Two tobacco genes induced by infection, elicitor and salicylic acid encode glucosyltransferases acting on phenylpropanoids and benzoic acid derivatives, including salicylic acid

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Abstract Two tobacco genes (*TOGT*) with homology to glucosyltransferase genes known to be induced by salicylic acid (SA) also responded rapidly to a fungal elicitor or to an avirulent pathogen. SA, although an efficient inducer, was shown not to be essential in the signal transduction pathway regulating *TOGT* gene expression during the resistance response. Recombinant TOGT proteins produced in *Escherichia coli* exhibited low, but significant, glucosyltransferase activity towards SA, but very high activity towards hydroxycoumarins and hydroxycinnamic acids, with glucose esters being the predominant products. These results point to a possible important function in defense of these glucosyltransferases in conjugating aromatic metabolites prior to their transport and cross-linking to the cell wall.

Key words: Glucosyltransferase; Phenylpropanoid; Salicylic acid; Hypersensitive response; *Nicotiana tabacum*

1. Introduction

Resistance of plants to pathogens is often associated with a hypersensitive response (HR) which is characterized by the formation of necrotic lesions at the site of infection and the restriction of pathogen spread. Early events occurring during the HR are the production of active oxygen species, ion fluxes across the plasma membrane, production of signal molecules such as salicylic acid (SA), reinforcement of the cell wall by cross-linking of cell wall proteins and deposition of related wall-bound phenolics [1]. Many early induced defense responses are thought to be controlled by transcriptional activation of defense-response genes, including the phenylpropanoid biosynthetic genes involved in SA biosynthesis and lignin production [2]. The late events associated with defense responses are the coordinated activation of a set of host genes encoding the pathogenesis-related (PR) proteins including the antimicrobial proteins chitinases and glucanases [3].

SA was shown to accumulate in plants undergoing an HR [4] and has been involved in the expression of localized hypersensitive resistance. Indeed, transgenic tobacco expressing the bacterial *nahG* gene encoding SA hydroxylase (which oxidizes SA to an inactive compound, catechol) exhibits a weakened localized resistance [5]. Little is known about the various components of the SA signal transduction pathway. Many reports point to the role of SA in the induction of defense-related genes, particularly those encoding PR proteins [3], but SA is also known to regulate the expression of alternative oxidase [6], manganese SOD [7], nopaline synthase [8], the 35S promoter of CaMV [9] and to induce the cellular protectant glutathione *S*-transferase genes [10]. A SA-activated protein kinase has been purified from tobacco cell suspensions and the encoding gene assigned to the MAP kinase gene family as a new member [11].

Recently, two related genes induced by SA (*IS5a* and *IS10a*) were isolated by differential display from cycloheximide-pretreated SA-induced tobacco BY-2 cells [12]. From the predicted amino acid sequences, they were found to have similarity to glucosyltransferases, but the actual substrates were not defined. Glucosyltransferases, catalyzing the conjugation of glucose from sugar nucleotide to phenolic compounds, are of widespread occurrence in plants [13]. Glucosides might serve as transport forms of lignin monomers into the cell wall [14] and as intermediates towards impregnation of the cell wall with cinnamate derivatives after elicitor treatment of cell suspension cultures [15]. Conjugation reactions catalyzed by glucosyltransferases are providing a means of detoxification of exogenously applied compounds, but may also play a key role in regulating the levels of plant hormone molecules and signaling compounds such as SA, jasmonic acid and auxin by converting the active molecules to inactive conjugated forms [16,17].

Here we report the cloning from tobacco Samsun NN of two genes (*TOGT*) encoding glucosyltransferases (TOGT) homologous to *IS5a* and *IS10a* [12] and show that they are induced in tobacco cells not only by SA treatment but also by a fungal elicitor, as well as in tobacco leaves during the HR of tobacco to tobacco mosaic virus (TMV). Moreover, we show that induction of the *TOGT* genes after elicitor treatment was not suppressed in transgenic tobacco expressing the *nahG* gene. Thus, though *TOGT* genes are induced by SA, it appears that SA may not be essential in the transduction pathway leading to *TOGT* expression in elicited cells. Recombinant TOGT proteins were produced in *Escherichia coli* and their activity assayed towards a broad range of putative substrates. We found a high TOGT activity for hydroxylated coumarins and (hydroxy)cinnamic acids and a low activity for SA. Taken together these results suggest that these UDP-glu-

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Abbreviations: TOGT, tobacco glucosyltransferase; HR, hypersensitive response; SA, salicylic acid; TMV, tobacco mosaic virus

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cose:hydroxycoumarin/(hydroxy)cinnamic acid glucosyltransferases may have an important role in resistance-related aromatic metabolism.

2. Materials and methods

2.1. Chemicals

Hydroxycinnamic acids (*trans*-isomers) and hydroxycoumarins were obtained from Sigma (Germany). Chlorogenic acid, benzoic and cinnamic acids were purchased from Fluka (Switzerland), auxin (indole-3-acetic acid) from Merck (Germany) and esculin (esculetin-7-*O*-glucoside) from Roth (Germany). Salicylic acid β-*O*-D-glucoside, salicyloyl glucose ester, *p*-hydroxybenzoic acid β-*O*-D-glucoside, *p*-hydroxybenzoyl glucose ester and *m*-hydroxybenzoic acid β-*O*-D-glucoside were kindly provided by Dr. S. Tanaka (University of Kyoto, Japan). All other glucosides were generous gifts from Dr. P. Desbordes (Rhône-Poulenc Agrochimie, Lyon, France). [7-^{14}C]Salicylic acid (2.0 GBq/mmol) and UDP-D-[U-^{14}C]glucose (10.6 GBq/mmol) were obtained from NEN (UK).

2.2. Biological materials and treatments

Nicotiana tabacum cv. Samsun NN and transgenic NahG-10 plants (kindly provided by Dr. J. Ryals, Ciba-Geigy, USA) were maintained in growth chambers at 25°C with a 16 h photoperiod. For TMV infection, *N. tabacum* cv. Samsun NN was inoculated by rubbing fully expanded leaves with a virus suspension (0.2 μg/ml). For treatment with elicitor, tobacco leaves were infiltrated with 50 nM β-megaspermin, a proteinaceous HR-inducing elicitor isolated from *Phytophthora megasperma* [18]. At different times after infection or elicitor treatment, treated leaf tissue was harvested, quickly frozen in liquid nitrogen and stored at −70°C. BY-cultured tobacco cells (derived from *N. tabacum* cv. Bright Yellow) were grown at pH 5.8 in MS medium supplemented with 3% sucrose, 1 mg/l thiamine, 0.1 g/l myoinositol, 0.2 mg/l 2,4-D, 0.2 g/l KH_2PO_4, maintained in darkness on a shaking platform at 25°C. Seven-day-old BY cells were induced either with 100 μM SA or with 50 nM β-megaspermin. Cells were harvested by vacuum filtration, washed with 3% sucrose, rapidly frozen in liquid nitrogen and stored at −70°C before analysis.

2.3. Molecular cloning of TOGT genes from N. tabacum cv. Samsun NN

Genomic DNA was extracted from 5 g tobacco leaves as described by Jaeck et al. [19]. Glucosyltransferase coding sequences [12] were amplified by PCR from tobacco genomic DNA using the following primers: 5′-TOGTCCCCCGGTOGTCTAGACATGGTOGTCAGCTCCATWTTTTC-3′ and 5′-GCCGCTCGAGCTCTTAATGACCATOGTAGAACTATATG-3′. These primers were determined from GenBank databases for amplifying the glucosyltransferase cDNAs *IS5a* and *IS10a* (accession numbers: U32644 and U32643, respectively). The expected 1.4 kb DNA fragments were amplified, cloned and analyzed in the pGEM-T vector (Promega). These fragments represented two closely related genes which could be distinguished by a *Sal*I restriction analysis before sequencing.

2.4. RNA analysis

Total RNA was extracted from 0.3 g plant material. RNA extraction, Northern blotting and hybridization procedures have been described previously [18]. The different probes used were as follows: the tobacco cDNA clones encoding PAL (phenylalanine ammonia-lyase [20]), PR-1a (provided by Dr. J. Bol, University of Leiden, The Netherlands) and a cDNA clone encoding the 18S rRNA from *Oryza sativa* (a gift from Dr. N. Chaubet, IBMP, Strasbourg, France). For reverse transcription (RT)-PCR analysis, cDNAs were synthesized from 2 μg of RNA for 1 h at 37°C in 20 μl PCR buffer (20 mM Tris-HCl pH 8.4, 2.5 mM $MgCl_2$, 50 mM KCl, 0.1 mg/ml BSA) complemented with 1 mM dNTPs, 50 pmol oligodT primers and 14 units reverse transcriptase (M-MuLV RT, Promega). PCR reactions were linear between 0.05 μg and 0.4 μg of first-strand cDNA and were carried out in quantitative conditions from 0.1 μg newly synthesized cDNAs in 50 μl PCR buffer complemented with 0.2 mM dNTPs, 20 pmol primers amplifying both *TOGT* genes and 0.2 unit Taq polymerase (Gibco-BRL). One half of the PCR products was analyzed on 1% agarose gel, the other half was digested with the *Sal*I restriction enzyme and analyzed by Southern blot. Signals were quantified using a Fujix Bio-Imager Analyser (BAS 1000 MacBas).

2.5. Salicylic acid analysis

Total SA was quantified using HPLC because it exhibited a similar pattern of accumulation compared to free SA in tobacco leaves infiltrated with the elicitor and allowed an enhanced detectability [18].

2.6. Production and purification of the recombinant TOGT proteins

*Xba*I-*Xho*I 1.4 kb fragments encoding TOGT proteins were ligated into *Xba*I-*Xho*I-digested pGEX-KG expression vectors (Pharmacia Biotech) harboring the glutathione *S*-transferase (GST)-encoding sequence. Recombinant protein production was performed in *E. coli* $DH_5\alpha$ transformed by electroporation with the expression vectors. The transformed bacterial cells were grown at 37°C in Luria-Bertani medium containing 100 μg/ml ampicillin until they reached OD_{600} values of 0.6–0.8. 1 mM isopropyl β-D-thiogalactoside (IPTG) was added to induce TOGT proteins. The bacteria were grown at 18°C for 12 h and then harvested by centrifugation and resuspended in 20 ml of lysis PBS buffer (150 mM NaCl, 16 mM Na_2HPO_4, 4 mM NaH_2PO_4) containing 0.1 mM PMSF, 10 mM DTT. Batch purification with glutathione Sepharose 4B (Pharmacia Biotech), fusion-protein cleavage by thrombin and recombinant protein purification were performed according to the manufacturer's instructions.

2.7. Standard enzyme assays and reaction product identification

The enzymatic assays were carried out at 37°C for 60 min in 50 μl of reaction mixture containing 0.1 M KH_2PO_4/K_2HPO_4 pH 6.0, 10 μg recombinant enzyme and either 200 μM unlabeled substrate and 110 μM UDP-[^{14}C]glucose (5.6 kBq), or 180 μM [7-^{14}C]SA (18.4 kBq) and 200 μM unlabeled UDP-glucose. Incubations were terminated by the addition of 50 μl methanol. Reaction products were analyzed on silicagel TLC plates (Merck, Germany) developed in the following solvents: (I) *n*-butanol:acetic acid:water (4:1:1, v/v/v) for the detection of benzoic and (hydroxy)cinnamic acid glucosides, (II) chloroform:methanol (70:30, v/v) for the glucosides of coumarins and (III) chloroform:methanol:acetic acid (75:20:5, v/v/v) for auxin glucoside. Radioactivity on TLC plates was visualized and quantified with a Bio-Imager Analyser. Relative activity of TOGTs versus various substrates was normalized to incorporation of radioactivity into scopoline, the glucoside of scopoletin, taken as 100%. Compounds were identified by combination of co-chromatography on silicagel TLC plates with authentic compounds, where available, and analysis by HPLC [13] with a photodiode array detector (maxplot between 230 and 350 nm, Waters Millenium software). Further identification was based on the coincidence of the retention time of the acid-hydrolyzed product and the aglycone, coupled to the loss of radioactivity bound to the corresponding glucoside.

3. Results and discussion

3.1. Cloning and genomic sequence analysis of the Samsun NN tobacco homologues of IS5a and IS10a

Due to the high sequence homology (95% identity at the

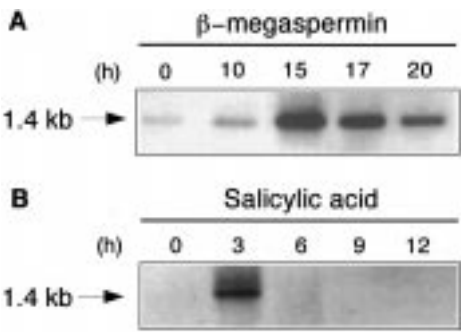

Fig. 1. RT-PCR analysis of *TOGT* induction in tobacco BY cells treated with 50 nM β-megaspermin (A) or 100 μM SA (B). RT-PCR reactions were repeated two times in separate samples to generate the results presented.

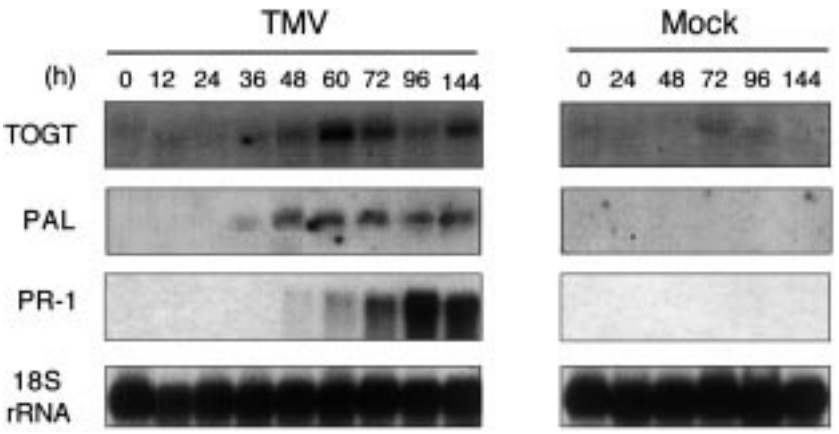

Fig. 2. Comparative time course of *TOGT*, *PAL* and *PR-1* mRNA accumulation in Samsun NN tobacco leaves inoculated with TMV. At the times indicated (in hours) after inoculation, leaf tissue was harvested from TMV-infected and mock-inoculated leaves. Total RNA was extracted and analyzed by Northern blotting. The same blot was successively hybridized with probes specific for *TOGT*, *PAL*, *PR-1* and *18S* RNA. The positions of the 1.4 kb bands are shown on the left.

nucleotide level) between *IS5a* and *IS10a* [12], a PCR approach using the same couple of primers was performed to amplify the coding regions of their homologues in Samsun NN tobacco. Genomic DNA and cDNA PCR amplifications revealed in both cases a 1.4 kb fragment suggesting that these two genes, distinguished by a *Sal*I restriction analysis and called *TOGT1* and *TOGT2*, do not contain any intron (data not shown). These results were confirmed by sequencing genomic clones. The *TOGT1* and *TOGT2* genomic sequences were almost identical to *IS5a* and *IS10a* cDNA sequences except for three and one nucleotide(s), respectively. These differences do not affect the open reading frame but slightly modify the amino acid content of the predicted proteins. TOGT1 (53.6 kDa) contains Phe^{6}, Val^{343} and Ile^{363} instead of Ile^{6}, Ile^{343} and Val^{363} as found in IS5a amino acid sequence. Compared to IS10a, TOGT2 (53.4 kDa) contains Glu^{394} instead of Lys^{394}.

3.2. *TOGT* gene expression analyses

To determine whether *TOGT* genes could play a role in plant defense responses, we studied *TOGT* gene expression in tobacco cells treated with elicitor or SA and in tobacco leaves during the HR to TMV. RT-PCR analyses performed on tobacco cells revealed one transcript of 1.4 kb (Fig. 1). Treatment of tobacco cells with the elicitor induced the accumulation of the *TOGT* transcripts (Fig. 1A), peaking 15 h after treatment and remaining at high levels throughout the experiment. In contrast, *TOGT* mRNAs accumulated more rapidly and transiently after SA induction (Fig. 1B). In the control cells, there was no detectable accumulation of mRNAs. Quantitative RT-PCR coupled with a discriminating *Sal*I restriction and Southern blot analyses revealed that both *TOGT1* and *TOGT2* genes were expressed to the same extent in SA-treated (52% and 48%, respectively) or elicitor-treated cells (58% and 42%, respectively), suggesting that these genes are co-regulated under the two induction conditions.

To investigate further the involvement of *TOGT* genes in defense responses, we used tobacco plants reacting hypersensitively to TMV. The kinetics of *TOGT* mRNA accumulation after TMV infection was compared with those of typical defense genes such as *PAL* and *PR-1*. During an HR, *PAL* is activated earlier than *PR-1* [3,18,20]. *TOGT* and *PAL* mRNAs started to accumulate at approximately the same time after infection while PR-1 transcripts accumulation was delayed (Fig. 2). There was no induction in mock-inoculated plants. The rather rapid activation of the *TOGT* genes is consistent with a role in early events occurring during the resistance response.

As exogenous SA was shown to induce *TOGT* gene expression and since SA is known to be produced in vivo during the HR induced by TMV infection or elicitor treatment [4,18], we addressed the question whether SA was the intermediate signal required for the induction of the *TOGT* genes during the HR. The approach to down-regulate SA during an HR con-

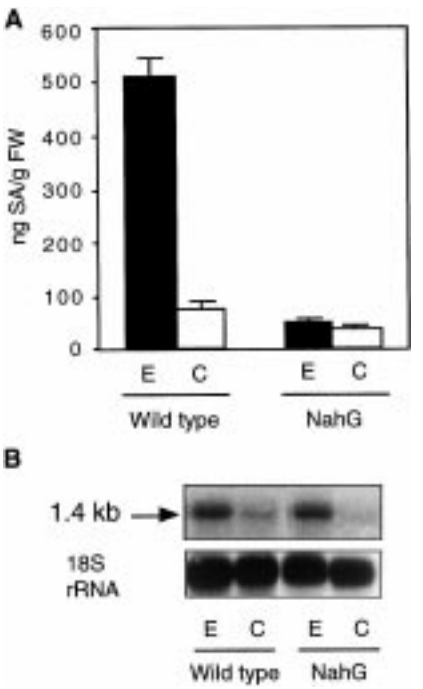

Fig. 3. Levels of total salicylic acid (A) and of *TOGT* mRNAs (B) in wild-type and transgenic NahG tobacco leaves reacting hypersensitively to the elicitor β-megaspermin. Leaves were harvested 9 h after infiltration with either elicitor (E) or water (C) as a control. Values are means with standard deviation from duplicates.

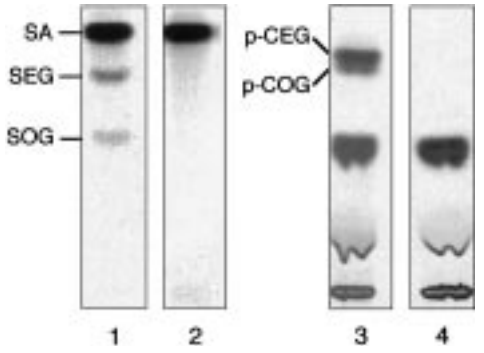

Fig. 4. Detection by TLC and Bio-Imaging of TOGT1-catalyzed conjugation of salicylic (lane 1) and *p*-coumaric (lane 3) acids to the corresponding phenolic glucosides. SEG: salicyloyl glucose ester, R_f 0.71; SOG: salicylic acid β-*O*-D-glucoside, R_f 0.54; *p*-CEG: *p*-coumaroyl glucose ester, R_f 0.76; *p*-COG: *p*-coumaric acid β-*O*-D-glucoside, R_f 0.73. TLC plates were developed in solvent I. Lanes 1 and 3 correspond to the complete reaction mixtures with [^{14}C]SA and UDP-[^{14}C]glucose as labeled substrates, respectively. Unlabeled UDP-glucose and unlabeled *p*-coumaric acid were omitted in lanes 2 and 4, respectively.

sisted in treating transgenic tobacco plants expressing the bacterial *nahG* gene encoding salicylate hydroxylase [5] with β-megaspermin. Abundance of *TOGT* mRNAs and levels of SA were measured into the HR lesions 9 h after treatment where *PAL* transcript accumulation and SA level were maximum [21]. As expected, elicitor-treated NahG plants had reduced SA levels when compared with the elicited wild-type plants (Fig. 3A). However, the elicitor-stimulated accumulation of *TOGT* transcripts in NahG tobacco was not significantly different from that in wild-type (Fig. 3B). This result indicated that, even though the *TOGT* genes were induced by exogenous SA, their induction during an HR is SA-independent. Similarly, a tomato *Twi1* gene with homology to *TOGT1* and *TOGT2*, and also induced during HR or by exogenous SA, has been recently shown to be induced by wounding and remained wound-inducible in tomato transgenic lines expressing *nahG* [22].

3.3. Catalytic activity of TOGT proteins

TOGT1 and TOGT2 proteins were produced by heterologous expression in *E. coli* as GST fusion proteins. After purification by glutathione Sepharose affinity chromatography, cleavage of the fusion protein led to the expected 53 kDa and 26 kDa proteins corresponding to TOGT and GST, respectively (data not shown).

Since TOGT proteins had similarity to glucosyltransferases, some of which are known to act on aromatic substrates such as flavonols and auxin [23,24] and since they were efficiently induced upon SA treatment, SA appeared a likely candidate for a role of preferential substrate. This would represent an important function of the enzyme because conjugation to glucose is known to be the major mechanism for controlling free SA levels, the actual active signal, and hence for regulating the numerous SA-dependent defense responses [16]. Assays to measure SA glucosylation activity using either [7-^{14}C]SA and unlabeled UDP-glucose (Fig. 4) or unlabeled SA and UDP-[^{14}C]glucose (data not shown) both revealed that TOGT1 indeed significantly catalyzed the transfer of the glucosyl moiety onto the carboxyl and the hydroxyl groups. When assaying a range of phenolic compounds and phenolic acids known to be abundant and/or increased in response to pathogens in tobacco and other plant species [25], SA appeared as a rather poor substrate, as well as other related benzoic derivatives, except *p*-hydroxybenzoic acid (Fig. 5). Among the phenylpropanoid-derived compounds scopoletin and esculetin were the best substrates, followed by cinnamic and the hydroxycinnamic acids, *p*-coumaric, *o*-coumaric, caffeic and ferulic acids. While caffeic acid was efficiently glucosylated, chlorogenic acid, one of its abundant conjugated forms, was not. Interestingly, coniferyl alcohol, a ubiquitous monomer of polymerization into lignin, was a good substrate. The same relative activities towards scopoletin and SA were observed with the TOGT2 protein (data not shown). The fact that TOGT1 acted preferentially on aromatic substrates of the phenylpropanoid types was confirmed by assaying auxin. No detectable activity was measured, whereas a maize glucosyltransferase with similarity to tobacco TOGTs, efficiently glu-

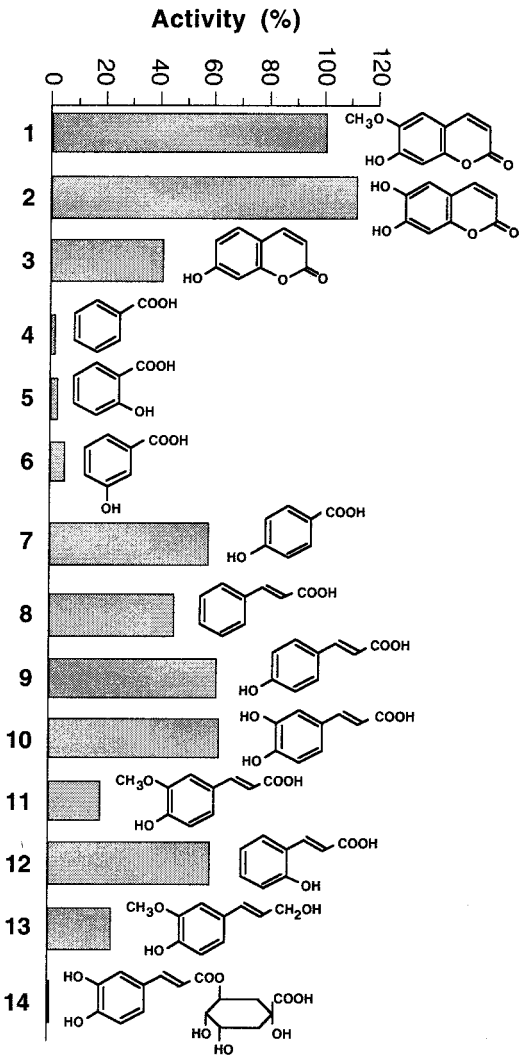

Fig. 5. Glucosyltransferase activity of recombinant TOGT1 protein against various phenylpropanoid and benzoic derivatives. (1) scopoletin; (2) esculetin; (3) umbelliferone; (4) benzoic acid; (5) salicylic acid; (6) *m*-hydroxybenzoic acid; (7) *p*-hydroxybenzoic acid; (8) cinnamic acid; (9) *p*-coumaric acid; (10) caffeic acid; (11) ferulic acid; (12) *o*-coumaric acid; (13) coniferyl alcohol; (14) chlorogenic acid. Activities are given relative to the conjugation of scopoletin taken as 100%. Structural formulas of the substrates are shown.

cosylated auxin [24]. Moreover, TOGT introduced the glucosyl moiety from UDP-glucose onto both carboxyl and hydroxyl groups not only of SA but also of other aglycones having two reactive groups such as *p*-coumaric acid (Fig. 4). However, glucose esters appeared to be the predominant forms of the glucosides, as determined in comparison with the authentic products by TLC in the different solvent systems.

Since these SA- and pathogen-inducible glucosyltransferases are apparently not involved in regulating SA levels, the question then becomes: what is their role in defense responses? Glucose conjugation might serve to activate the acyl moiety for *trans*-esterification into the cell wall for cross-linking polysaccharides and/or for anchoring of phenolic polymers into lignin-like material [26,27]. In bean, the elicitation during defense responses of UDP-glucose:cinnamate glucosyltransferase activity is associated with the appearance of cinnamates esterified to cellulosic and hemicellulosic components [28]. The accumulation of the cell wall-bound residues was preceded by the appearance of glucosides in the cells. Elicitor treatment of cultured parsley cells stimulated UDP-glucose:cinnamate, UDP-glucose:*p*-coumarate, and UDP-glucose:ferulate glucosyltransferases prior to the incorporation of these hydroxycinnamic acids into the cell wall [24]. In tobacco cell cultures, the release of scopoletin into the medium after methyl jasmonate elicitation involved the intermediate formation of the glucoside scopoline [29]. Furthermore, the formation of cinnamoyl alcohol glucosides may be important in transport to the cell wall, because the unglycosylated precursors like coniferyl alcohol are not very soluble in water [14].

In conclusion, the characterization of the catalytic activity of the proteins encoded by the SA-induced *TOGT1* and *TOGT2* genes indicates that these glucosyltransferases are not acting preferentially on SA, thus do not seem to regulate the levels of this critical signal. However, a glucosyltransferase, more specific for SA, is likely to occur, given previous descriptions of such specific activity [16], but has still to be characterized. Instead, our results point to a different but very important role of the *TOGT1* and *TOGT2* genes in disease resistance upstream in the mechanism of reinforcement of the plant cell wall by deposition of phenylpropanoid derivatives. The fact that *Twi1*, a highly homologous gene of tomato, is rapidly and strongly induced by wounding [22] also points to a possible role in rapid wound-healing by deposition of phenolics. The exact role of the *TOGT* genes in these two processes is now under investigation.

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References

[1] Hammond-Kosack, K.E. and Jones, J.D.G. (1996) Plant Cell 8, 1773–1791.
[2] Ni, W., Fahrendorf, T., Murray Ballance, G., Lamb, C.J. and Dixon, R.A. (1996) Plant Mol. Biol. 30, 427–438.
[3] Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahl-Goy, P., Métraux, J.-P. and Ryals, J.A. (1991) Plant Cell 3, 1085–1094.
[4] Métraux, J.P., Signer, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, J., Raschdorf, K., Schmid, E., Blum, W. and Inverardi, B. (1990) Science 250, 1004–1006.
[5] Delaney, T.P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., Ward, E. and Ryals, J. (1994) Science 266, 1247–1250.
[6] Rhoads, D.M. and McIntosh, L. (1993) Plant Mol. Biol. 21, 615–624.
[7] Bowler, C., Alliotte, T., De Loose, M., Van Montagu, M. and Inzé, D. (1989) EMBO J. 8, 31–38.
[8] Kim, S.-R., Kim, Y. and An, G. (1993) Plant Physiol. 103, 97–103.
[9] Quin, X.-F., Holuigue, L., Horvath, D. and Chua, N.-H. (1994) Plant Cell 6, 863–874.
[10] Boots, K.J.M., van der Zaal, B.J., Velterop, J., Quint, A., Mennes, A.M., Hooykaas, P.J.J. and Libbenga, K.R. (1993) Plant Physiol. 102, 513–520.
[11] Zhang, S. and Klessig, D.F. (1997) Plant Cell 9, 809–824.
[12] Horvath, D.M. and Chua, N.-H. (1996) Plant Mol. Biol. 31, 1061–1072.
[13] Tabata, M., Umetani, Y., Ooya, M. and Tanaka, S. (1988) Phytochemistry 27, 809–813.
[14] Whetten, R. and Sederoff, R. (1995) Plant Cell 7, 1001–1013.
[15] Nicholson, R.L. and Hammerschmidt, R. (1992) Annu. Rev. Phytopathol. 30, 369–389.
[16] Enyedi, A. and Raskin, I. (1993) Plant Physiol. 101, 1375–1380.
[17] Sembdner, G., Atzorn, R. and Schneider, G. (1994) Plant Mol. Biol. 26, 1459–1481.
[18] Baillieul, F., Genetet, I., Kopp, M., Saindrenan, P., Fritig, B. and Kauffmann, S. (1995) Plant J. 8, 551–560.
[19] Jaeck, E., Martz, F., Stiefel, V., Fritig, B. and Legrand, M. (1996) Mol. Plant-Microbe Interact. 9, 681–688.
[20] Pellegrini, L.O.G., Rohfritsch, O., Fritig, B. and Legrand, M. (1994) Plant Physiol. 106, 877–886.
[21] Dorey, S., Baillieul, F., Pierrel, M.-A., Saindrenan, P., Fritig, B. and Kauffmann, S. (1997) Mol. Plant-Microbe Interact. 10, 646–655.
[22] O'Donnell, P.J., Truesdale, M.R., Calvert, C.M., Dorans, A., Roberts, M.R. and Bowles, D. (1998) Plant J. 14, 137–142.
[23] Ralston, E.J., English, J.J. and Dooner, H.K. (1988) Genetics 119, 185–197.
[24] Szerszen, J.B., Szczyglowski, K. and Bandurski, R.S. (1994) Science 265, 1699–1701.
[25] Hahlbrock, K. and Scheel, D. (1989) Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 347–369.
[26] Kauss, H., Franke, R., Krause, K., Conrath, U., Jeblick, W., Grimmig, B. and Matern, U. (1993) Plant Physiol. 102, 459–466.
[27] Li, S.-M., Wang, Z.-X., Wemakor, E. and Heide, L. (1997) Plant Cell Physiol. 38, 844–850.
[28] Edwards, R., Mavandad, M. and Dixon, R. (1990) Phytochemistry 29, 1867–1873.
[29] Sharan, M., Taguchi, G., Gonda, K., Jouke, T., Shimosaka, M., Hayashida, N. and Okazaki, M. (1998) Plant Sci. 132, 13–19.