Title:

Cloning and characterization of a glucosyltransferase that reacts on 7-hydroxyl group of flavonol and 3-hydroxyl group of coumarin from tobacco cells

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Short title: Flavonoid 7-*O*-glucosyltransferase gene from tobacco

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Abbreviations: A5GT; anthocyanin 5-*O*- glucosyltransferase; F3GT, flavonoid 3-*O*-glucosyltransferase; F7GT, flavonoid 7-*O*-glucosyltransferase; GTase, glycosyltransferase; OH, hydroxyl group, PSPG box, plant secondary product glucosyltransferase signature.

Abstract

In higher plants, secondary metabolites are often converted to their glycoconjugates by glycosyltransferases (GTases). We cloned a cDNA encoding GTase (*NtGT2*) from tobacco (*Nicotiana tabacum* L.). The recombinant enzyme expressed in *Escherichia coli* (rNTGT2) showed glucosylation activity against several kinds of phenolic compound, particularly the 7-hydroxyl group of flavonoids and 3-hydroxycoumarin. The K_m values of kaempferol and 3-hydroxycoumarin with rNTGT2 are 6.5 μM and 23.6 μM, respectively. The deduced amino acid sequence of NTGT2 shows 60-70 % identity to that of anthocyanin 5-*O*-glucosyltransferase (A5GT); rNTGT2 did not show activity against the anthocyanins tested. *NtGT2* gene expression was induced by treating tobacco cells with plant hormones such as salicylic acid. We consider that *NtGT2* gene might have evolved from the same ancestral gene as the *A5GT* genes to the stress-inducible GTases that react on several phenolic compounds.

Key words: *Nicotiana tabacum* L. cv Bright Yellow; glucosyltransferase; flavonoid 7-*O*-glucosyltransferase; 3-hydroxycoumari; kaempferol; phenolic compound; plant-hormone-inducible

Introduction

In higher plants, secondary metabolites are often converted to their glycoconjugates, which are then accumulated and compartmentalized in vacuoles [1-3]. Such glycosylation reactions are catalyzed by glycosyltransferases (GTases). To date, many genes encoding GTases that catalyze mainly hydroxyl groups or carboxyl groups of secondary products were isolated from several plants (for review, [4, 5]). Plant GTases are highly conserved in their C-terminus, named the PSPG box (plant secondary product glucosyltransferase signature, [6]), forming a super-gene family. The *Arabidopsis* genome project showed that about 107 putative *GTase* genes exist in a plant species [7]; however, the functions of many of these genes remain to be investigated. Further studies are required to clarify the relationships between the structures and functions of GTases and to understand their roles in plants.

Among the substrates of plant GTases, flavonoids are the most extensively investigated. The genes encoding flavonoid 3-*O*-glycosyltransferase (F3GT), anthocyanin 5-*O*-glycosyltransferase (A5GT), and flavonoid 7-*O*-glycosyltransferase (F7GT) were reported. The *F3GT* gene was first reported in maize [8], and later found in several plant species [9-11]. The amino acid sequences of F3GTs were highly conserved among these plant species, and many of them were reported to be associated with anthocyanin biosynthesis. The gene encoding A5GT, which catalyzes the introduction of the second glucose moiety to the anthocyanin substrate, was first reported by Yamazaki et al. [12]. The substrate specificity of the enzyme was quite strict and anthocyanin 3-*O*-glucoside is the only major substrate of the enzyme [11, 12].

Although flavonoid 7-*O*-glucoside is one of the major flavonoid glycosides naturally produced in plants (see a list of flavone and flavonol glycosides in a review, [13]), there

are few studies on the enzyme activity and genes of F7GT. To date, two candidate *F7GT* genes from *Scutellaria baicalensis* (baicalein 7-*O*-glucosyltransferase gene, [14]) and *Dorotheanthus bellidiformis* (betanidine 5-*O*-glucosyltransferase (B5GT) gene, [15]) were reported. The amino acid sequences of these GTases showed about 60 % homology to each other, forming one family. Therefore, the enzymes expressed from these genes and their homologues are considered as GTases that probably possess glucosylation activity against the 7-hydroxy group (OH) of flavonoids (see review, [4]).

The reported members of this GTase family are baicalein 7-*O*-glucosyltransferase, B5GT, salicylate-inducible GTases (IEGT [16]) and their homologues (TOGT [17]) from tobacco. B5GT was reported to be able to catalyze the glucosylation of 4'-OH or 7-OH of quercetin, but the activity against 7-OH was 10-fold lower than that against 4'-OH [15]. IEGT catalyzes the glucosylation of flavonoids, but the reaction on 7-OH was weak (D. Horvath, personal communication). TOGT was reported to be a phenylpropanoid GTase. Recently, an antisense suppression study on *TOGT* gene has revealed that its product is involved in the metabolism of a coumarin derivative, scopoletin [18]. Since the enzymes classified into this family seem to have different substrate specificities, it may leave some room for consideration regarding the genes of F7GT.

In tobacco, several glucosides of phenolic compounds have been identical, such as coumarin glucosides, flavonol glucosides, anthocyanin glucosides, and salicylate glucosides. Moreover, several GTase genes have been reported, namely, genes of IEGT, *TOGT*, jasmonate-inducible GTase gene (*JIGT*) [19], and salicylic acid GTase gene [20]. In our previous studies, the glucosylation activity in cultured tobacco cells was investigated [21-23]. We isolated GTase genes (*NtGT1a*, *NtGT1b* and *NtGT3*) whose

encoding enzymes have glucosylation activity against many kinds of phenolics, such as flavonoids, coumarins and naphthols [22, 23]. These enzymes glucosylate mainly on 3-OH of flavonoids. We report here the molecular cloning of a GTase gene (*NtGT2*) from tobacco and the characterization of the recombinant enzyme of NTGT2 (rNTGT2). The results revealed that rNTGT2 has strong activity against 7-OH of flavonoids as well as against several phenolic compounds. The expression of the gene in tobacco is also discussed.

Materials and Methods

Culture of plant cells. Cells of T-13 habituated calli of *Nicotiana tabacum* L. cv. Bright Yellow were used and maintained as described previously [21].

Chemicals. Cyanidin 3-*O*-glucoside was kindly donated by Dr. M. Yamazaki (Chiba University). Kaempferol-3-*O*-glucoside, cyanidin and some coumarin derivatives were obtained from Extrasynthèse (Genay, France).

Screening and sequencing of glucosyltransferase genes. A partial-length cDNA of *NtGT2* was obtained by the screening of the tobacco cDNA library [22] using a fragment of the *Perilla* glucosyltransferase gene (3R4, [12]) containing the PSPG box. To obtain the full-length cDNA of *NtGT2*, the cDNA library (100,000 plaques) was screened with the *Nco*I fragment of *NtGT2* (678 bp) in accordance with the standard method [24]. Two full-length clones that corresponded to *NtGT2* were isolated, and cDNAs were sequenced using a DNA sequencer (Genetic Analyzer 310, Applied Biosystems, Foster City, CA).

Expression of recombinant NTGT2 enzyme (rNTGT2) in E. coli. The fragment containing the coding region of *NtGT2* was then subcloned into the *Bam*HI / *Xba*I site of the pET28c (+) expression vector (Novagen, Madison, WI), yielding pEGT2. *E. coli* BL21 (DE3) (Novagen) transformed with pEGT2 was cultured at 25 ºC for 1 day and then the expression of rNTGT2 was induced for 3 hours by adding isopropyl β-*D*-thiogalactopyranoside (400 μM) to the culture. The crude enzyme was extracted from the cells in accordance with the manufacturer's instruction using 50 mM Tris-HCl (pH 8.0) containing 5 mM 2-mercaptoethanol as an extraction buffer. The enzyme was extremely unstable; the half-life of the activity was about 4-6 hours after extraction in the standard extraction buffer. To prevent this decline in the activity, we added UDP-glucose (1 mM) to the extraction buffer. This improvement of the extraction conditions enabled us to obtain enzymological data. The recombinant enzyme was purified using nickel-equilibrated iminodiacetic acid-Sepharose 6B (Amersham Bioscience, Tokyo, Japan) in accordance with the manufacturer's instruction except that 1 mM UDP-glucose was added to each buffer used for the column purification.

Enzyme reaction of rNTGT2. Affinity-purified enzymes were used to determine substrate specificity and enzymatic parameters. The enzyme reaction for the determination of the substrate specificity of rNTGT2 using UDP- [^{14}C] glucose was performed as described previously [22]. For the reaction with the anthocyanin substrate, the enzyme was extracted using 50 mM Tris-HCl (pH 8.0) containing 10 mM 2-mercaptoethanol, according to Yamazaki et al. [12]. For the determination of the K_m values of the substrates, the concentrations of kaempferol and 3-hydroxycoumarin were

varied from 1 to 30 μM, and 2 to 500 μM, respectively, at a fixed UDP-glucose concentration of 1 mM. For the determination of the K_m values of UDP-glucose, its concentration was varied from 2 to 500 μM at a fixed 3-hydroxycoumarin concentration of 500 μM. The enzymatic reaction was performed in 100 μL of 50mM Potassium-Phosphate (pH 8.0), containing 5 mM β-mercaptoethanol, 0.01 % BSA, substrates and the recombinant enzyme. After incubation for 5 min at 30 °C, the reaction was stopped by adding 10 μL of 1M HCl, and then the reaction mixture was subjected to HPLC.

HPLC conditions. HPLC was performed using an ODS column (4.6 mm i.d. x 150 mm: LUNA 5u C18(2), Phenomenex, Torrance, CA) with an LC10Avp system (Shimadzu, Kyoto, Japan). Kaempferol and its glucoside were separated as described previously [23]. For the separation of 3-hydroxycoumarin and its glucoside, the column was eluted with solvent A (40 % methanol in 0.1 % HCOOH) for 12 min at a flow rate of 1 mL / min at 40 °C. The glucosides of kaempferol and 3-hydroxycoumarin were detected at 365 nm and 310 nm, respectively, using a diode array detector (SPD-M10Avp, Shimadzu).

Identification of products of NTGT2 enzyme reaction. Kaempferol, quercetin and 3-hydroxycoumarin were glucosylated by the reaction of rNTGT2 using UDP-glucose as a substrate. The reaction products were purified by the preparative HPLC, which was performed using an ODS column (7.8 mm i.d. x 300 mm: μBondapak C_{18}, Waters, Milford, MA) with the LC10Avp system. For the separation of glucosides from their aglycons, the column was eluted with solvent A for 10 min (3-hydroxycoumarin) or 16

min (flavonoids) at a flow rate of 2 mL / min at 40 °C, and the eluate was fractionated. The glucoside in water was extracted using ethyl acetate, and then used for the NMR analysis. The ^{1}H NMR spectrum was recorded in CD_3OD with a Varian Unity plus 500 spectrometer operating at 500 MHz. MS was recorded on a JEOL JMS DX-303 spectrometer. 3-Hydroxycoumarin 3-*O*-β-D-glucopyranoside; positive ion FAB-MS (*m/z*): 325 $[M+H]^+$. ^{1}H NMR (500 MHz, CD_3OD) δ : 7.54 (1H, *dd*, *J* = 1.6, 7.8 Hz, H-5), 7.48 (1H, *s*, H-4), 7.48 (1H, *ddd*, *J* = 1.6, 7.1, 8.3 Hz, H-7), 7.34 (1H, *ddd*, *J* = 1.1, 3.8, 7.5 Hz, H-6), 7.32 (1H, *dd*, *J* = 1.1, 7.5 Hz, H-8), 5.01 (1H, *d*, *J* = 7.5 Hz, H-1'), 3.94 (1H, *dd*, *J* = 2.2, 12.0 Hz, H-6'), 3.69 (2H, *d*, *J* = 6.2, 12 Hz, H-6'), 3.56 - 3.37 (4H, *m*, H-2', H-3', H-4', H-5').

Detection of NtGT2 expression in tobacco. Total RNA was extracted from T-13 cells treated with 2,4-D (5 μM), salicylic acid (50 μM), or methyl jasmonate (25 μM) by the phenol-SDS method as described previously [23]. To detect the organ-specific expression, total RNA was extracted from the flowers, leaves, stems, and roots of tobacco (*Nicotiana tabacum* L. cv. Bright Yellow-4). cDNAs were synthesized from these RNAs using a TrueScript II Reverse Transcriptase RNase H^- system (Sawady Technology, Tokyo, Japan) with an oligo-dT primer at 50 ºC for 60 min. One micro-liter of a cDNA sample from the RT reaction was used for PCR using *Ex Taq* (Takara Bio, Kusatsu, Japan) under the following conditions: 95 ºC for 5 min, 25 cycles of 95 ºC for 30 sec, 50 ºC for 1 min, and 72 ºC for 2 min, and followed by a final extension at 72 ºC for 7 min with the primers of NtGT2-720 5'- TATAGCTTTGCTCTACC -3' and NtGT2-1119n 5'- CAACTCCATCATACAAC -3' for the 399-bp fragment of *NtGT2,* Act-fw 5'- GATTGGAATGGAAGCTG -3' and Act-rv 5'-

CCTCCAATCCAAACACT -3’ for the 231-bp fragment of the consensus region from tobacco actin (GenBank accession numbers, **X63603**, **U60489**, **U60491**, **AF154640**). The PCR product was analyzed by electrophoresis in 1.5 % agarose gel.

Results

Cloning of NtGT2 and deduced structure of NTGT2.

In this work, we attempted to isolate flavonoid glucosyltransferases from tobacco. The GTase gene obtained (1637 bp, *NtGT2*, Genbank accession no. **AB072919**) was shown to encode a protein (NTGT2) of 470 amino acid residues. The calculated molecular mass of NTGT2 was 52.8 kDa. The protein showed 60-70 % identity with A5GTs from *Petunia*, *Verbena* and *Perilla* [11, 12]. The protein also showed 86 % and 50 % identities with GTases of unknown function, namely, the cold-induced GTase from potato (SsCIGT, Genbank Accession **AY033489**) and jasmonate-induced GTase from tobacco (JIGT) [19], respectively. The alignment of the amino acid sequences of NTGT2 and A5GT homologues is shown in Fig.1. NTGT2 and A5GT homologues were highly conserved in many parts besides the PSPG box; these were not conserved between NTGT2 and F7GT or F3GT (Fig. 1). The molecular phylogenetic tree constructed based on the deduced amino acid sequences of plant GTases is shown in Fig. 2. The amino acid sequence identities between NTGT2 and other GTases are also shown. Based on its sequence homology, NTGT2 is considered to belong to the group of A5GTs.

Properties of recombinant NTGT2 enzyme.

The recombinant enzyme of NTGT2 (rNTGT2) was expressed in *E. coli*. The enzyme did not react with cyanidin-3-*O*-glucoside or cyanidin (data not shown). Then, several phenolic compounds, namely, flavonoids, hydroxycoumarins, naphthols, hydroxycinnamate and hydroxybenzoate were tested as the substrates of the reaction with UDP-^{14}C-glucose (Fig. 3). All of the compounds possess phenolic OH, which

would be the site of glucosylation. Naphthols are not natural substrates, but they are useful as model substrates for some GTases [22].

Among the compounds tested, kaempferol (3, 5, 7, 4'-tetrahydroxyflavone) and 3-hydroxycoumarin were the best acceptors of glucose. Quercetin (3, 5, 7, 3', 4'-pentahydroxyflavone) was also glucosylated by this enzyme, but the yield was lower than that with kaempferol. Kaempferol-3-*O*-glucoside was also glucosylated to some extent by this enzyme. When the reaction was performed using flavonoids containing a single OH in the structure, 7-hydroxyflavone was a good sugar acceptor, and 6-hydroxyflavone was a less effective sugar acceptor. Besides 3-hydroxycoumarin, other hydroxycoumarins tested also served as acceptors. Naphthols were catalyzed at 1-OH, but 1/3 less than at the 2-OH. The enzyme also reacted on 2-coumaric acid and 4-coumaric acid, but not on salicylic acid. These results suggest that rNTGT2 has a broad substrate specificity.

The enzyme activity against kaempferol and 3-hydroxycoumarin was further analyzed. The optimum pH of the enzyme reaction was 8.0, when kaempferol was used as a substrate. The HPLC profile of the product of enzyme reaction on kaempferol is shown in Fig. 4. A single glucoside peak was found under this condition, suggesting the high regiospecificity of the enzyme. To confirm the site of glucosylation by rNTGT2, the glucosylated product of kaempferol was purified by preparative HPLC. The glucosylated product of kaempferol was identified as kaempferol 7-*O*-β-glucopyranoside by the comparisons of its FAB-MS, ^{1}H-NMR and ^{13}C-NMR spectra with those described in the literature [25]. The site of glucosylation, 7-OH, was finally confirmed by the ^{1}H-Detected Heteronuclear Multiple Bond Connectivity experiment, in which, the correlation of the anomeric proton (δ_H 5.05) with C-7

(δ 164.3) was observed. Quercetin was also determined to be glucosylated at 7-OH using the same procedure. A single peak was also detected by HPLC analysis as a product of the enzyme reaction with 3-hydroxycoumarin. The product was also purified and determined as 3-hydroxycoumarin 3-*O*-β-D-glucopyranoside by ^{1}H-NMR and FAB-MS spectroscopy.

The K_m values of kaempferol (7-OH) and 3-hydroxycoumarin (3-OH) with rNTGT2 were calculated based on the Lineweaver-Burk plot (Table 1). High concentrations (beyond 50 μM) of kaempferol inhibited the enzyme activity (data not shown). This inhibition was not observed when 3-hydroxycoumarin was used as the substrate. For the determination of enzymatic parameters, therefore, the reaction of kaempferol was performed at concentrations between 1 and 30 μM. Since the enzyme was very unstable, the V_{max} value differed for each reaction. The enzyme reaction with each substrate was therefore performed simultaneously under the same conditions to compare the reactivity of the enzyme.

Expression of NtGT2 in tobacco.

The expression of some GTase genes can be induced by plant hormones, such as salicylic acid [16, 17, 22], auxin [16, 22] or jasmonate [19], and many of them show a broad substrate specificity. Since NTGT2 also shows a broad substrate specificity, RT-PCR was performed to determine the expression pattern of *NtGT2* mRNA in plant-hormone-treated tobacco cells. *NtGT2* mRNA was weakly expressed in the tobacco habituated callus T-13 without plant hormones (Fig. 5A). When the cells were treated with salicylic acid, the accumulation of *NtGT2* mRNA increased. The accumulation somewhat increased following the addition of 2,4 -dichlorophenoxyacetic

acid, an artificial auxin, or methyl jasmonate, suggesting that the gene product expression is controlled by plant hormones similar to other GTases with a broad substrate specificity. The organ-specific accumulation of *NtGT2* mRNA in tobacco plant was also investigated by RT-PCR analysis. The result (Fig. 5B) showed that *NtGT2* mRNA was expressed in many parts of tobacco plant, particularly in the leaves and flowers.

Discussions

Recently, many GTase genes have been isolated from diverse plant species, and they could be classified into some gene families based on their amino acid sequences [5]. However, it is still difficult to deduce the substrates of gene products from their sequences. In the present study, we demonstrated NTGT2, newly isolated from cultured tobacco cells as the homologue of A5GT, regiospecificially glucosylated 7-OH of flavonol such as kaempferol and quercetin.

Considering the fact that NTGT2 is very similar to A5GT in terms of the primary structure (Fig 1), we tested its activity against cyanidin and cyanidin-3-*O*-glucoside, which are naturally occurring anthocyanins (about > 90%) in tobacco flowers [26]. The rNTGT2 enzyme hardly reacted on the compounds under conditions that would be favorable for other A5GTs. It showed a strong activity against kaempferol, a flavonoid aglycon (Table 1), which is definitely different from those of A5GTs whose substrate specificity is strictly limited to anthocyanin 3-*O*-glucosides or its derivatives [11, 12]. NTGT2 would be a type of enzyme with a broad substrate specificity that is not involved in the biosynthesis of anthocyanins.

The K_m values of kaempferol and 3-hydroxycoumarin with rNTGT2 were 6.5 μM and 23.6 μM, respectively (Table 1). These values were the same as other plant GTases reported [14, 23, 27], suggesting that these substrates are reasonable sugar acceptors for the enzyme. Kaempferol but not 3-hydroxycoumarin is a natural compound in tobacco. With the comparison of V_{max}/K_m value between the reactions, it is conceivable that kaempferol is better acceptor of the NTGT2 reaction.

There are some reports concerning the K_m values of flavonoids with F7GTs; that of 7-OH of baicalein with a recombinant enzyme of baicalein 7-*O*-glucosyltransferase is 137 μM [14], and those of naringenin and hesperetin with a flavanone-specific F7GT purified from seedlings of grapefruit are 62 μM and124 μM, respectively [28]. The K_m value of kaempferol with rNTGT2 is 6.5 μM, indicates its sufficient affinity to the substrate. Moreover, the rNTGT2 enzyme hardly reacted on other OHs of kaempferol (Fig. 4), suggesting that the regiospecificity of the glucosylation is strictly determined. These results indicate that NTGT2 is a type of F7GT, however its amino acid sequence was not highly homologous to that of the reported F7GT, such as baicalein 7-*O*-glucosyltransferase.

The GTase superfamily is divided into 13 to 14 families based on their sequence homology [7], but GTases that utilize the same substrate are often distributed in some families. In the case of *Arabidopsis*, 48 GTases that belong to six GTase families were revealed to have glucosylation activity against hydroxycoumarins, which are not natural substrates of *Arabidopsis* [29]. NTGT2 seems to have the same ancestral gene as that of the A5GT family, and may have acquired F7GT activity along its course of evolution. It is difficult to assume that NTGT2 shows F7GT activity based on the sequence data,

which suggest that ‘putative GTases’ annotated only by the sequence homology would be needed to examine their enzymatic activities biochemically.

The *NtGT2* gene is expressed in the leaves and flowers, and is inducible by plant hormones (Fig. 5). As few flavonoids were found in the leaves of normal tobacco BY-IV plants (data not shown), NTGT2 might play some roles other than flavonol accumulation in the leaves. We consider that the role of NTGT2 would be the glucosylation of several phenolic compounds as in the case of NTGT1 and NTGT3. They are inducible by plant hormones or naphthols, react on 3-OH of flavonoids and several phenolic compounds including naphthols, and belong to a GTase-family different from those of other reported F3GTs [23]. The amino acid sequence of NTGT2 showed 86 % and 50 % identities with stress inducible GTases of unknown functions, namely SsCIGT (cold-induced) from potato and JIGT (jasmonate-induced) from tobacco. They might have evolved from the same ancestral gene of *NtGT2*, and acquired roles associated with stress responses.

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Table. 1 Kinetic parameters of the recombinant NTGT2 enzyme.

Substrates	K_m [a] (μM)	V_{max} (nmol · min^{-1} mg^{-1})	V_{max} / K_m
kaempferol (7-OH)	6.5 ± 0.5	563 ± 31	86
3-hydroxycoumarin	23.6 ± 2.6	932 ± 49	39
UDP-glucose	43.2 ± 3.9	939 ± 45	22

[a] The K_m values of the substrates were determined at a fixed UDP-glucose concentration of 1 mM. The K_m values of UDP-glucose were determined at a fixed 3-hydroxycoumarin concentration of 500 μM. Results represent the mean of three independent replicates ± SD.

Figure legends

Figure 1 Multiple alignments of the deduced amino acid sequences of plant glucosyltransferases. The bar indicates the conserved region among plant secondary product glucosyltransferase (PSPG box). Species, abbreviations and Genbank accession nos. of the sequences are: NTGT2, this study (**AB072919**); SsCIGT, cold-induced glucosyltransferase from *Solanum* (**AY033489**); PhA5GT and PfA5GT, anthocyanin 5-*O*-glucosyltransferase from *Petunia* (**AB027455**) and *Perilla* (**AB013596**); NtJIGT, jasmonate-inducible glucosyltransferase from tobacco (**AB000623**); PhF3GT, flavonoid 3-*O*-glucosyltransferase from *Petunia* (**AB027454**); SbF7GT, flavonoid 7-*O*-glucosyltransferase from *Scutellaria* (**AB031274**).

Figure 2 Molecular phylogenetic tree constructed based on the deduced amino acid sequences of plant glycosyltransferases. The tree was constructed by the neighbor-joining method using ClustalX [30]. Lengths of lines indicate the relative distance between nodes. Amino acid sequence identities between NTGT2 and other GTases were calculated using Genetyx-SV/RC ver. 10.1 (Genetyx, Tokyo, Japan). F3GT, flavonoid 3-*O*-glycosyltransferase; A5GT, anthocyanin 5-*O*-glucosyltransferase; F7GT, flavonoid 7-*O*-glucosyltrasferase. Species, abbreviations and Genbank accession nos. of the sequences are: PvZXT, zeatin xylosyltransferase from *Phaseolus* (**AF116858**); GtF3GT, PhF3GT, and ZmF3GT, flavonoid 3-*O*-glucosyltransferases from *Gentiana* (**D85186**), *Petunia* (**AB027454**), and maize (**X13502**); VmF3GalT and PhF3GalT, flavonol 3-*O*-galactosyltransferase from Vigna (**AB009370**) and *Petunia* (**AF165148**); SbHMNGT, p-hydroxymandelonitrile-*O*-glucosyltransferase from *Sorghum* (**AF199453**); ZmBX8, benzoxazinoids glucosyltransferase from *Zea*

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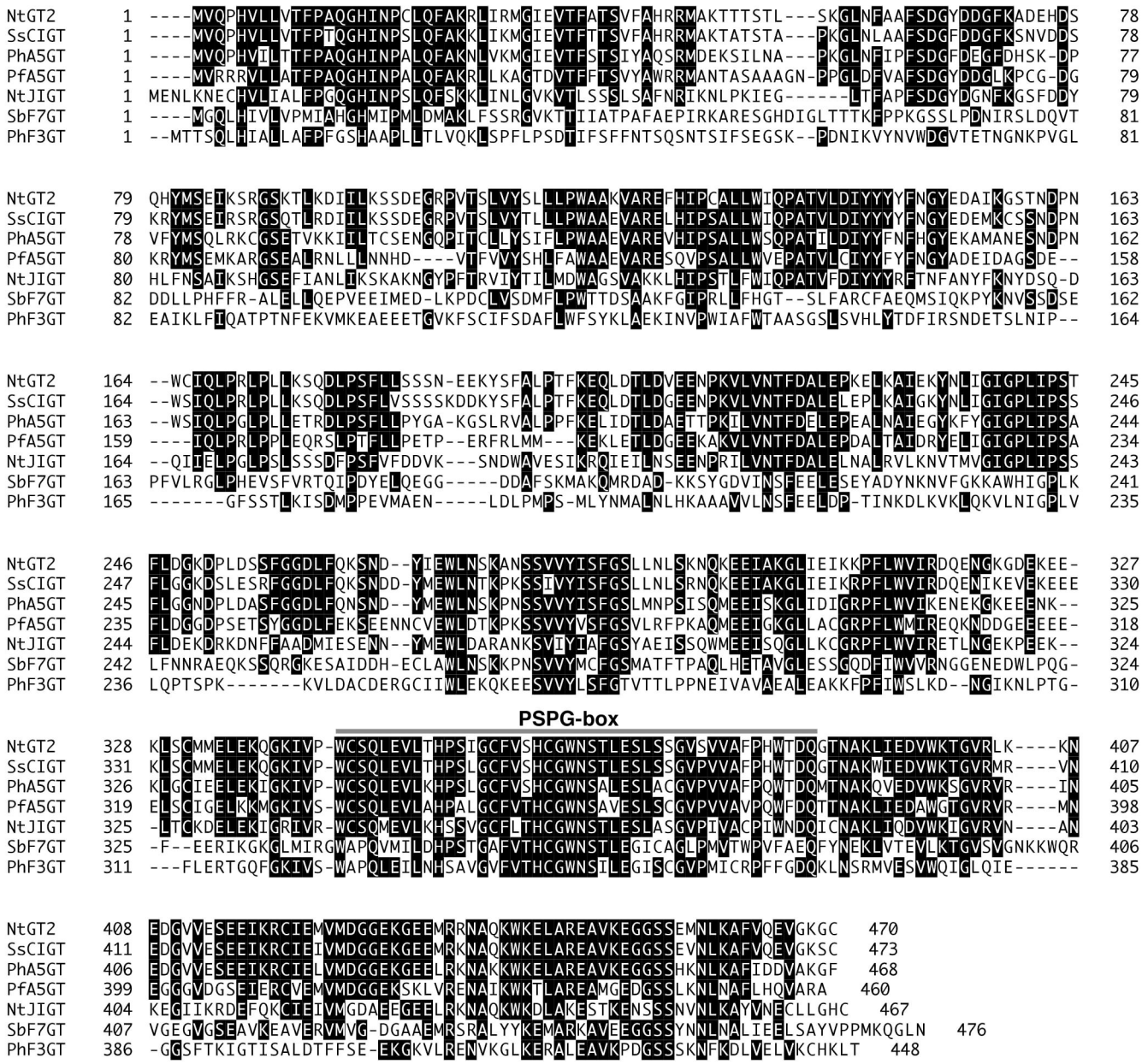

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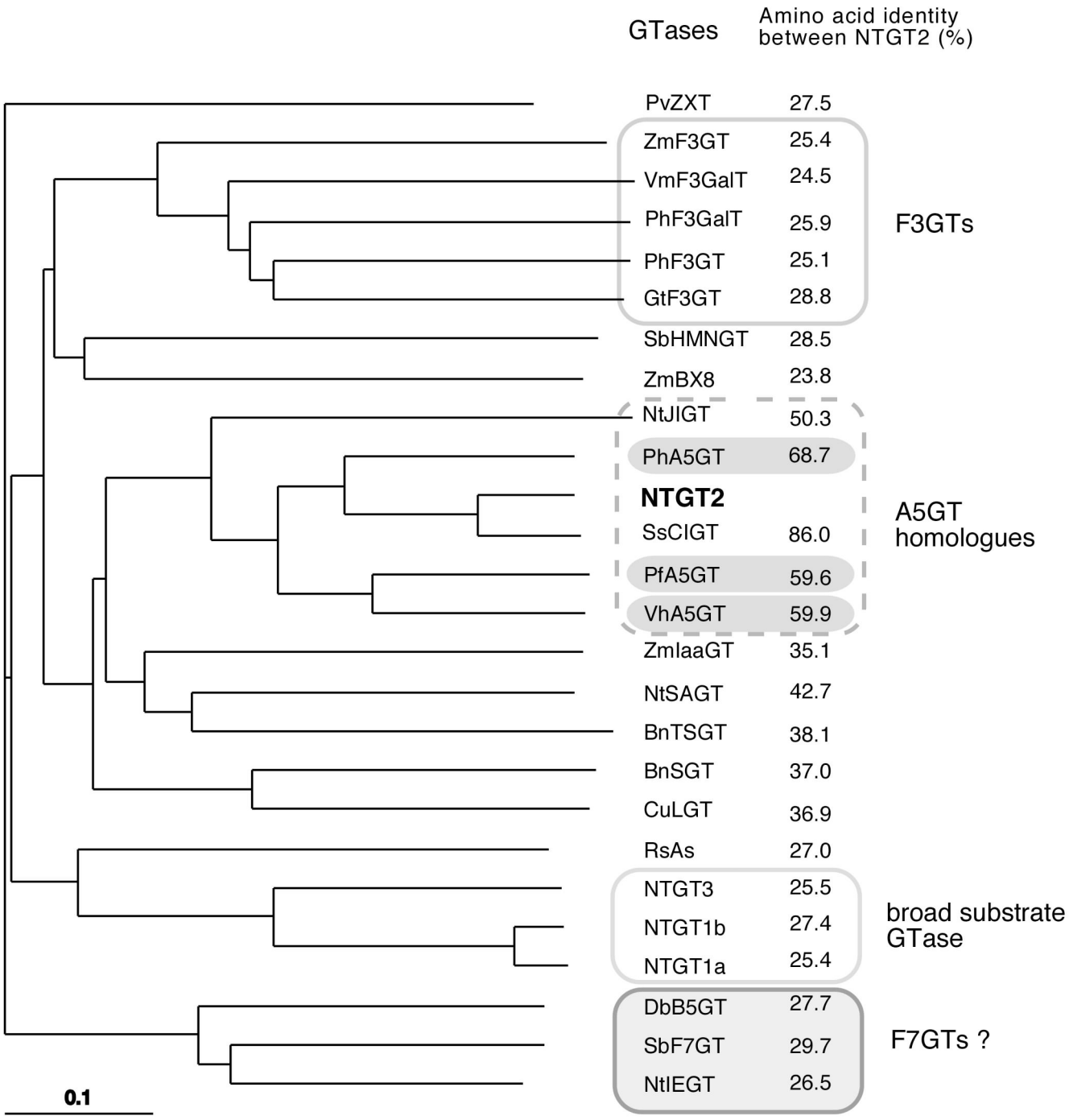

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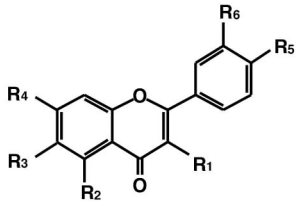
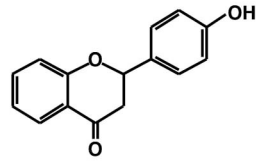
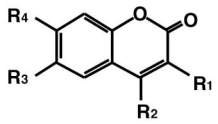
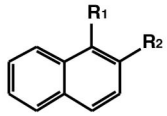
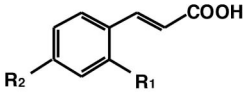
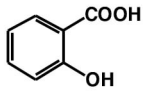
Substrate		Relative activity (%)
Flavonoids		
 flavones	kaempferol (R1=R2=R4=R5=OH, R3=R6=H)	100
	quercetin (R1=R2=R4=R5=R6=OH, R3=H)	19 ± 4
	3-hydroxyflavone (R1=OH, R2=R3=R4=R5=R6=H)	12 ± 2
	5-hydroxyflavone (R1=R3=R4=R5=R6=H, R2=OH)	6 ± 3
	6-hydroxyflavone (R1=R2=R4=R5=R6=H, R3=OH)	24 ± 2
	7-hydroxyflavone (R1=R2=R4=R5=R6=H, R4=OH)	53 ± 13
	kaempferol-3-*O*-glucoside (R1=OGlc, R2=R4=R5=OH, R3=R6=H)	45 ± 4
 4'-hydroxyflavanone	4'-hydroxyflavanone	30 ± 2
Hydroxycoumarins		
 coumarins	scopoletin (R1=R2=H, R3=OMe, R4=OH)	11 ± 3
	esculetin (R1=R2=H, R3=R4=OH)	26 ± 3
	3-hydroxycoumarin (R1=OH, R2=R3=R4=H)	310 ± 32
	4-hydroxycoumarin (R1=R3=R4=H, R2=OH)	4 ± 1
Other Phenolics		
 naphthols	1-naphthol (R1=OH, R2=H)	44 ± 15
	2-naphthol (R1=H, R2=OH)	14 ± 2
 cinnamic acids	2-coumaric acid (R1=OH, R2=H)	41 ± 22
	4-coumaric acid (R1=H, R2=OH)	43 ± 15
 salicylic acid	salicylic acid	n.d.

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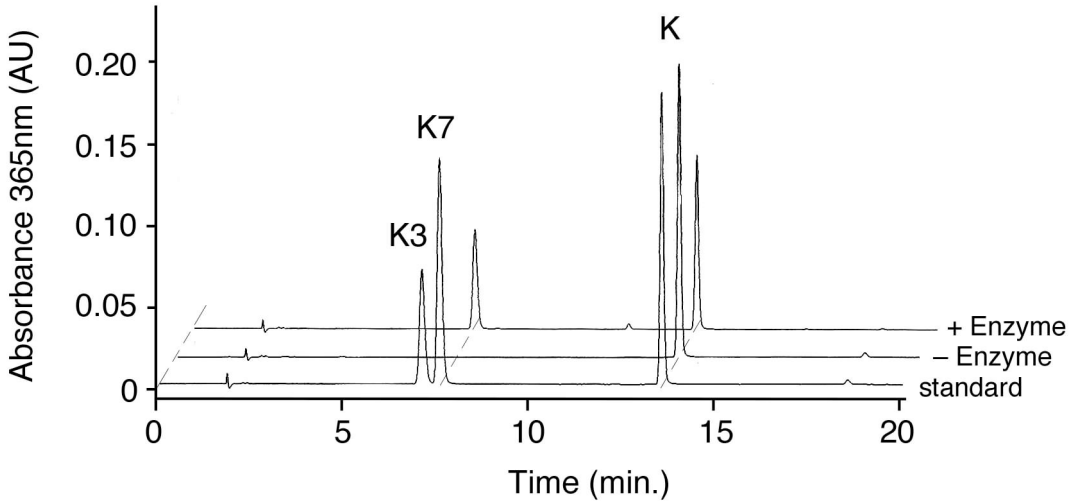

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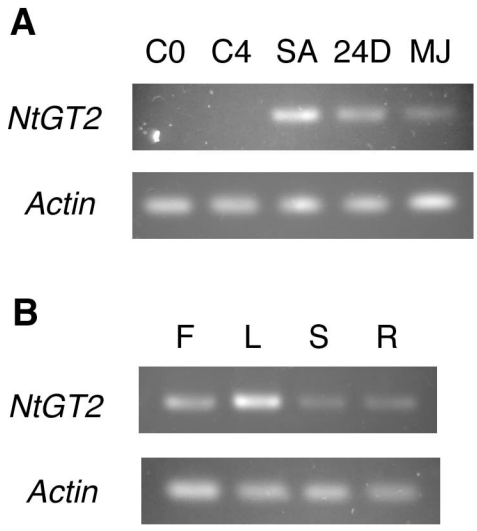

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