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Purification and cDNA cloning of a wound inducible glucosyltransferase active toward 12-hydroxy jasmonic acid

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ABSTRACT

Tuberonic acid (12-hydroxy *epi*-jasmonic acid, TA) and its glucoside (TAG) were isolated from potato leaflets (*Solanum tuberosum* L.) and shown to have tuber-inducing properties. The metabolism of jasmonic acid (JA) to TAG in plant leaflets, and translocation of the resulting TAG to the distal parts, was demonstrated in a previous study. It is thought that TAG generated from JA transmits a signal from the damaged parts to the undamaged parts by this mechanism. In this report, the metabolism of TA in higher plants was demonstrated using [12-^{3}H]TA, and a glucosyltransferase active toward TA was purified from the rice cell cultures. The purified protein was shown to be a putative salicylic acid (SA) glucosyltransferase (OsSGT) by MALDI-TOF-MS analysis. Recombinant OsSGT obtained by overexpression in *Escherichia coli* was active not only toward TA but also toward SA. The OsSGT characterized in this research was not specific, but this is the first report of a glucosyltransferase active toward TA. mRNA expressional analysis of *OsSGT* and quantification of TA, TAG, SA and SAG after mechanical wounding indicated that *OsSGT* is involved in the wounding response. These results demonstrated a crucial role for TAG not only in potato tuber formation, but also in the stress response in plants and that the SA glucosyltransferase can work for TA glucosylation.

1. Introduction

Plants suffer many environmental stresses, such as via drought, extreme temperatures, wounding, pathogenesis, and herbivore attack. As these cannot be avoided by movement and escape, plants must build up unique and sophisticated defense systems. Jasmonic acid (JA, **2**, Fig. 1) is a plant hormone reported to be involved in the plant response triggered by abiotic and biotic stresses, including wounding, insect herbivory, and necrotrophic pathogens. When plants are exposed to such stresses, JA (**2**) is immediately biosynthesized through the octadecanoid pathway (Fig. 1A) (Schaller et al., 2005), and this induces expressions of defense-related genes such as those encoding proteinase inhibitors (PIs) (Farmer and Ryan, 1992; Farmer et al., 1992). This defense response is set in motion systemically, not only at the wound site but also in the undamaged distal leaves (Green and Ryan, 1972). Green and Ryan proposed that specific signals generated at the wound site travel through the plant to the distal parts and activate PIs and other genes in undamaged leaves. The intercellular signal for wound-induced PI gene expression has been proposed to be systemin, an 18-amino-acid peptide derived from proteolytic cleavage of a larger precursor protein called prosystemin (Pearce et al., 1991). Li et al. (2002) reported, however, that systemin is an upstream component in the wound-induced response, and concluded that (pro)systemin activates JA (**2**) biosynthesis, and that JA (**2**) or its related compounds may act as mobile signaling molecules used to activate the systemic response as observed during the grafting experiment using tomato JA signaling mutants (Li et al., 2002; Schilmiller and Howe, 2005).

The existence of JA-amino conjugates is well known in the plant kingdom. *Arabidopsis* JAR1 has been characterized as an enzyme which produces JA-amino conjugates such as JA-Ile, and has been shown to be required for activation of JA (**2**) signaling (Staswick and Tiryaki, 2004). Recently, Thines et al. (2007) and Chini et al. (2007) reported that jasmonate ZIM-domain (JAZ) repressors are

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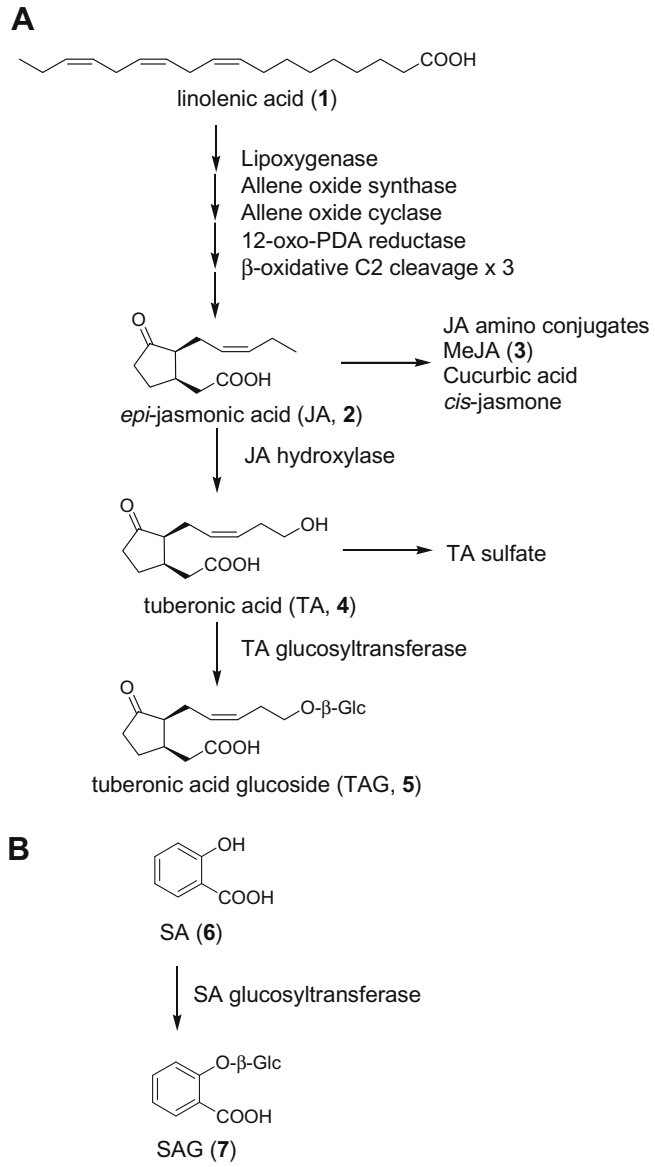


Fig. 1. (A) Biosynthesis of jasmonic acid (**2**) and related compounds. Starting from α-linolenic acid (**1**), JA (**2**) is biosynthesized via peroxidation with lipoxygenase, epoxidation with allene oxide synthase, cyclization with allene oxide cyclase, reduction with OPDA reductase and three cycles of β-oxidative C2 cleavage. JA (**2**) is metabolized to TA (**4**) by hydroxylation at the C-12 position, and then glucosylated to produce TAG (**5**) or sulfated to produce TA-sulfate. There are many other metabolites of JA (**2**), such as JA-amino conjugates, JA methyl ester (**3**, MeJA), cucurbic acid, and *cis*-jasmone. (B) Metabolism of SA (**6**) to SAG (**7**).

key regulators of jasmonate signaling. The crucial role of JA-Ile in *Arabidopsis* in acquired resistance was reported to be as an activator of the interaction of two proteins, SCF^{COI1} E3 ubiquitin ligase and JAZ (Thines et al., 2007). Binding of SCF^{COI1} to JAZ was not activated by JA (**2**), MeJA (**3**) or 12-oxo-phytodienoic acid, but, after binding through JA-Ile, JAZ was ubiquitinated and degraded with the 26S proteasome to de-suppress the jasmonate signaling pathway. Thus, JA-Ile is thought to be a key substrate in the JA-dependent systemic response. However, Wang et al. (2008) recently reported that attempts to recover [$^{13}C_6$]JA-Ile in the systemic leaves after feeding the wounded leaves with [$^{13}C_6$]Ile were not accomplished in tobacco.

Other candidates for the mobile signal for systemic response are tuberonic acid (TA, 12-hydroxyjasmonic acid, **4**) and its glucoside (TAG, **5**). These compounds were first isolated from potato leaflets (*Solanum tuberosum* L.) as tuber-inducing substances (Yoshihara et al., 1989). In a previous study using [^{14}C]JA (**2**), the metabolism of JA (**2**) to TAG (**5**) and translocation of the biosynthesized TAG (**5**) to distal parts were confirmed (Yoshihara et al., 1996). The metabolism of 8-{3-oxo-2-*cis*-[(*Z*)-2-pentenyl cyclopentyl]}octanoic acid (OPC-8:0) to TAG (**5**) has also been demonstrated using deuterated OPC-8:0 (Matsuura and Yoshihara, 2003). These results indicated that TAG (**5**) might be a crucial substrate for transmitting environmental signals to the underground organs. However, there is no experimental evidence to show any clear physiological function of TAG (**5**). In order to being to establish a function for either TA (**4**) or TAG (**5**), we considered that the characterization and functional analysis of TA glucosyltransferase were required.

Glucosylation is known to be an important step in controlling the activity of plant hormones. In case of abscisic acid (ABA), glucosylation is an inactivating step which regulates hormone level (Priest et al., 2006). The characterization of ABA glucosyltransferase was reported by Xu et al. (2002). There have also been reports on glucosyltransferase toward plant hormones such as salicylic acid (SA, **6**) (Lee and Raskin, 1999; Lim et al., 2002), indole-3-acetic acid (IAA) (Jackson et al., 2001), and *cis*-zeatin (Martin et al., 1999). A multifunctional glucosyltransferase which is active toward plant hormones such as ABA, IAA, SA (**6**), and JA (**2**) was found in immature seeds of *Ipomoea nil* (Suzuki et al., 2007). However, there have been no reports concerning TA glucosyltransferase as yet.

Here, we report the universal occurrence of TA (**4**) and TAG (**5**) and the enzymatic generation of TAG (**5**) in higher plants, and demonstrate that endogenous amounts of TA (**4**) and TAG (**5**) in rice and tobacco are increased after mechanical wounding. A protein (OsSGT), which shows TA glucosyltransferase activity, was also purified from rice cell culture extract, and the involvement of *OsSGT* in wounding stress response was demonstrated.

2. Results

2.1. Quantification of endogenous amounts of TA (*4*) and TAG (*5*) in higher plants

In order to investigate the occurrence and levels of TA (**4**) and TAG (**5**) in planta, endogenous amounts of both were determined using Ultra performance liquid chromatography (UPLC) MS/MS together with a multiple reaction monitoring (MRM) analytical method. Extracts from *Nicotiana tabacum*, *Oryza sativa*, *Glycine max*, *S. tuberosum*, *S. lycopersicum, Zea mays, Arabidopsis thaliana* and *Albizzia julibrissin* were prepared and analyzed (Table 1). Almost all of the plant species examined contained TA (**4**) and TAG (**5**), with *A. julibrissin*, in particular, containing large amounts of each. *N. tabacum*, however, had no detectable TA (**4**) and TAG (**5**) under normal growing conditions, but after mechanical wounding, endogenous amounts of these compounds reached detectable levels.

2.2. Quantification of JA (*2*), TA (*4*) and TAG (*5*) in rice after mechanical wounding stress

It is well known that endogenous amounts of JA (**2**) are increased by either mechanical wounding or herbivore attack. The endogenous amounts of TA (**4**) and TAG (**5**) in rice were quantified after mechanical wounding stress in order to determine whether they were also involved in the wounding stress response. In addition to JA (**2**), the amounts of TA (**4**) and TAG (**5**) were found to increase to about 2–4 times the levels present prior to wounding after 5 h from the treatment (Fig. 2), which indicated the involvement not only of JA (**2**) but also of TA (**4**) and TAG (**5**) in wounding

Table 1
Quantification of endogenous amounts of TA (**4**) and TAG (**5**) in various higher plants. Endogenous amounts of TA (**4**) and TAG (**5**) in eight higher plant species were measured using a UPLC-MS/MS system with deuterated compounds employed as internal standards. Data are mean ± SD (n = 3).

		TAG (**5**) (pmol/g FW)	TA (**4**) (pmol/g FW)
Nicotiana tabacum	(Normal)	n.d.[a]	n.d.[a]
	(3 h after wounding)	805.70 ± 168.39	716.81 ± 376.11
Oryza sativa		365.79 ± 91.53	85.81 ± 8.95
Glycine max		121,059.59 ± 24647.67	48,827.43 ± 1641.59
Solanum tuberosum		17,507.72 ± 4432.12	378.03 ± 139.60
Solanum lycopersicum		206.48 ± 39.38	403.10 ± 121.24
Zea mays		10,230.57 ± 1349.74	3,429.20 ± 787.61
Arabidopsis thaliana		n.d.[a]	n.d.[a]
Albizzia julibrissin		738,295.34 ± 25619.17	33,044.25 ± 8845.13

[a] n.d., not detected.

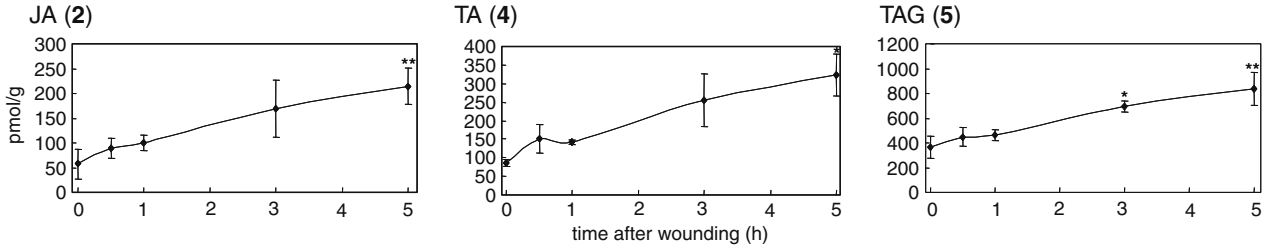
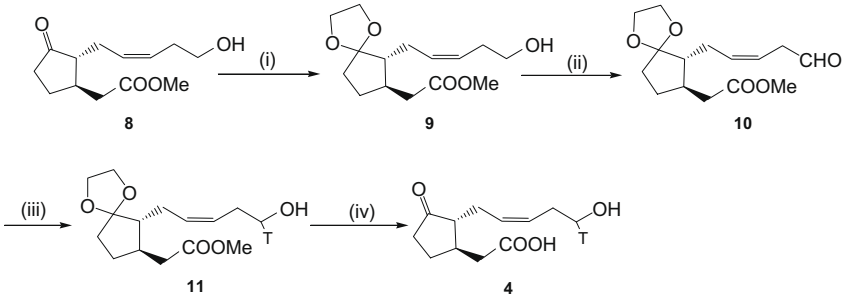


Fig. 2. Endogenous amounts of JA (**2**), TA (**4**) and TAG (**5**) after mechanical wounding in rice. Mechanical wounding stress was inflicted on the rice plant and the endogenous amounts of JA (**2**), TA (**4**) and TAG (**5**) were quantified after 0.5, 1, 3 and 5 h using UPLC-MS/MS. Data are mean ± SD (n = 3). Asterisks denote significant difference between control and each hour (Student's test $^{*}P < 0.05$; $^{**}P < 0.01$).



Scheme 1. Synthesis of tritium-labeled TA (**4**): (i) ethylene glycol, toluene, reflux; (ii) Dess–Martin periodinane; (iii) $NaB[^3H]_4$, EtOH; (iv) HCl/MeOH, then 0.5% KOH in MeOH–H_2O (1:1, v/v).

stress response. Increases in TA (**4**) and TAG (**5**) were also detected in *N. tabacum* after mechanical wounding (Table 1).

2.3. Enzymatic activity converting TA (*4*) to TAG (*5*) in plants

In order to establish the universality of the enzymatic activity that converts TA (**4**) to TAG (**5**), [12-^{3}H]TA (**4**) was synthesized as shown in Scheme 1. The enzymatic activity that generates TAG (**5**) from TA (**4**) was investigated using synthesized [12-^{3}H]TA (**4**). *S. tuberosum*, *N. tabacum*, *A. thaliana*, *O. sativa*, *G. max* and *Salvia officinalis* were selected as test plants. The leaf was harvested, and the part of petiole was dipped into H_2O containing [12-^{3}H]TA (**4**). After 12 h treatment, the leaf was extracted with MeOH–H_2O (6:4, v/v), and then the extracts were analyzed using a HPLC equipped with a radio analyzer. Peaks due to TAG (**5**) and TA (**4**) were detected at about 17 min and 23 min (Fig. 3A–F), respectively; retention times corresponded to those of corresponding authentic compounds as detected by the UV absorbance at 210 nm. In order to confirm the peak at 17 min as TAG (**5**), that part of the solution was collected and hydrolysis was carried out using a β-glucosidase. After hydrolysis the product was analyzed again, and only a peak corresponding to TA (**4**) was observed (data not shown).

2.4. Translocation of TAG (*5*) or TA (*4*) in potato

In order to examine the metabolism of TA (**4**) to TAG (**5**) and the translocation of TAG (**5**) to the distal parts of the plant, a mixture of [12-^{3}H]TA (**4**) and anhydrous lanolin was pasted onto one of the lower leaflets of the potato plant. After growing for 3 days, the upper leaves, which had not been treated with [12-^{3}H]TA (**4**), were cut, and then analyzed using a HPLC equipped with a radio analyzer. As shown in Fig. 3G, TAG (**5**) was detected in the extracts obtained from the leaflets that had not been treated with [12-^{3}H]TA (**4**). The same experiment was carried out for tobacco plants, and the metabolism and translocation of the compound were also confirmed (data not shown). There are two possibilities to explain this result, one is that TA (**4**) is transported to other plant parts and then converted to TAG (**5**), the other is that TAG (**5**) generated from TA (**4**) in the same sight is transmitted

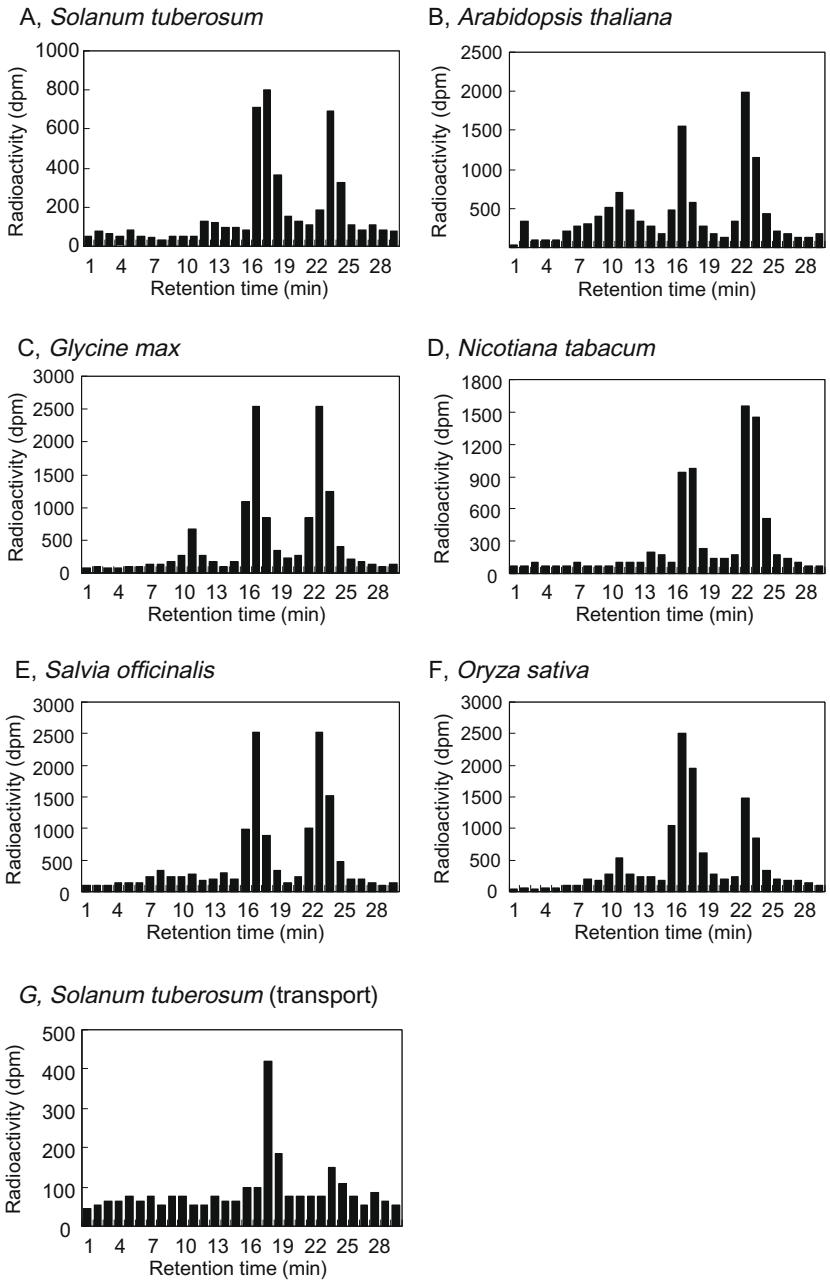


Fig. 3. Metabolism of [12-^{3}H]TA (**4**) in higher plants and transport of [12-^{3}H]TAG (**5**) in potato. Metabolism of [12-^{3}H]TA in (A) *Solanum tuberosum*, (B) *Arabidopsis thaliana*, (C) *Glycine max*, (D) *Nicotiana tabacum*, (E) *Salvia officinalis* and (F) *Oryza sativa*, respectively. Transport of [12-^{3}H]TAG (**5**) in potato (G). Each plant was grown in the water containing [12-3H]TA (**4**) for 12 h. After extraction and purification through a C_{18} cartridge column, samples were analyzed using a HPLC equipped with a radioanalyzer. The peaks at 17 min and 23 min are due to TAG (**5**) and TA (**4**), respectively. For transport tests, potato plant leaf was treated with [12-^{3}H]TA (**4**). Then leaves which were not treated with [12-^{3}H]-TA (**4**) were extracted and analyzed.

to distal leaflets. However, it was thought that the latter would be reasonable due to the small amounts of [12-^{3}H]TA (**4**) in the distal leaflets (Fig. 3G).

2.5. Enzymatic conversion of TA (*4*) to TAG (*5*) using a crude enzyme extract

In order to investigate glucosyltransferase activity *in vitro*, a crude enzyme extract was prepared from rice cell cultures. UDP-glucose was used as a glucose donor as for other plant glucosyltransferases (Jackson et al., 2001; Lee and Raskin, 1999; Martin et al., 1999; Xu et al., 2002). The crude enzyme extract was prepared from rice cell culture, and the enzymatic reaction was carried out using TA-*d*5 (**4**, Matsuura et al., 2000) as a substrate. After the reaction, the supernatant was analyzed by UPLC-MS/MS to detect TAG-*d*5 (**5**). The peak of TAG-*d*5 (**5**) was observed at t_R = 1.90 min, detected by the daughter ion, *m/z* 210.8, in the MRM analysis (Fig. 4A). The production of TAG-*d*5 (**5**) was not observed when the reaction was carried out without either TA-*d*5 (**4**) or using boiled extract (Fig. 4B and C). Glucosyltransferase activity was activated by addition of divalent metal ions such as Mg^{2+}, Mn^{2+} and Co^{2+}, but completely inhibited by Zn^{2+}. A crude enzyme extract prepared from potato node stem also showed TA glucosyltransferase activity (Fig. 4D).

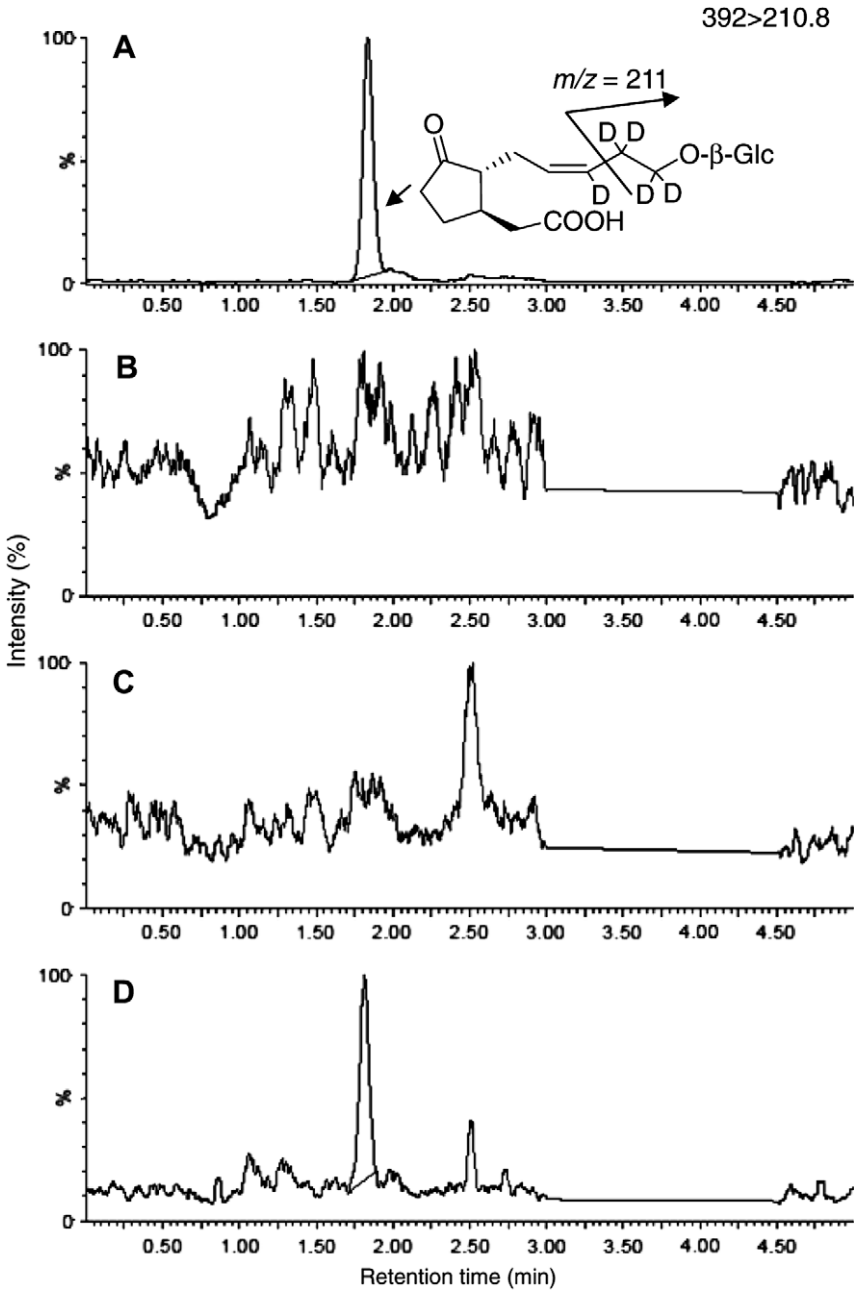


Fig. 4. UPLC-MS/MS analysis of glucosyltransferase assay products. TAG-*d*5 (**5**) was detected by the daughter ion, *m/z* 210.8 derived from *m/z* 392, in the MRM analysis. (A) Reaction with TA-*d*5 (**4**), UDP-glucose, and crude enzyme extract (B) reaction without TA-*d*5 (**4**); (C) reaction using boiled extract; (D) reaction using crude enzyme prepared from potato node stem.

2.6. Purification of TA glucosyltransferase and MALDI-TOF-MS analysis of purified protein

As TA glucosyltransferase activity was detected using a crude enzyme extract derived from the rice cell cultures, purification of the enzyme was accomplished using a series of column chromatographic steps. Rice cell cultures were selected for plant material not only based on the enzymatic activity, but also due to the availability of the full genome information for rice. A crude enzyme extract prepared from the rice cell cultures were purified through three steps of column chromatography namely, DEAE anion exchange, hydroxyapatite and Blue Sepharose. The TA glucosyltransferase was bound by the affinity column, Blue Sepharose, and eluted with UDP-glucose. The result of SDS–PAGE analysis for each step is given in Fig. 5A. Since the molecular mass of the plant UDP-glucose glucosyltransferases are typically 50 kDa, the 50 kDa band was cut from the gel and treated with trypsin, and the products of tryptic digestion were analyzed by MALDI-TOF-MS. The data so obtained was entered into the MASCOT Database, and a putative salicylic acid glucosyltransferase (OsSGT, AK064395) showed a high score for a homologous sequence (Fig. 6, sequence coverage = 19%). Although proteins of 66 kDa and 30 kDa were also detected on the SDS gel (line 5) and were analyzed by MALDI-TOF-MS, they did not score high for any glucosyltransferases (66 kDa; heat shock protein, AK065431, 30 kDa; fructose-1,6-bisphosphatase, AK070516).

2.7. cDNA cloning and functional expression of OsSGT

The putative salicylic acid glucosyltransferase of the rice (*OsSGT*) was amplified by RT-PCR and ligated into an expression

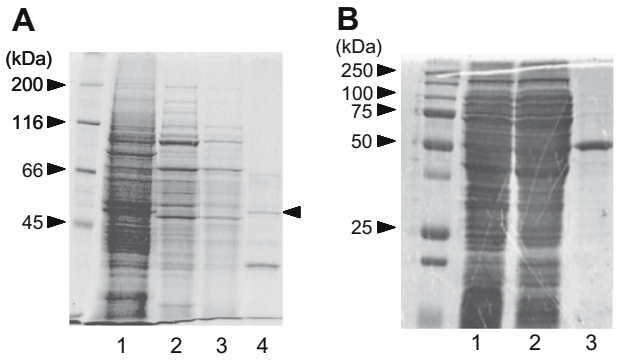


Fig. 5. (A) SDS–PAGE analysis of active fractions in each rice glucosyltransferase purification step (lane 1, crude extract; lane 2, DEAE; lane 3, hydroxyapatite; lane 4, Blue Sepharose, The arrow at the right of the figure shows the 50 kDa protein which matched with a putative salicylic acid glucosyltransferase AK064395 by MALDI-TOF-MS analysis). (B) SDS–PAGE analysis of recombinant protein at each purification step (lane 1, crude protein; lane 2, DEAE; lane 3, Ni-NTA).

vector pQE30 (Qiagen) to obtain the expression vector pQE30-OsSGT. This vector was transformed into *Escherichia coli* M15, and the OsSGT was expressed as a N-terminal His-tag fused protein. The crude extract containing the recombinant protein was next successively subjected to DEAE anion exchange and Ni-NTA agarose column chromatographic steps to afford the pure protein (Fig. 5B), which possessed glucosyltransferase activity toward TA (**4**). The effect of divalent metal ions was also investigated; the activity was activated by Mg^{2+}, $Mn^{2+}$and Co^{2+} (each 10 mM, Mg^{2+}; 153 ± 11%, Mn^{2+}; 208 ± 33%, Co^{2+}; 222 ± 10% compared with control, respectively) and completely inhibited by Zn^{2+} (10 mM).

The substrate specificity of the glucosyl acceptor was examined using salicylic acid (SA, **6**,), because the gene was expected to encode a SA glucosyltransferase (Fig. 1B). It was found that the purified enzyme also showed SA glucosyltransferase activity. The K_m values toward TA (**4**), SA (**6**) and UDPG were 0.4, 2.1 and 0.4 mM, respectively (Table 2). However, the V_{max} value toward SA (**6**) was considerably higher than that toward TA (**4**) (Table 2). Tobacco SA glucosyltransferase has been reported to be active toward both the hydroxyl group and the carboxylic acid group of SA (**6**) (Lee and Raskin, 1999). The OsSGT obtained from the rice cell cultures also showed glucosyltransferase activity toward the carboxylic acid group of SA (**6**), but did not show activity toward the carboxylic acid group of JA (**2**) (data not shown).

Table 2
Michaelis–Menten kinetics (K_m and V_{max} values) of recombinant OsSGT. The amounts of the reaction products were calculated using TAG and SAG as internal standards.

	K_m (mM)	V_{max} (pkat/mg protein)
TA (**4**)	0.4	0.9
SA (**6**)	2.1	1534.7
UDPG	0.4	–

2.8. mRNA expressional analysis of OsSGT

Semi-quantitative RT-PCR was carried out to check the mRNA expression levels of *OsSGT* in the rice cell cultures and the rice plants. The rice cell cultures were treated independently with 1 mM JA (**2**), TA (**4**) and SA (**6**), and the expression level of *OsSGT* was analyzed after 1, 3 and 7 h, respectively (Fig. 7A). For treatment with JA (**2**) and TA (**4**), the maximum expression levels were at 1 and 3 h, respectively, whereas treatment with SA (**6**) gave a continuous level from 1 h onwards. These figures clearly established that expression of *OsSGT* was induced by treatment with JA (**2**), TA (**4**) and SA (**6**).

Mechanical wounding stress was inflicted on rice plants using sandpaper, and the expression levels were analyzed after 1, 3 and 5 h, respectively. It was found that the expression of *OsSGT* in rice was increased 3 h after treatment (Fig. 7B), which corresponded to the time in which endogenous TAG (**5**) increased (Fig. 2). To confirm that SA (**6**) and SAG (**7**) are not involved in the wounding response, endogenous amounts of SA (**6**) and SAG (**7**) were quantified after mechanical wounding. As shown previously, increases in the endogenous amounts of TA (**4**) and TAG (**5**), which were well correlated with mRNA expression, were observed (Figs. 1 and 7B); however, no increases in SA (**6**) and SAG (**7**) occurred after 3–5 h (Fig. 8). These results suggested that gene expression of this enzyme was stimulated by wounding stress to convert TA (**4**) into TAG (**5**).

3. Discussion

We report here that the presence of TA (**4**) and TAG (**5**) is common in higher plants and that the endogenous amounts of these compounds were increased by mechanical wounding in rice as well as tobacco (Table 1 and Fig. 2). This result corresponds well with a recent report by Miersch et al. (2008) in which the endogenous amounts of TA (**4**) and TAG (**5**) in 12 higher plants were ana-

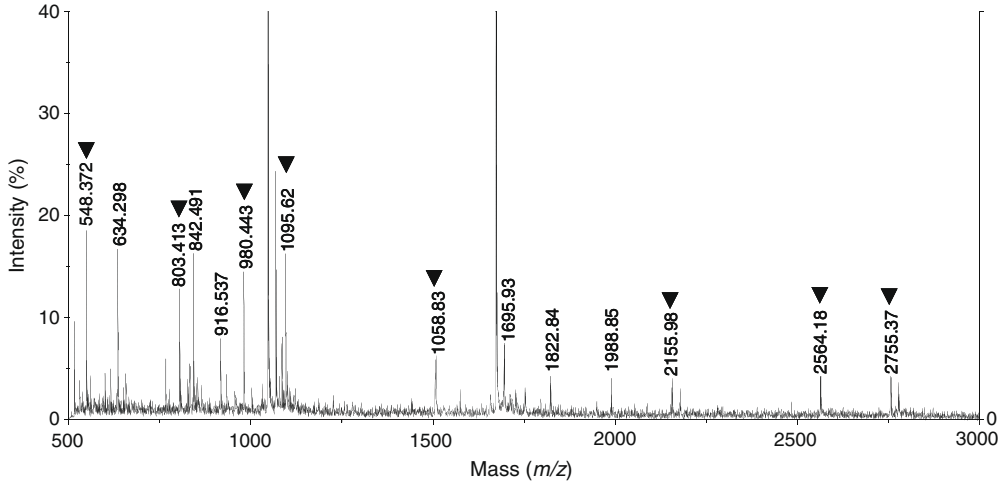


Fig. 6. MALDI-TOF-MS spectrum of the 50 kDa protein after purification with Blue Sepharose (black arrows show ions matched with information from the database entry of AK064395).

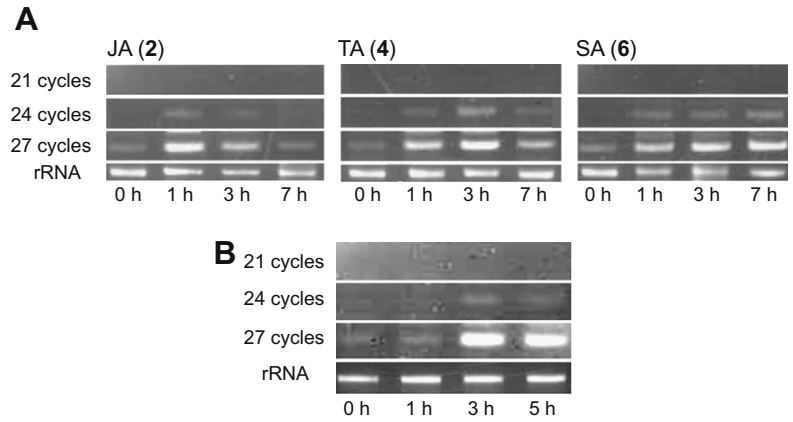


Fig. 7. Semi-quantitative RT-PCR analysis of *OsSGT* transcription amount using total RNA extracted from (A) rice cell culture treated with 1 mM JA (**2**), TA (**4**) or SA (**6**), and (B) rice plant after mechanical wounding using sandpaper.

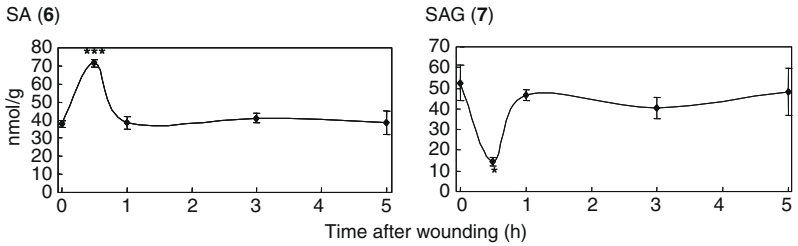


Fig. 8. Endogenous amounts of SA (**6**) and SAG (**7**) after mechanical wounding in rice. Mechanical wounding stress was inflicted on the rice plant and the endogenous amounts of SA (**6**) and SAG (**7**) were quantified using UPLC-MS/MS. Data are mean ± SD ($n = 3$). Asterisks denote significant difference between control and each hour (Student's test $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

lyzed to prove the existence of these compounds in almost all plants. They also demonstrated increases in the endogenous amounts of these compounds after mechanical wounding in tomato. They showed that TA (**4**) and its sulfate down-regulated JA (**2**) biosynthesis and some defense-related genes, and indicated that these compounds contribute to a partial switch-off in jasmonate signaling. There is a possibility that TAG (**5**) also has the same function, but its function has not yet been fully elucidated. Our results nevertheless indicated translocation of TAG (**5**) through the plant body (Fig. 3G). Although it was supposed that TAG (**5**), due to its mobility, acts as a mobile signaling molecule that puts in motion a systemic response, the results reported by Miersch et al. (2008) do not support this hypothesis. However, it is thought that TA (**4**) or its metabolites have a crucial function in JA (**2**) signaling, and therefore, this work on the characterization of TA glucosyltransferase is expected to be important in elucidating the functions of TA (**4**) and TAG (**5**) in the JA (**2**) signaling pathway.

In this study, the universal existence of TA glucosyltransferase was also demonstrated (Fig. 3A–G). TA glucosyltransferase activity was detected using a crude enzyme extract from rice cell cultures, in which UDP-glucose acts as a glucose donor (Fig. 4). The purification of UDP-glucose glucosyltransferase from a rice cell culture extract was carried out, and MALDI-TOF-MS analysis of a 50 kDa protein obtained by our research showed this protein to be a putative salicylic acid glucosyltransferase (OsSGT, AK064395, Fig. 6). Recombinant OsSGT showed glucosyltransferase activity toward TA (**4**). However, this protein was also active toward SA (**6**). Although the K_m value toward TA (**4**) was lower than that toward SA (**6**), the V_{max} value for SA (**6**) was considerably higher than that for TA (**4**) (Table 2). However, these values for TA (**4**) probably do not reflect the real affinity of the enzyme for its substrates, because the synthetic TA (**4**) used in this study contained four stereoisomers due to the presence of two chiral carbons at C-3 and C-7 of the cyclopentanone ring. Additionally, the concentration of the naturally occurring *cis* isomer is likely reduced even further by rearrangement *in vitro* to the more thermodynamically stable *trans* form. Some enzymes which catalyze the reactions of jasmonates recognize only the *cis* isomer which is a naturally biosynthesized form (Schulze et al., 2007; Schaller et al., 2000). If the OsSGT characterized in this study predominantly recognizes *cis*-TA (**4**) as a substrate, this may be a reason for its relative lack of reactivity toward TA (**4**) compared to SA (**6**). Thus, it will be interesting to see whether the OsSGT purified in this study works in a stereoselective manner. Also there is a possibility that there are other co-factors to activate of this enzyme as TA glucosyltransferase. Thus now we cannot conclude that OsSGT works as TA glucosyltransferase *in vivo*.

OsSGT showed glucosyltransferase activity toward the carboxylic acid group of SA (**6**), similarly to tobacco SA glucosyltransferase (Lee and Raskin, 1999). In *Arabidopsis*, two glucosyltransferases, *UGT74F1* and *UGT74F2*, were characterized as SA glucosyltransferases; *UGT74F1* is specific toward hydroxyl group, while *UGT74F2* is active toward both hydroxyl group and carboxylic acid (Lim et al., 2002). OsSGT, which is active toward both the hydroxyl group and the carboxylic acid of SA (**6**), is thought to be an orthologue of *UGT74F2*. Interestingly, OsSGT did not show activity toward the carboxylic acid of JA (**2**).

mRNA expressional analysis showed that the *OsSGT* was induced by mechanical wounding or treatment with JA (**2**), TA (**4**) or SA (**6**) (Fig. 7). The quantification analysis of JA (**2**), TA (**4**), TAG (**5**), SA (**6**) and SAG (**7**) after wounding showed increases in the endogenous amounts of JA (**2**), TA (**4**) and TAG (**5**) 3–5 h after treatment, which corresponded with the inducing time of mRNA expression, but the amounts of SA (**6**) and SAG (**7**) were not

increased during this time (Figs. 2 and 8). This result suggested a possibility that *OsSGT* acts as a TA glucosyltransferase when the plant is exposed to wounding stress. The endogenous amount of SA (**6**) was found to increase 0.5 h after wounding, while that of SAG (**7**) decreased at the same time period (Fig. 8). This result suggested that SA (**6**) might be produced by hydrolysis of SAG (**7**), which supported the hypothesis that the latter is a storage form for spontaneous generation of SA (**6**) (Henning et al., 1993).

4. Concluding remarks

In this paper, the universal existence of TA (**4**) and TAG (**5**) along with TA glucosyltransferase activity in plants was demonstrated, and a rice glucosyltransferase active toward TA (**4**) and SA (**6**) was characterized. It is well known that SA (**6**) is a signaling molecule for biotrophic stress responses, on the other hand, JA (**2**) is a signal for abiotic and biotic stresses and necrotrophic pathogens. The function of these signaling systems is thought to be independently put in motion, and some researchers have reported the existence of an antagonistic relationship between JA (**2**) and SA (**6**) signaling systems (Takahashi et al., 2004; Niki et al., 1998).

Although, *OsSGT* is predicted to encode an SA glucosyltransferase based on amino acid sequence similarity, our report is first to demonstrate that it is active toward SA (**6**) and TA (**4**), and, furthermore, is induced by wounding stress. These results suggest the multifunctionality of this enzyme, and it is noteworthy that one enzyme metabolizes the key compounds which work between antagonistic signaling system.

Here, we cannot provide the clear function of *OsSGT in vivo*. In order to investigate in more detail the function of *OsSGT*, a mutant must be constructed in which this gene is over-expressed or deficient. Anyway our results suggest the crucial role of TA (**4**) and TAG (**5**) not only in potato but also in higher plants in wounding response signaling pathway.

5. Experimental

5.1. General

UPLC was performed on a Waters ACQUITY UPLC system equipped with a binary solvent delivery manager and a sample manager. MS was performed on a Waters Micromass Quattro Premier tandem quadrupole mass spectrometer. The UPLC/MS system was controlled by MassLynx 4.0. NMR spectra were recorded using a JNM-EX 270 FT-NMR system (^{1}H at 270 MHz, JEOL).

5.2. Plant material

Plants (*N. tabacum*, *O. sativa*, *G. max*, *S. tuberosum*, *S. lycopersicum*, *Z. mays*, *A. thaliana*, *A. julibrissin* and *S. officinalis*) were grown in a greenhouse for about 2 weeks before being used for either quantification analyses or mRNA extractions. To obtain potato (*S. tuberosum*) node stems, plants were grown under constant dark conditions at 25 °C for about 2 weeks. Rice cell cultures (*O. sativa* L. cv. Nipponbare) were grown in 100-ml Erlenmeyer flasks containing 50 ml culture medium {145 mg of KNO_3, 23 mg of $(NH_4)_2SO_4$, 46 mg of KH_2PO_4, 17 mg of $CaCl_2 \cdot 2H_2O$, 19 mg of $MgSO_4 \cdot 7H_2O$, 2.5 mg of Fe–Na–EDTA, 0.22 mg of $MnSO_4 \cdot 4H_2O$, 0.075 mg of $ZnSO_4 \cdot 7H_2O$, 0.08 mg of H_3BO_3, 0.04 mg of KI, 0.05 mg of thiamine HCl, 0.025 mg of pyridoxine, 0.025 mg of nicotinic acid, 0.1 mg of glycine and 0.05 mg of 2,4-dichlorophenoxyacetic acid}. The cell culture was incubated with rotary shaking at 25 °C for 10 days and used for enzyme preparation and total RNA extraction.

5.3. Quantification of endogenous amounts of JA (2), TA (4), TAG (5), SA (6) and SAG (7)

5.3.1. Extraction procedure

Each plant material (0.5–1.0 g) was harvested and immediately frozen using liquid N_2. The frozen material was crushed and extracted with MeOH–H_2O (7:3, v/v, 0.1% AcOH, 50 ml) for 24 h. Each mixture was filtered to give a crude extract. Before purification, 600 ng of deuterium-labeled JA (**2**), TA (**4**), TAG (**5**), SA (**6**) and SAG (**7**) were added as internal standards. The volatile components of each extract were removed under reduced pressure, and to each residue was added H_2O (10 ml). Each mixture was put onto the cartridge column of Bond Elut C_{18} (Varian), this being successively washed with H_2O, and then eluted with MeOH–H_2O (10 ml, 7:3, v/v). The MeOH–H_2O fraction was evaporated *in vacuo*, and the residue was dissolved in MeOH–H_2O (0.5 ml, 1:1, v/v). A portion of mixture (5 μl) was subjected to UPLC-MS/MS MRM analysis.

5.3.2. UPLC conditions

UPLC separation was performed on a Waters ACQUITY ethylene-bridged (BEH) C_{18} column (1.7 μm, 2.1 × 100 mm) at 38 °C. The analytes were eluted from the column with a mixed solvent of MeOH–H_2O (1:4, v/v) with 0.05% AcOH (solvent A) and MeOH with 0.05% AcOH (solvent B) using a linear gradient mode. In the analyses for JA (**2**) and SA (**6**), from 0 s to 0.2 min, the ratio of A and B was 70:30, and from 0.2 min to 2 min, the ratio was changed linearly from 70:30 to 10:90. The ratio of 10:90 was then maintained from 2 min to 3 min. Finally, the column was eluted with 0:100 A:B from 3.1 min to 4 min with a flow rate of 0.3 ml/min. In the analyses for SAG (**7**) and SA-glucose ester (**12**), the ratio of A and B was 90:10 from 0 s to 0.2 min, which was changed linearly from 90:10 to 30:70 between 0.2 min and 2 min; the ratio of 30:70 was then maintained from 2 min to 3 min. Finally, the column was eluted with 0:100 A:B from 3.1 min to 4 min with a flow rate of 0.25 ml/min. In the analyses for TA (**4**) and TAG (**5**), the ratio of A and B was 90:10 from 0 s to 0.2 min, changing linearly from 90:10 to 10:90 between 0.2 min and 2 min; the ratio of 10:90 was then maintained from 2 min to 3 min. Finally, the column was eluted with 0:100 A:B from 3.1 min to 4 min, with a flow rate of 0.25 ml/min.

5.3.3. MS conditions

All MS optimization experiments were performed in MS scan mode and in product scan mode. All quantifications were performed in MRM mode. The tune page parameters and conditions for each of the MRM transitions were optimized by infusing the neat standard solution into the mass spectrometer at 10 μg/ml. To ensure that the tune page parameters were compatible with the UPLC flow during tuning, a UPLC flow of 0.3 ml/min (A:B, 1:1) was introduced into the mass spectrometer simultaneously by utilizing a T unit (Upchurch Scientific). For MRM data collection during the UPLC experiments, the capillary voltage was 3.0 kV, the source temperature was 120 °C, the desolvation temperature was 350 °C, the desolvation gas flow was 800 L/h, and the cone gas flow was 50 L/h. During each UPLC injection, the mass spectrometer was set to collect data in MRM mode using electrospray ionization (ESI) in negative ion mode. The MRM condition of each compound was as follows: SA (**6**) {$[M-H]^-$: 136.83, transition ion (*m/z*): 92.50, cone voltage: 26 V, collision energy: 16 eV}; SA *d*-4 (**6**) {$[M-H]^-$: 140.83, transition ion (*m/z*): 96.63, cone voltage: 26 V, collision energy: 16 eV}; SAG (**7**) {$[M-H]^-$: 298.90, transition ion (*m/z*): 136.63, cone voltage: 48 V, collision energy: 18 eV}; SAG *d*-4 (**7**) {$[M-H]^-$: 302.97, transition ion (*m/z*): 140.70, cone voltage: 48 V, collision energy: 18 eV}; TA (**4**) {$[M-H]^-$: 224.97, transition ion (*m/z*): 58.70, cone voltage: 32 V, collision energy: 28 eV}; TA *d*-5 (**4**) {$[M-H]^-$: 229.97, transition ion (*m/z*): 58.70, cone voltage: 32 V,

collision energy: 28 eV}; TAG (**5**) {[M-H]$^-$: 387.03, transition ion (*m/z*): 206.70, cone voltage: 56 V, collision energy: 50 eV}; TAG *d*-5 (**5**) {[M-H]$^-$: 392.03, transition ion (*m/z*): 210.80, cone voltage: 56 V, collision energy: 50 eV}; SA-glucose ester (**12**) {[M-H]$^-$: 299.23, transition ion (*m/z*): 136.705, cone voltage: 93 V, collision energy: 18 eV}.

5.4. Synthesis of [^{3}H]-TA (**4**)

5.4.1. [2-(5-Hydroxy-pent-2-enyl)-3-oxo-cyclopentyl]-acetic acid methyl ester (**9**)

Ethylene glycol (16 ml) and *p*-TsOH (40 mg) were added to a solution of TA methyl ester (**8**, 800 mg, 3.4 mmol in 100 ml of toluene), and the mixture was heated until reflux began this being maintained for 6 h. The resulting reaction product was then purified by silica gel column chromatography (CC) (100 g, EtOAc-hexane, 1:1, v/v) to give the protected TA methyl ester (**9**, 950 mg, 3.36 mmol, 98%).

^{1}H NMR (270 MHz, $CDCl_3$) δ 5.52 (1H, m), 5.40 (1H, m), 3.85 (4H, m), 3.67 (3H, s), 3.61 (2H, m), 3.42 (2H, d, J_H = 7 Hz), 2.54 (1H, m), 2.31 (2H, m), 2.22 (1H, m), 2.12 (1H, m), 1.90 (1H, m), 1.72 (2H, m), 1.27 (2H, m).

5.4.2. [6-(5-Hydroxy-pent-2-enyl)-1,4-dioxa-spiro[4.4]non-7-yl]-acetic acid methyl ester (**10**)

A solution of Dess–Martin periodinane (0.3 M, 0.3 ml in CH_2Cl_2) was added to a solution of compound **9** (120 mg, 0.42 mmol, 1 ml of CH_2Cl_2), and the mixture was stirred for 6 h at −40 °C. The product was purified by silica gel CC (20 g, EtOAc-hexane, 1:1, v/v) to give compound **10** (12 mg, 0.042 mmol, 10%).

^{1}H NMR (270 MHz, $CDCl_3$) δ 9.66 (1H,s), 5.70 (1H, m), 5.57 (1H, m), 3.84 (4H, m), 3.64 (3H, s), 3.42 (2H, d, J_H = 7 Hz), 2.53 (1H, m), 2.25 (2H, m), 2.04 (2H, m), 1.90 (1H, m), 1.73 (2H, m)1.29 (2H, m).

5.4.3. [6-(5-Oxo-pent-2-enyl)-1,4-dioxa-spiro[4.4]non-7-yl]-acetic acid methyl ester (**11**)

Na[^{3}H]$_4$ (18.5 MBq) was added to a solution of compound **10** (12 mg, 0.042 mmol in 2 ml EtOH) and the mixture was stirred for 3 h. The reaction mixture was diluted with 0.1 N HCl (20 ml) and extracted with EtOAc (20 ml × 3). The EtOAc fraction was evaporated *in vacuo* to give compound **11**, which was used without following purification.

5.4.4. 12-[^{3}H]- Tuberonic acid (**4**)

Compound **11** was dissolved in HCl methanolic solution (2 ml, 0.5 mol/l, Wako) and stirred for 1 h. After evaporation *in vacuo*, the sample was dissolved in 0.5% KOH in MeOH–H_2O (10 ml, 1:1, v/v). After stirring for 12 h, the reaction mixture was subjected to Amberlite cation exchange CC to afford a neutralized solution, which was evaporated *in vacuo* to dryness. The resulting residue was dissolved with MeOH (5 ml) to give a radioactive solution (2.5 MBq/ml) containing [12-^{3}H]TA (**4**).

5.5. Metabolism of [12-^{3}H]TA (**4**)

The leaf was detached, and the part of petiole was dipped into H_2O (1 ml) containing [12-^{3}H]TA (**4,** 50 kBq). At 12 h following the treatment, the leaf was extracted with MeOH–H_2O (6:4, v/v, 20 ml). The sample was passed through a Bond Elut C_{18} cartridge column and concentrated *in vacuo*. The residue was dissolved with 500 μL of MeOH–H_2O (1:1, v/v) and analyzed with a HPLC (Tsk-gel 80Ts, φ4.6 × 250 mm, Tosoh, MeOH–H_2O, 3:7, v/v, containing 0.1% AcOH, 0.8 ml/min) equipped with a radio analyzer. The hydrolysis of [^{3}H]TAG (**5**) was carried out using commercially available β-glucosidase (1 mg, Sigma) (Matsuura et al., 2000).

5.6. TA glucosyltransferase assay

The glucosyltransferase assay mix (100 μL) contained 1 mM TA-*d*5 (**4**), 5 mM UDP-glucose, 10 mM $MgCl_2$ and crude enzyme. The reaction was carried out at 30 °C for 1 h and stopped by adding 100 μL of MeOH, and then non-labeled TAG (**5**, 20 ng) was added as an internal standard. The reaction mixture was centrifuged at 15,000*g* for 5 min, and the supernatant was analyzed by UPLC-MS/MS. The amount of the reaction product was calculated by comparison of the peak areas of TAG-*d*5 (**5**) and non-labeled TAG (**5**). The SA glucosyltransferase assay was carried out in the same manner, except that SA-*d*4 (**6**) was used as a substrate.

5.7. Purification of TA glucosyltransferase

Frozen plant material (rice cell culture, 30 g) was pulverized in liquid N_2 using a mortar and pestle, and resuspended in 60 ml of buffer A (50 mM Tris buffer (pH 7.8) containing 1 mM DTT and 15% (w/v) glycerol). The tissue slurry was removed by centrifugation at 15,000*g* for 20 min to afford a supernatant, which was subjected to DEAE anion exchange CC (30 ml, DEAE 650 M, Tosoh), which was equilibrated with buffer A. The column was washed with buffer A (200 ml), and then the protein was eluted with a 0–0.5 M NaCl gradient in buffer A. The active fractions were collected and dialyzed to the buffer B {5 mM phosphate (pH 7.0) containing 1 mM DTT and 15% (w/v) glycerol}. The active fractions from DEAE column were subjected to hydroxyapatite CC (10 ml, Nihon Chemical) which was equilibrated in buffer B. The column was washed with buffer B (100 ml), and then the protein was eluted with 5–200 mM phosphate buffer {pH7.0, 1 mM DTT, 15% (w/v) glycerol} to give the active fractions, which were dialyzed in buffer A. The active fractions from hydroxyapatite chromatography were subjected to Blue Sepharose CC (10 ml, GE Healthcare), which was equilibrated with buffer A. The column was washed with buffer A (50 ml), and the protein was eluted with buffer A containing 5 mM UDP-glucose (50 ml).

5.8. MALDI-TOF-MS analysis

The active fractions from Blue Sepharose chromatography were concentrated by ultrafiltration, and the sample was subjected to SDS–PAGE. The bands at 30, 50 and 66 kDa were excised from the gel and destained twice with CH_3CN–50 mM NH_4HCO_3 (1:1, v/v). The gels were dried and treated with trypsin solution (10 μg/ml) on ice for 30 min and at 37 °C for 12 h. The digested peptides were eluted twice with 100 μL of CH_3CN–H_2O (3:1, v/v, 0.1% TFA). After concentration, the sample was loaded onto the plate using ZipTip C_{18} (Millipore) and analyzed by MALDI-TOF-MS in positive ion mode. The results were searched with the MASCOT database (http://www.matrixscience.com/).

5.9. cDNA cloning of putative salicylic acid glucosyltransferase (OsSGT, AK064395)

Total RNA was extracted from the rice cell culture, and reverse-transcription (invitrogen, M-MLV reverse transcriptase) was carried out according to the manual to yield cDNA. PCR was carried out with cDNA prepared from total RNA from rice cell culture using forward primer (SGT-1F, 5′-GTATCACAGTTCACAAAAG CAGCACAGAGC-3′) and reverse primer (SGT-1R, 5′-GACTGTGCCCA TTTCATTGAATATAGTGGG-3′) designed based on the database entry of AK064395. The PCR reaction was optimized for a 50 μL of reaction mixture per tube containing 0.2 mM dNTP mix, 0.5 μM of RTAG-1F, 0.5 μM of RTAG-1R, 1 μL of cDNA, 25 μL of PCR buffer, and 0.5 μL of KOD FX DNA polymerase (Toyobo). The reaction temperature was set to 98 °C for 10 s, 65 °C for 5 s, and 68 °C for 1 min 30 s, with 30

cycles. The PCR product was analyzed by gel electrophoresis and visualized after ethidium bromide staining. The gene fragment was cut from the gel and ligated into cloning vector pBluescript II SK to obtain the plasmid pBS-OsSGT.

5.10. Expression of OsSGT in E. coli

After sequence analysis of pBS-OsSGT, PCR was carried out using a primers containing a restriction enzyme site, forward primer (STG-2F with BamH I site, 5′-GTCGCAGGATCCGCGAGCTCA-GAGCGC-3′) and reverse primer (STG-2R with Hind III site, 5′-CACCTGTCTCAAAAGCTTAA ATTTTCG-3′). The composition of PCR was as described before. After restriction with BamH I and Hind III, the gene fragment was ligated into the expression vector pQE30 (Qiagen) to obtain the plasmid pQE30-OsSGT, which was transformed into *E. coli* M15. A single colony was grown overnight in 20 ml of LB broth containing 100 μg/ml of ampicillin and 25 μg/ml of kanamycin at 37 °C. A 10 ml aliquot of the culture was inoculated into 1 L of fresh LB broth containing 100 μg/ml of ampicillin and 25 μg/ml of kanamycin at 22 °C. After the culture had grown to a cell density of OD_{600} = 0.8–1.0, the fusion protein was induced by adding IPTG at 0.1 mM. After further incubation for 5 h, the cells were collected by centrifugation at 7000 *g* for 20 min, then washed with buffer A. The cells were resuspended in buffer A and burst by ultrasonication. The soluble proteins collected by centrifugation (10,000*g* for 20 min) were subjected to DEAE anion exchange CC (20 ml). The column was washed with buffer A (100 ml) and the proteins were eluted with a gradient of 0–0.5 M NaCl in buffer A. The active fractions were collected and dialyzed in buffer C (buffer A containing 50 mM imidazole and 0.3 M NaCl). The sample was subjected to Ni-NTA CC (2 ml) and washed with buffer C (20 ml). The His-tag fused protein was eluted with a gradient of 50–500 mM imidazole gradient in buffer A containing 0.3 M NaCl. After desalting, the protein was used for activity tests.

5.11. Semi-quantitative RT-PCR analysis of OsSGT

Leaves of rice plants which had been growing for about 2 weeks were rubbed with sandpaper. After 1, 3 and 5 h, total RNA was extracted from the plant using a FastPure RNA Kit (Takara), and RT-PCR was carried out with a one step RT-PCR kit (Takara) using the primers SGT2F (5′-GGCGAACACGGAGGACAGCCCGGCG-3′) and SGT2R (5′-CTCG GCGTCGCCACGCTGCCGAAGG-3′). The program was set to initial reverse-transcription for 30 min at 50 °C followed by various numbers of cycles (21, 24 and 27 cycles) at 98 °C for 10 s, 60 °C for 30 s and 68 °C for 30 s. The PCR products were analyzed by gel electrophoresis and visualized after ethidium bromide staining.

The rice cell culture was treated with 1 mM JA (**2**), TA (**4**) or SA (**5**). After 1, 3 and 7 h, total RNA was extracted using same kit and RT-PCR was carried out by the same procedure as mentioned previously.

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References

Chini, A., Fonseca, S., Fernandez, G., Adie, B., Chico, J.M., Lorenzo, O., Garcia-Casado, G., Lopez-Vidriero, I., Lozano, F.M., Ponce, M.R., Micol, J.L., Solano, R., 2007. The JAZ family of repressors is the missing link in jasmonate signaling. Nature 448, 666–671.

Farmer, E.E., Ryan, C.A., 1992. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell 4, 129–134.

Farmer, E.E., Johnson, R.R., Ryan, C.A., 1992. Regulation of expression of proteinase inhibitor genes by methyl jasmonate and jasmonic acid. Plant Physiol. 98, 995–1002.

Green, T.R., Ryan, C.A., 1972. Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. Science 18, 776–777.

Henning, J., Malamy, J., Grynkiewicz, G., Indulski, J., Klessig, D.F., 1993. Interconversion of the salicylic acid signal and its glucoside in tobacco. Plant J. 4, 593–600.

Jackson, R.G., Lim, E., Li, Y., Kowalczyk, M., Sandberg, G., Hoggett, J., Ashford, D.A., Bowls, D.J., 2001. Identification and biochemical characterization of an *Arabidopsis* indole-3-acetic acid glucosyltransferase. J. Biol. Chem. 276, 4350–4356.

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, L., Li, C., Lee, G.I., Howe, G.A., 2002. Distinct roles for jasmonate synthesis and action in the systemic wound response of tomato. Proc. Natl. Acad. Sci. USA 99, 6416–6421.

Lim, E., Doucet, C.J., Li, Y., Elias, L., Worrall, D., Spencer, S.P., Ross, J., Bowles, D.J., 2002. The activity of *Arabidopsis* glycosyltransferases toward salicylic acid, 4-hydroxybenzoic acid, and other benzoates. J. Biol. Chem. 277, 586–592.

Martin, R.C., Mok, M.C., Mok, D.W.S., 1999. Isolation of a cytokinin gene, *ZOG1*, encoding zeatin *O*-glucosyltransferase from *Phaseolus lunatus*. Proc. Natl. Acad. Sci. USA 96, 184–289.

Matsuura, H., Ohmori, F., Kobayashi, M., Sakurai, A., Yoshihara, T., 2000. Qualitative and quantitative analysis of endogenous jasmonoids in potato plant (*Solanum tuberosum* L.). Biosci. Biotechnol. Biochem. 64, 2380–2387.

Matsuura, H., Yoshihara, T., 2003. Metabolism of deuterium-labeled jasmonic acid and OPC 8:0 in the potato plant (*Solanum tuberosum* L.). Biosci. Biotechnol. Biochem. 67, 1903–1907.

Miersch, O., Neumerkel, J., Dippe, M., Stenzel, I., Wasternak, C., 2008. Hydroxylated jasmonates are commonly occurring metabolites of jasmonic acid and contribute to a partial switch-off in jasmonate signaling. New Phytol. 177, 114–127.

Niki, T., Mitsuhara, I., Seo, S., Otsubo, N., Ohashi, Y., 1998. Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. Plant Cell Physiol. 39, 500–507.

Pearce, G., Strydom, D., Johnson, S., Ryan, C.A., 1991. A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. Science 23, 895–897.

Priest, D.M., Ambrose, S.J., Vaistij, F.E., Elias, L., Higgins, G.S., Ross, A.R.S., Abrams, S.R., Bowles, D.J., 2006. Use of the glucosyltransferase UGT71B6 to disturb abscisic acid homeostasis in *Arabidopsis thaliana*. Plant J. 46, 492–502.

Schaller, F., Biesgen, C., Müssig, C., Altmann, T., Weiler, E.W., 2000. 12-Oxophytodienoate reductase 3 (OPR3) is the isoenzyme involved in jasmonate biosynthesis. Planta 210, 979–984.

Schaller, F., Schaller, A., Stintzi, A., 2005. Biosynthesis and metabolism of jasmonates. J. Plant Growth Regul. 23, 179–799.

Schulze, B., Dabrowska, P., Boland, W., 2007. Rapid enzymatic isomerization of 12-oxophytodienoic acid in the gut of lepidopteran larvae. CHEMBIOCHEM 8, 208–216.

Schilmiller, A.L., Howe, G.A., 2005. Systemic signaling in the wound response. Curr. Opin. Plant Biol. 8, 369–377.

Staswick, P.E., 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.

Suzuki, H., Hayase, H., Nakayama, A., Yamaguchi, I., Asami, T., Nakajima, M., 2007. Identification and characterization of *Ipomoea nil* glucosyltransferase which metabolizes some phytohormones. Biochem. Biophys. Res. Commun. 361, 980–986.

Takahashi, H., Kanayama, Y., Zheng, S.M., Kusano, T., Hase, S., Ikegami, M., Shah, J., 2004. Antagonistic interactions between the SA and JA signaling pathways in *Arabidopsis* modulate expression of defense genes and gene-for-gene resistance to cucumber mosaic virus. Plant Cell Physiol. 45, 803–809.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S.Y., Howe, G.A., Browse, J., 2007. JAZ repressor proteins are targets of the SCF^{COI1} complex during jasmonate signaling. Nature 448, 661–666.

Yoshihara, T., Omer, E.A., Koshino, H., Sakamura, S., Kikuta, Y., Koda, Y., 1989. Structure of a tuber-inducing stimulus from potato leaves (*Solanum tuberosum* L.). Agric. Biol. Chem. 10, 2835–2837.

Yoshihara, T., Amanuma, M., Tsutsumi, T., Okumura, Y., Matsuura, H., Ichihara, A., 1996. Metabolism and transport of [2-^{14} C](±) jasmonic acid in the potato plant. Plant Cell Physiol. 37, 586–590.

Wang, L., Allmann, S., Wu, J., Baldwin, I.T., 2008. Comparisons of LIPOXYGENASE3- and JASMONATE-RESISTANT4/6-silenced plants reveal that jasmonic acid and jasmonic acid-amino acid conjugates play different roles in herbivore resistance of *Nicotiana attenuate*. Plant Physiol. 146, 904–915.

Xu, X., Nakajima, M., Suzuki, Y., Yamaguchi, I., 2002. Cloning and characterization of the abscisic acid-specific glucosyltransferase gene from adzuki bean seedlings. Plant Physiol. 129, 1285–1295.