Arabidopsis thalianaにおけるジャスモン酸経路がサツマイモネコブセンチュウ侵入に及ぼす影響

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Jasmonic acid signaling pathway of *Arabidopsis thaliana* is important for root-knot nematode invasion

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We investigated the interaction between infection of the root-knot nematode (RKN), *Meloidogyne incognita*, and activation of the jasmonic acid (JA) signaling pathway in *Arabidopsis thaliana*. We monitored the RKN infection response of various *Arabidopsis* mutants. In addition, we analyzed effects of methyl jasmonate (MeJA) treatment on RKN infection. The number of invading juveniles in the wild type (WT) was significantly reduced by the application of MeJA. RKN infection in the defective salicylic acid mutant (*npr1-1*) was similar to that of WT. JA over expresser mutants (*sa2-1* and *sa2-7*) had a lower number of juveniles in roots than WT without MeJA treatment, and the repressing effect was not enhanced by MeJA treatment. A negative correlation was found between the activation of the JA related pathway and RKN infection. However, the repressing effect on RKN infection was not changed though JA related genes expressed more than a certain level. RKN infection was also repressed in the JA insensitive mutant (*coi1-1*). This result might suggest that the COI-1 signaling pathway related to plant reactions such as production of metabolites and gene expression is important for RKN infection into roots. These results also indicated that RKN may require the JA pathway for infection and RKN infection may be suppressed when the JA pathway is activated. Nematol. Res. 41 (1), 9-17 (2011)

Key words: gene expression, *Meloidogyne incognita*, methyl jasmonate, signaling pathway

INTRODUCTION

Plants live in complex environments in which they interact with many organisms such as herbivores, pathogens, microbes, and symbionts. These interactions may be very diverse in nature; some interactions are obviously beneficial to the plant (though not necessary). One of the best examples of a beneficial interaction is the plant–pollinator interaction: in obligatory out-crossing plant species, pollinators are often actively attracted to plants by specific volatile and visual cues (Shi *et al.*, 2008; Teichert *et al.*, 2008). Other biotic interactions are detrimental such as attacks by herbivores or pathogens. Consequently, plants have evolved intricate defense mechanisms to protect themselves against detrimental organisms.

The plant hormones jasmonic acid (JA), ethylene (ET) and salicylic acid (SA) mediate a variety of physiological processes, including the basal plant defense responses induced by various biotic stresses such as fungal, bacterial and viral infections (Tuzun and Bent, 2006). The hormonal defense responses in plants are classified into two major pathways: the SA pathway and the JA/ET pathway (Thomma *et al.*, 2001). The SA-mediated defense response provides resistance to biotrophic fungi and bacteria such as *Peronospora parasitica* and *Pseudomonas syringae* (Uknes *et al.*, 1992). Activation of the SA-mediated defense response is related to the hypersensitive response which triggers systemic acquired resistance. In contrast, the JA/ET-mediated pathway appears to regulate resistance against necrotrophic fungi such as *Alternaria brassicicola* and *Botrytis cinerea* (Thomma *et al.*, 1998).

In addition, there are at least two modes of interaction between JA and ET, one synergistic and the other antagonistic (Pieterse *et al.*, 2006). Expression of ß-chitinase (*chiB*) and plant defensin 1.2 (*PDF1.2*), marker genes of the JA/ET pathway, is induced by application of either JA or ET, and a synergistic effect occurs when both hormones are applied. However, expression of vegetative storage protein 2 (*VSP2*) and lipoxygenase 2 (*LOX2*), marker genes of the JA pathway, is induced by JA but not by ET. The effect of JA on the expression of these genes is antagonistically reduced by ET treatment (Lorenzo and Solano, 2005). Campbell *et al.* (2002) reported that expression of some defense-related genes might be regulated through the coordination of multiple signaling cascades. Thus, it is important to examine the involvement of each hormone in each biotic stress response.

It is known that plants have induced resistance responses to root-knot nematodes (RKN) when JA or SA is applied to the plant (Branch *et al.*, 2004; Cooper *et al.*, 2005), but there is little information about interactions between

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the detailed functions of JA and SA and nematode infection. Plant-parasitic nematodes parasitize roots and/or stems of various host plants, affecting the growth and development of the plants (Wyss *et al.*, 1992; Wyss and Zunke, 1998). Among them RKN, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 has a wide host range of over 700 species and varieties (Goodey *et al.*, 1965). RKN are sedentary endoparasitic nematodes, and the second stage juveniles are the infective stage that penetrate behind the root tips and migrate to the vascular cylinder, where they start a close interaction with the host plant. In susceptible hosts, RKN induce a series of complicated changes in the plant cell architecture. Cells undergo repeated karyogenesis without cytogenesis in response to nematode secretions of the esophageal glands. The feeding sites of RKN are known as giant cells and serve as metabolic sources to RKN (Williamson and Gleason, 2003). Cells around the giant cell undergo concurrent hyperplasia and hypertrophy, which are manifested as galls on plant roots. Female nematodes become saccate, reproduce parthenogenetically, and lay eggs on the root surface in a protective gelatinous matrix (Williamson and Gleason, 2003).

In this study, we focused on JA pathways and analyzed the function of JA in the response of *Arabidopsis thaliana* to *M. incognita* infection. We investigated changes in RKN infection of *Arabidopsis* by methyl jasmonate (MeJA) treatment. In addition, we also analyzed RKN infection of the mutants *coi1-1* (JA-insensitive), *npr1-1* (SA-insensitive), *sa2-1* (over expresser of JA) and *sa2-7* (over expresser of JA). The object of this research was to clarify the importance of JA-pathways for RNK infection.

MATERIALS AND METHODS

Plant materials:

Plants (*A. thaliana* ecotype Col-0) were grown in sand as described previously (Weigel and Glazebrook, 2002). Sand with a particle size range of 0.2-0.4 mm in diameter was obtained by wet sieving soil to remove stones, silt and clay. The ecotype Col-0 is defined as the wild type (WT) in this study. Several mutants related to plant hormone biosynthesis and signaling (*coi1-1*, *npr1-1*, *sa2-1*, and *sa2-7*) were used in this study. The mutant *coi1-1* is coronatine insensitive (Xie *et al.*, 1998). The mutant *npr1-1* is a non-expresser of pathogenesis-related genes 1 (Cao *et al.*, 1997). Mutants of *sa* (*sa2-1* and *sa2-7*) are over expressers of JA-related genes (Abe *et al.*, 2003). *Arabidopsis* seeds were sown on sterile sand in pots, moistened, and placed in a cold room at 4°C in the dark to synchronize germination. The pots were then transferred to 22°C with a long-day photoperiod (16 hr light/8 hr dark). Plants at the four-leaf stage were transferred individually to a pot (220 cm^3) filled with sand (approximately 350 g) and grown to the rosette stage.

Identification of *coi1-1* plants:

Homozygous *coi1-1* plants were selected by PCR analysis followed by *Bsm* I digestion. Genomic DNA was extracted from a single rosette leaf of each plant and purified with a DNeasy Plant Kit (Qiagen) and the PCR was performed with ExTaq DNA polymerase (Takara). Nucleotide sequences of primers were as follows: forward primer, 5'-GGA AAC AGG AGC CCG AGA TC-3'; reverse primer, 5'-TGG ATG TTT CTC GGA GCA GC-3'. Amplified DNA was treated with *Bsm* I, which digests DNA from the *coi1-1* mutant only. The reaction mixture was analyzed by agarose gel electrophoresis.

Nematode cultures:

A strain of *M. incognita*, designated MAFF108302 (accession number of NIAS Genbank; http://www.gene.affrc.go.jp/about_en.php), was used for bioassays. This strain was a cloned population that was established from a single female. The nematodes were maintained on tomato plants (cv. Fukuju No.2) and the egg masses were removed with tweezers from infected roots. Then, the collected egg masses were incubated in a small volume of distilled water and infective second-stage juveniles, just after hatching, were collected in the liquid suspension. Nematodes obtained were re-suspended in a suitable volume of distilled water and quantified by counting numbers of nematodes in aliquots of the sample with a light microscope. In this study, the concentration of the nematode suspension was about 200 RKN/ml.

Effects of MeJA application on *M. incognita* invasion of plants:

The local effects of MeJA on RKN invasion were evaluated on *Arabidopisis* plants which had been treated with either MeJA or the carrier solution (five plants/treatment). MeJA (Wako) was dissolved in ethanol at 100 mM and diluted in water to obtain 0.1 mM MeJA solutions. The ethanol carrier was used as the control treatment consisting of a 5% ethanol solution. A plant grown in the pots described above was transferred into a cylindrical acryl chamber (1,000 cm^3) containing 100 ml of the MeJA solution or the same amount of control solution. About 200 second-stage juveniles were injected into the sand surrounding the roots of each plant 48 hr after the plant was transferred and plants remained in the chamber for a total of 9 days. Plants were kept under greenhouse conditions (22°C; 16 hr light/8

hr dark) throughout the assay. The roots of plants were washed 7 days after inoculation to examine RKN invasion. The root fresh weight, shoot fresh weight and shoot height were measured and leaf number of each plant was counted. Then, RKN in roots were stained with acid fuchsin and counted (Byrd *et al.*, 1983). The experiment was conducted twice with 5 replicates (plants) each for a total of ten replicates.

Quantification of plant gene expression using qRT-PCR:

The root samples were collected 48 hr after treatment with MeJA solution and expression of the genes shown in Table 1 was examined. In addition, to investigate the effect of RKN infection combined with MeJA application on the plant gene expression, we inoculated the surrounding sand with about 200 RKN 48 hr after MeJA treatment and the roots were sampled 7 days (168 hr) after inoculation. After sampling, the plant roots were frozen in liquid nitrogen for further analyses. Total RNA (2 μg) isolated by using Trizol reagent (Invitrogen) was treated with RNase-free DNase (Takara) to eliminate genomic DNA contamination. First-strand cDNA was synthesized with random oligo-hexamers using Superscript III reverse transcriptase according to the manufacturer's instructions (Invitrogen). Quantitative reverse transcriptional PCR was carried out with SYBR Green PCR Master Mix (Applied Biosystems) using the first-strand cDNA as a template on a sequence detector system (ABI Prism 7900HT, Applied Biosystems). Expression of *CBP20* was used for normalization as a standard control gene. Nucleotide sequences of the gene-specific primers are described in Table 1. Each treatment had five replicates (plants) and experiments were performed twice. A logarithmic scale was used for the relative gene expression.

Statistical analyses:

The number of RKN detected in roots was used for measuring the effect of applying MeJA solution to plant mutant varieties on nematode infection (Sokal and Rohlf, 1995). Two-way ANOVA followed by Tukey's test and Student's t-test were used to estimate the influence of MeJA on differences in RKN infection. The mean of the dependent variables (*i.e.* number of nematodes in the root) was logarithmically transformed to obtain normality and homoscedasticity. These analyses were performed using JMP (Version 7.0.1, SAS Institute Inc., Cary, NC, USA).

RESULTS

Nematode infection:

MeJA treatment and/or RKN infection did not cause any significant differences in plant growth parameters such as shoot height, shoot weight, number of leaves, and root weight. Two hundred RKN were inoculated to each plant pot but very few juveniles, about 5 to 10% of the initial number, were observed in the roots.

When WT was treated with MeJA, the number of invading juveniles was about half of that in control plants (Fig. 1). The number of invading juveniles in *npr1-1* without MeJA treatment was not different from that in WT plants and the number was reduced by MeJA to a similar level of that in MeJA-treated WT plants. There was a significantly ($P < 0.001$) lower number of juveniles in roots of untreated *coi1-1* than that in WT (Fig. 1). No reduction in number of juveniles was observed in roots of *coi1-1* when the mutant was treated with MeJA. To further assess the role of JA related resistance to RKN we used the mutants *sa2-1* and *sa2-7* which over-express JA-related genes to compare nematode infection with WT and the other mutants. There was a significantly ($P < 0.001$) lower number of nematode juveniles, about 1/4 of that of WT, in both untreated *sa* mutants than that in WT (Fig. 1). There was no difference in nematode numbers between *sa2-1* and *sa2-7* with or without MeJA treatment. The main effects (mutants and MeJA treatment) and their interaction were significant for number of RKN in roots (Fig. 1, Table 2).

Table 1. List of primers used for qRT-PCR analysis of jasmonic acid-pathway-related genes and gene for normalization.

Locus Tag	Gene name	Sense Primer(5'-3')	Anti-sense Primer(5'-3')
At5g24770	*VSP2*; vegetative storage protein 2	GTT AGG GAC CGG AGC ATC AA	AAC GGT CAC TGA GTA TGA TGG GT
At3g45140	*LOX2*; lipoxygenase 2	TTG CTC GCC AGA CAC TTG C	GGG ATC ACC ATA AAC GGC C
At1g32640	*AtMYC2; Arabidopsis thaliana* MYC 2	CTA AAC CAA AGA TTC TAC GCG TTA C	GTT CTT GAT TTG GAG TTT CTC TGA C
At5g12140	*AtCYS1*; cysteine-type endopeptidase inhibitor 1	GAT GAG CAT AAC AAG AAC GAG AAC T	TCA TAG ACC TTA TTG GTC TCA CCA T
At5g44420	*PDF1.2*; plant defensin 1.2	CCA TCA TCA CCC TTA TCT TCG C	TGT CCC ACT TGG CTT CTC G
At3g12500	*chiB*; β-chitinase	ACG GAA GAG GAC CAA TGC AA	GTT GGC AAC AAG GTC AGG GT
At2g14610	*PR1;* pathogenesis-related genes 1	GTT GCA GCC TAT GCT CGG AG	CCG CTA CCC CAG GCT AAG TT
At3g57260	*BGL2*; β-glucosidase 2	GCC GAC AAG TGG GTT CAA GA	AAC CCC CCA ACT GAG GGT T
At5g44200	*CBP20;* cap-binding protein 20	CCT TGT GGC TTT TGT TTC GTC	ACA CGA ATA GGC CGG TCA TC

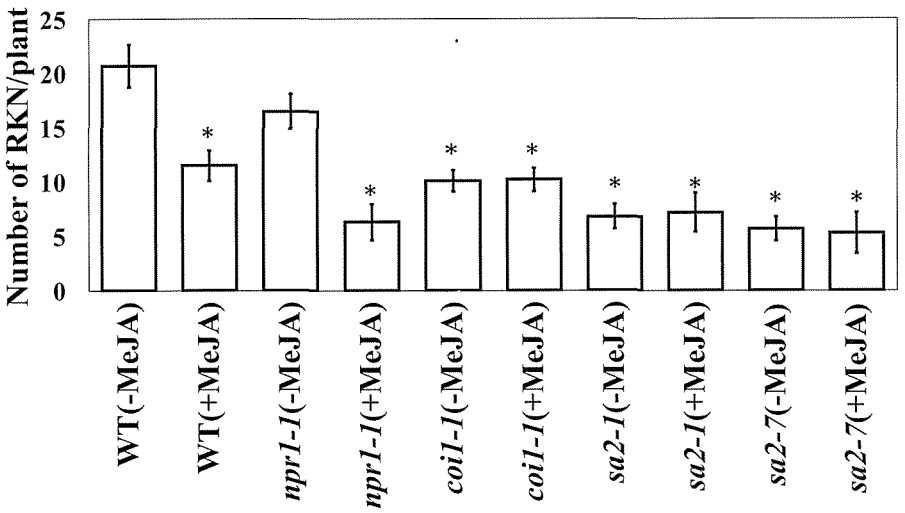

Fig. 1. The number of *Meloidogyne incognita* (RKN) detected 1 week after inoculation in roots of wild type (WT) and mutants of *Arabidopsis* treated with methyl jasmonate (MeJA) or control solution. Each value and error bar represents the mean of 10 replications and standard deviation, respectively. All means marked with an asterisk (*) were significantly lower than the wild type without MeJA treatment (according to Tukey's HSD test).

Table 2. *F*-statistics from two-way ANOVA for significant effects and interaction of the *Arabidopsis* mutant and methyl jasmonate (MeJA) treatment on number of *Meloidogyne incognita* juveniles in roots one week after inoculation.

	df	sum of squares	*F*-value	*P*-value	
Arabidopsis mutant	4	2.80	27.09	< 0.0001	*
MeJA treatment	1	0.65	25.08	< 0.0001	*
Arabidopsis mutant × MeJA treatment	4	0.70	6.75	< 0.0001	*

*: Test significant at the 1% level ($P < 0.01$).

Expression of marker genes involved in JA-related pathway:

We analyzed the JA-, ET- or SA-inducible expression of several marker genes in *Arabidopsis* roots. *AtMYC2*, *AtCYS1*, *VSP2*, and *LOX2* are marker genes of the JA pathway. Expression of these genes did not change between before and after MeJA treatment in *coi1-1* (Fig. 2). *AtMYC2* and *AtCYS1*were induced in WT and other mutants by MeJA treatment. *VSP2* and *LOX2* were also induced in WT and *npr1-1* by MeJA treatment, but there was little change in the expression of these genes before and after the application of MeJA in *sa2-1* and *sa2-7* (Fig. 2). This induction was not observed in WT without MeJA treatment. Similarly, the expression of *chiB* and *PDF1.2*, marker genes of the JA/ET pathway, was also induced in WT and the mutant by MeJA treatment except for *coi1-1*, but expression of the SA-inducible genes *PR1* and *BGL2* was not induced by MeJA treatment (Fig. 2) in any of the plants. Expression of *VSP2* and *chiB*, marker genes of the JA and JA/ET pathways, respectively, was induced less in the JA-insensitive *coi1-1* mutant than in WT plants treated with MeJA (Fig. 2). These marker genes in *sa2-1* and *sa2-7* without MeJA were expressed at higher levels than those in untreated WT. The expression level of these genes in the *sa*-mutants was almost the same as or higher than that of MeJA-treated WT. On the other hand, the gene expression tendency of the root was hardly changed by RKN infection for all mutants except *sa2-1* (data of only *BGL2*, *LOX2* and *chiB* shown in Fig. 3). Some expression patterns of *LOX2* and *chiB* differed between *sa2-1* with and without RKN inoculation.

DISCUSSION

RKN infection decreased in WT and *npr1-1* plant roots when MeJA was applied (Fig. 1). RKN infection was also lower than WT plants in both *sa2-1* and *sa2-7* with and without MeJA treatment. In these interactions, where the nematode infection was lower, activation of (genes involved in) JA and JA/ET pathways was high except for *coi1-1* (Fig. 2). There might be a negative correlation between the activation of JA related pathways and RKN infection of roots. It is known that the reproduction of RKN is repressed when the plant is treated with MeJA (Cooper *et al.*, 2005). We

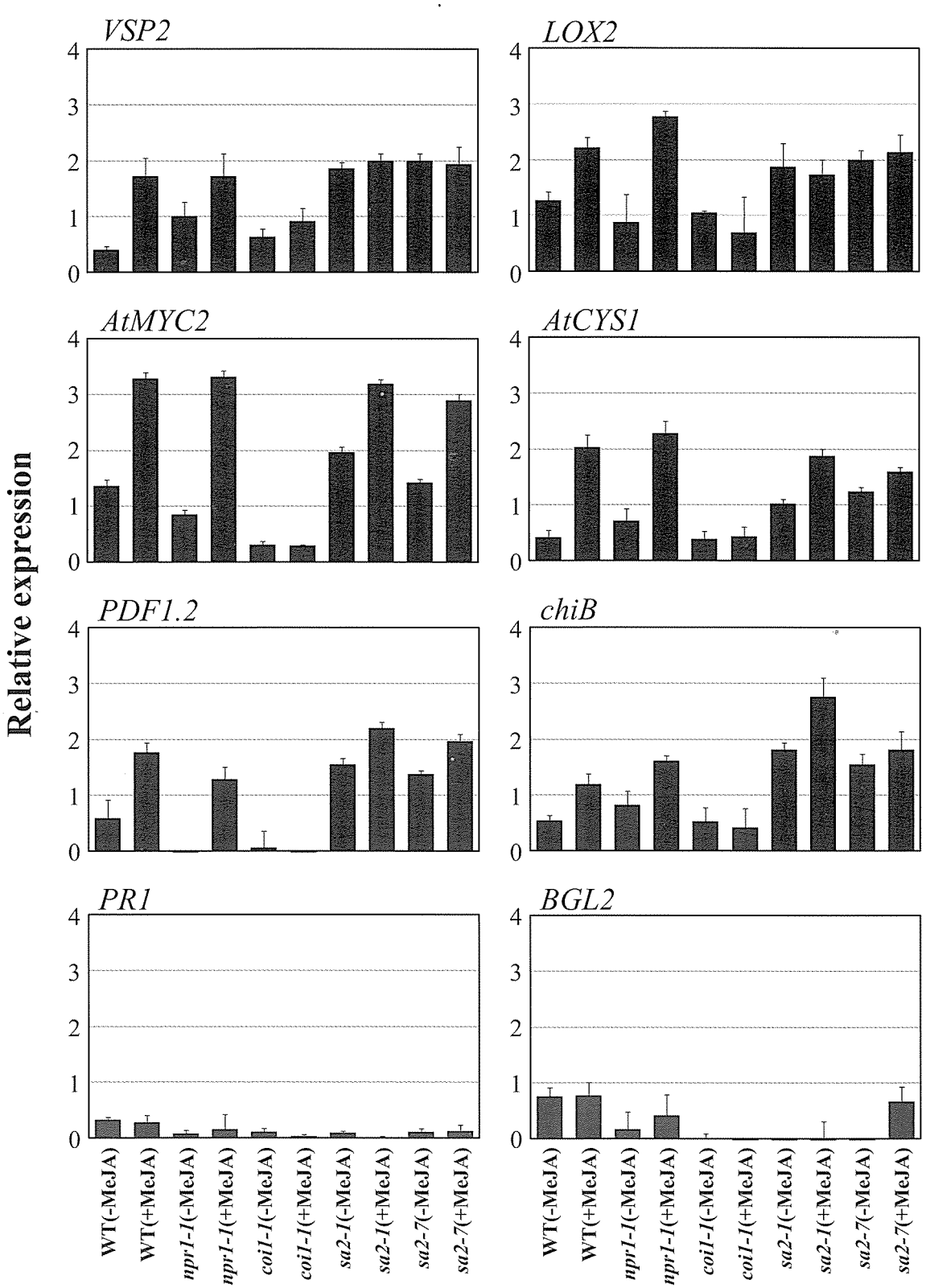

Fig. 2. Expression of marker genes for JA-, JA/ET and SA- signals in roots of wild type (WT) and mutants of *Arabidopsis* treated with methyl jasmonate (MeJA) or a control solution. Total RNA was prepared from the roots of the plants 48 hr after MeJA application. Each value is the mean of 10 replicates, and represents the degree of expression with a relative value. The relative expression is shown with a logarithmic scale.

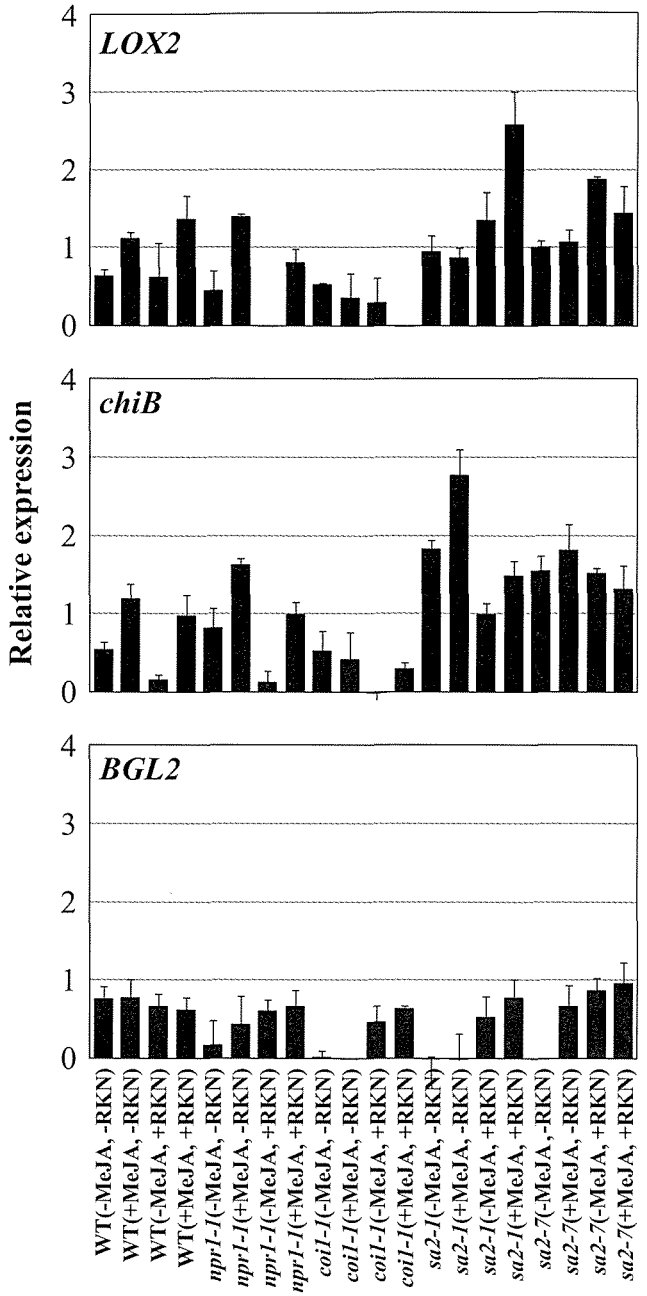

Fig. 3. Comparison of marker genes expression for JA-, JA/ET and SA- signals in roots of wild type (WT) and mutants of *Arabidopsis* treated with methyl jasmonate (MeJA) or a control solution and then inoculated with 200 or 0 root-knot nematodes (RKN), *Meloidogyne incognita*. Total RNA was prepared from the roots of the plants 168 hr after inoculation. Plants were inoculated 48 hr after MeJA application. Each value is the mean of 10 replicates, and represents the degree of expression with a relative value. The relative expression is shown with a logarithmic scale.

found that the RKN infection was repressed when genes involved in JA pathways were highly expressed. In addition, the RKN repressing effect was not changed in JA overexpresser mutants with or without MeJA treatment. This result suggested that a minimum expression level of certain genes may be important for repressing RKN infection (Figs. 1, 2).

In this study *AtCYS1*, one of the genes of cysteine proteinase inhibitor, was induced in WT and *npr1-1* by MeJA treatment. The expression levels of *AtCYS1* in *sa2-1* and *sa2-7* were high before MeJA treatment, and were enhanced by MeJA application. Gene expression of cysteine proteinase inhibitors is closely related to the regulation of cysteine proteinase inhibitor proteins (Zhao *et al.*, 1996). Cysteine proteinase inhibitors have been shown to affect nematode resistance, and have been used to protect various plant species including alfalfa, banana, rice, and tomato against a wide range of nematodes with diverse feeding strategies (Atkinson *et al.*, 1996, 2004; Samac and Smigocki, 2003; Urwin *et al.*, 1995, 1997). These reports assumed repression of nematode parasitism was caused by an increase in cysteine proteinase inhibitor proteins, but our results indicated that RKN root invasion declines when cysteine proteinase inhibitor proteins increase in the plant root. Fujimoto *et al.* (2011) also observed a similar result in tomato. RKN invasion of tomato roots was lower when genes of proteinase inhibitors and cysteine proteinase inhibitor in roots were expressed after foliar application with MeJA. Metabolites in root exudates might change when cysteine proteinase inhibitor proteins increase in roots. These responses are well known as an inducible direct defense against insect herbivores (Chen, 2008) but are not known for roots. In addition, RKN infection was low when *AtMYC2* was expressed in roots in our study. The transcriptional factor MYC2 regulates metabolites in plants (Dombrecht *et al.*, 2007). It is known that RKN respond to metabolites in root exudates (Zhao *et al.*, 2000). RKN might perceive changes in metabolites from roots that reduce nematode invasion.

Susceptibility of the *coi1-1* mutant (without MeJA) to RKN infection was lower than that of the WT (Fig. 1). This result might indicate that JA signaling pathways have a very important role when RKN invade the plant root. RKN infection was repressed by mutants with a defective JA-pathway but we could not specify which pathway is the most important for RKN infection. Bhattarai *et al.* (2008) also observed similar results. They showed that RKN reproduction in the *jai1* mutant, which is able to produce endogenous JA but is impaired in JA perception, was significantly lower than that in WT. On the other hand, the SA-signaling

pathway might be unrelated when RKN is infected. The tendency of RKN infection in *npr1-1* was similar to that of WT. Sanz-Alferez *et al.* (2008) also observed similar results. These reports might also suggest that RKN need intact JA pathway when they parasitize. In addition, Zhao *et al.* (2003) reported that *Pseudomonas syringae* required an intact COI-1 signaling pathway for infection. It is interesting to speculate that, similar to *P. syringae*, RKN may produce a JA functional analog like the phytotoxin coronatine and may target the COI-1 signaling pathway (Bender *et al.*, 1999; Weiler *et al.*, 1994).

The gene expressions in the roots were not changed by the invasion of RKN in most cases, except for *sa2-1* (Fig. 3). We could not specify the gene expression of the feeding site because we had extracted RNA from the entire root. However, some gene expression changed after RKN infection in *sa2-1*. This result might show that the plant gene expression changes when RKN infect roots but more detailed research will have to be done in the future to understand this interaction. Gene expression induced by nematode infection may be specifically regulated at the nematode feeding site. Gheysen and Fenoll (2002) showed that nematode invasion induces changes in plant gene expression, but the majority of up-regulated genes seem to be induced through the establishment of a nematode feeding site (Bar-Or *et al.*, 2005; Bhattarai *et al.*, 2008; Puthoff *et al.*, 2003).

The lower RKN infection on *coi1-1* plants might indicate that the nematode is able to manipulate plant defense responses to its advantage by leveraging the existing cross talk between JA and SA signaling pathways (Bostock, 2005; Rojo *et al.*, 2003). The *coi1-1* mutant does not have a good balance between JA and SA signaling pathways because *coi1-1* is a JA insensitive mutant. RKN infection was also repressed on other mutants when JA-responsive genes were activated. The balance between JA and SA signaling pathways was disrupted before RKN inoculation because JA-responsive genes were expressed strongly in the plant except for *coi1-1* treated or untreated with MeJA. Our results indicate that RKN infection of the plant might be inhibited when the balance between JA and SA signaling pathways is disrupted.

Our results suggest that activation of JA related pathways of the plant is important for repressing RKN infection. In addition, the intact JA pathway may have an important role in RKN root invasion. RKN infection may be suppressed by the activation of the JA pathway. However, we consider the activation of JA related pathways to be one of the key reactions accelerating secondary reactions effective in nematode resistance, such as changes in metabolite release from the root. We also consider this secondary reaction to be important for repressing RKN infection. RKN may be repelled by substances exuded from the root; some research showed that exogenous application of MeJA to roots of oat and spinach and to shoots of tomato enhanced resistance to parasitic nematodes, possibly by elevating the level of compounds toxic to nematodes like phytoectosteroids, flavonoids and proteinase inhibitors (Soriano *et al.*, 2004a and 2004b; Cooper *et al.*, 2005). Jasmonate in plants is closely associated with the production of secondary metabolites such as flavonoids (Gundlach *et al.*, 1992). Emigration of second-stage juveniles of *M. incognita* and *M. javanica* have also been observed out of the roots of peanut and tomato resistant genotypes (Choi *et al.*, 1999; Kouassi *et al.*, 2005). Our future experiments will evaluate the metabolites in root exudates related to RKN infection.

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LITERATURE CITED

Abe, H., Urao, T., Ito, T., Seki, M., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2003) Arabidopsis AtMYC2 (bHLH) and AtMYB2(MYB) function as transcriptional activators in abscisic acid signaling. Plant Cell 15, 63-78.

Atkinson, H. J., Urwin, P. E., Clarke, M. C. and McPherson, M. J. (1996) Image analysis of the growth of *Globodera pallida* and *Meloidogyne incognita* on transgenic tomato roots expressing cystatins. Journal of Nematology 28, 209-215.

Atkinson, H. J., Grimwood, S., Johnston, K. and Green, J. (2004) Prototype demonstration of transgenic resistance to the nematode *Radopholus similis* conferred on banana by a cystatin. Transgenic Research 13, 135-142.

Bar-Or, C., Kapulnik, Y. and Koltai, H. (2005) A broad characterization of the transcriptional profile of the compatible tomato response to the plant parasitic root knot nematode *Meloidogyne javanica*. European Journal of Plant Pathology 111, 181-192.

Bender, C. L., Alarcon-Chaidez, F. and Gross, D. C. (1999) *Pseudomonas syringae* phytotoxins: Mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. Microbiology and Molecular Biology Reviews 63, 266-292.

Bhattarai, K. K., Xie, Q. G., Mantelin, S., Bishnoi, U., Girke, T., Navarre, D. A. and Kaloshian, I. (2008) Tomato sus-

ceptibility to root-knot nematodes requires an intact jasmonic acid signaling pathway. Molecular Plant-Microbe Interactions 21, 1205-1214.

Bostock, R. (2005) Signal crosstalk and induced resistance: Straddling the line between cost and benefit. Annual Review of Phytopathology 43, 545-580.

Branch, C., Hwang, C. F., Navarre, D. A. and Williamson, V. M. (2004) Salicylic acid is part of the *Mi-1*-mediated defense response to root-knot nematode in tomato. Molecular Plant-Microbe Interactions 17, 351-356.

Byrd, D. W. Jr., Kirkpatrick, T. and Barker, K. R. (1983) An improved technique for clearing and staining plant tissues for detection of nematodes. Journal of Nematology 15, 142-143.

Campbell, M. A., Fitzgerald, H. A. and Ronald, P. C. (2002) Engineering pathogen resistance in crop plants. Transgenic Research 11, 599-613.

Cao, H., Glazebrook, J., Clarke, J. D., Volko, S. and Dong, X. (1997) The Arabidopsis *NPR1* gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell 88, 57-63.

Chen, M. S. (2008) Inducible direct plant defense against insect herbivores: A review. Insect Science 15, 101-114

Choi, K., Burow, M. D., Church, G., Burow, G., Paterson, A. H., Simpson, C. E. and Starr, J. L. (1999) Genetics and mechanism of resistance to *Meloidogyne arenaria* in peanut germplasm. Journal of Nematology 31, 283-290.

Cooper, W. R., Jia, L. and Goggin, L. (2005) Effects of jasmonate-induced defenses on root-knot nematode infection of resistant and susceptible tomato cultivars. Journal of Chemical Ecology 31, 1953-1967.

Dombrecht, B., Xue, G. P., Sprague, S. J., Kirkegaard, J. A., Ross, J. J., Reid, J. B., Fitt, G. P., Sewelam, N., Schenk, P. M., Manners, J. M. and Kazan, K. (2007) MYC2 differentially modulates diverse jasmonate-dependent functions in *Arabidopsis*. Plant Cell 19, 2225-2245.

Fujimoto, T., Tomitaka, Y., Abe, H., Tsuda, S., Futai, K. and Mizukubo, T. (2011) Expression profile of jasmonic acid-induced genes and the induced resistance against the root-knot nematode (*Meloidogyne incognita*) in tomato plants (*Solanum lycopersicum*) after foliar treatment with methyl jasmonate. Journal of Plant Physiology 168, 1084-1097.

Gheysen, G. and Fenoll, C. (2002) Gene expression in nematode feeding sites. Annual Review of Phytopathology 40, 191-219.

Goodey, J. B., Franklin, M. T. and Hooper, D. J. (1965) The nematode parasites of plants catalogued under their hosts. 3rd edition. Commonwealth Agricultural Bureaux, Slough, 214 pp.

Gundlach, H., Muller, M. J., Kutchan, T. M. and Zenk, M. H. (1992) Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. Proceedings of the National Academy of Sciences of the United States of America 89, 2389-2393.

Kouassi, A. B., Kerlan, M. C., Sobczak, M., Dantec, J. P., Rouaux, C., Ellisseche, D. and Mugniery, D. (2005) Genetics and phenotypic characterisation of the hypersensitive resistance of *Solanum sparsipilum* to *Meloidogyne incognita*. Nematology 7, 213-225.

Lorenzo, O. and Solano, R. (2005) Molecular players regulating the jasmonate signalling network. Current Opinion in Plant Biology 8, 532-540.

Puthoff, D. P., Nettleton, D., Rodermel, S. R. and Baum, T. J. (2003) *Arabidopsis* gene expression changes during cyst nematode parasitism revealed by statistical analyses of microarray expression profiles. Plant Journal 33, 911-921.

Rojo, E., Solano, R. and Sanchez-Serrano, J. J. (2003) Interactions between signaling compounds involved in plant defense. Journal of Plant Growth Regulation 22, 82-98.

Samac, D. A. and Smigocki, A. C. (2003) Expression of oryzacystatin I and II in alfalfa increases resistance to the root-lesion nematode. Phytopathology 93, 799-804.

Sanz-Alferez, S., Mateos, B., Alvarado, R. and Sanches, M. (2008) SAR induction in tomato plants is not effective against root-knot nematode infection. European Journal of Plant Pathology 120, 417-425.

Shi, J., Luo, Y. B., Bernhardt, P., Ran, J. C., Liu, Z. J. and Zhou, Q. (2008) Pollination by deceit in *Paphiopedilum barbigerum* (Orchidaceae): a staminode exploits the innate colour preferences of hoverflies (Syrphidae). Plant Biology 11, 17-28.

Sokal, R. R. and Rohlf, F. J. (1995) Biometry third edition. W. H. Freedman and Company, New York, 887 pp.

Soriano, I., Asenstorfer, R., Schmidt, O. and Riley, I. (2004a) Inducible flavone in oats (*Avena sativa*) is a novel defence against plant parasitic nematodes. Phytopathology 94, 1207-1214.

Soriano, I., Riley, I., Potter, M. and Bowers, W. (2004b) Phytoectysteroids: A novel defence against plant parasitc nematodes. Journal of Chemical Ecology 30, 1885-1899.

Teichert, H., Do¨tterl, S., Zimma, B., Ayasse, M. and Gottsberg, G. (2008) Perfume-collecting male euglossine bees as pollinators of a basal angiosperm: the case of *Unonopsis stipitata* (Annonaceae). Plant Biology 11, 29-37.

Thomma, B., Eggermont, K., Penninckx, I., Mauch-Mani, B.,

Vogelsang, R., Cammue, B. P. A. and Broekaert, W. F. (1998) Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. Proceedings of the National Academy of Sciences of the United States of America 95, 15107-15111.

Thomma, B. P., Penninckx, I. A., Broekaert, W.F. and Cammue, B. P. (2001) The complexity of disease signaling in *Arabidopsis*. Current Opinion in Immunology 13, 63-68.

Tuzun, S. and Bent, E. (2006) Multigenic and Induced Systemic Resistance in Plants. Springer, New York. 521 pp.

Uknes, S., Mauch-Mani, B., Moyer, M., Potter, S., Williams, S., Dincher, S., Chandler, D., Slusarenko, A., Ward, E. and Ryals, J. (1992) Acquired resistance in *Arabidopsis*. Plant Cell 4, 645-656.

Urwin, P. E., Atkinson, H. J., Waller, D. A. and McPherson, M. J. (1995) Engineered oryzacystatin-I expressed in transgenic hairy roots confers resistance to *Globodera pallida*. Plant Journal 8, 121-131.

Urwin, P. E., Lilley, C. J., McPherson, M. J. and Atkinson, H. J. (1997) Resistance to both cyst and root-knot nematodes conferred by transgenic Arabidopsis expressing a modified plant cystatin. Plant Journal 12, 455-461.

Weigel, D. and Glazebrook, J. (2002) *Arabidopsis*, A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York, 448 pp.

Weiler, E. W., Kutchan, T. M., Gorba, T., Brodschelm, W., Niesel, U. and Bublitz, F. (1994) The *Pseudomonas* phytotoxin coronatine mimics octadecanoid signalling molecules of higher plants. FEBS Letters 345, 9-13.

Williamson, V. M. and Gleason, C. A. (2003) Plant-nematode interactions. Current Opinion in Plant Biology 6, 327-333.

Wyss, U., Grundler, F. M. W. and Munch, A. (1992) The parasitic behavior of second stage juveniles of *Meloidogyne incognita* in root of *Arabidopsis thaliana*. Nematologica 38, 98-111.

Wyss, U. and Zunke, U. (1998) Observations on the behavior of second juveniles of *Heterodera schachtii* inside host roots. Revue de Nématologie 9, 153-165.

Xie, D. X., Feys, B. F., James, S., Nieto-Rostro, M. and Turner, J. G. (1998) *COI1*: An *Arabidopsis* gene required for jasmonate-regulated defense and fertility. Science 280, 1091-1094.

Zhao, Y., Botella, M. A., Subramanian, L., Niu, X., Nielsen, S. S., Bressan, R. A. and Hasegawa, P. M. (1996) Two wound-inducible soybean cysteine proteinase inhibitors have greater insect digestive proteinase inhibitory activities than a constitutive homolog. Plant Physiology 111, 1299-1306.

Zhao, X., Schmitt, M. and Hawes, M. C. (2000) Species dependent effects of border cell and root tip exudates on nematode behavior. Phytopathology 90, 1239-1245.

Zhao, Y., Thilmony, R., Bender, C. L., Schaller, A., He, S. Y. and Howe, G. A. (2003) Virulence systems of *Pseudomonas syringae* pv. *Tomato* promote bacterial speck disease in tomato by targeting the jasmonate signaling pathway. Plant Journal 36, 485-499.

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英文論文（本報・短報）の和文摘要

植物のジャスモン酸（JA）経路がサツマイモネコブセンチュウ（RKN）の侵入に及ぼす影響をシロイヌナズナの多様な植物ホルモン合成経路変異体を用いて評価した。野生株（WT）とサリチル酸経路欠損体（*npr1-1*）はジャスモン酸メチル（MeJA）に浸漬すると、無処理時に比べRKNの侵入が有意に低下した。JA経路過剰発現体（*sa2-1*、*sa2-7*）におけるRKNの侵入数は、MeJAを処理した場合も無処理と変わらず、ともにWTへのMeJA処理時と同等であった。RKNの侵入が抑制されたとき、植物のJA経路が活化されており、JA経路の活化とRKNの侵入との間に負の相関があることが判明した。JA経路欠損体（*coi1-1*）ではMeJAを処理してもJA関連遺伝子は発現しなかったが、RKNの侵入はWTのMeJA処理時と同等であった。これらの結果から、植物への侵入の際にRKNがJA経路に影響を受けることが示唆された。