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Molecular cloning and characterization of one member of 3β-hydroxy sterol glucosyltransferase gene family in *Withania somnifera*

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Abstract

Sterol glycosides are constituents of plant cell membranes. Glucosylations of the sterols are catalyzed by sterol glucosyltransferases (SGTs), which are members of family 1 glycosyltransferases. We have identified the family of *SGT* genes expressed in the leaves of a medicinal plant *Withania somnifera*. One member (*SGTL1*) of this gene family was cloned. The full-length cDNA sequence of *SGTL1* represents 2532 bp, comprising untranslated regions (UTRs) of 337 and 89 bp at the 5′ and 3′ ends, respectively. The amino acid sequence deduced from the 2103 bp open reading frame (ORF) showed homology (67–45%) to the reported plant SGTs. The presence of two putative transmembrane domains suggested the association of SGTL1 with membrane. The *SGTL1* was expressed in *Escherichia coli* and recombinant enzyme from the supernatant was partially purified and biochemically characterized. The relative activity and kinetic properties of SGTL1 for different sterols were compared with a recombinant SGT (GenBank Accession No. Z83833) of *Arabidopsis thaliana* (*At*SGT). Both the recombinant enzymes showed activity with 3-β-OH sterols. The distribution of *SGTL1* transcript in *W. somnifera*, as determined by quantitative PCR, showed higher expression in roots and mature leaves. Expression of the *SGTL1* transcript in the leaves of *W. somnifera* was enhanced following the application of salicylic acid. In contrast, it decreased rapidly on exposure of the plants to heat shock, suggesting functional role of the enzyme in biotic and abiotic stresses.

Keywords: *Withania somnifera*; Sterol glucosyltransferase; UGTs; Substrate specificity; Secondary metabolism; Salicylic acid; Heat shock

Glycosyltransferases (GTs)[2] constitute a superfamily of enzymes that catalyze conjugation of carbohydrate moieties to oligo/polysaccharides, proteins, lipids, terpenoids, flavanoids, alkaloids and other small molecules. Over 12,000 sequences encoding GTs are available in CAZy database (http://afmb.cnrs-mrs.fr/CAZY). The superfamily consists of 78 families based on sequences, signature motifs, stereochemistry of glyco-linkages, substrate specificity, nature and range. GTs that use UDP-activated sugars as donors and various types of small molecules as acceptors are called UDP-glycosyltransferases (UGTs) and represent family 1 GTs. Such UGTs are present commonly in plants and animals, but have been reported in a few cases only in microorganisms. In higher plants, UGT catalyzed glycosylation constitutes a prominent terminal modification in the biosynthesis of secondary metabolites and generates diverse natural glycosides [1]. They also glycosylate xenobiotics to deal with environmental challenges through detoxification process. The UGTs possess substrate specificity based on regioselective recognition of chemical sub-structures. Biological functions of glycosylations in plants include storage, inter and intracellular transport of metabolites, regulation of homeostasis of hormones, etc. UGTs glycosylating at –OH, –COOH, $–NH_2$, –SH and C–C groups in secondary metabolites have been reported [2–6].

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[2] *Abbreviations used:* GT, Glycosyltransferases; SGT, sterol glucosyltransferases; UGTs, UDP-glycosyltransferases; UTRs, untranslated regions; SA, salicylic acid; RACE, rapid amplification of cDNA ends; MOPS, 3-(*N*-morpholino) propanesulfonic acid; ORF, open reading frame.

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The UGTs are classified based on conserved domain in their C-terminal. The gene family comprises 112 full-length sequences and 8 pseudogenes with frame-shift mutations in *Arabidopsis thaliana* [7]. However, limited information is available concerning function of individual UGTs, as biochemical analyses of individual enzyme for substrate range and specificity have been reported in a few cases only [8–12].

Most of the higher plant sterols possess β-OH group at C-3 (A-ring) and are transformed into their glycoconjugates by sterol glucosyltransferases (SGTs). A large group of glycosterols called saponins, comprising glycosylated triterpenoids, steroids and steroidal alkaloids, occurs in many plants. These phytochemicals contain sugar chain coupled to C-3 hydroxyl group and possess antifungal activity [13]. The sugar chain is critical for membrane permeabilization and biological activity [14,15]. Synthesis, transport and functions of steroids and their esters have been studied extensively in animals, plants and yeast [16–18] but such information on sterol glycosides is limited.

In plants, sterols are biosynthesized by mevalonate [19] and non-mevalonate pathways [20]. They occur in highly diversified skeletal and structural forms that are finally glycosylated. Some of these (e.g. sitosterol, stigmasterol, brassinosteroids) are ubiquitous in plants whilst others (e.g. withanolides, limonoids) are highly restricted in occurrence. *Withania somnifera* (*Solanaceae*) is a medicinal plant known for several pharmacological properties attributed to its characteristic steroidal compounds, called withanolides and glycowithanolides [21–24]. However, biosynthetic pathway of withanolides, their functions in *W. somnifera* and metabolic step(s) leading to their glyco-transformations are unknown. One of the reasons for limited knowledge of their functions is non-availability of the relevant enzymes and genes. We report the identification of a member (*SGTL1*) of *SGT* family in *W. somnifera*. The deduced amino acid sequence of the gene has been compared with known plant SGTs. Full length cDNA of *SGTL1* has been cloned and expressed in *Escherichia coli*. Functional activities of the encoded protein have been compared with those of *A. thaliana*. The expression profiles of the *SGTL1* in different organs and its physiological modulation by stress have been examined.

Materials and methods

Plant material

Withania somnifera plants were grown in a green house under 16 h day/8 h night cycle. For expression studies, seeds were germinated in pots and the seedlings were collected after 2 weeks. The young and mature leaves, roots and stems were collected from 4 weeks old plants. For salicylic acid (SA) treatment, leaf discs from 4 weeks old plants grown in green house were used.

Chemicals

General chemicals and reagents were obtained from Sigma–Aldrich. Molecular biology reagents and kits were from New England Biolabs, Stratagene, Applied Biosystem, Invitrogen, Qiagen, Clontech, etc. Uridinediphosphate D-glucose [U-^{14}C] was from Amersham Biosciences. Sequences of primers used in this study are listed in Supplementary Table 1.

Cloning of SGT cDNA

Total RNA from *W. somnifera* leaves was extracted by hot phenol method [25] and the first strand cDNA was synthesized using Superscript II RNase H$^-$ reverse transcriptase (Invitrogen), according to the manufacturer's instructions. Sequences of degenerate primers F6A (forward) and R17 (reverse) were designed (Supplementary Table 1) from the conserved domains identified in SGTs [26] and used for PCR with 1/10th volume of cDNA reaction. The PCR parameters were: initial denaturation at 94 °C for 3 min, followed by 35 cycles at 94 °C (1 min), annealing at 57 °C (2 min), extension at 72 °C (2 min) and final extension at 72 °C for 5 min. The RT-PCR products were electrophoresed on 1.2% agarose gel. The resolved fragments were purified and cloned in pBluescript SK$^+$ vector following standard protocols [27].

Sequence analyses and comparisons

The cloned fragments were sequenced by dideoxynucleotide chain termination method using cycle sequencing kit in an ABI Prism 377 genetic analyzer. The nucleotide and amino acid sequence similarity searches were made using BLASTN and BLASTP. The partial sequences were designated as *SGTL1*, *SGTL2* and *SGTL3*.

Rapid amplification of cDNA ends (RACE)

The RACE primers for *SGTL1* were designed from the available partial cDNA sequences using Primer Express software. The PCR for 3′ RACE was performed using 3′RACE kit (Invitrogen). The 5′ RACE product was obtained using BD SMART RACE cDNA amplification kit (Clontech). PCR products from 5′ and 3′ RACE were cloned, sequenced and analyzed to obtain full length *SGTL1*. Amino acid sequence of *SGTL1* was deduced and subjected to multiple sequence alignment with plant SGTs using clustalW.

Heterologus expression and purification of SGT in E. coli

For expression in *E. coli*, the ORF of *SGTL1* was PCR amplified with L_1ORF1F and L1ORF1R as forward and reverse primers, respectively. The amplified 2.1 kb ORF was cloned at *Nco*I and *Sal*I sites in pET21d expression vector (Novagen) and the construct was used to transform *E. coli* BL21 codon plus RIL strain (Stratagene). *E. coli* transformed with the vector alone served as control. Transformants were cultured at 37 °C in LB liquid medium containing 50 μg ml^{-1} chloramphenicol and 100 μg ml^{-1} ampicillin. The *AtSGT* (Z83833) cloned in SK$^+$was a kind gift from Prof. D.C. Warnecke [28]. It expresses a fusion protein with a few amino acid residues of β-galactosidase fused at the N-terminal of the SGT. It was used for comparative observations on enzyme activity and kinetics. *E. coli* DH5α strain was transformed with this construct and the transformants were cultured at 37 °C in LB liquid medium containing 100 μg ml^{-1} ampicillin. After the culture reached OD_{600} 0.4–0.6, isopropyl 1-thio β-D galactoside (IPTG) was added to the final concentration of 1 mM in SGTL1 and *At*SGT expressing cultures. The induced bacteria were further cultured overnight at 16 °C, harvested by centrifugation and suspended in 20 mM Tris–HCl, pH 8.0, containing 10% glycerol, 2.5 mM DTT, 1 mM EDTA and 1 mM PMSF. The suspension was sonicated, centrifuged (12,000*g*, 20 min) and the supernatants used for partial purification of the recombinant SGTL1/*At*SGT proteins. The extract was chromatographed through Superdex 200 gel filtration column (pre-equilibrated and eluted with 20 mM Tris–HCl containing 2.5 mM DTT, 150 mM NaCl at flow rate 1.0 ml min^{-1}). Active fractions were pooled and analyzed on SDS–PAGE. SGTL1 showed the expected protein band with a few contaminating proteins. In case of *At*SGT, a distinct band was not visible at this stage. Thus, the pool of *At*SGT was further

chromatographed through a pre-equilibrated (20 mM Tris–HCl and 2.5 mM DTT) anion exchange column (Q-Sepharose Fast Flow) and eluted with a linear gradient (0.1–1 M) of NaCl in the above buffer at a flow rate of 1.0 ml min^{-1}. The active fractions eluted between 0.3 to 0.35 M NaCl were pooled. The partially purified enzyme preparations were used for determining activity and kinetics.

SGT assay

SGT activity was assayed radiometrically using UDP-[U-^{14}C] glucose as per following reaction.

$$\text{Sterol} + \text{UDP} - [\text{U} - {}^{14}\text{C}]\ \text{glucose} \xrightarrow{\text{SGT}} \text{UDP} + [\text{U} - {}^{14}\text{C}]\ \text{sterol glucoside}$$

A priori, assay conditions were optimized for pH, temperature, linearity with time and protein concentration. The optimized standard reaction mixture (100 μl) contained 20 mM Tris–HCl, pH 8.0, 0.7 mM sterol (aliquoted from an ethanolic stock solution of the lipophilic substrates), 1.5 μM UDP-[U-^{14}C]-glucose (specific activity 304 mCi $mmol^{-1}$) and enzyme preparation. The reaction was run at 37 °C for 15 min followed by recovery of the glycosylated reaction product by extraction in ethyl acetate (800 μl). Radioactivity in the ethyl acetate extract was measured in liquid scintillation counter (Wallac) using Optiphase Hisafe 3′ (Wallac) as scintillant. For kinetic studies, the substrate concentration was altered as per needs of the experiment. Data obtained were processed using Excel programme to generate the best fitting trend-lines for the Lineweaver–Burk double reciprocal plots.

Analysis of SGTL1 transcript by real time PCR

Withania somnifera seedlings, leaves (young and mature), stem and roots were analyzed for relative expression of *SGTL1* transcript using real time PCR. Total RNA isolated from the organs was used for reverse transcription as mentioned earlier. The probe and primers for *SGTL1* transcript analysis were designed from 5′ end of the ORF as this region lacks similarity with the reported glucosyltransferases (Supplementary Table 1 for primer and probe sequences). The *SGTL1* gene expression was normalized to *ubiquitin* (AJ309010) as an internal (housekeeping gene) control. The reverse transcription efficiencies of *SGTL1* and *UB* were almost equal as analyzed by comparing the C_T values [29] on ABI prism 7700. Multiplex PCR mixture (25 μl) contained 12.5 μl TaqMan master mix, 1 μl cDNA, 200 nM of RTL1F and RTL1R (forward and reverse primers respectively for *SGTL1*), 100 nM each of UBF and UBR (forward and reverse primers, respectively for *UB*), 56 nM *SGTL1* (RTL1P) and 28 nM *UB* (UBP) TaqMan MGB probes (Applied Biosystem).

The modulation of expression of *SGTL1* by salicylic acid (SA) and heat shock was studied by real time PCR. The leaf discs were floated on 3-(*N*-morpholino) propanesulfonic acid (MOPS) buffer, pH 7.5, containing 1 mM SA for different time intervals and analyzed for *SGTL1* expression. For monitoring the modulation of *SGTL1* expression by heat shock, the plants were shifted from 25 to 42 °C and their leaves were sampled at different time intervals. All the experiments were repeated thrice and the data were analyzed statistically (±SD and two tailed student's *t*-test).

Results and discussion

Withania somnifera is a medicinal plant rich in a wide variety of glycosterols. No gene from this plant has yet been studied. Cloning of genes and characterization of proteins involved in steroidal transformations can reveal new variants in this plant. This communication reports the first study on sterol glucosyltransferases (SGTs) from this plant.

Identification of SGT gene family in the leaves of W. somnifera

Degenerate primers were designed from homologous regions among the known SGTs in *Arabidopsis thaliana* (Z83833), *Avena sativa* (Z83832), *Candida albicans* (AF091398), *Dictyostelium discoideum* (AF098916), *Pichia pastoris* (AF091397) and *Saccharomyces cerevisiae* (U17246). The forward and reverse primers were designed from the putative sterol binding (PSBD) and UDP-sugar binding domains, respectively [26]. Leaf cDNA was used as template in degenerate PCR. It resulted in the amplification of about 700 bp fragment which was cloned in pBluescript SK^+ vector. Sequencing of multiple clones identified three different cDNA sequences (*SGTL1*, *SGTL2* and *SGTL3*) containing conserved domains of SGT family (Supplementary Fig. 1). The cloned partial *SGTL2* and *SGTL3* sequences were submitted to GenBank (Accession Nos. DQ356888 and DQ356889, respectively) whilst *SGTL1* was cloned in full length (DQ356887) and studied in detail. The presence of a gene family for SGTs in *W. somnifera* suggests differential functionality of the members in sterol modifications and cellular physiology.

Cloning and analysis of full-length SGTL1 cDNA sequence

The full-length *SGTL1* cDNA was obtained by 3′ and 5′ RACE. The sequences of 3′ RACE (0.4 kb including the poly A sequence) and 5′ RACE (1.4 kb including the ATG initiation codon) were used again to clone the full-length cDNA (2532 bp). The *SGTL1* contained an ORF of 2103 bp encoding 701 amino acids followed by a stop codon. The UTRs of 337 and 89 bp were present at the 5′ and 3′ ends, respectively. Conserved PSBD and UGT prosite motifs were present in the deduced amino acid sequence of *SGTL1* (Fig. 1). Two putative transmembrane domains (from 369 to 387 and 406 to 432 amino acid residues, shown as dotted lines in Fig. 1) were detected in SGTL1 by tmap (http://www.bioweb.pasteur.fr/seqanal/interfaces/tmap.html). The presence of putative transmembrane domains suggested the association of SGTL1 with membranes. Free sterols are typical constituents of eukaryotic membranes and their transformation into glycosides by SGTs have been reported to occur in plasma membrane [30] and tonoplast [31]. Accordingly, SGTL1 may be involved in catalysing the glucosylation of membrane sterols.

The clustalW alignment of deduced amino acid sequence of *SGTL1* with other plant SGTs showed its significant similarity to SGTs from *Arabidopsis* (At1g43620, 67%; Z83833, 45%), *Oryza*, 58%; *Avena*, 50% and *Panax*, 48%. Among these, only *Arabidopsis* Z83833 and *Avena* Z83832 have been characterized so far [28]. The conserved PSBD and UGT prosite motifs in SGTL1 were 52% and 89% similar, respectively in the aligned sequences. The plant secondary product glycosyltransferase (PSPG) motif is a modification of UGT prosite. The membrane bound

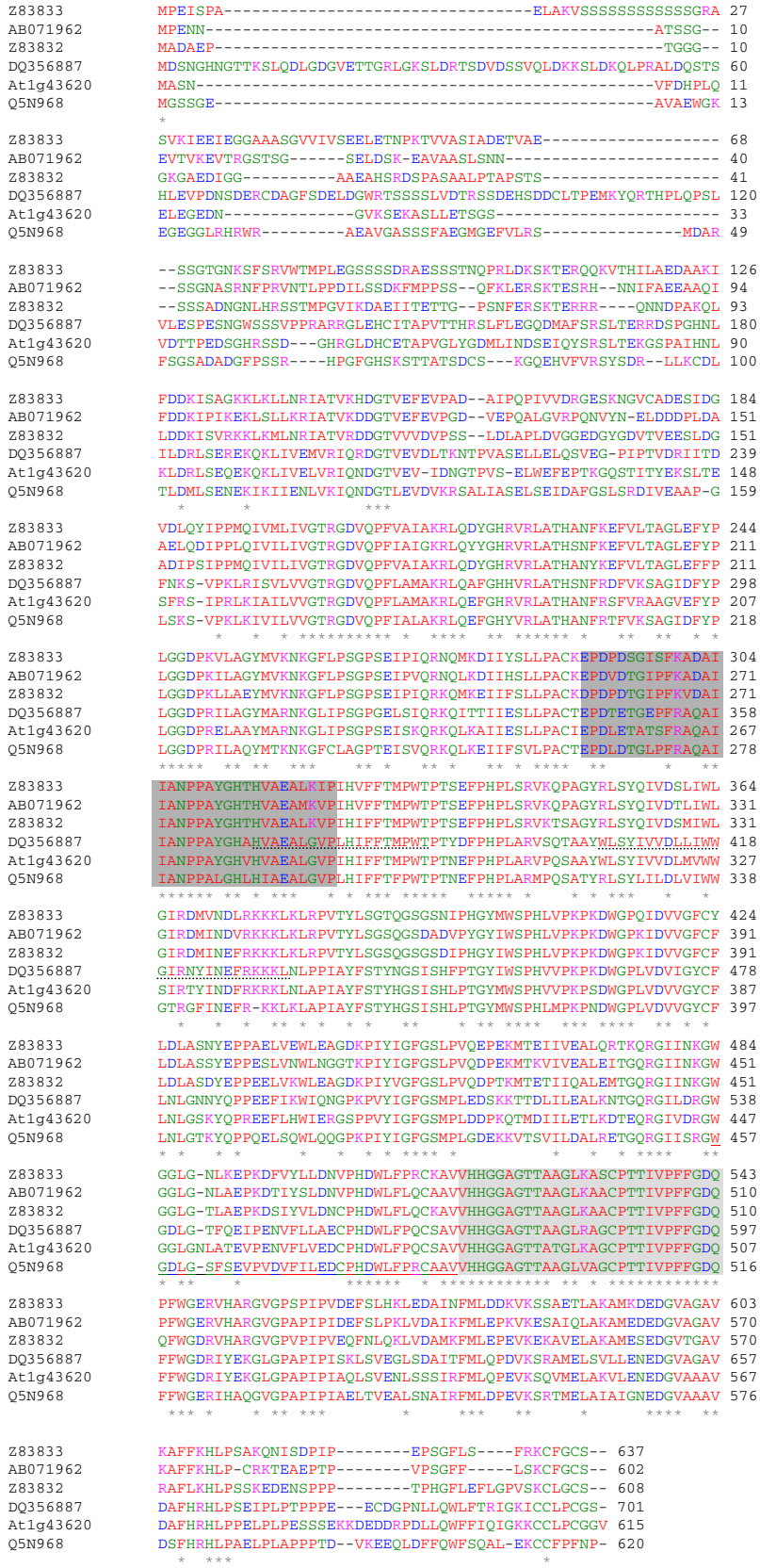

Fig. 1. Alignment of *Withania somnifera* SGTL1 amino acid sequence with plant sterol glucosyltransferases using ClustalW. Sequences of SGT in *Arabidopsis thaliana* (Z83833, At1g43620), *Panax ginseng* (AB071962), *Avena sativa* (Z83832), *Withania somnifera* (DQ356887) and *Oryza sativa* (Q5N968) can be retrieved from EMBL database. Conserved domains are shown in boxes. Two putative transmembrane domains are shown as dotted lines. Underline indicates the additional stretch from the UGT prosite motif. The asterisks indicate common residues.

plant SGTs differ significantly in the PSPG motif by incorporation of additional residues within the PSPG motif compared to the cytosolic GTs [7]. This additional sequence stretch was found in the SGTL1 (underlined in Fig. 1). The major differences between the SGTL1 and other SGTs are seen in N-terminal part of the protein. Similarity was higher in the middle and C-terminal part (shown by asterisks in Fig. 1). It suggests that SGTL1 may be involved mainly in transformation of the membrane located primary sterols rather than in the cytosolic secondary metabolites. The SGTL1 did not show significant alignment with other plant SGTs like *St*SGT [32], *Sa*GT4A, *Sa*GT4R and *Sa*GT6 [33].

Biochemical comparison of recombinant SGT from W. somnifera and A. thaliana

To express the recombinant SGTL1, its ORF was amplified, sub-cloned into SK^+/pET21d and used for expression in *E. coli*. The *SGTL1* cloned in $SK^+$with the few residues of β-galactosidase, expressed very poorly in DH5α (data not shown), presumably because codons in *SGTL1* are not preferred in *E. coli*. Thus, the *SGTL1* cloned in pET21d was transformed into *E. coli* BL21 codon plus RIL strain. This strain contains extra copies of tRNAs for Arg (R), Ile (I) and Leu (L). The BL21 transformants gave IPTG inducible expression of SGTL1 as analyzed by the enzyme activity and SDS–PAGE. In sonicated *E. coli* cell free extract, only 30% activity was associated with 100,000*g* membrane fraction and major activity (70%) was in the soluble fraction. Thus, in spite of the presence of transmembrane residues in SGTL1, association with membrane was loose in *E. coli*. Similar results have been reported in case of a membrane bound enzyme, cholesterol-α-glucosyltransferase from *H. pylori* expressed in *E. coli* where 50% of the enzyme was present in soluble fraction [34]. Recombinant SGTL1 in the supernatant was partially purified by gel filtration chromatography resulted in 3.73-fold purification. The SDS–PAGE showed induction of a 78 kDa protein, which agreed with the calculated molecular mass of SGTL1 (Fig. 2). The induced level of expression of the recombinant *At*SGT in *E. coli* DH5α was low. In order to enrich the enzyme, the crude extract was further subjected to gel filtration and ion exchange column chromatography. At this stage, 4.05-fold purification was achieved. In case of *At*SGT, only a faint band corresponding to the SGT was visible along with other contaminating proteins on SDS–PAGE (data not shown). The partially purified SGTL1 and *At*SGT were compared for activity and kinetic parameters.

Structures of different sterol substrates used in this study are given in Supplementary Fig. 2. The activity obtained for SGTL1 with dehydroepiandrosterone was considered as 100%. The relative activities with different sterols were plotted for each enzyme as shown in Fig. 3. The deacetyl 16-DPA, transandrosterone, 3-β-hydroxy-16, 17-α-epoxy-pregnenolene, pregnenolene, stigmasterol, β-sitosterol, ergosterol, brassicasterol and solasodine gave activities with SGTL1. In addition to these, *At*SGT also showed activity with 5-α-cholestan-3β-ol and cholesterol (Fig. 3). No activity was detected in *E. coli* harbouring the vector alone. Products from the reaction mixtures of SGTL1 and *At*SGT were

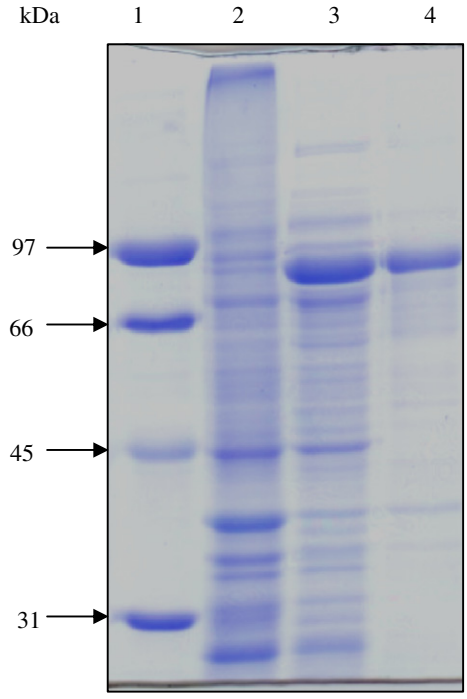

Fig. 2. SDS–PAGE analysis of the recombinant SGTL1 expressed in *E. coli* BL21 codon plus RIL strain. After IPTG induction, crude homogenate from the *E. coli* was fractionated by gel filtration chromatography and analyzed on 10% SDS–PAGE. Molecular weight markers (lane 1), crude homogenates from uninduced (lane 2) and induced (lane 3) cultures and the fraction after gel filtration (lane 4) are shown.

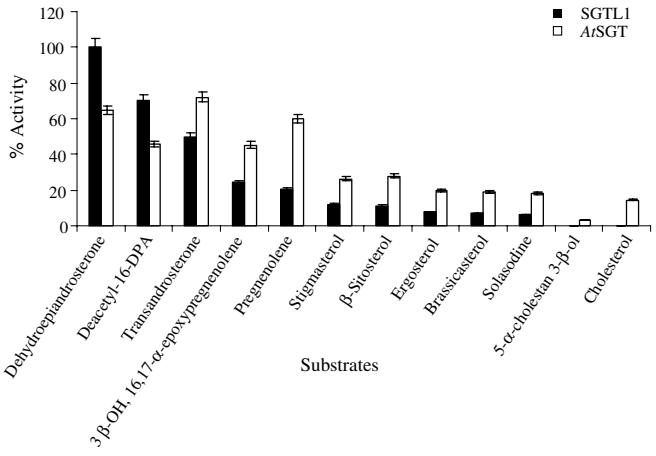

Fig. 3. The relative activity of partially purified SGTL1 and *At*SGT expressed in *E. coli*. The activity was observed at 1.5 μM UDP-[U-^{14}C]-glucose and 0.7 mM of the respective sterol. The activity of SGTL1 with dehydroepiandrosterone is taken as 100%. Relative activities with other sterols are plotted for each enzyme. Activity with 5-α-cholestan-3β-ol and cholesterol was undetectable in SGTL1. Values are the means ± standard deviation of three independent experiments.

confirmed by radio-TLC (data not shown). Sterols with higher activities (Fig. 3) for SGTL1 and *At*SGT were further used for kinetic studies. The Table 1 shows the efficiency (V_{max}/K_m) of the two partially purified enzymes for different sterols. For the tested sterols, both the recombinant enzyme preparations showed activity only with sterols having hydroxyl group at C-3 position. Among the sterols used, SGTL1 was more active towards sterols without side chain such as dehydroepiandrosterone, deacetyl 16-DPA, transandrosterone, 3-β-hydroxy-16,17-α-epoxy pregnenolene and pregnenolene as compared to sterols with side chain (Table 1). Among the above sterols, SGTL1 gave higher V_{max}/K_m value with dehydroepiandrosterone and transandrosterone as compared to *At*SGT. The other sterols showed nearly similar V_{max}/K_m values. This may be due to the keto group present at the C-17 position in dehydroepiandrosterone and transandrosterone while it is present at the C-21 position in the other sterols (Supplementary Fig. 2). These results suggest the role of chemical groups attached at other positions in determining enzyme efficiency of SGTL1. It has earlier been reported that membrane bound SGTs glucosylate primary sterols preferably. However, cytosolic SGTs are specific for secondary metabolites. Solasodine was the only secondary metabolite accepted as substrate by both the enzymes. Since the GTs possess regiospecificity for substrates, the activity with solasodine may be due to its structural similarity with primary sterols.

The comparison of K_m values (Table 1) indicated that SGTL1 had more affinity for sterols with methyl groups in the side chain at C-24 position (ergosterol and brassicasterol). However, the affinity of SGTL1 was significantly lowered with the replacement of methyl group at C-24 in the side chain by ethyl group (stigmasterol and sitosterol). Thus, the alkyl group at C-24 in sterols appears to be favourable for the binding to SGTL1 than *At*SGT. However, in these cases, V_{max} values were reduced substantially. The alignment of PSBD (putative domain for sterol binding) of SGTL1 and *At*SGT showed a difference of 30% (Fig. 1). The differences in the binding affinity of SGTL1 and *At*SGT with sterols having alkyl group in the side chain could be a manifestation of the differences in their PSBD regions. It may be possible that some of the differences in the specificities for substrates could arise due to the presence of a few residues of β-galactosidase at N-terminal of *At*SGT. However, it has been reported that the substrate specificity of recombinant *Avena* SGT (Z83832) having 31 amino acid residues of β-galactosidase at the N-terminal does not differ from the plant purified protein [28]. Hence, the amino acids at N-terminal may not seriously influence the catalytic functions of SGT.

Both the enzymes utilized only UDP-glucose. UDP-galactose could not serve as the sugar donor. This specificity was consistent with the recent demonstration that the last amino acid of the PSPG motif in glycosyltransferases controlled relative specificity for UDP glucose or UDP galactose. A glutamine (Q) in glucosyltransferases and histidine (H) in galactosyltransferases is critical to such specificity [35]. The presence of glutamine as the last amino acid in UGT prosite motif in SGTL1 (Fig. 1) corroborates the above functionality.

Expression analysis of SGTL1 transcript in W. somnifera

Quantitation of the *SGTL1* transcript revealed that it was expressed at significantly higher level in roots (3.13-fold) and leaves (2.21-fold) as compared to that in stem (Fig. 4a). Developmentally, *SGTL1* transcript accumulated at a higher level in mature leaves (2.41-fold) than in the young leaves and seedlings (Fig. 4b). These results are in agreement with more accumulation of glycosteroids in roots [24] and leaves [36].

The incubation of leaf discs in the presence of salicylic acid resulted in transient accumulation of *SGTL1* transcript. It increased gradually to reach the maxima of 2.15-fold at 12 h followed by a decline (Fig. 4c). The SA signaling is known to induce defense-related genes, particularly those

Table 1
Kinetic comparison of recombinant *W. somnifera* SGTL1 and *At*SGT for different substrates

Substrates	SGTL1 (K_m in μM)	SGTL1 (V_{max} in pmol mg^{-1} min^{-1})	SGTL1 (V_{max}/K_m)	*At*SGT (K_m in μM)	*At*SGT (V_{max} in pmol mg^{-1} min^{-1})	*At*SGT (V_{max}/K_m)
UDP-glucose	8	8	1	25	10	0.4
Dehydroepiandrosterone	40	7.2	0.12	43	9.4	0.05
Deacetyl 16-DPA	10	2.5	0.25	7	1.7	0.24
Transandrosterone	25	1.8	0.07	59	1.8	0.03
3-β-hydroxy 16,17-α-epoxypregnenolene	10	0.6	0.06	17	1.5	0.09
Pregnenolene	6	0.8	0.13	8	1.2	0.15
Stigmasterol	27	0.6	0.02	43	0.7	0.02
β-sitosterol	40	0.8	0.02	59	0.6	0.01
Ergosterol	4	0.2	0.05	35	1.2	0.03
Brassicasterol	4.5	0.2	0.04	30	0.9	0.03
Solasodine	7	0.5	0.07	3	0.4	0.13
5-α-chol estan-3-β-ol	ND	ND	ND	63	0.2	0.003
Cholesterol	ND	ND	ND	56	0.4	0.007

The values of V_{max} and K_m are the means of three separate experiments. The standard deviation is below 5% in all the cases. ND: not determined.

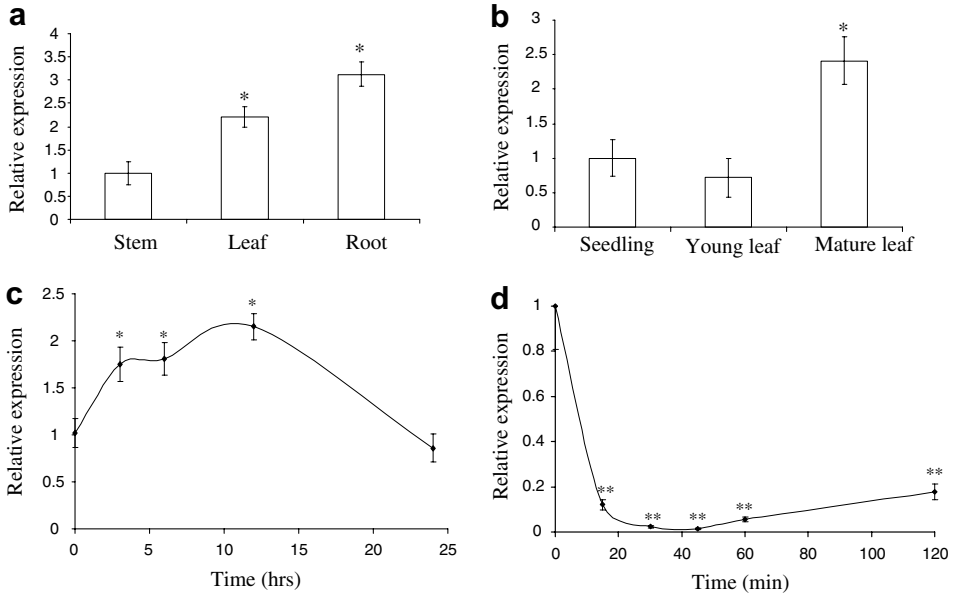

Fig. 4. The expression of *SGTL1* transcript determined by real time PCR, taking *UB* as endogenous reference. Relative expression of *SGTL1* in (a) stem, leaf and root; (b) seedling, young leaf and mature leaf; (c) SA treated and non-treated leaf discs at different time intervals; (d) leaves subjected to heat shock (by shifting plants from 25 to 42 °C) for different time intervals. Asterisks indicates (*), significant ($p < 0.05$) and (**), highly significant ($p < 0.01$) mean difference.

encoding pathogenesis related proteins [37], cellular protectant glutathione-*S*-transferase, cytochrome P_{450}, ABC transporters and glucosyltransferases. Also, saponins have been shown to accumulate in response to pathogen attack [38]. Saponins aggregate with sterols in pathogen membrane, leading to the formation of membrane pores and thus, the loss of membrane integrity [39]. The increase in expression of *SGTL1* in the presence of SA indicates that it may play a role in defense response in *W. somnifera*.

A decrease in the transcript level was observed within 15 min when the plants were shifted from 25 to 42 °C. The level of *SGTL1* transcript declined by ten fold, became undetectable at 45 min and then began to recover slowly (Fig. 4d). Earlier reports from *Myxoamoeba* and human fibroblastoma cell lines showed the activation of sterol glucosyltransferase and the production of sterol glucoside following heat stress. The sterol glucosides have been reported to induce the signal transduction pathway, leading to the synthesis of heat shock proteins during heat stress [40,41] in animal cells. Transcriptional analysis using microarray comprising a large set of genes including 109 glycosyltransferases suggested the role of glycosylations in defense response of *A. thaliana* [42]. Similarly, our study suggests diverse functions of the glycosylated sterols in plants in response to different stresses.

Conclusion

This study identifies SGT gene family in *W. somnifera* and characterization of one member (*SGTL1*) of this family. It is expressed ubiquitously in different parts of the plant. The presence of putative transmembrane domains in the deduced amino acid sequence and its preference for glucosylation of membrane sterols suggest its membrane functionality. The partially purified recombinant SGT from *W. somnifera* was specific to the sterols having hydroxyl group at C-3 position. Stress modulation of *SGTL1* expression suggests its putative functional recruitment under environmental challenge(s). The stress response, substrate specificity and localization of different members of SGT in *W. somnifera* need to be examined to attribute specific functions to different members of the family. Characterization of novel sterol glucosyltransferases in *W. somnifera* will provide the basis for genetic manipulation of these genes and contribute to the elucidation of glycosterol functions in plants.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.abb.2007.01.024

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