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Sterol Glycosyltransferases—The Enzymes That Modify Sterols

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Abstract Sterols are important components of cell membranes, hormones, signalling molecules and defense-related biotic and abiotic chemicals. Sterol glycosyltransferases (SGTs) are enzymes involved in sterol modifications and play an important role in metabolic plasticity during adaptive responses. The enzymes are classified as a subset of family 1 glycosyltransferases due to the presence of a signature motif in their primary sequence. These enzymes follow a compulsory order sequential mechanism forming a ternary complex. The diverse applications of sterol glycosides, like cytotoxic and apoptotic activity, anticancer activity, medicinal values, anti-stress roles and anti-insect and antibacterial properties, draws attention towards their synthesis mechanisms. Many secondary metabolites are derived from sterol pathways, which are important in defense mechanisms against pathogens. SGTs in plants are involved in changed sensitivity to stress hormones and their agrochemical analogs and changed tolerance to biotic and abiotic stresses. SGTs that glycosylate steroidal hormones, such as brassinosteroids, function as growth and development regulators in plants. In terms of metabolic roles, it can be said that SGTs occupy important position in plant metabolism and may offer future tools for crop improvement.

Keywords Adaptive response · Brassinosteroids · Cellular homeostasis · Detoxification · Glycosyltransferases · Hormonal regulation · Insect resistance · Medicinal plant · Sterols stress

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Introduction

Bacteria, fungi, animals and plants are a valuable source of numerous metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives. The immense heterogeneity of metabolites is a result of diverse modification mechanisms, which are undergoing inside the organisms. Many of these metabolites are made, in attempt to adapt rapidly to the external environmental conditions and maintain their cellular homeostasis. This adaptability depends on their growth and developmental stages and evolution of mechanisms that regulate metabolic functions. Studies suggest that glycosylation is one of the mechanisms by which organisms modify hormones, secondary metabolites, biotic and abiotic chemicals and toxins in the environment to maintain their cellular homeostasis. The identification of large multigene families of glycosyltransferases (GTs), which recognise these diverse molecules, adds to this fact. This review focuses on glycosyltransferases that recognise small molecules such as sterols especially in plants and describes their functions pertaining to their biological activities, structural features and response to stress. The sterol glycosyltransferases are grouped into family 1 of the 90 distinct families, which describe GTs (http://afmd.cnrs-mrs.fr/CAZY/acc.html). Most of the GT family 1 members are defined by the presence of a carboxyl terminal consensus sequence termed as signature motif (Fig. 1), which is involved in the interaction of the enzyme with the activated sugar donor [1–4]. The signature motif can be identified in the enzyme sequences of animals, plants, fungi and bacteria [5, 6]. In *Arabidopsis thaliana*, the gene family comprises 112 full-length sequences and eight pseudogenes with frame-shift mutations [3].

The activated sugar donor of plant GTs is commonly UDP-glucose (UDP-Glc) although UDP-rhamnose (UDP-Rha), UDP-galactose (UDP-Gal), UDP-xylose (UDP-Xyl) and UDP-glucuronic acid (UDP-GicUA) also act as activated sugars [7–9]. Glycosylation occurs at –OH, –COOH, $–NH_2$, –SH and C–C groups and more than one sugar may be attached [10, 11]. The membrane transport systems recognise the glycosyl residues and facilitate the transport of glycosides, which is reflected by the fact that many glycosylated molecules are found in vacuole compartments [12]. Studies do show ATP binding cassette (ABC) transporters in the tonoplast membranes, which transport both endogenous and xenobiotic glycosides. Glycosides and glycosinolates of small molecules have also been identified in the apoplast [13, 14]. Diverse glycosylated molecules accumulate in the vacuolar and apoplastic space, and there is also clearance of these molecules. The above points indicate that GTs are involved in detoxification. It can be concluded that the glycosyltransferases modify small lipophilic molecules, which leads to a change in their participation in cellular metabolism.

Sterol Glycosyltransferases

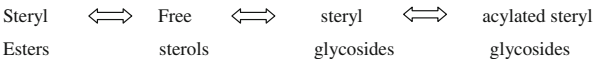
In contrast to vast literature available on glycosyltransferases of diverse molecules, there are few reports on sterol glycosyltransferases. Sterols are a part of vast family of isoprenoids and are precursors of many steroid hormones, defense-related compounds, such as

Fig. 1 Signature sequence of glycosyltransferases

[FW]-x(2)-[LIVMYA]-[LIMV]-x(4-6)-[LVGAC]-[LVFYA]-[LIVMF]-[STAGCM]-[HNQ]-[STAGC]-G-x(2)-[STAG]-x(3)-[STAGL]-[LIVMFA]-x(4)-[PQR]-[LIVMT]-x(3)-[PA]-x(3)-[PA]-x(3)-[DES]-[QEHN]

saponins, signalling molecules and are important structural components of cell membranes [15]. Sterols have been shown to regulate development and gene regulation in *Arabidopsis* [16]. A group of oxidised sterols called brassinosteroids function as plant growth regulators and development in plants [17]. In plant cells, sterols are synthesised primarily in the endoplasmic reticulum using the mevalonate pathway of isoprenogenesis by phenyl precursors from cytosol. Some contribution of the plastid localised 1-deoxy-D-xylulose 5-phosphate (DOXP) pathway of isoprenogenesis has also been suggested [18]. DOXP pathway is an alternative pathway by which plants, bacteria, such as *Mycobacterium*, or protozoans synthesise isoprene units such as isopentenyl pyrophosphate and dimethylallyl phosphate. These isoprene units lead to the biosynthesis of 2,3-oxidosqualene, which serves as the common progenitor of different classes of sterols. Sterol pathway (Fig. 2a, b) has been extensively reviewed [19].

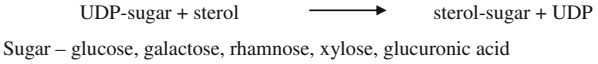
Sterols occur either in free form or as steryl conjugates.



The ratio of sterol and their modified counterparts varies when plant adapts to environmental conditions, which lays down an area to be explored by looking for enzymes involved in sterol modifications. Sterol glycosides have been extensively reviewed by Grille et al. [20], where a number of medicinal, cytotoxic, apoptotic, anti-stress, anti-insect, anti-fungal and anti-cancer properties have been attributed, though in most of the cases sterol glycosyltransferase enzymes have not been reported. Sterol glycosides are highly bioactive food components and laboratory mice fed on sterol glycosides lead to either amyotrophic lateral sclerosis or Parkinsonism pathologies [21]. Hence, understanding the processes involved in sterol glycoside production is of immense human importance. In addition, the consumption of seeds of the cycad palm (*Cycas micronesica*), containing high sterol glycoside levels, has been linked to an unusual human neurological disorder, amyotrophic lateral sclerosis-parkinsonism in people of Guam [22]. SGTs provide a way to look into diverse sterol modifications, which is helpful in understanding plant mechanisms.

Glycosylation

Most of the higher plant sterols posses a β-OH group at C-3 position and are structurally diversified through a variety of transformations including desaturation, chain-elongation, cyclisation, esterification, epoxidation, hydroxylation and glycosylation. Amongst them, the cytochrome P_{450}-dependent oxidation and glycosylation represent the most predominant transformations [23]. Glycosylation not only stabilises the products but also modulates their physiological activities and governs intracellular distribution [24]. Glycosylation enhances water solubility of otherwise lipophilic membrane sterols and therefore can lead to a change in cellular mobility, fluidity, permeability, hydration and phase behaviour [25]. SGTs catalyse the transfer of sugar molecules on to sterols at different positions such as C-3, C-17 or C-27.



Sterol glycosides with di-, tri- and terta-glucoside residues have also been reported though they are rare in occurrence [26]. Sterol glycosides can be acylated,

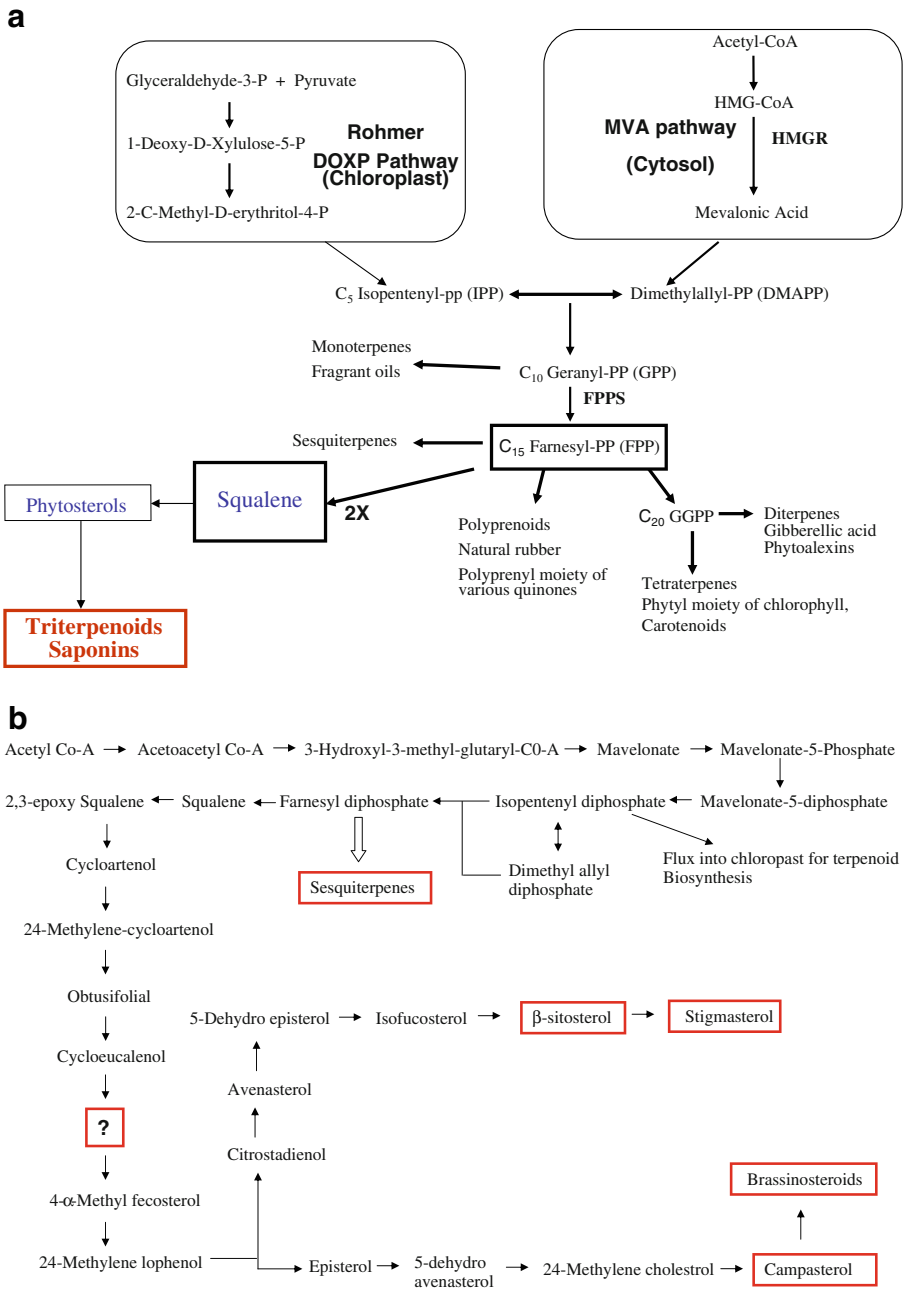


Fig. 2 **a** Synthetic pathway of various classes of phytosterols in plants. **b** The sterol pathway

polyhydroxylated or sulphated and the primary alcohol of the carbohydrate unit attached to acylated sterol glycoside can be further esterified with fatty acids (most common type of modification).

Characteristic Structural Features

Structural information is important in fundamental understanding of protein evolution and understanding the catalytic mechanisms. Initially, only three GT1 protein structures were available, and all of those were bacterial GTs involved in antibiotic synthesis [27–29]. The structure consisted of two Rossmann folds, each constructed with a central sheet of several β strands flanked on either side by α helices.

Results from the co-crystallisation of these proteins with their ligands indicated that residues in the N-terminal half of the protein were responsible for acceptor binding, whereas those in the C-terminal half were involved mainly in donor interactions. However, the bacterial sequences are substantially different from those of plant and mammalian enzymes and are not classified in the same subset of family 1, since they lack the 44-amino acid consensus sequence.

From the plants, two GT1 enzymes have been crystallised, and their 3D structure has been solved independently by two research groups [30, 31]. In both the studies, results show that plant proteins also contain two Rossmann folds and acceptors bind to residues in the N-terminal half, whereas activated donor sugars bind mainly to the C-terminal region. The UGT71G1 enzyme from *Medicago truncatula* was co-crystallised with donor UDP-Glc and the structure was resolved at 2.6 Å [31]. The *Vitis vinifera* GT1 enzyme was co-crystallised with UDP-2-deoxy-2-fluoro-Glc, and the structure was resolved at 1.9 Å. The structure of both GTs clearly illustrates the role of the signature motif in activated sugar-donor binding.

Mechanism of Action

Most biochemical reactions involve two substrates, and these are often transfer reactions of one type or another (including oxidation/reduction reactions). The reaction mechanism may be a sequential one, where both substrates bind to the enzyme to form a ternary complex before the first product is formed or it may be non-sequential. SGTs have been reported to follow a compulsory-order sequential mechanism forming a ternary complex [32–34]. The analysis of kinetic mechanism of two purified enzymes from *Withania somnifera*, one for 3-β hydroxy position and other for 27-β hydroxyl position of β-sitosterol and testosterone, respectively, was performed. For both enzymes, varying non-saturating concentrations of sterols in the presence of several sub-optimal concentrations of UDP-glucose and vice versa was used. The Lineweaver–Burk plots (Fig. 3) of initial velocity vs. substrate concentration showed that

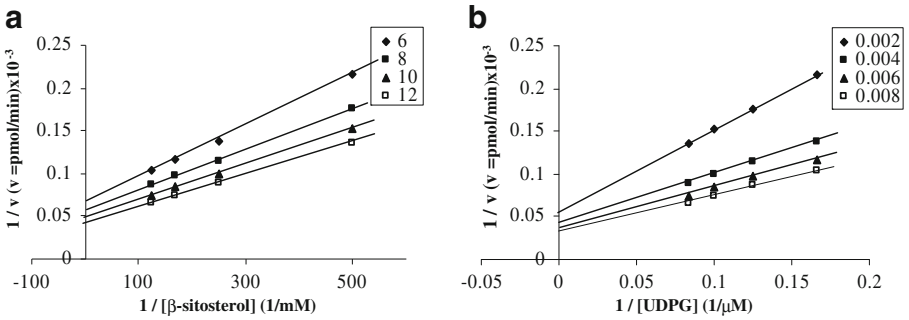


Fig. 3 Enzyme kinetics of *W. somnifera* cytosolic sterol glycosyltransferase

lines in the plots converged to the left of the vertical axis in the second quadrant. This is the characteristic of an enzyme that acts via the formation of a ternary complex [35].

Thus, both UDP-glucose and sterol substrates are required to bind to the enzyme at the same time to catalyse the reaction. Ternary complex formation is entirely in agreement with the theory that UDP-glucuronosyl transferases posses two major functional domains, a theory proposed as a result of sequence homology studies made on these enzymes [36, 37]. Product inhibition studies suggested that the reaction followed a compulsory order ternary complex mechanism in which UDP-glucose was the first binding substrate. This ternary complex mechanism is in agreement with the limited work carried out on other enzymes [38, 39].

Localisation of SGTs

Both membrane-bound and cytosolic forms of SGTs have been reported. A membrane-bound UDP-glucose:sterol glycosyltransferase has been purified from *Avena sativa* [40] and expressed in *Escherichia coli* [41]. In *Calendula officinalis* 2-week-old seedlings, UDPG: sterol glycosyltransferase has been reported to be localised in the membrane structures, separated from chloroplast and mitochondria and consisting probably of fragments of Golgi apparatus [15]. A sterol glycosyltransferase UGT80A2 has been found in plasma membrane, endoplasmic reticulum membrane, golgi vesicles and, occasionally, the vacuolar membrane tonoplast [39, 41–43]. Glycosyltransferases function in the cytosol, but this may involve association with the cytosolic face of the membrane compartments or location within the multiprotein complexes [44].

Although the GT transfer reactions are cytosolic, the glycoside products that are formed gain access to the membrane bound transport systems that recognise the glycosyl residues, e.g. ATP-binding cassette transporter (ABC) systems implicated in glycosylated xenobiotic transport [45, 46]. Studies have documented the accumulation of glycosylated compounds in vacuolar compartment [12]. Glycosides and glycosidases of small lipophilic molecules have also been identified in the apoplast [13, 14]. In contrast, the human glycosyltransferase have a signal sequence involved in cotranslational translocation into the rough endoplasmic reticulum, as well as a transmembrane spanning domain and an ER retention signal [47]. These enzymes therefore clearly function in the ER, necessitating the transport of nucleotide sugar from the cytosol into the ER lumen for the transfer reaction [48].

Occurrence of SGTs

SGTs from Bacteria and Viruses

Steryl glycosides are mostly membrane lipids that are synthesised by all plants, most fungi, slime moulds, and some animals [41, 49, 50]. The biosynthesis of sterol glycosides was first reported in *Mycoplasma gallinarum*, where the cholesteryl glycoside was synthesised by the transfer of glucose from uridine-5′-diphosphoglucose to membrane bound sterol [51]. The enzyme activity was associated with the membrane and treatment of the membrane to remove endogenous sterol inactivated the enzyme. Sterol glycosides have also been reported from *Spiroplasma citri* [52], *Acholeplasma axanthum* [53], *Borrelia hermsi* [54] and *Helicobacter pylori* [55]. The presence of sterol glycosides in bacteria indicates that these bacteria contain sterol glycosyltransferases.

A cholesterol alpha-glycosyltransferase has been cloned from *H. pylori* [56]. *H. pylori* infection causes gastric pathology such as peptic ulcers and carcinoma. Cholesterol

glycosylation by SGT of *H. pylori* promotes immune invasion by the pathogen [57]. When the gene coding for this enzyme was deleted, there was loss of cholesteryl glycoside and its derivatives. The mutant lacking the cholesteryl glycosides showed impaired infection. The cholesterol-α-glycosyltransferase also showed sequence similarities with bacterial diacylglycerol α-glycosyltransferase. Such diacylglycerol α-glycosyl and galactosyl transferases have been identified in *Acholeplasma laidlawii*, *Streptococcus pneuminiae*, *Deinococcus radiodurans* and *Thermotoga maritime* [58, 59].

Sterol glycosyltransferases have been reported from several baculoviruses, where these enzymes glycosylate ecdysteroids [60]. Baculoviruses are invertebrate-specific pathogens that have been described in more than 800 species of insects. The ecdysteroid UDP-glucosyltransferases (EGT) catalyse the conjugation of sugars (glucose or galactose) from UDP-sugars to ecdysteroid molting hormones, which makes the hormone inactive in the infected larvae [38]. As many as 27 baculovirus EGTs have been reported, some of which are given in Table 1. An active EGT disrupts the hormonal balance of the host larvae and prevents insect larvae from moulting. Since this gene plays an important role in the regulation of host development, it can be potential tool for pest management.

SGTs from Fungi and Slime Moulds

Fungal *sgt*s have been reported from *Candida bogoriensis* [61]; *Pythium sylvaticum* [62]; *Sachharomyces cerevisiae*, *Candida albicans*, *Pichia pastoris*, and slime mould *Dictyostelium discoidium* [50]. SGTs play different functional roles in *P. pastoris* and *Yarrowia lipolytica* [63]. *P. pastoris* is a methylotropic yeast and a sterol glycosyltransferase has been shown to be involved in vacuole-dependent selective degradation of peroxisomes (pexophagy) in response to glucose or ethanol. Upon induction of pexophagy, the enzyme was found to reside in proximity with the vacuolar membrane and associated with a novel membranous structure, essential for the pexophagic process. A mutant, defective in the UGT51 gene was also defective in pexophagy and did not contain the catalytic product of sterol glycosyltransferase–ergosterol glycoside.

In alkane-utilising yeast *Yarrowia lypolytica*, the UGT51 enzyme is required for utilisation of decane and not for pexophagy. A mutant defective in UGT51 gene of this yeast is not defective in pexophagy but is severely affected in the assimilation of decane. It was demonstrated that sterol glycoside accumulates in *P. pastoris* under stress conditions, such as heat shock or excess ethanol [49]. Hard surface contact has been known to be necessary to induce infection structure (appresorium) in many phytopathogenic fungi. One of the genes induced by hard surface contact of the conidia of *Colletotrichum gloeosporioides chip6* encodes a protein with homology to sterol glycosyltransferases [64]. When expressed in *E. coli*, this enzyme caused glycosylation of cholesterol. Disruption of this gene causes reduction in virulence on its natural host. Heat stress induces the glycosylation of membrane sterols in myxamoebae of a true slime mould, *Physarum polycephalum* [65, 66]. The above examples elucidate that many fungi contain sterol glycosides and sterol glycosyltransferases with different functions.

Animal SGTs

SGTs have been reported from snake epidermis [67], chicken epidermis [68], rat liver [69], and humans [70, 71]. Glucuronidation is a major pathway for the inactivation and excretion of both endobiotic compounds such as bilirubin and steroids as well as xenobiotic compounds including drugs, carcinogens and other environmental pollutants [72]. Two rat

Table 1 Sterol glycosyltransferases from bacteria, fungi and slime moulds, animals and plants

Group of organism	Name of the gene/enzyme	Source	Significance of the gene	Reference
Bacteria	UDP-G:sgt[a]	*Mycoplasma gallinarum*	Biosynthesis of cholesteryl glucoside	[51]
	UDP-G:sgt	*Spiroplasma citri*	Synthesis of sterol glucosides	[52]
	UDP-G:sgt	*Acholeplasma axanthum*	Synthesis of cholesterol glucosides	[53]
	UDP-G:sgt	*Borrelia hermsi*	Synthesis of cholesterol glucosides	[54]
	α-Cholesterol glycosyltransferase (*HP0421*)	*Helicobacter pylori*	Synthesis of cholesterol glucosides	[56]
Virus	Ecdysteroid UDP-gt	Baculovirus	Glycosylation of ecdysteroid	[38]
	Ecdysteroid UDP-gt	Nucleopolyhedro virus from *Epiphyas postvittana*	Glycosylation of ecdysteroid	[145]
	Ecdysteroid UDP-gt	Single-enveloped nucleopolyhedrovirus from *Helicoverpa armigera*	Glycosylation of ecdysteroid	[60]
	Ecdysteroid UDP-gt	Granulovirus from *Epinotia aporema*	Glycosylation of ecdysteroid	[146]
	Ecdysteroid UDP-gt	nucleopolyhedrovirus from *Anticarsia gemmatalis*	Glycosylation of ecdysteroid	[147]
	Ecdysteroid UDP-gt	Multicapsid nucleopolyhedrovirus from *Spodoptera frugiperda*	Glycosylation of ecdysteroid	[148]
	Eecdysteroid UDP-gt	Granulovirus from *Adoxophyes orana*	Glycosylation of ecdysteroid	[149]
Fungi and slime moulds	UDP-G:porifera sgt	*Physarum polycephalum*	Biosynthesis of poriferasterol glucoside in heat stress	[66]
	UDP-G:sgt (*UGT51B1*)	methanol-utilising yeast *Pichia pastoris*	required for pexophagy	[50] [63]
	UDP-G:sgt (*UGT51*)	alkane-utilising yeast *Yarrowia lipolytica*	required for utilisation of decane, but not for pexophagy.	[63]
	UDP-G:sgt	*Candida albicans, Pichia pastoris, Pichia anomala, Sordaria macrospora, Pyrenophora teres, Ustilago maydis, Acremonium chrysogenum, Penicillium olsonii and Rhynchosporium secalis*	Biosynthesis of sterol glycosides, acylated sterol glycosides and cerebrosides	[49]

	UDP-G:sgt	*Candida bogoriensis*	Glycosylation of ergosterol and cholesterol	[61]
	UDP-G:sgt	*Pythium sylvaticum*	Glycosylation of cholesterol	[62]
	UDP-G:sgt (*UGT51*, *UGT51C1*, *UGT51B1*, *UGT52*)	*Saccharomyces cerevisiae, Candida albicans, Pichia pastoris, and Dictyostelium discoideum*	Glycosylation of mostly the membrane bound lipids cholesterol, sitosterol, and ergosterol	[50]
	UDP-G:sgt (*chip6*)	*Colletotrichum gloeosporioides* conidia	Hard surface contact induced gene which induces formation of appressorium. Biosynthesis of cholesterol glycoside	[64]
Animals	UDP-G:sgt	Snake epidermis	Biosynthesis of glycosylsterol and acylglycosylsterol (cholestrol)	[67]
	UDP-G:sgt	Chicken epidermis	beta-D-glucosylsterols and 6-O-acyl-beta-D-glucosylsterols	[68]
	UDP-glucuronyltransferase	rat liver	Biosynthesis of testosterone and oestrone glycosides	[69]
	UDP-G:sgt	human fibroblasts	Glycosylation of cholesterol	[115, 116]
Plants	UDP-G:sgt	etiolated oat shoots (*Avena sativa* L. cv Alfred)	Glycosylates membrane sterols:[beta]-sitosterol, cholesterol, stigmasterol, and ergosterol.	[40]
	UDP-G:sgt (*UGT73C5*)	*Arabidopsis thaliana*	Glycosylates brassinosteroids which regulate growth and development	[137]
	UDP-G:sgt (*sgt* L1)	*Withania somnifera*	Glcosylates 3beta-hydroxy sterols such as sitosterol and stigmasterol which are membrane sterols	[34]
	Membrane-bound UDP-G:sgt	*Solanum melongena*	Glycosylates 22-oxycholesterol	[84, 85, 150]
	Solanidine UDP-G:gt	*Solanum tuberosum*	Glycosylates solanidine and accumulates in response to injury	[86]
	SGT2	*Solanum tuberosum*	Reduce SGA accumulation	[151]
	UDP-G:sgt (*SaGT4A*)	*Solanum aculeatissimum*	Involved in steroid saponin biosynthesis	[87]
	UDPG:ginsenoside Rd-Glucosyltransferase	*Panax ginseng*	Biosynthesis of ginsenoside Rb_1 from ginsenoside Rd	[93]
	UDP-G:sgt	*Withania somnifera*	Glycosylates 27-beta-OH steroidal lactones which are medicinally important	[33]

Table 1 (continued)

Group of organism	Name of the gene/enzyme	Source	Significance of the gene	Reference
	UDP-G:sgt	*Withania somnifera*	Glycosylates 3-beta-OH steroidal lactones which are medicinally important	[32]
	UDP-glucuronic acid:soyasapogenol glucuronosyltransferases (*UGASGT*)	Germinating seeds of *Glycine max* [L.] Merr.	Key enzyme in saponin biosynthesis	[152]
	UDP-G:sgt	Fruits of *Tribulus terrestris*	Involved in steroidal saponin biosynthesis which are medicinally importat	[153]
	UDP-G:sgt	From Turkish *Astragalus* species	Glycosylates cycloartane-type triterpenes which are medicinally important	[154]
	UDP-G:sterol β-D-glucosyltransferase	Etiolated maize coleoptiles	Glycosylation of membrane sterols	[24]
	UDP-G:sgt	*Arabidopsis thaliana*	Glycosylation of membrane sterols	[41]
	UDP-glucose:saponin glucosyltransferase (*UGT73K1, UGT71G1*)	*Medicago truncatula*	UGT73K1 with specificity for hederagenin and soyasapogenols B and E, and UGT71G1 with specificity for medicagenic acid.	[90]
	UDP-G:saponin glucosyltransferase	Oat and tomato	Biosynthesis of glycosylated saponins which are antimicrobial	[98]
	UDPG-ginsenoside:Rd glycosyltransrerase	*Panax notoginseng*	Biosynthesis of ginsenosides	[139]
	UDP-glucose dependent glucoceramide synthase	*Gossypium hurisutum*	Biosynthesis of sterol glucosides	[88]
	CesA glycosyltransferases	Cotton fibres	Sitosterol-β-glucoside	[92]

UDP-G:sgt UDP-glucose:sterol glycosyltransferase, *UDP-gt* UDP-glucosyltransferase

liver steroid UDP-glucuronosyltransferases have been purified and their enzymatic mechanism of glucuronidation has been reported [73]. Recently, several human UGT complementary DNAs (cDNAs) have been cloned, and on the basis of evolutionary divergence, have been split into two families termed UGT1 and UGT2 [74].

A single UGT1 gene on choromosome 2 encodes human phenol and bilirubin UGTs [70, 71]. The UGT2 gene family, which contains odorant and steroid metabolising isoforms in contrast with UGT1 family, is composed of independent genes [36, 75], which are clustered on human choromosome 4 [76]. Human bilirubin UGT is involved in ethinyloestradiol glucuronidation, and also it is capable of the glucuronidation of endogenous oestrogens (3-oestradiol estriols) and a diverse variety of xenobiotic compounds (phenols, anthraquinones and flavones) of which many are found in our diet, e.g. eugenol (cloves), galletes (food preservatives), emodin (rhubarb) and naringenin (grape fruit), indicating the functional diversity of this enzyme.

Plant SGTs

Apart from membrane sterols, plants contain triterpenoids, steroids or steroidal alkaloids, which exist in numerous glycoforms, regioselectively glycosylated at different positions [23, 77–79]. Various membrane-bound UDP-sterol:glycosyltransferases have been studied in plants [24, 40, 80]. Their intracellular localisation in the plasma and vacuolar membranes as well as in the Golgi apparatus reflects the distribution of sterol glycosides and acylated sterol glycosides in plant cell [24, 81, 82]. A membrane-bound sterol glycosyltransferase has been cloned and characterised from *Arabidopsis thaliana* and *Avena sativa* [40]. The enzyme was expressed in *E. coli*, and it exhibited in vitro enzyme activity for membrane associated sterols. In *Arabidopsis thaliana, SGT* mutants have been generated by insertional inactivation of the gene. Mutations in the UDP-glucose:sterol glucosyltransferase in *Arabidopsis thaliana* caused transparent testa phenotype and suberisation defect in seeds [83].

A membrane-bound UDP-glucose:sterol glycosyltransferase from *Solanum melongena* (eggplant) leaves was partially purified and its specificity as well as molecular and kinetic properties were defined [84, 85]. Among a wide spectrum of 3-OH steroids (i.e. typical plant sterols, androstane, pregnane and cholestane derivatives, steroidal alkaloids and sapogenins) and triterpenic alcohols, the highest activity was found with 22-oxycholesterol. UDP-glucose appeared to be the best sugar donor. The enzyme preparation was also able to utilise UDP-galactose, TDP-glucose and CDP-glucose as a sugar source for sterol glycosylation, but at distinctly lower rates.

A potato *sgt* was identified from screening a wound-induced potato cDNA library in yeast and selecting clones on the basis of higher toxicity of a steroidal alkaloid aglycone relative to its glycoside [86]. In vitro assays with the recombinant Sgt1 indicated that it could transfer glucose to a number of acceptors with higher activity towards tomatidine and solasodine as compared with solanidine (Fig. 4). Another *Solanum* species SGT (SAGT4A)

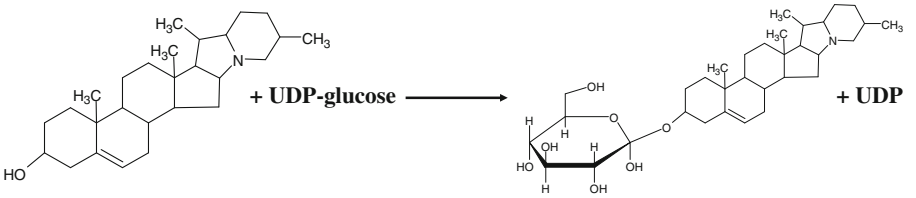


Fig. 4 Biosynthesis of γ-chaconine by solanidine UDP-glucose glucosyltransferase

catalysed the 3-o-glycosylation of steroidal sapogenins, such as nuatigenin, as well as steroidal alkaloids such as solanidine, using UDP-glucose as donor [87]. In *Gossypium hirsutum*, a UDP-glucose-dependent glucoceramide synthase has been reported, which also synthesises sterol glucosides in plants [88].

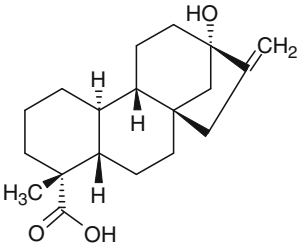
Methyl jasmonate, an elicitor involved in pathogen response, has been shown to induce the accumulation of saponins in cell suspension culture of *Medicago truncatula* [89]. Transcriptome analysis has revealed a number of potential GT transcripts upregulated in response to methyl jasmonate. Two *SGT*s UGT73K1 and UGT73G1 were functionally characterised in vitro from *M. truncatula*, using UDP-Glc, UDP-Gal or UDP-GlcUA as donors and a wide variety of saponin aglycone and phenolic acceptors [90]. Both GTs used UDP-Glc as donor and glycosylated a number of triterpenoids. The sweet honey leaf (*Stevia rebaudiana*) accumulates a mixture of intensely sweet compounds in its leaves (Fig. 5). These are different diterpenoid glycosides in which the extent and regioseletivity of glycosylation influences the taste perception. The three GTs (UGT74G1, UGT76G1 and UGT85C2) were identified and cloned from *Stevia* leaves, and their regioselective glycosylation of steviol was confirmed through in vitro analysis of the recombinant enzymes [91]. Sterol glucosides can serve as primers for cellulose synthesis in plants, which shows the importance of SGT's for plants [92]. Sitosterol-β-glucoside was co-purified with cellulose fragments in herbicide-treated cotton fibres. The three important steps in cellulose synthesis are glucan initiation, elongation and termination. The glucan polymerisation is initiated by CesA glycosyltransferases, which use sitosterol-β-glucoside as primer. In addition, the herbicides that inhibit cellulose synthesis (2-6-dichlorobenzonitrile) also pharmacologically inhibit sitosterol-β-glucoside biosynthesis [92].

The purified cytosolic sterol glycosyltransferase that glycosylates C-3β hydroxy group of ginsenosides has been reported from *Panax ginseng* [93]. Two sterol glycosyltransferases specific for 3β-OH and 27β-OH position have been purified and characterised from *Withania somnifera* in our laboratory [32, 33]. A gene coding for 3-β-hydroxy position of sterols has also been cloned and expressed in *E. coli* [34].

Functional Significance of SGTs

Role in Biotic Stress—Bacterial and Fungal Resistance

In comparison to antifungal properties of steroidal glycoalkaloids (saponins), there are only a few reports on antibacterial activity. In potato, the tuber glycoalkaloid content was linked to resistance to bacterial ring rot [94] or soft rot [95]. Downregulation of a pathogen-responsive tobacco UDP-Glc:phenylpropanoid glycosyltransferase reduces scopoletin



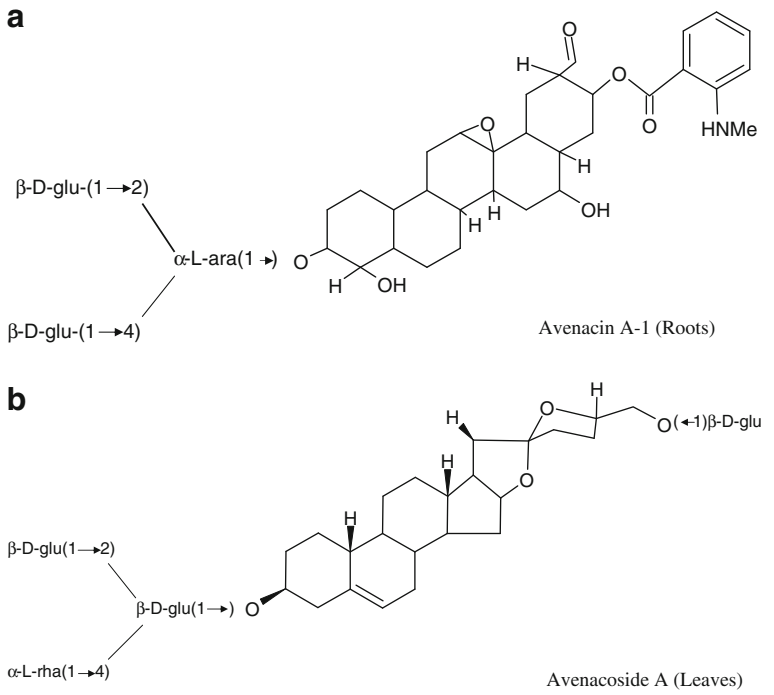
Steviol

Fig. 5 Sweet compound in *Stevia*

glycoside accumulation, enhances oxidative stress and weakens virus resistance [96]. Tomatine was reported to have some antibacterial effects on Gram-positive bacteria that infect humans [97]. Avenacosides A and B are well-characterised steroidal saponins in oat plants that have sugar chains at C-3 and C-26 carbons (Fig. 6a, b). They lack antimicrobial activity but can be converted into their biologically active forms by removing the C-26 sugar. The avenacosides glycosylated at C-3 position are active against the oat root pathogen *Gaeumannomyces gramines* [98].

Avenacosides A-1 from oat roots (a triterpenoid saponin) and the steroidal glycoalkaloids α-tomatine and α-chaconine (from tomato and potato, respectively) contain α-1,2-linked C-rhamnose molecule at the C-3 position and provide resistance against *Gibberella pulicaris* [99]. The glycosyltransferase SAGT4, involved in steroid saponin biosynthesis in *Solanum aculetissimun*, catalyses the 3-o-glycosylation of steroidal sapogenins, such as, diosgenin, nuatigenin and tigogenin [87]. The response of this enzyme to wounding stress indicates the involvement of SAGT4 in plant defense system. This enzyme also glycosylates steroidal alkaloids such as solanidine, solasodine and tomatidine. The potato GT (SAGT1) identified by screening a wound-induced cDNA library in yeast suggested the role of Sgt in stress [86]. The enzyme (SAGT1) glycosylated tomatidine, solasodine and solanidine, the metabolites active in defense mechanism (Fig. 7). UGT73C5 which glycosylates brassinosteroids in *Arabidopsis thaliana* also glycosylates fungal mycotoxin de-oxynivalenol (DON). Hence, it provides protection against the pathogen *Fusarium* by detoxifying DON [100].

The major mechanism of antifungal activity of saponins is apparently due to their ability to complex with sterols in fungal membranes and cause loss of membrane integrity [101], although the precise mechanism is not fully understood. Electron microscopic analysis and



Figs. 6 **a**, **b** Avenacosides from oat plant

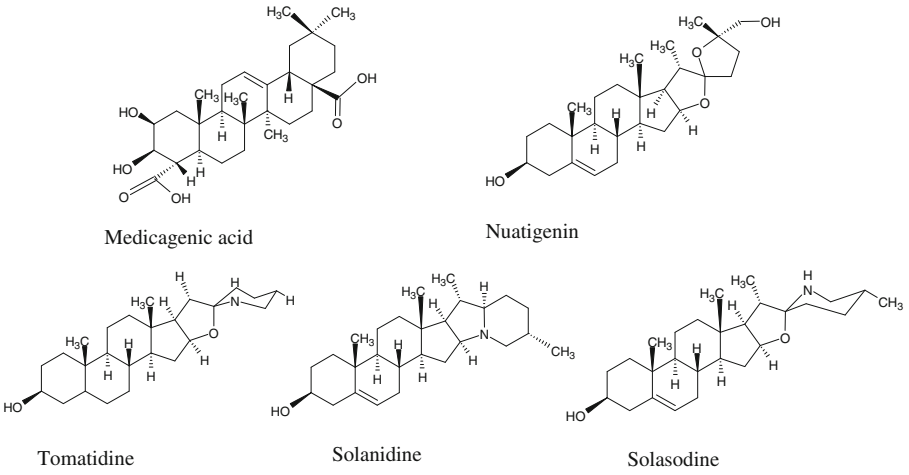


Fig. 7 Metabolites active in defense mechanism

electrical conductivity measurements suggest the formation of transmembrane pores [101, 102]. Aggregation of saponin sterol complexes in the membrane may be mediated by interaction between the sugar residues of the saponin molecules. The sugar chain attatched to C-3 is usually critical for both the membrane permeabilising and antifungal properties of saponins, and removal of these sugar residues often results in loss of biological activity [102–104]. Some pairs of steroidal glycoalkaloids that have a common aglycone but differ in composition of their sugar chains show synergism in their membranolytic and antifungal activity, indicating that some kind of complementation occurs between carbohydrate moieties [105]. This synergism is particularly effective at ratios that are similar to those in which steroidal glycoalkaloids occur naturally [106]. Furthermore, 14 schidigera saponins that have a single sugar chain were identified from stems of *Yucca schidigera*, and the range of their anti-yeast activities depending upon the sugar moiety was determined [107].

Role in Insect Resistance

In Colorado potato, the steroidal glycoalkaloid tomatine is associated with field resistance [108]. Furthermore, in studies using synthetic diets, it was shown that increased concentrations of tomatine caused retarded growth and delayed development of beetles feeding on Colorado potato, from egg stage to adults [109]. It was concluded that the tetra saccharide moiety of tomatine was crucial for insecticidal activity because of its membranolytic action. The type of glycoalkaloid was also an important factor in resistance to potato leaf hopper. When separate glycoalkaloids were studied, tomatine caused the greatest mortality of potato leaf hopper compared with other glycoalkaloids such as solanine, chaconine, leptine I and II, solasonine and solamargine [110]. Solamargine and chaconine caused the second greatest mortality. Flanders et al. [108] also suggested that tomatine was connected with field resistance to potato leaf hopper.

Role in Abiotic Stress—Heat Stress

Sterol glycosides have been considered to have a role in temperature stress [111], since they are important in membrane fluidity and permeability [40, 50, 112], and there is a

phospholipid dependence of UDP-glucose:sterolglycosyltransferase [113]. In comparison to normal sterols, sterol glycosides and acylated sterol glycosides exchange more slowly between the monolayer halves of a bilayer, which could serve to regulate free sterol content and its distribution [39, 50]. This can provide insight into the role of sterol glycosides in the plasma membrane. Plants adapt to environmental heat stress by synthesising heat shock proteins. It is well known that transcriptional induction of heat shock genes is mediated by the heat shock transcription factors (HSFs) in eukaryotes [114]. The HSFs are activated by multimerisation and phosphorylation, bind to the promoters of heat shock genes and stimulate their transcription. The activation of sterol glycosyltransferase and the production of sterol glycoside are important events in the signal transduction system to induce the synthesis of heat shock proteins. Heat stress induces rapid glycosylation of membrane sterols in myxoamoebae of true slime mould *P. polycephalum* [66, 115, 116].

When heat shock was given to human foetal lung fibroblast cells, there was a change in the composition of membrane lipids. There was a heat-induced expression of cholesteryl glycoside, which suggests the involvement of steryl glycoside in heat shock responses in mammalian cells. This phenomenon is also followed by activation of calcium-dependent protein kinase followed by the HSP induction in *P. polycephalum* cells. The activity of UDP-glucose:poriferasterol glycosyltransferase also increased rapidly after heat shock. It was also observed that HSP 70 was induced when cholesteryl glycosides were added to the culture medium of TIG-3 cells without heat stress. Hence, steryl glycoside is indirectly considered to enhance high temperature tolerance and participate in the activation of HSFs. Recently, two enzymes of *SGT* gene family from *W. somnifera* have been characterised [32–34]. Both the enzymes exhibited a rapid in vivo response to high temperature and salicylic acid treatment, suggesting a physiological role in biotic and abiotic stress. Glycosylation of sterols may alter the distribution of 3β-OH sterols in membrane rafts, and these may be involved in the transmission of heat shock signals in cells, by change in membrane functions like fluidity, phase transition, etc. The membrane rafts rich in 3β-OH sterols have been reported in plants [117]. Hence, increase in the level of *SGT*s during heat stress shows their role in perceiving heat stress.

Cold Stress

The adaptation of plants towards cold stress involves complex phenomena such as modulating the fluidity of biomembranes and synthesis and accumulation of low molecular weight and high molecular weight cryoprotectants [118–120]. The plasma membrane is regarded as key site of injury during freeze thaw stress in herbaceous plants [111, 121]. Alterations that contribute to increased freezing tolerance include increased level of fatty acid desaturase in membrane phospholipids and change in the level and type of membrane sterols and cerebrosides [122]. A difference in the proportion of glycosylated versus acylated sterols has been reported [111]. It has been observed that sterol content of the plasma membrane changes in response to environmental conditions and alterations in the sterol compositions of plasma membranes may play a role in cold acclimation process [123]. This is supported by the fact that SGT's, e.g. UGT80B1 transcript has been slightly upregulated in cold stress, as shown by gene expression data form micro-array experiments (genome cluster database). The proportion of phospholipids increase from 46.8% to 57.1 mol% of the total lipids with increase in the proportion of di-unsaturated species of phasphatidylcholine and phasphatidylethanolamine [124]. The proportion of cerebrosides decreases from 7.3% to 4.3 mol% with decrease in the proportion of free sterols from 37.7% to 31.2 mol%. These studies indicate that the level of sterols and their modified

counterparts varies in response to cold stress. Hence, Sgts, which bring about the modification of sterols, play an important role in coping up with heat and cold stress.

Some heat shock proteins such as Hsp70 have been reported to function as molecular chaperons during cold stress [125]. Upregulation of Hsp70s at low temperature may be related to an increased demand for molecular chaperone function at low temperature, and Hsp70s could bind unfolded or non-native cold labile proteins. Furthermore, cholesteryl glycosides have been reported to enhance the expression of heat shock proteins. Hence, an increase in the expression of *SGT*s may be related to the increase in the production of sterol glycosides, which behave as messengers in triggering heat shock proteins, which help the plant in adaptation to heat and cold stress. Treatment with methyl jasmonate and methyl salicylate in tomato and peppers reduced chilling injury, enhanced transcript level of heat shock proteins, PR proteins and alternative oxidase [126]. The same treatment also enhances the expression of *SGT*s, which indicate the involvement of *SGT*s in stress. Expression of a 70-kDa spinach endoplasmic reticulum heat shock protein during cold acclimation has been reported [127]. Coordinated and non-coordinated expression of the stress 70 family and other molecular chaperones have been reported at high and low temperature in spinach and tomato [128]. Increase in the expression of *SGT*s in cold and heat stress and cholesteryl glycosides triggering the expression of heat shock proteins indicate that *SGT*s are playing an important role in both heat and cold stress.

Role of SGTs in Hormonal Regulation

Steroid hormones play a conserved role in regulating development in eukaryotic organisms ranging from fungi and plants to insects, vertebrates and mammals. Sterols function as biosynthetic precursors of steroid hormones, such as glycocorticoids, androgens and estrogens in animals, ecdysteroid in insects, anthridiol and oogoniol in fungi and brassinosteroids in plants [129, 130]. Sgts that glycosylate steroidal hormones play an important role in regulating the activity of steroids in plants, mammals and insects. Brassinosteroids (BRs) are plant-specific polyhydroxylated derivatives of 5α-cholestane structurally similar to cholesterol-derived animal steroid hormones and ecdysteroids from insects [131, 132]. Like their animal counterparts, BRs (Fig. 8) have been shown to regulate gene expression, stimulate cell division and differentiation and modulate reproductive biology [133].

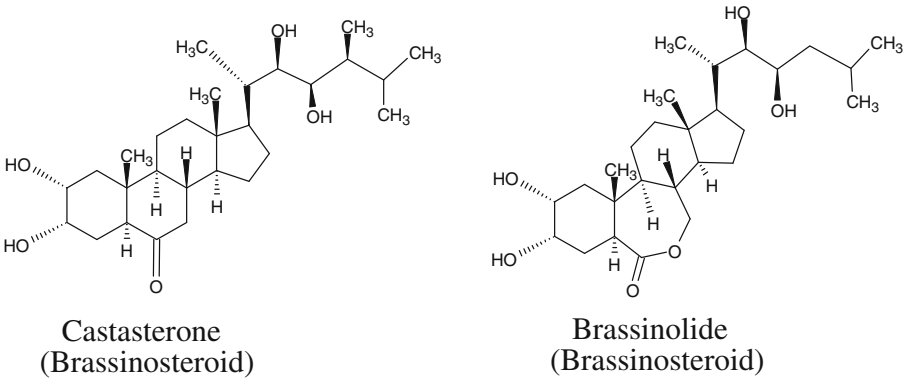


Fig. 8 Structure of brassinosteroids

BRs also mediate growth response unique to the plants, including the promotion of cell elongation in the presence of a complex cell wall and influencing multiple development responses to darkness and light. The phenotypic mutants have been described extensively and include extreme dwarfism, altered leaf morphology, reduced fertility or male sterility, delayed senescence and altered vascular development, implicating BRs in all of these developmental processes [129]. One of the ways by which plants maintain hormonal homeostasis is by conjugating the hormones with fatty acids, glucose or disaccharides [133, 134]. Enzymes involved in glycosylation of plant hormones, such as auxins, cytokinins and abscisic acid, belong to a subset of family 1 UDP-glycosyltransferases (UGTs) defined by the presence of a C-terminal consensus sequence [10]. Proteins of this class have also been shown to accept mammalian and insect steroid hormones as substrates. In mammals, the typical donor sugar is UDP-glucuronic acid, and the conjugation of androgens and estrogens by members of UGT subfamily 2B is thought to regulate hormone activity [135, 136]. The UDP-glycolsyltransferase UGT73C5 of *Arabidopsis thaliana* catalyses 23-O glycosylation of the BRs brassinolide and catasterone [137]. Transgenic plants over-expressing UGT73C5 displayed BR-deficient phenotypes and contained reduced amount of BRs. The phenotype, which was already apparent in the seedlings, could be rescued by application of BR.

Role in Medicinally Important Plants

Panax ginseng is a medicinal plant that contains diverse ginsenosides, which are cardioprotective, antifatigue, antitumor, hepatoprotective and immunomodulatory. The genes involved in the biosynthesis of ginsenosides and other secondary metabolites were estimated by the treatment of hairy roots with methyl jasmonate and analysis of the transcript. Among the genes were glycosyltransferase, oxidosqualene cyclase and cytochrome P_{450} [138]. A glycosyltransferase UDPG-ginsenoside:Rd glycosyltransrerase has been purified from suspended cells of *P. notoginseng* [139]. The effect of sugar positions in ginsenosides on inhibition of Na(+)/K(+) ATPase suggests that the enzymes involved in sterol transformation determine the level of pharmacologically important bioactive metabolites in *P. ginseng* [140]. *W. somnifera*, which is equated to ginseng in its medicinal properties, is a rich source of a variety of glycosylated steroidal lactones called withanosides in roots [141, 142] and leaves [143]. Withanosides are steroidal lactones with one or more glucose units attached to C-3 or C-27 positions. Recently, Ahuja et al. [144] have isolated four glycowithanolides, viz., withanoside IV (WSG-3), withanoside VI (WSG-3A), physagulin D (WSG-P) and withastraronolide (WSC-O), from multiple shoot cultures of *W. somnifera*. A novel sterol glycosyltransferase specific to β-OH position has recently been reported from our group [32]. The enzyme showed broad sterol specificity, glycosylating a variety of sterols and steroidal sapogenins with β-OH group at C-17, C-21 and C-27 positions. Two Sgts glycosylating sterols at C-3-OH have also been reported from our group [33, 34]. Increase in the expression of both *sgt*s on salicylic acid treatment suggests their possible role in stress. In the light of above points, it can be postulated that sterols and their modified counterparts play a crucial role in plant defense mechanism, and they are diversified by sterol modifying enzymes.

This review has emphasised the range of acceptors for Sgts and their regioselectivity. These features provide a basis for application of SGTs in crop plants. These may involve, for example, changed sensitivity to hormones and their agrochemical analogs and changed tolerance to biotic abiotic stresses. Increasing the glycosylation of secondary metabolites,

may lead to increased yield of the corresponding glycosides. It may be possible to increase the flux of a molecule by glycosylating it and removing the product from the reaction mix. In terms of metabolic engineering, the SGTs could prove to have utility in crop improvement.

Conclusion

The principle feature of Sgts is their ability to glycosylate sterol acceptors in a regioselective manner. Sterols are major components of plant cell membranes and exist as sterol glycosides or acylated sterol glycosides. The ratio of sterols and their modified counterparts varies when plant perceives stress conditions like heat and cold. Many secondary metabolites are derived from the sterol pathway, which are important in defense mechanisms against pathogens. Sterol glycosides such as cholesterol glycosides act as important signalling molecules in triggering the expression of heat shock proteins. Sgts that glycosylate steroidal hormones, such as brassinosteroids, play an important role in regulating the level of hormones in planta. Some Sgts that catalyse the glycosylation of endogenous substrates also glycosylate foreign acceptors. Many sterols and steroidal molecules attribute medicinal properties to the plant as in the case of *W. somnifera* and *P. ginseng*. This fascinating group of enzymes represent an area to study various aspects of plant metabolism such as stress, plant defense mechanism and medicinal properties with coming years to provide still more clarity about these enzymes.

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