

Jasmonic acid carboxyl methyltransferase: A key enzyme for jasmonate-regulated plant responses

Hak Soo Seo*, Jong Tae Song*, Jong-Joo Cheong*, Yong-Hwan Lee*, Yin-Won Lee*, Ingyu Hwang*, Jong Seob Lee†, and Yang Do Choi*‡

*School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea; and †School of Biological Sciences, Seoul National University, Seoul 151-742, Korea

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Methyl jasmonate is a plant volatile that acts as an important cellular regulator mediating diverse developmental processes and defense responses. We have cloned the novel gene *JMT* encoding an *S*-adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase (JMT) from *Arabidopsis thaliana*. Recombinant JMT protein expressed in *Escherichia coli* catalyzed the formation of methyl jasmonate from jasmonic acid with K_m value of 38.5 μ M. *JMT* RNA was not detected in young seedlings but was detected in rosettes, cauline leaves, and developing flowers. In addition, expression of the gene was induced both locally and systemically by wounding or methyl jasmonate treatment. This result suggests that *JMT* can perceive and respond to local and systemic signals generated by external stimuli, and that the signals may include methyl jasmonate itself. Transgenic *Arabidopsis* overexpressing *JMT* had a 3-fold elevated level of endogenous methyl jasmonate without altering jasmonic acid content. The transgenic plants exhibited constitutive expression of jasmonate-responsive genes, including *VSP* and *PDF1.2*. Furthermore, the transgenic plants showed enhanced level of resistance against the virulent fungus *Botrytis cinerea*. Thus, our data suggest that the jasmonic acid carboxyl methyltransferase is a key enzyme for jasmonate-regulated plant responses. Activation of *JMT* expression leads to production of methyl jasmonate that could act as an intracellular regulator, a diffusible intercellular signal transducer, and an airborne signal mediating intra- and interplant communications.

Emission of volatiles is among the diverse mechanisms plants use to withstand various environmental stresses. The plant volatiles may be attractive for pollinators and seed-dispersing animals or repellent for a wide range of herbivores. Accumulating evidence suggests that the synthesis of volatiles is also induced by certain external challenges of pathogens and herbivorous animals (1). Not only the attacked plant but also neighboring plants are affected, becoming more attractive to herbivore predators and less susceptible to invaders (2).

A variety of methyl esters of secondary metabolites constitute an important portion of the plant volatiles. For instance, methyl jasmonate (MeJA) is a fragrant compound initially identified from flowers of *Jasminum grandiflorum* (3), and that is ubiquitously distributed in the plant kingdom (4). MeJA and its free acid jasmonic acid (JA), collectively referred to as jasmonates, are important cellular regulators mediating diverse developmental processes, such as seed germination, flower and fruit development, leaf abscission, and senescence (5). In addition, jasmonates induce plant defense responses against a group of pathogens (6) and mechanical or herbivorous insect-driven wounding (7). In particular, MeJA has become a strong candidate for airborne signals that mediate interplant communication for defense responses (8).

Jasmonates are synthesized in plants via the octadecanoid pathway (9, 10). Briefly, linolenic acid is oxygenated by lipoxygenase (LOX), and then converted to 12-oxo-phytodienoic acid (12-oxo-PDA) by allene oxide synthase (AOS) and allene oxide

cyclase. Jasmonic acid is synthesized from the 12-oxo-PDA through reduction and three steps of β -oxidation. Cellular organelles such as plastids (11) or peroxisomes (12) are regarded as the primary site(s) of JA biosynthesis. In addition, a cytoplasmic pathway has also been described (13). JA is then catabolized further to form its volatile counterpart MeJA and numerous conjugates (4).

Because of the similarity of JA to the animal prostaglandin in structure and biogenesis, this compound has been regarded as the primary intracellular transducer. However, it has been observed that a number of biosynthetic intermediates, isomers, derivatives, and metabolites of the octadecanoid pathway are also powerful cellular regulators, depending on biological system (4, 10). For instance, the octadecanoid precursors of JA, including linolenic acid, 13(*S*)-hydroperoxylinolenic acid and 12-oxo-PDA (14), and a hexadecanoid dinor-oxo-PDA (15) are known to have biological activities in the jasmonate-regulated responses. In addition, a component of flower volatiles *cis*-jasmone is induced on damage to plant vegetative tissue (16, 17), and has roles as an insect semiochemical and in plant defense (18). The exact role and relative importance of each jasmonate derivative for the cellular responses has not been clearly defined.

Activation of JA biosynthesis is not enough to explain the complexity in biological roles of this family of compounds. It was reported that overexpression of the plastidic flax *AOS* cDNA caused an increase in JA levels in transgenic potato plants, but this increase did not activate jasmonate-responsive genes (11). Moreover, overexpression of a cytoplasm-localized flax *AOS* (13) or the *Arabidopsis AOS* cDNA (19) failed to alter the basal level of jasmonates in the transgenic tobacco and *Arabidopsis*. Thus, there might be additional key regulatory points for either the accumulation of jasmonates in the cytoplasm or the generation of signal transducers other than JA.

MeJA could be a candidate for such intra- and intercellular signal transducers mediating jasmonate-responsive plant responses, because it can diffuse through the membranes. Characterization of cellular component(s) catalyzing the formation of MeJA would help clarify jasmonate-mediated plant responses.

We have identified an enzyme that catalyzes the methylation of JA to form MeJA. Previously, a floral nectary-specific gene *NTRI* was isolated from *Brassica campestris* (20), which has structural features similar to those of *SAMT* encoding an *S*-

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Abbreviations: JMT, *S*-adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase; MeJA, methyl jasmonate; JA, jasmonic acid; SAM, *S*-adenosyl-L-methionine; AOS, allene oxide synthase; 12-oxo-PDA, 12-oxo-phytodienoic acid; RT-PCR, reverse transcription-PCR.

Data deposition: The nucleotide sequences of *JMT* have been deposited in the GenBank database (accession nos. AY008434 for cDNA and AY008435 for genomic DNA).

*To whom reprint requests should be addressed. E-mail: choiyngd@snu.ac.kr.

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adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase (21). An *Arabidopsis* *NTR1* homolog, *JMT*, has been cloned to perform molecular biological studies in more detail. Characteristics of *JMT* indicated that the gene encodes an *S*-adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase (*JMT*), a key enzyme for the jasmonate-regulated plant responses. We propose a possible role of MeJA as an intracellular regulator and a long distance signal mediating intra- and interplant communications.

Materials and Methods

Cloning of *JMT*. The gene *JMT* was isolated from a cDNA (22) and a genomic library of *A. thaliana* (Columbia ecotype) by screening with the *Brassica* *NTR1* (20). The genomic library was constructed into the *Xho*I site of partially filled λ FixII vector (Stratagene, Heidelberg, Germany). Inserts in the phage DNAs hybridizing with the *NTR1* probe were isolated and subcloned into pBluscriptSK(+)^{II} vector. Nucleotide sequences of the overlapping partial clones were determined by using an automated DNA sequencer (Applied Biosystems, model PRISM 377).

Purification of Recombinant *JMT* Protein. *JMT* cDNA was inserted into the pGEX-2T vector (Amersham Pharmacia) at the *Eco*RI site and fused with glutathione-*S*-transferase gene (*GST*). The recombinant was transformed into *E. coli* BL21. Expressed *GST*-*JMT* fusion proteins were purified by using glutathione-agarose beads (Sigma). Further purification was conducted by using a Superdex 300 h 10/30 column on the Superdex 200 FPLC (Amersham Pharmacia) to electrophoretic homogeneity. Fractions exhibiting *JMT* activity were pooled and dialyzed against the Superdex 200 buffer containing 50 mM Tris·HCl (pH 7.5), 100 mM KCl, 5 mM EDTA, and 10 mM β -mercaptoethanol. Purified enzymes were stored at 4°C for 2 months without significant loss of the activity.

Protein concentrations were determined by the method of Bradford (23) by using BSA as a standard.

Assays of *JMT* Activity and Jasmonate Contents. *JMT* activity was determined by measuring the production of MeJA from 1 mM JA and 1 mM *S*-adenosyl methionine (SAM) including 6.4 μ M [¹⁴C]SAM. The 50- μ l assay buffer contained 50 mM Tris·HCl (pH 7.5), 100 mM KCl, and 10 mM β -mercaptoethanol. The reaction mixture was incubated at 20°C for 30 min, and MeJA was extracted with 100 μ l of ethyl acetate. Amounts of MeJA synthesized were determined either by counting the radioactivity in the organic phase by using a liquid scintillation counter or by GC-MS.

For GC-MS analyses, total jasmonates were extracted from the enzyme assay mixture or *Arabidopsis* leaves with 1:2 (vol/vol) mixture of hexane/dichloromethane as described (15). The concentrated samples were separated into MeJA and JA fractions by isocratic silical gel HPLC. MeJA fractions were analyzed directly by GC-MS as described (7), and JA fractions were analyzed after methylation with diazomethane. Total jasmonates were the sum of results obtained from both fractions. Recovery of the standard 9,10-dihydromethyljasmonate added before leaf grinding ranged between 30% and 40%.

In the kinetics studies, appropriate enzyme concentration (20 pmol) and incubation times were taken at which the reaction velocity was linear during the incubation time. K_m and V_m values for each substrate were calculated from Lineweaver-Burk plots.

Reverse Transcription (RT)-PCR and Blot Analyses. For Northern blot analysis, 5 μ g of total RNA isolated from leaves of *Arabidopsis* was electrophoresed on a formaldehyde agarose gel. Alternatively, 2 μ g of total RNA was amplified by RT-PCR, and the products were analyzed by Southern blotting.

		Motif I	
<i>JMT</i>	MEVMR-VLHNN-KGANGETSYAKNSIQSNII SLGRRVMDALKKLMNRNS-EISSIGIADLGCSSGPN	66	
<i>NTR1</i>	MEVMR-VLHNN-KGANGETSYAKNSIQSNII SLGRRVMDALKKLMNRNS-EILSFGIADLGCSSGPN	66	
<i>SAMT</i>	MDVRO-VLHNN-KGANGETSYAKNSIQSNII SLGRRVMDALKKLMNRNS-EILSFGIADLGCSSGPN	66	
<i>BAMT</i>	MKVMKLLCNIIAGDGETSYANNISGLQVMMSKSLHVLDELTKDIIGDHVGFKPKVMMDGCSGPN	69	
		* * * * *	
<i>JMT</i>	LLSISNIVDTIHNLCPLDRP-VPELRSVSLNDLPSNDFNYICASLPEFYDRVK---NYESLGFGRGGG	131	
<i>NTR1</i>	LLSISNIVETIHNLCPLDRP-VPELRSVSLNDLPSNDFNYIFASLPEFYDRVNRKRDNKMSLGFHGSG	134	
<i>SAMT</i>	LFATVELIKTVEELRKKMGRENSPEYQIFLNDLPGDNFNAIFRSLPIENDVGG-----	119	
<i>BAMT</i>	LLVMSGIINTIEDLYTEKNIINELPEFEVFLNDLPGDNFNLKLL--SHENGN-----	120	
<i>JMT</i>	ESCFYSAVPGSFYGRLLFRRSLHFVHSSSSLHMLSDVPCREAEKED-RTITADLENMCKIYLSKTSPPKS	199	
<i>NTR1</i>	GPCFYSAVPGSFYGRLLFRRSLHFVHSSSSLHMLSDVPCREAEKED-RTITADLDNRGKIYLSKTSPPKS	203	
<i>SAMT</i>	-VCFINGVPGSFYGRLLFRRSLHFVHSSSSLHMLSDVPGSVEKED-RTITADLENMCKIYLSKTSPPKS	175	
<i>BAMT</i>	--CFYVPGSFYGRLLPKKSLHFAYSSYIHLSDVPGSVEKED-----NNRQNIYMATSPPE	176	
		* * * * *	
		Motif III	
<i>JMT</i>	AHKAYALQFOTDFVFLRSRSEELVPGGRMVLDFLGRSSDPDTEESCOWELLAAQALMSMAKGIIEE	268	
<i>NTR1</i>	AHKVYALQFOTDFVFLRSRSEELVPGGRMVLDFLGRSSDPDTEESCOWELLAAQALMSMAKGIIEE	272	
<i>SAMT</i>	VLNAYIKQFQEDHALFLRCRAQEVVPGGRMVLDFLGRSSDPDTEESCOWELLAAQALMSMAKGIIEE	244	
<i>BAMT</i>	VYKAYAQYERDFSTFLKRGEEIVPGGRMVLDFLGRSSDPDTEESCOWELLAAQALMSMAKGIIEE	245	
		* * * * *	
<i>JMT</i>	EKIDAFNAPYYAASPEELKMIKEEGSFSIDRLEISPIDWEGGSISEESYDLAIRSKPEALASGRVRNS	337	
<i>NTR1</i>	ENIDAFNAPYYAASPEELKMIKEEGSFSIDRLEISPIDWEGGSISDDSYDI-VRFKPEALASGRVAK	340	
<i>SAMT</i>	EKMDFKIPQYTPSPTEVEAEILKEGSFLDHIIEASEIYWSCSTKGGGGG-----VEEYGVNAR	306	
<i>BAMT</i>	DDLYSFNIPISYPCREVEAAIILEGGSFIDRLERFVRVCDASDYTDODDGG-DPSIFGQRSGKRVAD	313	
<i>JMT</i>	TIRAVVEPMLPEPTFGENVDELFEYAKIVGEYFYVSSPRYAVIVLSLVRTG-	389	
<i>NTR1</i>	TIRAVVEPMLPEPTFGQKVMDELFEYAKIVGEYFYVSSPRYAVIVLSLRLMG-	392	
<i>SAMT</i>	CMRAVAEPLLDLHFGEAIIEDVFHRYKLLIIEKMSKTEKFIINIVLSLIRKSD	359	
<i>BAMT</i>	CVRAIITERNLASHFGSTIMDLLFGKYAKKIVEHLSVENSYSYFIVLSLRR--	364	

Fig. 1. Comparison of the *JMT* amino acid sequence and related methyl transferases. *JMT*, *Brassica* *NTR1* (20), *C. breweri* *SAMT* (21), and *A. majus* *BAMT* (27) were aligned with the CLUSTAL X. Shaded boxes represent amino acid residues that are identical among the three proteins. Gaps (as hyphens) are introduced to maximize the alignment. Amino acid residues identical to those of the motifs I and III that are conserved in *O*-methyltransferases for the binding of methyl donor are indicated by asterisks.

For genomic Southern blot analysis, 5 μ g of *Arabidopsis* genomic DNA was digested with *Hind*III and electrophoresed. Blots were probed with the random primer-extended *JMT* cDNA.

Transgenic *Arabidopsis*. Full-length *JMT* cDNA was digested with *Afl*3 and inserted into the *Sma*I site of pBI121 (CLONTECH) in sense orientation under the control of the cauliflower mosaic virus 35S (CaMV 35S) promoter. The construct was transformed into 6-week-old *Arabidopsis* via *Agrobacterium tumefaciens* C58C1 after the floral dip transformation procedure (24). The plants were grown at 22°C and 54% relative humidity and exposed to cycles of white light (16 h) and darkness (8 h). Five independent T2 plants were selected and used for further analysis. *JMT* mRNA expression levels of the transgenic lines were similar to each other. Thus, the line 1 plants were chosen for further experiments.

Plant Treatments and Inoculation. Wound treatment was carried out on leaves of 5-week-old *Arabidopsis* by cutting with scissors and pinching with forceps. MeJA, ethylene or 12-oxo-PDA was applied at a concentration of 50 μ M. Samples were collected 2 h after wounding, or 48 h after the chemical treatments. During the treatments, plants were kept in an open area, but the treated leaves were enclosed in a plastic bag to separate from distal leaves.

For the pathogen-resistance tests, 7-week-old *Arabidopsis* plants were inoculated with the virulent pathogen *B. cinerea*. Conidial spores (1×10^5 per ml) suspended in potato dextrose broth were sprayed onto the plants until droplets ran off. Inoculated plants were incubated at 100% relative humidity for 1 day, transferred into growth chamber maintaining 90% relative humidity, and grown further for 2 days.

Results

Structural Features of *JMT*. The *JMT* cDNA and genomic DNA sequence revealed a putative 45-kDa polypeptide consisting of 389-aa residues spanning 4 exons (Fig. 1). The *JMT* protein

Table 1. Relative methyltransferase activity of *Arabidopsis* JMT on jasmonic acid and related substrates

Substrates	Relative activity, %
(±) Jasmonic acid	100
Dihydrojasmonic acid	18
Linolenic acid	<1
12-oxo-phytodienoic acid	<1
Salicylic acid	<1
Benzoic acid	<1
Cinnamic acid	<1

All substrates were tested at a 1 mM concentration, and methyltransferase activity was determined by measuring the radioactivity of transferred [¹⁴C]methyl group from SAM. The relative activity of JMT with jasmonic acid was set arbitrarily at 100%. Values are the averages of three independent measurements.

contains motifs that are conserved among *O*-methyltransferases (25, 26), including the binding sites (the motifs I and III) of SAM, a well-known methyl donor in plant cells. In particular, the JMT protein showed 85, 43, and 40% amino acid sequence similarities with *B. campestris* NTR1, an orthologue of JMT (20), *Clarkia breweri* SAMT, a SAM:salicylic acid carboxyl methyltransferase (21), and *Antirrhinum majus* BAMT, a SAM:benzoic acid carboxyl methyltransferase (27), respectively. The SAM-binding motifs of JMT and NTR1 occur in the N-terminal portion of the protein, instead of C-terminal side as observed in other *O*-methyltransferases. In addition, the number of amino acid residues between the two motifs is also beyond the range that has been observed in other SAM-using *O*-methyltransferases. JMT lacks an organelle-specific transit signal peptide and hydrophobic regions long enough to be integrated in membrane, and thus appears to be a cytoplasmic enzyme.

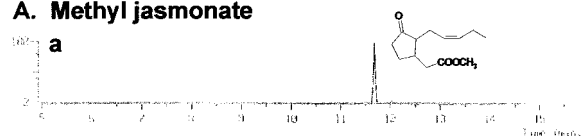
Enzyme Activity of JMT Protein. To test the enzyme's activity, affinity-purified recombinant JMT was incubated with various putative substrates in the presence of SAM (Table 1). JA reacted with JMT to produce its methyl ester, MeJA. GC-MS analysis demonstrated that the reaction product from JA was authentic MeJA (Fig. 2). In contrast, 9,10-dihydrojasmonic acid was a poor substrate for JMT, and JMT did not convert 12-oxo-PDA, salicylic acid (SA), benzoic acid, linolenic acid, and cinnamic acid to the corresponding methyl esters (Table 1).

Optimal conditions for MeJA production by JMT were determined (data not shown). Highest enzyme activity was observed at between pH 7.0–8.0, at 20°C, and in the presence of 100 mM KCl. Presence of divalent ions such as Ca²⁺, Cu²⁺, Fe²⁺, Mg²⁺, Mn²⁺, and Zn²⁺ in the reaction mixture was inhibitory for the JMT enzyme activity. Approximately 80% of the enzyme activity was abolished by divalent ions at 5 mM concentration. At the same concentration, monovalent ions such as Na⁺ and NH₄⁺ also exhibited inhibitory effect, reducing the MeJA production by 50–60%.

Under the standard enzyme reaction conditions described in *Materials and Methods*, kinetic parameters for the two substrates, JA and methyl donor SAM, were calculated from Lineweaver-Burk plots. *K_m* values of JMT for JA and SAM were 38.5 μM and 6.3 μM, respectively (Table 2). Turnover number *k_{cat}* values for the two substrates were 25 S⁻¹ and 70 S⁻¹, respectively.

Expression Pattern of JMT in *Arabidopsis*. RT-PCR analysis revealed that *JMT* was expressed differentially in different organs, developmental stages, and in response to various stimuli. *JMT* RNA was not detected in young seedlings but was expressed in most parts of mature plants, particularly in rosettes, cauline leaves, and flowers (Fig. 3A). Expression of *JMT* in the flower was temporally regulated with the highest expression during the

A. Methyl jasmonate



B. JMT reaction product

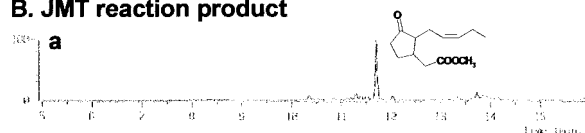


Fig. 2. Assay of JMT activity by GC-MS analysis. (A) MeJA standard. (B) Reaction product from JA by JMT (10 pmol) under the standard assay condition (*Materials and Methods*). The reaction products were extracted with ethyl acetate and analyzed by GC-MS. (a) Mass chromatograms; (b) mass spectrums. The mass of MeJA is 224 Da.

opening of flower at the stage 13 (28); expression was reduced abruptly on flower opening (Fig. 3B). *JMT* expression was induced by wounding or MeJA treatment but not by ethylene (Fig. 3C), exhibiting similar to the expression pattern of the JA-inducible gene *JR2* (29). *JMT* was not induced when plants were treated with both MeJA and ethylene, whereas *PDF1.2* was synergistically induced by this treatment. JMT was also induced in distal leaves systemically by wounding or MeJA treatment, but not by 12-oxo-PDA (Fig. 3D).

Transgenic *Arabidopsis* Overexpressing *JMT*. Full-length JMT cDNA (1.5 kb) was fused with the CaMV 35S promoter, and transformed into *Arabidopsis* to generate transgenic plants overproducing the JMT. Genomic Southern blot analysis confirmed successful integration of the transgene in the genome of the transgenic plants (1.2-kb *Hind*III fragment in the line 1) where the endogenous gene (6.5-kb fragment) is present as a single copy (Fig. 4A). In Northern blot analysis, *JMT* cDNA probe detected 1.5-kb transcripts overexpressed in leaves of the transgenic plants (Fig. 4B). We have analyzed five independent T2 plants. Phenotypic characteristics such as mRNA expression levels, MeJA concentrations, and responses to the various treatments were similar in all lines.

GC-MS analysis revealed that the transgenic plants contained

Table 2. Kinetic parameters of JMT

Substrate	<i>K_m</i> , μM	<i>V_m</i> , nmol/min	<i>k_{cat}</i> , s ⁻¹	<i>k_{cat}</i> / <i>K_m</i> , μM ⁻¹ ·s ⁻¹
S-adenosyl methionine	6.3 ± 0.7	84 ± 8.2	70 ± 6.8	11.1 ± 0.9
(±) Jasmonic acid	38.5 ± 5.8	30 ± 3.6	25 ± 3.0	0.7 ± 0.05

The values are the average of three independent measurements. Each value was obtained by Lineweaver-Burk plots, which were linear within experimental error.

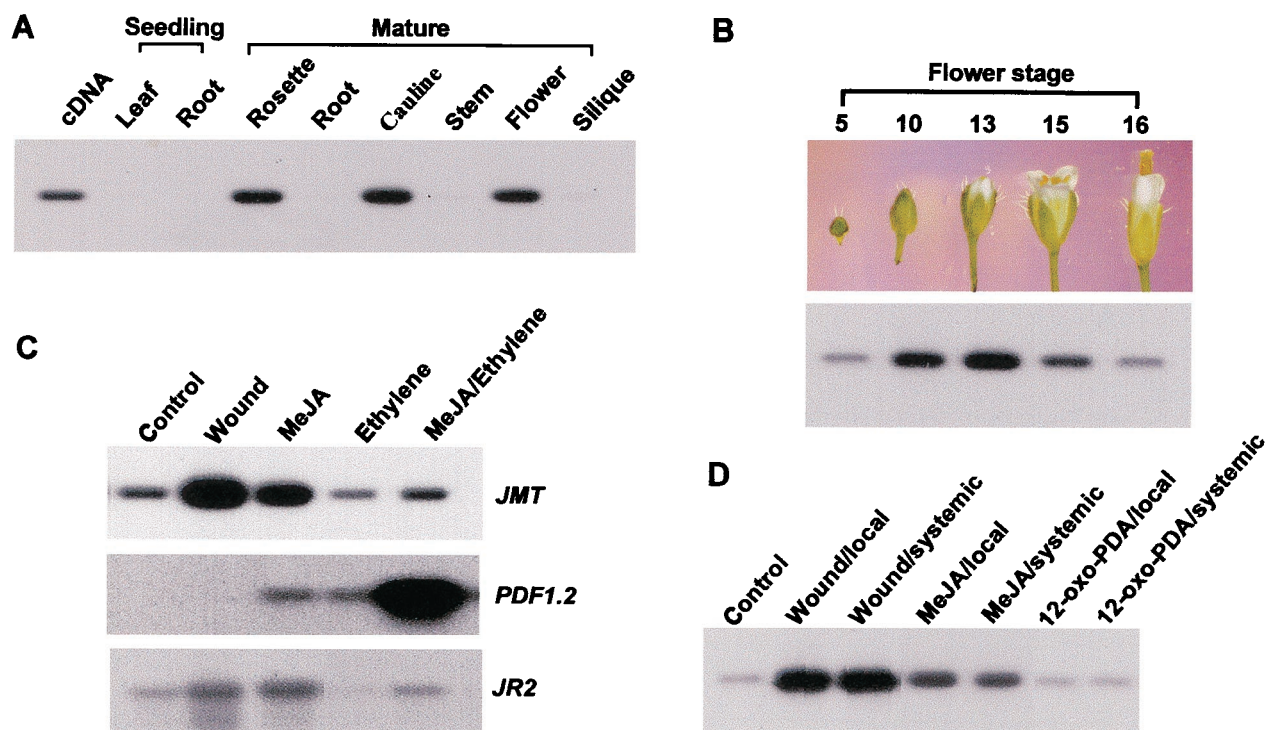


Fig. 3. Analysis of *JMT* gene expression by RT-PCR. Two micrograms of total RNA isolated was amplified by RT-PCR, and the products were analyzed by using a *JMT* gene-specific probe. (A) Organ- and developmental stage-specific expression. (B) Stage-specific expression during the flower development. Flower stages are designated according to Bowman (28). Stage 13 is at the anthesis. (C) Local expression induced by various treatments. Expression of *PDF1.2* and *JR2* genes are also analyzed as references. (D) Systemic expression induced by wounding or MeJA. The treated leaves (local) were enclosed in a plastic bag to be separated from distal leaves (systemic) and the plants were kept in an open area.

about 3-fold higher level of MeJA, whereas JA content was not altered (Table 3). This result is consistent with the enzymatic activity of *JMT* observed *in vitro* (see Fig. 2).

The transgenic *Arabidopsis* exhibited phenotypes visually indistinguishable from those of nontransformed plants. In the transgenic plants, however, expression of jasmonate-responsive genes (6, 29) such as *Lox1*, *AOS*, *JR2*, *JR3*, *DAH1*, *PDF1.2*, and *VSP* was elevated in the absence of wounding or jasmonate treatment (Fig. 5). In contrast, SA-responsive genes (6) such as *PR1*, *PR5*, and *ST* were not induced in these plants. This result is reminiscent of the gene expression pattern induced by externally applied jasmonates.

The transgenic plants exhibited elevated resistance to the virulent pathogen *B. cinerea* (Fig. 6). After 3 days of inoculation, wild-type *Arabidopsis* plants exhibited severe symptom of disease, whereas the transgenic plants did not.

Discussion

An enzyme catalyzing the methylation of JA to form MeJA has been identified in this study. The *Arabidopsis* gene *JMT*

Table 3. Levels of JA and MeJA in wild and transgenic *Arabidopsis* plants, ng/g fresh wt

Compound	Wild	Transgenic
Jasmonic acid	26.53 ± 8.24	23.72 ± 7.82
Methyl jasmonate	19.37 ± 4.87	58.50 ± 9.65
Total jasmonate	45.90 ± 13.11	82.22 ± 17.47

JA and MeJA in rosette leaves of wild-type and transgenic (line 1) *Arabidopsis* were separated by HPLC and quantified with GC-MS (7). Total JA is the sum of JA and MeJA. Values represent the mean ± SD of three independent experiments. JA and MeJA levels in other transgenic lines (2 through 5) were similar to that of line 1 plants within experimental errors.

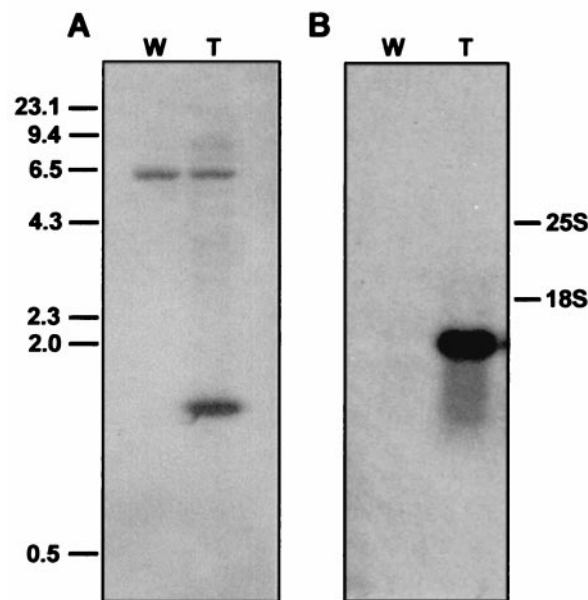


Fig. 4. Southern and Northern blot analyses of transgenic plants overexpressing *JMT*. (A) Five micrograms of *Arabidopsis* genomic DNA isolated from leaves of wild type (W) or transgenic *JMT* (T) plants (line 1) was digested with *Hind*III and electrophoresed. Sizes of the markers in kilobase pairs are indicated on the left side of the gel. (B) Five micrograms of total RNA isolated from wild-type (W) or transgenic (T) plants (line 1) was electrophoresed on a formaldehyde agarose gel. Location of 25S and 18S rRNAs are shown on the right. Blots were probed with a random primer-extended cDNA. *JMT* mRNA expression levels of other transgenic lines (2 through 5) were similar to that of line 1 plants.

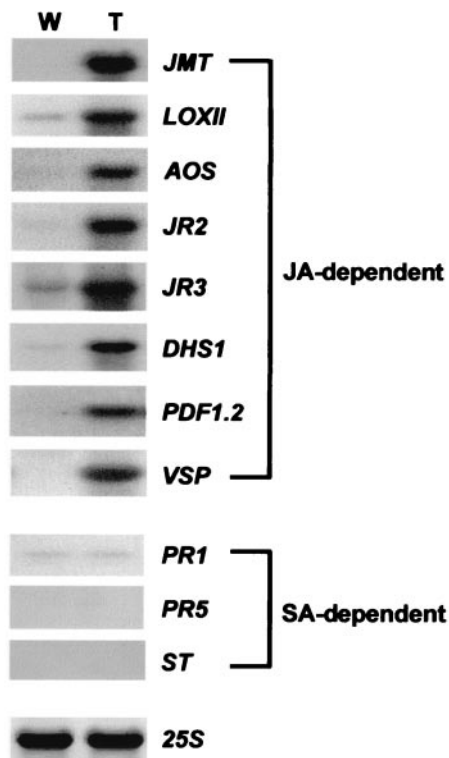


Fig. 5. Expression analysis of jasmonate-responsive genes in transgenic *Arabidopsis*-overexpressing *JMT* gene. Five micrograms of total RNA was isolated from rosette leaves of wild-type (W) and transgenic (T) *Arabidopsis* line 1, respectively. Northern blots were hybridized with each of the random primer-extended cDNA clones as indicated. *LOXII*, lipoxygenase II; *AOS*, allene oxide synthase; *JR2*, jasmonate response protein 2; *JR3*, jasmonate responsive aminohydrolase; *DHS1*, 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase; *PDF1.2*, plant defensin 1.2; *VSP*, vegetative storage protein, *PR1*, pathogen related protein 1; *PR5*, acidic thaumatin-like protein; *ST*, sulfonylesterase; *25S*, 25S ribosomal RNA. Expression patterns of the genes in other transgenic lines (2 through 5) were similar to that of line 1 plants.

encodes a protein homologous to a new class of carboxyl methyltransferases, such as the *B. campestris* floral nectary-specific NTR1 (20), the *C. breweri* SAMT (21), and the *A. majus* BMT (27) (Fig. 1). The recombinant *JMT* specifically converts JA into MeJA *in vitro* (Table 1 and Fig. 2). The kinetic parameters of *JMT* (Table 2) were comparable to those of SAMT (21) and BMT (30). Increased level of MeJA in the transgenic plants overexpressing *JMT* (Table 3) indicate that the protein encoded by *JMT* is indeed a SAM:jasmonic acid carboxyl methyltransferase.

It was found in this study that substrate specificity and kinetic characteristics of the recombinant NTR1 protein of *B. campestris* (20) were almost identical to those of *JMT* (data not shown), suggesting that these two enzymes are orthologues. It appears that *JMT* and NTR1 catalyze MeJA production in the cytoplasm, because these proteins lack apparent organ-specific transit signal peptide and hydrophobic regions long enough to be integrated in membranes (Fig. 1). In fact, an immuno-localization experiment demonstrated that NTR1 was located in the cytoplasm of *Brassica* cells (20).

JMT was expressed differentially in different organs at particular developmental stages and induced by wounding (Fig. 3). The expression pattern of *JMT* is consistent with previous observations of jasmonate distribution among developing tissues and of jasmonate-responsive defense responses (5). *JMT* was activated when *Arabidopsis* tissues were treated with exogenous

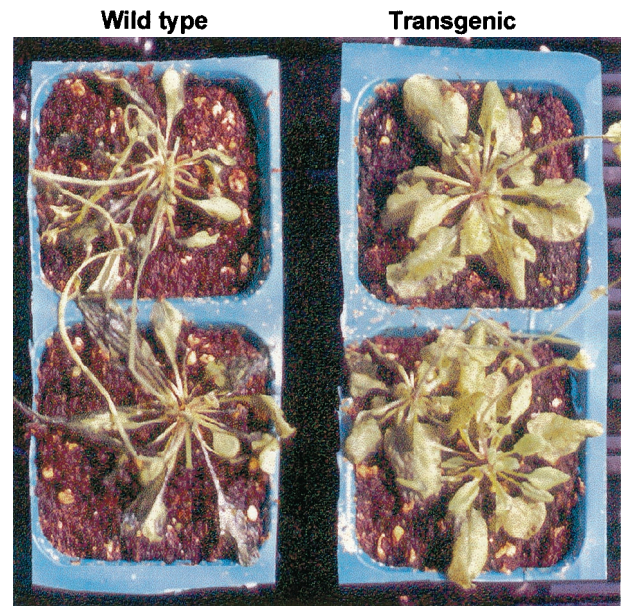


Fig. 6. Resistance of the transgenic *Arabidopsis* against a fungal pathogen, *B. cinerea*. Seven-week-old *Arabidopsis* plants were inoculated with the virulent pathogen *B. cinerea*. Conidial spores (1×10^5 per ml) suspended in potato dextrose broth were sprayed on plants until droplets ran off. Inoculated plants were grown further for 3 days. Left, wild-type *Arabidopsis*; right, transgenic *Arabidopsis* (line 1) constitutively expressing *JMT* gene. Degree of resistance of other transgenic lines (2 through 5) to the fungal infection was similar to that of line 1 plants.

MeJA (Fig. 3C). Thus, MeJA can amplify *JMT* expression induced at the developmental stages and by external stimuli including wounding. Ethylene inhibited *JMT* expression. In contrast, *PDF1.2* encoding a defense protein defensin (31–33) was synergistically induced by MeJA and ethylene treatments, as was reported for a number of plant defense genes (34, 35). These results suggest that the synergistic effect exerted by ethylene on the activation of *PDF1.2* is not by the enhancement of MeJA synthesis but rather, as proposed (31), by a cross-talk between the independent signaling pathways derived from them.

JMT was also induced systemically in distal leaves after wounding or MeJA treatment (Fig. 3D). *JMT* might be activated in the distal leaves by a long distance signal. Systemin (36, 37) and electrical signals (38) have been proposed to mediate the systemic induction of jasmonate synthesis in response to wounding. In addition, MeJA itself may act as such a systemic signal. It was suggested that systemic accumulation as well as movement among plants of jasmonates occur via the vapor phase in the form of MeJA (8, 39). Alternatively, MeJA may diffuse to distal parts of the plant through phloem to act as a long distance intercellular transducer, as observed in the study of systemin transport (36). Recently, a putative systemin receptor has been identified (40, 41). The systemin-receptor binding increases severalfold in response to MeJA (41). Thus, it is possible that MeJA and systemin migrate through phloem together exerting a synergistic mode of action.

It has been assumed that JA is the primary intracellular signal transducer and that exogenously applied or airborne MeJA is converted to JA in plant tissue. As described, however, MeJA may have its own role in developmental processes and defense responses. We tested this hypothesis by generating transgenic *Arabidopsis* that constitutively express the *JMT* gene to provide elevated levels of MeJA in the cytoplasm. The transgenic plants contained elevated level of MeJA without altering JA content (Table 3). In the transgenic plants, expression of various jas-

monate-responsive genes was elevated in the absence of wounding or jasmonate treatment (Fig. 5). Expression of *PDF1.2* is known to be responsible for plant defense against a group of pathogenic fungi (31–33). Indeed, the transgenic plants exhibited enhanced resistance to the virulent pathogen *B. cinerea* (Fig. 6).

It has been suggested that AOS is a major control point in the octadecanoid signaling pathway (42, 43). However, overexpression of the plastidic flax *AOS* cDNA in transgenic potato plants was not enough to trigger a constitutive expression of wound- or jasmonate-responsive genes, even though the plants contained 6- to 12-fold increased levels of JA (11). The transgenic plants responded to wounding by activating the expression of the genes responsible for the stresses. It was thus proposed that mechanical damage or water stress facilitates the movement of JA sequestered in the chloroplast to the cytoplasm where its receptors are presumably present. However, free acid JA might not be able to move across the cellular membrane without a carrier because of its acidic nature, as observed in a study of intracellular distribution of abscisic acid (44). It was also proposed that such external stimuli might activate an alternative JA biosynthetic pathway located in the cytoplasm. However, JA levels in the transgenic plants overexpressing cytoplasm-localized AOS were not increased unless the tissues were damaged (13, 19). Thus,

even though AOS is a control point in the biosynthesis of jasmonates, there might be other regulatory enzymes or mechanisms for the activation of jasmonate-responsive genes. As shown in the present study with the transgenic *Arabidopsis*, expression of *JMT* can induce genes such as *LOXII* and *AOS* (Fig. 5). This induction may provide more substrate for MeJA synthesis and further induce jasmonate-responsive genes.

We propose here that *JMT* is a key enzyme for the jasmonate-regulated plant responses and that MeJA can act as an intracellular regulator, a diffusible intercellular signal transducer, or an airborne signal mediating intra- and interplant communications. Some signals generated during an early event of developmental processes or defense responses may activate *JMT* that can self-amplify, stimulate, or regulate its own expression, propagating the MeJA-mediated cellular responses throughout whole plants.

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