blade, primary signals were generated in the wounded roots, and then rapidly transferred to the shoots. In shoots of seedlings with wounded roots, primary signals were converted to chemical signals, JA and OPDA. Increase of JA and OPDA content activate the expression of *RtS* genes in the shoots after 30min and 360 min of root-wounding , respectively.

Figure 2. Visualization of wounding-induced root-to-shoot communication of *AtERF13* expression. (A) Quantitative analysis of luciferase activity of the *AtERF13* promoter::*Luc* plants in shoots of seedlings with wounded roots and wounded leaves (local response). Proteins were extracted from each sample, and the luciferase activity was quantified. Fold changes are relative to those of untreated control samples. Each bar shows mean of three independent samples. Error bars represent the standard deviation (n = 3). Similar results were obtained from two independent *AtERF13* promoter::*Luc* transgenic lines (#8 and #10). (B)

Visualization of wounding-induced root-to-shoot communication of *AtERF13* expression. Two-week-old *AtERF13* promoter::*Luc* #8 seedlings were sprayed with luciferin (Sigma-Aldrich). After 30 min of spraying, roots of seedlings were wounded. Luminescence from seedlings was continuously captured by a charge couple device (CCD) camera. In the right panel, luminescence is shown in the seedling at 0, 30 and 120 min after wounding of the roots. In the left panel, luminescence is shown in a mock-treated seedling.