Transcript Profile of Transgenic *Arabidopsis* Constitutively Producing Methyl Jasmonate

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Using an Affymetrix GeneChip® containing 8300 oligonucleotide probes, we measured transcript levels in transgenic *Arabidopsis* overexpressing the jasmonate carboxyl methyltransferase gene (*AtJMT*). When compared with wild-type plants, 5-week-old transgenics exhibited significant alterations (more than a two-fold increase or decrease) in the expression levels of 168 genes. Among them, 80 were up-regulated, including those involved in defense, oxidative stress-tolerance, and senescence. In contrast, the expression of 88 genes, including those that function in photosynthesis and cold/drought-stress responses, was significantly down-regulated. Thus, endogenous generation of methyl jasmonate through *AtJMT*-overexpression modified the transcript levels of genes previously identified as being jasmonate-responsive. This result confirms that MeJA formation is a key control point for jasmonate-responsive gene expression in plants.

Keywords: gene transcript profile, jasmonic acid methyltransferase, methyl jasmonate, microarray, transgenic *Arabidopsis*

Jasmonates are a group of plant cellular regulators that mediate diverse developmental processes, including seed germination, flower/fruit development, leaf abscission, and senescence (Creelman and Mullet, 1997; Howe and Schilmiller, 2002). In addition, jasmonates activate gene expression for plant defense mechanisms in response to insect-driven wounding and a variety of pathogens (Wasternack and Hause, 2002; Farmer et al., 2003).

Jasmonic acid (JA), the best studied jasmonate, is synthesized from linolenic acid via the octadecanoic pathway, and then further catabolized to form various derivatives by methylation, oxidation, hydroxylation, glycosylation, and amino acid conjugation (Beale and Ward, 1998). As one of the JA derivatives, methyl jasmonate (MeJA) is formed by JA carboxyl methyltransferase (JMT), becoming a constituent of floral scent in developing flowers. MeJA has also been identified as a vital regulator that mediates diverse cellular responses, possibly via gene-activation control and systemic long-distance signaling (Cheong and Choi, 2003).

Identification of the genes encoding JA carboxyl methyltransferase has provided basic information on the role(s) of this volatile phytohormone (Song et al., 2000; Seo et al., 2001; Song et al., 2005; Barkman, 2006). Transgenic *Arabidopsis* overexpressing the *AtJMT* gene contain three-fold more endogenous MeJA without altering the JA content (Seo et al., 2001). In those transgenic plants, various jasmonate-responsive genes are constitutively expressed in the absence of wounding or jasmonate treatment. As the level of defense gene transcripts increases, the transgenics exhibit enhanced resistance to a virulent fungal pathogen.

The means by which MeJA regulates these processes can be investigated by observing its activity on gene expression control in a wide range of jasmonate-responsive cellular metabolisms. Functional genomics, including microarray analysis, allows global and simultaneous analyses of expression profiles (Zhu et al., 2001; Cheong et al., 2002), providing the identification of numerous novel genes whose biological functions are not yet known.

In this study, we examined genes constitutively activated or repressed in *AtJMT*-transgenic plants, using an *Arabidopsis* genome microarray, and investigated the role of MeJA formation in activating jasmonate-responsive cellular metabolism.

MATERIALS AND METHODS

Plant Materials

Seeds of homozygote (T_4) *AtJMT*-overexpressing lines (Seo et al., 2001) and control wild-type *Arabidopsis* plants (ecotype Columbia) were sown on potting mix and grown for 5 weeks at 21°C to 23°C and 60% relative humidity, under a 16-h photoperiod from white light at 500 μmol m^{-2} s^{-1}. For our chemical treatments, 100 μM MeJA (in 0.1% ethanol) was applied to 5-week-old wild-type plants. The plants were kept in tightly sealed magenta vessels until the rosette leaves were harvested.

RNA Isolation and Biotinylation

Total RNAs were isolated and purified from the rosette leaves of individual plants, using the Concert™ Plant RNA Purification Reagent (Invitrogen, USA) and the RNeasy Mini Kit (Qiagen, USA). Total RNA (10 μg) was converted to first-strand DNA using SuperScript II reverse transcriptase (Invitrogen) and the T7-oligo $(dT)_{24}$ primer, which contained the T7 RNA polymerase promoter sequence (GenoTech, Korea). Double-strand cDNA was then synthesized using a Superscript Choice System (Invitrogen). Biotinylated complementary RNA (cRNA) was synthesized from 1 μg of cDNA by *in vitro* transcription, using the BioArray High Yield RNA transcript labeling kit (Enzo Diagnostics, USA).

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Table 1. Oligonucleotide primers and the sizes of their RT-PCR products.

Symbol	Gene name	AGI No.[a]	Primer sequence[b]	Size (bp)
JMT	Jasmonic acid carboxyl methyltransferase	At1g19640	F 5′-TGGGAACTCCTAGCTCAAGCTCTT-3′ R 5′-ACCGGTTCTAACGAGCGAAAGAAT-3′	420
PDF1.2	Plant defensin 1.2	At5g44420	F 5′-GTAATAATCATCATGGCTAAGT-3′ R 5′-GCACCAAAGATTATTGGTAGA-3′	277
JR3	Jasmonic acid responsive 3	At1g51760	F 5′-CATTAGGTCAAGTGAGCTCGA-3′ R 5′-CTCGCCATTGGTGAACGAGCT-3′	586

[a]Arabidopsis Genome Initiative number.
[b]F, forward primer; R, reverse primer.

Microarray Analysis

Microarray experiments were conducted with total-RNA preparations for each genotype, using a GeneChip® (Affymetrix, USA) that contained approximately 8300 gene probes synthesized *in situ*. Array hybridization was conducted as previously described (Zhu et al., 2001; Cheong et al., 2002). The microarray was scanned twice with an Agilent GeneArray Scanner (Affymetrix), and the intensities were averaged. Afterward, the images were processed and analyzed via GeneChip Suite 3.2 (Affymetrix). Functioning of each gene was predicted according to Affymetrix annotation, TIGR (The Institute for Genomic Research) definitions, and the NCBI (National Center for Biotechnology Information) database.

Blot Analyses

To examine the expression of jasmonate-inducible marker genes in our transgenic plants, each gene was amplified by RT-PCR from 2 μg of total RNA. The products were then analyzed via Southern blotting. Primer sets for PCR are listed in Table 1. For northern blot analysis, 10 μg of total RNA was loaded on a 0.8% formaldehyde agarose gel and blotted onto a nylon membrane. The blot was hybridized with each gene probe and washed at 65°C under stringent conditions. For the DNA probes, we used EST clones obtained from The Arabidopsis Information Resource (TAIR).

RESULTS AND DISCUSSION

Microarray Analysis

Total RNAs were extracted from four transgenic *Arabidopsis* plants that constitutively express *AtJMT* (Seo et al., 2001). These were tested by RT-PCR and Southern blot analysis (Fig. 1) to examine the expression levels of the transgene and two well-known *Arabidopsis* jasmonate-responsive genes: a defensin gene (*PDF1.2*) and *JR3* (*Jasmonate responsive3*). RNA preparations from a wild-type plant (W_4) and a transgenic plant (T_2) were selected for microarray analysis.

When compared with those in the wild-type plant, we found that 168 genes (2.0% of the 8300 probes) in the *AtJMT*-transgenic plant showed transcription levels that were altered either up or down by more than two-fold. The scanned images and transcript-level data were deposited in the ArrayExpress (http://www.ebi.ac.uk/arrayexpress) with an accession number of E-ATMX-4.

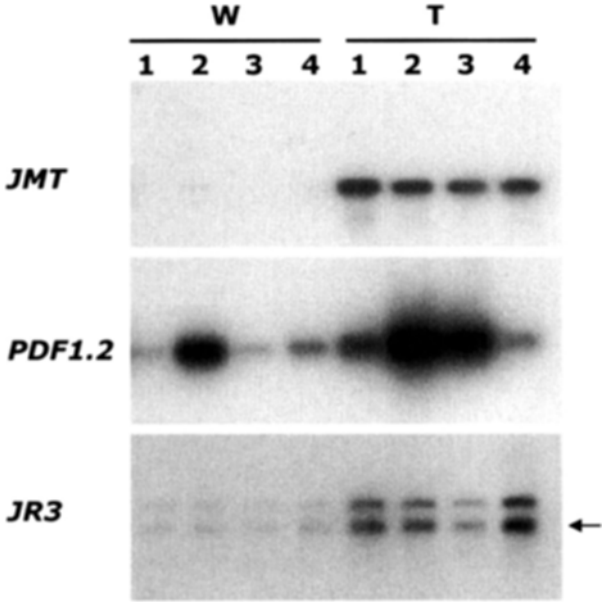

Figure 1. RT-PCR amplification and Southern blot analysis of jasmonate-responsive marker genes. Rosette leaves were harvested from 5-week-old *Arabidopsis* plants. Total RNA (2 μg) was amplified by RT-PCR using primer sets listed in Table 1. PCR products were probed by Southern blotting for transcripts of jasmonic acid carboxyl methyltransferase gene (*JMT*), a plant defensin gene (*PDF1.2*), and a jasmonate responsive gene (*JR3*). W and T denote RNA from wild-type and transgenic plants, respectively. Arrow indicates authentic *JR3* band.

Up-Regulated Genes

The transgenic plant contained 80 genes with up-regulated expression (Table 2). These included pathogenesis-related (PR) genes that putatively encode β-1,3-glucanase (PR-2), basic chitinase (PR-3), hevein-like protein (PR-4), and thaumatin (PR-5). Expression of lectin-like protein genes also increased. It has been reported that myrosinase-binding proteins are JA-inducible (Geshi and Brandt, 1998) and exhibit lectin activity (Taipalensuu et al., 1997), suggesting they are involved in plant defenses.

Expression of oxidative stress-tolerance genes (Mittler, 2002), including those for peroxidases and oxidases, was also enhanced in the transgenic plant, as was the expression for gene transcripts for glutathione *S*-transferases and thioredoxins. Serine acetyltransferase is known to play a major role in regulating sulfur assimilation and cysteine biosynthesis while also manipulating resistance to oxidative stress

Table 2. Genes up-regulated in *AtJMT*-transgenic *Arabidopsis*.

AGI number[a]	Description[b]	Fold[c]	AGI number	Description	Fold
Defense			At5g08790	ATAF2, NAM family TF	2.4
At1g75040	putative thaumatin protein	3.8	At5g47220	Ethylene response TF (AtERF2)	2.3
At1g75830	antifungal protein (PDF1.1)	3.5	At5g67300	AtMYB44 transcription factor	2.4
At2g32680	putative disease resistance protein	5.6	***Storage***		
At2g43570	putative endochitinase	3.2	At4g24350	putative storage protein	2.4
At2g43590	putative endochitinase	11.7	At4g24360	putative storage protein	2.9
At3g04720	hevein-like protein precursor (HEL)	18.8	At5g24780	vegetative storage protein Vsp1	1.5
At3g12500	basic chitinase	7.2	***Cell wall modification***		
At3g57260	beta-1,3-glucanase 2 (BG2)	20.7	At1g11580	putative pectinesterase	4.3
At4g16260	beta-1,3-glucanase class I precursor	15.2	At2g05540	putative glycine-rich protein	2.8
At3g15356	lectin-like protein	6.9	At2g43150	putative extensin	3.2
At3g16470	JA inducible myrosinase bind. prot.	3.4	At5g57550	endoxyloglucan transferase	3.6
Stress response			***Primary and second metabolism***		
At1g55920	serine acetyltransferase	2.2	At1g67980	caffeoyl-CoA 3-O-methyltransferase	2.4
At4g13830	putative DnaJ-like protein	2.0	At1g10070	branch-chain a.a. aminotransferase	4.4
At1g02920	glutathione *S*-transferase	2.7	At3g47340	glutamine-dep. asparagine synthetase	2.4
At1g08830	copper/zinc superoxide dismutase	6.7	At4g27450	probable asparagine synthase	4.6
At1g12520	Cu/Zn-superoxide dismutase	4.8	At5g05730	anthranilate synthase I-1 precursor	2.6
At1g16410	putative cytochrome P450	2.2	At5g18170	glutamate dehydrogenase	2.5
At1g23020	NADPH oxidase flavocytochrome	3.7	At5g54080	homogentisate 1,2-dioxygenase	3.1
At1g45145	putative thioredoxin protein	2.4	At1g06760	histone H1-1	3.2
At2g02930	glutathione S-transferase	3.7	At1g09430	similar to ATP-citrate-lyase	2.3
At2g28190	putative Cu/Zn superoxide dismutase	10.5	At1g29260	peroxisomal targeting receptor, Pex7p	2.1
At2g37130	putative peroxidase ATP2a	2.9	At3g50740	UTP-glucose glucosyltransferase-like	2.9
At4g02520	putative glutathione *S*-transferase	2.1	At4g27780	putative acyl-CoA binding protein	2.3
At4g37520	peroxidase, prxr2	3.2	At4g39980	aldolase (DHS1)	1.2
At2g47180	putative galactinol synthase	2.2	At5g54810	Trp synthase beta chain 1 precursor	2.4
Senescence			At1g03210	phenazine biosyn. protein phzC	2.0
At1g19670	coronatine-induced protein 1	8.0	At2g29350	putative tropinone reductase	2.8
At2g44790	phytocyanin (blue copper proteins)	4.0	At2g30140	putative glucosyltransferase	2.4
At4g02380	late embryogenesis 3 (SAG21)	2.5	At3g52070	putative squalene monooxygenase	2.5
At4g35770	senescence-associated protein sen1	11.0	At4g12480	pEARLI1	5.8
At5g20230	blue copper binding protein	10.1	At2g41180	similar to secretory IgA binding prot.	3.4
Growth and hormone metabolism			***Genes with unidentified function***		
At2g02040	histidine transport protein (PTR2-B)	2.2	At1g15350	unknown protein	2.6
At4g16370	isp4-like, oligopeptide transporter	2.6	At1g21000	unknown protein	3.5
At5g61520	monosaccharide transporter	2.5	At1g27020	unknown protein	2.6
At1g51760	IAA-Ala hydrolase (JR3)	4.4	At1g49470	unknown protein	2.7
Signal transduction and transcription			At2g16590	unknown protein	3.3
At3g13380	receptor protein kinase	2.8	At2g24550	unknown protein	3.1
At4g27300	putative receptor protein kinase	2.0	At2g29670	unknown protein	2.6
At5g65930	kinesin-like calmodulin-binding prot.	2.4	At4g26060	unknown protein	2.3
At2g01060	transfactor-like protein	2.0	At4g34560	unknown protein	4.3
At4g01720	WRKY transcription factor	3.0	At4g35750	unknown protein	2.8
At5g04340	putative C2H2 zinc finger TF	2.5			

[a]Arabidopsis Genome Initiative Number.
[b]Gene function predicted by Affymetrix annotation, TIGR definition, and NCBI database.
[c]Relative gene transcript levels compared with those of wild-type plants.

(Blaszczyk et al., 1999). DnaJ-like proteins are molecular chaperones as well as members of the family for heat shock proteins in eukaryotic cells (Cyr et al., 1994). Galactinol synthase plays a role in seed-desiccation tolerance by supplying a galactosyl donor to form oligosaccharides of the raffinose family (Downie et al., 2003). The blue-copper-binding pro-

Table 3. Genes down-regulated in *AtJMT*-transgenic *Arabidopsis*.

AGI number[a]	Gene description[b]	Fold[c]	AGI number	Gene description	Fold
Chlorophyll generation			***Growth and development***		
At1g03630	protochlorophyllide oxidoreductase	-3.7	At2g17230	putative phi-1-like protein	-3.1
At1g19150	PSI type II chl a/b-binding prot. Lhca2	-2.6	At5g43270	squamosa promoter binding prot-like 2	-2.3
At1g76100	Plastocyanin	-2.4	At5g20630	germin-like protein	-27.5
At2g40490	put. Uroporphyrinogen decarboxylase	-2.1	At4g12720	putative growth factor protein	-2.7
At3g27690	chlorophyll a/b-binding protein Lhcb2	-2.8	At2g42840	En/Spm-like transposon protein	-3.2
At3g51820	chlorophyll synthetase	-3.2	At1g08890	putative sugar transporter	-2.2
At4g27440	protochlorophyllide reductase precur.	-2.1	At1g77990	putative sulfate transporter	-4.3
Photosynthesis			***Signal transduction***		
At1g55490	rubisco binding-protein beta subunit	-3.7	At5g63300	nucleoside diphosphate kinase type 2	-2.0
At2g28000	rubisco binding-protein alpha subunit	-2.4	At1g72930	toll/interleukin-1 receptor-like (TIR)	-2.5
At2g36390	starch branching enzyme II	-3.0	At2g41090	calcium-binding protein CaBP-22	-2.2
At4g17090	beta-amylase	-2.3	At5g17520	root cap 1 (RCP1), a calcium-ATPase	-2.0
At5g19220	ADPG pyrophosphorylase large sub.	-2.1	At1g10200	putative transcription factor	-2.0
Abiotic stress			At5g03540	AT4, leucine zipper protein	-2.8
At1g20440	COR47	-2.5	***Cell wall modification***		
At2g42530	cor15b precursor	-4.4	At1g03870	fasciclin-like arabinogalactan-prot. 9	-2.4
At5g15960	cold and ABA inducible protein kin1	-2.1	At2g45470	fasciclin-like arabinogalactan protein	-3.0
At5g52310	low-temperature-induced protein 78	-2.8	At1g04680	putative pectate lyase A11	-2.8
At1g20450	ERD10 protein	-2.5	At1g10550	putative xyloglucan-specific glucanase	-2.1
At3g16370	proline-rich protein APG-like	-2.0	At1g13930	hydroxyproline-rich glycoprotein like	-3.3
Stress and defense			At1g70370	polygalacuronase isoenz. 1 beta sub.	-2.3
At2g02100	putative protease inhibitor II protein	-2.1	At1g78820	putative glycoprotein EP1	-2.3
At4g34150	putative elicitor-responsive protein	-2.5	At2g14890	putative proline-rich protein	-2.2
At4g27700	senescence-associated protein sen1	-3.4	At2g21140	putative proline-rich protein	-3.9
At1g14150	oxygen-evolving enhancer protein 3	-2.5	At2g26930	putative ripening-associated protein	-2.1
At2g40300	putative ferritin	-2.3	At4g00170	putative proline-rich protein	-4.2
At5g15350	copper binding protein – like	-2.3	At4g03210	put. xyloglucan endotransglycosylase	-3.8
Fatty acid metabolism			At4g12730	putative pollen surface protein	-2.1
At2g34770	fatty acid hydroxylase (FAH1)	-2.0	At4g29020	putative glycine-rich protein 5	-8.9
At2g38540	put. nonspecific lipid-transfer protein	-2.3	At4g37450	put. arabinogalactan protein AGP18	-2.1
At3g47860	outer membrane lipoprotein – like	-2.7	At4g37800	endo-xyloglucan transferase-like prot.	-2.4
At4g18970	lipase-like protein	-2.4	At5g10430	arabinogalactan-protein AtAGP4	-2.1
At5g05580	omega-3 fatty acid desaturase	-2.3	***Genes with unidentified function***		
Primary and secondary metabolism			At1g04430	putative ankyrin protein	-2.3
At1g11860	similarity to aminomethyltransferase	-2.0	At4g08685	pollen Ole e 1 allergen and extensin	-3.0
At1g35720	Ca^{2+}-dep. memb-binding prot. annexin	-2.1	At4g00430	transmembrane protein	-3.2
At2g28900	putative membrane channel protein	-2.0	At2g28410	GPI-anchored like protein	-2.2
At2g29650	inorganic phosphate cotransporter	-3.4	At2g34510	predicted GPI-anchored protein	-6.2
At2g30150	putative glucosyltransferase	-2.6	At2g35860	unknown protein	-2.0
At2g33800	30S ribosomal protein S5	-2.0	At2g37660	unknown protein	-2.1
At2g35370	glycine decarboxylase complex H	-2.0	At2g40330	unknown protein	-2.2
At2g43550	putative trypsin inhibitor	-2.3	At2g44670	unknown protein	-2.0
At3g47960	putative peptide transporter	-2.6	At2g45740	unknown protein	-2.3
At4g01900	nitrogen sensing protein GLB1	-2.0	At4g02530	unknown protein	-2.3
At4g13930	hydroxymethyltransferase	-2.2	At1g11850	unknown protein	-4.0
At4g17560	putative ribosomal protein L19 rplS	-2.3	At4g18030	unknown protein	-2.1
At4g26690	glycerophosphodiesterase (GPDL2)	-2.0	At4g28080	unknown protein	-2.2
At4g34240	putative aldehyde dehydrogenase	-2.0	At1g09310	unknown protein	-2.0

[a]Arabidopsis Genome Initiative Number.
[b]Gene function predicted by Affymetrix annotation, TIGR definition, and NCBI database.
[c]Relative gene transcript level compared with wild-type plants.

tein gene confers some resistance to aluminum toxicity, and increases resistance to oxidative stress induced by diamide (Ezaki et al., 2000).

That blue-copper-binding protein gene as well as *SAG21* (a senescence-associated gene) are among the senescence-related genes that are activated during ozone exposure in *Arabidopsis* (Miller et al., 1999). It has been reported that *SEN1* is regulated by MeJA-related signals that link defense and senescence (Schenk et al., 2005). We also found enhanced expression of the gene encoding a coronatine-induced protein (CORI1), which causes an increased breakdown of chlorophyll during senescence (Benedetti et al., 1998; Benedetti and Arruda, 2002). Coronatine is a microbial phytotoxin that mimics jasmonate-responsive gene activation when supplied to plants. In addition, gene transcripts encoding proteins involved in cell wall modification, storage, growth/development, and primary/secondary metabolisms showed enhanced accumulation in the transgenic plant. An additional 10 genes with unidentified function were up-regulated in that plant.

Down-Regulated Genes

In contrast, genes encoding proteins involved in photosynthesis, such as ribulose bisphosphate carboxylase/oxygenase (Rubisco), chlorophyll-constructing proteins, and light harvesting machineries, were all down-regulated (Table 3). This observation was consistent with previous reports for those individual genes (Wasternack and Hause, 2002). Gene transcripts encoding other proteins involved in growth/development, cell wall modification, signal transduction, and primary/secondary metabolisms also showed reduced accumulations in the transgenic plant.

It is notable that the expression of some cold-regulated (*cor*) genes and drought stress-related genes were repressed in the transgenic plant. It has long been recognized that salt, drought, and cold stresses cause increased biosynthesis and accumulation of ABA (Xiong et al., 2002). For example, the *kin1* gene is expressed at higher levels following treatment with low temperature, dehydration, or ABA (Wang et al., 1995). The ABA-inducible gene *ERD10* also responds rapidly to dehydration stress (Kiyosue et al., 1994). Our data suggest that MeJA-overproduction had a repressive effect on the expression of ABA-responsive genes in the transgenic plant. Anderson et al. (2004) have recently reviewed the mutual antagonistic interactions between abscisic acid and jasmonate-ethylene signaling pathways in defense-gene expression.

Gene Expression Patterns

We selected several genes identified in our microarray analysis and examined their constitutive expression in the transgenic plant, using either northern (Seo et al., 2001) or reverse northern (dot blot) (Jung et al., 2003) analyses. A number of the genes in this study also showed up- or down-regulated expression in the wild-type *Arabidopsis* when treated with 100 μM MeJA (Jung et al., 2007).

In addition, our northern blot analysis revealed that the transcription level of At4g24350, which encodes a storage protein belonging to the phosphorylase family, gradually increased following MeJA treatment (Fig. 2). At1g10200 represents a gene coding for a putative protein similar to the tobacco transcription factor Ntlim1, which is involved in lignin biosynthesis (Kawaoka et al., 2000). Its transcripts rapidly decreased within the first hour after MeJA treatment before gradually recovering to a normal level.

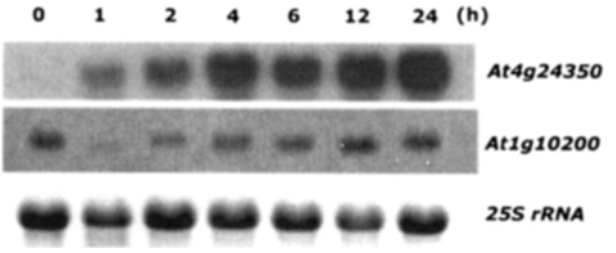

Figure 2. Northern blot analysis of gene expression after MeJA treatment. RNA was extracted from rosette leaves of 5-week-old *Arabidopsis* plants treated with 100 μM MeJA, and 10 μg of total RNA was loaded on 0.8% formaldehyde agarose gel. Blot was probed with putative storage protein (At4g24350) and gene similar to tobacco *Ntlim1* (At1g10200). Level of 25S rRNA was examined to verify equal loading.

Effect of *AtJMT*-Overexpression

The *AtJMT*-transgenic plant constitutively expressed numerous genes involved in jasmonate-dependent defense and developmental cellular metabolisms. Thus, endogenous generation of MeJA through *AtJMT*-overexpression activated a set of jasmonate-responsive genes that had previously been identified in experiments with external JA applications (Wasternack and Hause, 2002).

Seo et al. (2001) have reported that the *AtJMT*-transgenic plants contain three-fold greater levels of endogenous MeJA without altering their JA content (Seo et al., 2001). Thus, our microarray data support the hypothesis that MeJA is a more effective signal transducer than JA for jasmonate-mediated gene activation (Seo et al., 2001; Cheong and Choi, 2003). Alternatively, formation of the volatile compound MeJA may pull the JA-biosynthesis pathway forward, producing various biologically active oxylipins through different branches in the octadecanoic pathway. To date, about 20 naturally occurring jasmonates have been described (Gfeller and Farmer, 2004). In any case, MeJA formation is an important control point for activating jasmonate-responsive genes.

Constitutive expression of pathogenesis-related genes in the *AtJMT*-transgenic *Arabidopsis* is linked with enhanced resistance to fungal (Seo et al., 2001) and bacterial (Jung et al., 2003) pathogens. Cipollini (2007) have also explored the consequences of this *AtJMT*-overexpression on seed production and tolerance to defoliation. When one considers the enhanced expression of genes responsible for defenses against oxidative stress, one may suggest that transgenic plants overproducing MeJA could have improved tolerance to biotic and abiotic challenges. A detailed examination of the physiological nature of these transgenic plants is being conducted.

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