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Biochemical Characterization of an *Arabidopsis* Glucosyltransferase with High Activity toward Jasmonic Acid

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Biochemical characterization of the recombinant gene products from the *Arabidopsis* glucosyltransferase multigene family has identified one enzyme with high activity toward the plant cellular regulator jasmonic acid (JA). The protein, AtJGT1 (UDP-glucose:JA glucosyltransferase), also has significant activities with other substrates, such as dihydrojasmonic acid, indole-3-acetic acid (IAA), indole-3-propionic acid, and indole-3-butyric acid. The K_m values of AtJGT1 for JA or IAA are similar to those of an *Arabidopsis* IAA glucosyltransferase UGT84B1 previously reported. Northern blot analysis showed that *AtJGT1* is highly expressed in the leaves, but only slightly detectable in the roots, stems, and inflorescences. This study describes the first biochemical analysis of a recombinant glucosyltransferase with JA activity, and provides the foundation for future genetic approaches to understanding the role of JA-glucose in *Arabidopsis*.

Keywords: glucosyltransferase, hormone homeostasis, indole-3-acetic acid, jasmonic acid, JA conjugate

Glucosylation is a prominent modification in plant metabolism. This reaction involves the transfer of glucose from UDP-glucose to a second substrate, leading to the formation of either a glucose ester or a glucoside. In many cases, a number of plant products are glucosylated in the last step of their biosynthesis, including hormones, secondary metabolites involved in stress and defense responses, and xenobiotics such as herbicides (Sembdner et al., 1994). Consequently, glucosylation plays a crucial role in maintaining cellular homeostasis by regulating the level, activity, and location of key cellular metabolites.

Glucosyltransferases are found in many organisms. A signature motif thought to be involved in binding the UDP moiety of sugar nucleotides has been identified in a wide range of UDP-glucosyltransferases (UGTs). Using that motif to screen sequences in the genome and expressed sequence tag databases of *Arabidopsis*, a large multigene family has now been defined (Li et al., 2001).

Although plant hormones can be glucosylated to represent their storage forms, the role of the conjugates is not well defined. Despite the wide occurrence of glucosylated conjugates and the many studies of plant glucosyltransferases involved in hormone conjugation, none of the genes has been identified that encodes jasmonic acid glucosyltransferases (JA-UGTs), which form the glucosylated conjugate of jasmonic acid (JA) (Sembdner and Parthier, 1993).

JA is an important cellular regulator, activating diverse developmental processes, e.g., seed germination, flower and fruit development, leaf abscission, and senescence, as well as playing a role in defense responses against pathogens and insect-driven wounding (Sembdner and Parthier, 1993; Ji et al., 1995). Furthermore, JA may function in plant responses against various environmental stresses, such as drought, low temperature, osmosis, and high salt concentration.

Hormones, including JA and indole-3-acetic acid (IAA), exist as free acids; in their conjugated forms, they are linked to a wide variety of compounds, e.g., amino acids, peptides, and sugars (Cohen and Bandurski, 1982). Both the glucosyl and amino acid conjugates of IAA are inactive and are involved in regulating auxin homeostasis (Normanly, 1997), but the function of JA conjugates is unclear. Staswick and Tiryaki (2004) have shown that JA-isoleucine (an amino acid conjugate) is important to the activation of JA optimal signaling in *Arabidopsis*. Moreover, methyljasmonate (MeJA, the methyl ester of its free acid JA) is a potent signal to activate JA defense responses in *Arabidopsis* plants (Seo et al., 2001). These data suggest that JA-glucose itself may possibly be involved in the activation of the JA signaling pathway. Elevated or reduced JA-glucose levels may modulate the plant defense or development system. Therefore, the objective of this study was to isolate the *AtJGT1* gene encoding a protein with JA glucosyl-

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transferase activity from *Arabidopsis*.

MATERIALS AND METHODS

Expression and Purification of the Recombinant AtJGT1

The *AtJGT1* gene was amplified via polymerase chain reaction (PCR), using primers from the cDNA clone of *AtJGT1* (GenBank accession no. DQ158907) as template. Their sequences were: 5′-CCGCTCGAG**ATG**GGAGAGAAAGCGAAA-3 (underlining and bold symbols indicate the *Xho*I site and translation initiation codon, respectively) and 5′-AACCTCGAG**TTA**CCTCACAATTTTAGC-3′ (underlining and bold symbols indicate the *Xho*I site and translation stop codon, respectively). The PCR products of *AtJGT1* then were digested with *Xho*I and cloned into the expression vector pGEX-5X-1 with an N-terminal GST-tag (Amersham Biosciences, USA) cut with the appropriate restriction enzymes. The recombinant plasmids were transformed into *Escherichia coli* BL21 (DE3) pLysS. To express the recombinant enzymes, bacterial cultures were grown to an A_{600} of 0.7 to 0.9 in an LB medium containing 100 μg mL^{-1} ampicillin, then added at final concentration to 1 mM isopropyl-α-D-thiogalactopyranoside (IPTG). Incubation was continued at 20°C for an additional 12 h before the cells were harvested. The pellet was re-suspended in 0.1 culture volume of a solution containing 50 mM Tris·HCl buffer (pH 8.0), and subjected to two cycles of freezing and thawing. The crude extracts were treated with 10 μg mL^{-1} DNase and 20 mM $MgCl_2$ at RT for 30 min. After centrifugation, the supernatant was incubated with glutathione-agarose beads (Sigma, USA) at 4°C for 1 h, and the GST-fusion proteins were purified as described by Guan and Dixon (1991).

Enzyme Assay of the Recombinant AtJGT1 Protein

Protein concentrations were determined by the method of Bradford (1976), using BSA as a standard. Recombinant AtJGT1 activity was determined by measuring the concentration of the reaction product, JA- or another chemical-glucose conjugate, with UDP-[^{14}C]glucose as a substrate. The assay buffer contained 50 mM Tris·HCl (pH 7.0) and 5 mM KCl at 30°C, the temperature at which the recombinant proteins have their highest activities. The mixture samples were applied to a thin-layer plate (F254, Merck, USA) and developed with 1-butanol:acetic acid:water (4:1:1, v/v/v). The chromatogram was autoradiographed against the BAS-1500 system (Fuji, Japan). To quantify the signals, the NIH Image J program was used (US National Institutes of Health, http://rsb.info.nih.gov/ij/). Samples were also extracted with ethylacetate to separate the radio-labeled JA- or another chemical-glucose conjugate from a mixture containing UDP-[^{14}C]glucose. Radioactivities at the organic phase were measured with a liquid scintillation counter.

RNA Gel Blot Analysis

Total RNA was isolated as described by Kroczek and Siebert (1990). RNA (ca. 8 μg) was separated by electrophoresis on a 1% agarose gel, and hybridization was carried out according to the method of Sambrook et al. (1989). A gene-specific DNA probe for *AtJGT1* was made by using the random primer extension from the cDNA.

RESULTS

Isolation of *AtJGT1*

Of the numerous methyltransferases (MT), JA-MTs have higher homologies with those of salicylic acid (SA) (Song et al., 2000; Seo et al., 2001). This suggests that SA glucosyltransferases (SA-UGTs) may be more homologous to JA-UGTs than to other UGTs. Using searches with a protein basic local alignment search tool (BLAST), six clones out of the many UGTS were chosen for screening and isolation because of their previously reported greater homology with a tobacco SA-UGT (SAGT) gene (Lee and Raskin, 1999). The sequences were used to produce soluble recombinant fusion proteins with GST in *E. coli*, and analyzed for their activities in synthesizing the glucose ester of JA (Fig. 1). Only one sequence, named *AtJGT1*, had high activity toward JA (Fig. 2). As expected, one sequence, which was the most homologous to a tobacco SAGT, had significant activities toward SA and benzoic acid (data not shown). The amino acid sequence alignment of four proteins included two reported IAA-UGTs (Fig. 3). Therefore, AtJGT1 has higher homology with SAGT of tobacco than with UGT84B1 (*Arabidopsis* IAA-UGT) or iaglu (maize IAA-UGT).

Glucosyltransferase Activity of AtJGT1 Protein

To test for enzyme activity, the purified recombinant

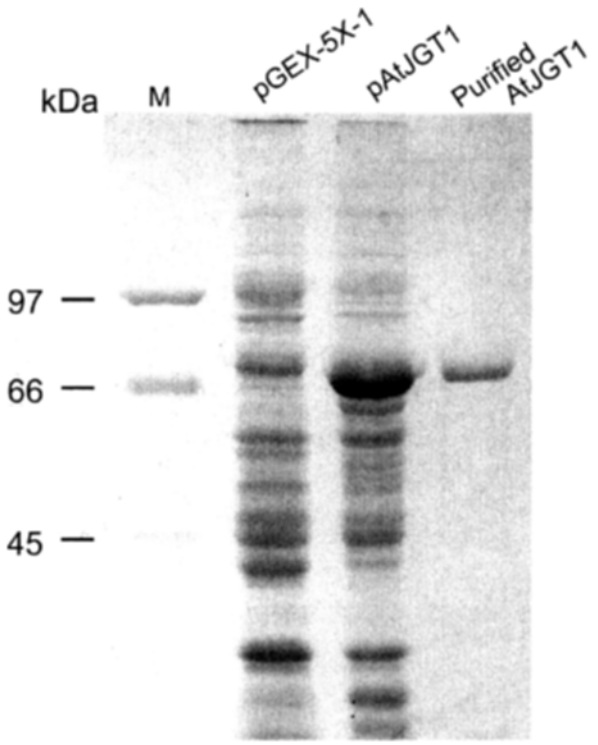

Figure 1. Purification of recombinant AtJGT1. Vector pGEX-5X-1 and recombinant plasmid pAtJGT1 were transformed into *E. coli* BL21, and their expressions were induced with IPTG. Recombinant AtJGT1 proteins were purified through a glutathione-agarose column and analyzed via 12.5% SDS-PAGE.

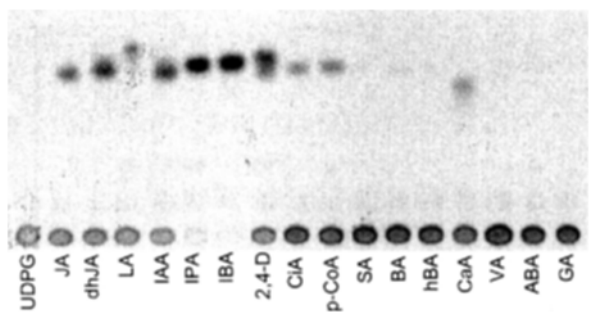

Figure 2. Synthesis of glucose conjugate(s) of JA or related substrates by recombinant AtJGT1. Reactions with UDP-[^{14}C]glucose and JA or other substrates were analyzed by TLC and autoradiographed. JA, jasmonic acid; dhJA, dihydrojasmonic acid; LA, linoleic acid; IAA, indole-3-acetic acid; IPA, indole-3-propionic acid; IBA, indole-3-butyric acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; CiA, cinnamic acid; *p*-CoA, *p*-coumaric acid; SA, salicylic acid; BA, benzoic acid; hBA, 4-hydroxybenzoic acid; CaA, caffeic acid; VA, vanillic acid; ABA, abscisic acid; GA, gibberellic acid (GA_4).

AtJGT1 was incubated, under standard assay conditions, with various putative substrates in the presence of UDPG, in which the glucosyl group was radio-

Figure 3. Sequence analyses of AtJGT1 and other known SA or IAA glucosyltransferases. **A,** Amino acid sequence alignment of 4 proteins using CLUSTALW program (http://clustalw.genome.jp/). Amino acid residues conserved in 3 or 4 sequences are indicated by dots or asterisks, respectively. **B,** Phylogenetic tree constructed from alignment in **(A)**. SAGT, a SA-UGT from tobacco (Lee and Raskin, 1999); iaglu, IAA-UGT from maize (Szerszen et al., 1994); UGT84B1, IAA-UGT from *Arabidopsis* (Jackson et al., 2001). GenBank accession number for $AtJGT_1$ is DQ158907.

labeled with [^{14}C]. Here, AtJGT1 showed activity toward JA or IAA to produce the JA- or IAA-glucose conjugate (Fig. 2). However, a range of compounds with structures similar to JA or IAA also had activities with UDPG. In addition, the recombinant AtJGT1 exhibited significant activities toward dihydroJA, IPA, and IBA, as well as JA and IAA (Table 1). One IAA-UGT (UGT84B1) with IAA glucosyltransferase activity has been reported previously in *Arabidopsis* (Jackson et al., 2001). The enzyme is also a broad substrate UGT with indole-3-propionic acid (IPA), indole-3-

Table 1. Relative activity of AtJGT1 with jasmonic acid and related substrates.

Substrates	Relative activity (% ± SD)
JA	100 ± 3
Dihydrojasmonic acid	141 ± 3
Linolenic acid	69 ± 5
IAA	182 ± 3
Indole-3-propionic acid	213 ± 2
Indole-3-butyric acid	248 ± 3
2,4-Dichlorophenoxyacetic acid	143 ± 2
Cinnamic acid	60 ± 1
p-Coumaric acid	83 ± 2
Caffeic acid	35 ± 2
Vanillic acid	<1
Salicylic acid	<1
Benzoic acid	4 ± 0.5
4-Hydroxybenzoic acid	3 ± 0.5
ABA	<1
GA_4	<1

Values are averages of three independent measurements. Each substrate was tested at a 2 mM concentration. The activity level of AtJGT1 with JA was set arbitrarily at 100%. SD indicates standard deviation.

butyric acid (IBA), cinnamic acid, and IAA. However, JA and dihydroJA were not tested here as potential substrates for UGT84B1.

Kinetic Parameters for the AtJGT1 Protein

To measure the K_m for each substrate, the concentration of one substrate was fixed at a saturated level and the concentration of the second substrate was varied. Lineweaver-Burk plots were drawn to obtain the K_m and V_{max} values. For example, the K_m values of AtJGT1 for JA and UDPG were 0.29 and 0.58 mM, respectively, whereas those for IAA and UDPG were 0.54 and 0.50 mM, respectively. UGT84B1 had K_m values (about 0.25 mM for IAA and UDPG) similar to those reported for AtJGT1 (Jackson et al., 2001). Turnover numbers (k_{cat} values) and catalytic efficiencies (k_{cat}/K_m) of the AtJGT1 enzyme also were calculated (Table 2).

Effect of Temperature, pH, or Salt on Enzyme Activity

AtJGT1 was incubated at various temperatures, from 10 to 45°C, under standard assay conditions. The optimum temperature for JA or IAA glucosyltransferase activity was 30°C; the ideal pH was 7.0 (Fig. 4A, B). The effects of various cations on AtJGT1 activity also were examined (Fig. 4C, D). Although six divalent ions -- Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, and Fe^{2+} -- significantly activated AtJGT1 activity at 5 mM each, three monovalent ions -- K^+, Na^+, and NH_4^+ -- were more effective. In particular, AtJGT1 required 5 mM K^+ to achieve the highest activity.

Expression of *AtJGT1* in *Arabidopsis* Plants

To analyze the expression pattern of *AtJGT1* in various tissues, northern blot analysis was performed (Fig. 5). The data clearly demonstrated that *AtJGT1* transcripts were abundant in the cauline and rosette leaves, but that expression was relatively low in the inflorescence.

DISCUSSION

JA is an important plant regulator that modulates diverse developmental processes and activates defense responses against various biotic and abiotic stresses. For example, MeJA and JA-isoleucine (a JA-amino acid conjugate) are critical to the activation of JA-signaling (Seo et al., 2001; Staswick and Tiryaki, 2004). This indicates that maintaining cellular homeostasis through the regulation of JA levels may be essential for plant development and stress responses. To address this possibility, the *AtJGT1* gene encoding a glucosyltransferase with JA activity was isolated. The database of *Arabidopsis thaliana* genes provides an opportunity to identify novel plant genes based on homology searches. Here, the gene (*SAGT*) encoding a tobacco SA-UGT (Lee and Raskin, 1999) was used because previous data showed that SA-MTs have

Table 2. Kinetic parameters of AtJGT1.

Substrate	Co-substrate	K_m (mM)	V_{max} (μM min^{-1})	k_{cat} (s^{-1})	k_{cat}/K_m (μ$M^{-1}s^{-1}$)
JA	UDPG (3 mM)	0.29 ± 0.05	0.26 ± 0.04	0.035	0.12
UDPG	JA (3 mM)	0.58 ± 0.08	0.37 ± 0.08	0.050	0.09
IAA	UDPG (3 mM)	0.54 ± 0.07	0.49 ± 0.07	0.067	0.12
UDPG	IAA (3 mM)	0.50 ± 0.08	0.40 ± 0.06	0.054	0.11

Values were obtained by Lineweaver-Burk plots, which were linear within the experimental error. The results shown represent the means of three independent experiments ± SD.

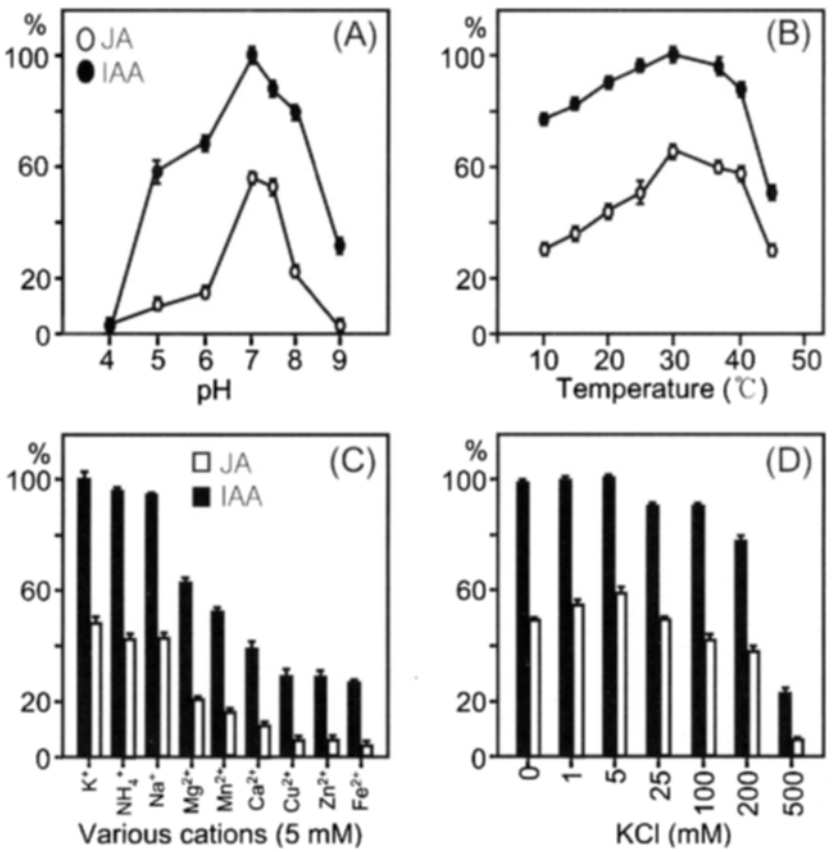

Figure 4. Optimum conditions for AtJGT1. Effects of pH **(A)**, temperature **(B)**, various cations **(C)**, and KCl concentration **(D)** on AtJGT1 activity. Recombinant AtJGT1 was reacted with 2 mM JA or IAA and 8.2 μM UDP-[^{14}C]glucose at 30°C for 30 min. Error bars represent ±SD.

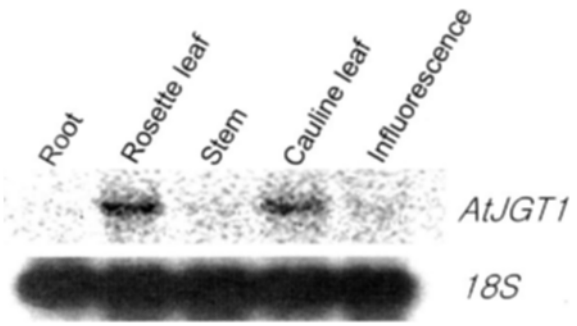

Figure 5. RNA expression of AtJGT1 in *Arabidopsis* (Col). Indicated tissues were used for RNA extraction and RNA gel blot analysis. 18S RNA was used as internal control for gel-loading.

higher homologies with JA-MTs than with other MTs (Song et al., 2000; Seo et al., 2001). Analysis of the recombinant proteins from six putative JA-UGT sequences then led to the identification of one JA-UGT from *Arabidopsis*.

This study involved the biochemical characterization of a recombinant protein using an *in vitro* assay. AtJGT1 exhibited significant activities with IAA, IBA, and JA, including their similar chemicals. As yet, the relationships of these activities to events within the plant are unknown. JA-, IAA-, and IBA-glucose conjugates have been identified in various species, including *Arabidopsis* (Epstein and Ludwig-Müller, 1993; Sembdner and Parthier, 1993; Tam et al., 2000). Here, although relative *in vitro* activity of AtJGT1 with IAA or IBA was approximately two-fold higher than that with JA, the enzyme *in planta* was able to glucosylate IAA, IBA, and JA altogether, depending on the cell specificity of expression, relative availability of substrate(s), and the relative compartmentalization of enzyme and substrate(s). Furthermore, the K_m values of AtJGT1 for IAA and JA were similar, suggesting that the two compounds could be substrates of AtJGT1. The biochemical data for AtJGT1 also indicated that AtJGT1 may provide possible crosstalk between JA and IAA homeostasis. Staswick et al. (2002) have reported that the auxin response mutant *axr1* is

defective in its JA response, implying another link between JA and IAA. This could provide a mechanism by which auxin influences the JA responses.

Until now, only two IAA-UGTs have been reported in maize and *Arabidopsis*. The maize enzyme iaglu has been analyzed only as a partial purified extract, and the recombinant product of that enzyme has not been purified or characterized (Szerszen et al., 1994). The *Arabidopsis* enzyme UGT84B1 has high activity toward IAA, and has now been biochemically characterized (Jackson et al., 2001). However, considerable evidences suggest that the roles, functions, or substrates of the AtJGT1 and UGT84B1 proteins may differ *in planta*. First, AtJGT1 had little homology with UGT84B1 (31% identity), but was more homologous to tobacco SAGT (47% identity). Second, this study revealed that the mRNA of *UGT84B1* was abundant in the inflorescence and siliques, but hardly detectable in the leaves, while AtJGT1 was highly expressed in the latter tissue (see also Jackson et al., 2001). Third, over-expression of UGT84B1 led to phenotypes that resembled those described previously for transgenic lines in which the IAA level was depleted, thereby indicating that the real substrate of UGT84B1 is IAA (Jackson et al., 2002).

To test this hypothesis, further studies of AtJGT1 *in planta* will be required to understand its contribution to JA or IAA homeostasis. The phenotypic characterization of transgenic *Arabidopsis* plants that either over-express *AtJGT1* or else have that gene knocked out by a T-DNA insertion will provide essential information concerning the functioning of AtJGT1.

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LITERATURE CITED

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248-254

Cohen JD, Bandurski RS (1982) Chemistry and physiology of the bound auxins. Annu Rev Plant Physiol 33: 403-430

Epstein E, Ludwig-Müller J (1993) Indole-3-butyric acid in plants: Occurrence, synthesis, metabolism, and transport. Physiol Plant 88: 382-389

Guan KL, Dixon JE (1991) Eukaryotic proteins expressed in *Escherichia coli*: An improved thrombin cleavage and purification procedure of fusion proteins with glutathione S-transferase. Anal Biochem 192: 262-267

Jackson RG, Kowalczyk M, Li Y, Higgins G, Ross J, Sandberg G, Bowles DJ (2002) Over expression of an *Arabidopsis* gene encoding a glucosyltransferase of indole-3-acetic acid: Phenotypic characterization of transgenic lines. Plant J 32: 573-583

Jackson RG, Lim EK, Li Y, Kowalczyk M, Sandberg G, Hoggett J, Ashford DA, Bowles DJ (2001) Identification and biochemical characterization of an *Arabidopsis* indole-3-acetic acid glucosyltransferase. J Biol Chem 276: 4350-4356

Ji JH, Lee JS, Lee JS, Kim WT (1995) Effects of methyl jasmonate on ethylene production in tomato (*Lycopersicon esculentum* Mill.) hypocotyl segments and fruits. J Plant Biol 38: 235-242

Kroczek RA, Siebert E (1990) Optimization of northern analysis by vacuum-blotting, RNA transfer, visualization and ultraviolet fixation. Anal Biochem 184: 90-95

Lee H, Raskin I (1999) Purification, cloning, and expression of a pathogen inducible UDP glucose: Salicylic acid glucosyltransferase from tobacco. J Biol Chem 274: 36637-36642

Li Y, Baldauf S, Lim E-K, Bowles DJ (2001) Phylogenetic analysis of the UDP glycosyltransferase multigene family of *Arabidopsis thaliana*. J Biol Chem 276: 4338-4343

Normanly J (1997) Auxin metabolism. Physiol Plant 100: 431-442

Sambrook J, Fritsch, EF, Maniatis T (1989) Molecular Cloning, A Laboratory Manual, Ed 2. Cold Spring Harbor Laboratory Press, New York

Sembdner G, Atzorn R, Schneider G (1994) Plant hormone conjugation. Plant Mol Biol 26: 1459-1481

Sembdner G, Parthier B (1993) The biochemistry and the physiological molecular actions of jasmonates. Annu Rev Plant Physiol Plant Mol Biol 44: 569-589

Seo HS, Song JT, Cheong JJ, Lee YH, Lee YW, Hwang I, Lee JS, Choi YD (2001) Jasmonic acid carboxyl methyltransferase: A key enzyme for jasmonate-regulated plant responses. Proc Natl Acad Sci USA 98: 4788-4793

Song JT, Seo HS, Song SI, Lee JS, Choi YD (2000) NTR1 encodes a floral nectary-specific gene in *Brassica campestris* L. ssp. *pekinensis*. Plant Mol Biol 42: 647-655

Staswick PE, Tiryaki I (2004) The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis*. Plant Cell 16: 2117-2127

Staswick PE, Tiryaki I, Rowe ML (2002) Jasmonate response locus JAR1 and several related *Arabidopsis* genes encode enzymes of the firefly luciferase superfamily that show activity on jasmonic, salicylic, and indole-3-acetic acids in an assay for adenylation. Plant Cell 14: 1405-1415

Szerszen JB, Szczyglowski K, Bandurski RS (1994) *iaglu*, a

gene from *Zea mays* involved in conjugation of growth hormone indole-3-acetic acid. Science **265**: 1699-1701

Tam YY, Epstein E, Normanly J (2000) Characterization of auxin conjugates in *Arabidopsis*: Low steady-state levels of indole-3-acetyl-aspartate, indole-3-acetyl-glutamate, and indole-3-acetyl-glucose. Plant Physiol **123**: 589-596