

Review

Emerging roles for conjugated sterols in plants

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ABSTRACT

In plants, sterols are found in free form (free sterols, FSs) and conjugated as steryl esters (SEs), steryl glycosides (SGs) and acyl steryl glycosides (ASGs). Conjugated sterols are ubiquitously found in plants but their relative contents highly differ among species and their profile may change in response to developmental and environmental cues. SEs play a central role in membrane sterol homeostasis and also represent a storage pool of sterols in particular plant tissues. SGs and ASGs are main components of the plant plasma membrane (PM) that specifically accumulate in lipid rafts, PM microdomains known to mediate many relevant cellular processes. There are increasing evidences supporting the involvement of conjugated sterols in plant stress responses. In spite of this, very little is known about their metabolism. At present, only a limited number of genes encoding enzymes participating in conjugated sterol metabolism have been cloned and characterized in plants. The aim of this review is to update the current knowledge about the tissue and cellular distribution of conjugated sterols in plants and the enzymes involved in their biosynthesis. We also discuss novel aspects on the role of conjugated sterols in plant development and stress responses recently unveiled using forward- and reverse-genetic approaches.

1. Introduction

Sterols are essential components of eukaryotic cell membranes that determine their physicochemical properties and, consequently, their biological function [1]. However, important new roles for sterols have been discovered in recent years. For instance, sterols interact with sphingolipids in the plasma membrane (PM) to form liquid-ordered microdomains (lipid rafts) that are involved in different biological processes [2,3]. Sterols also serve as precursors for the biosynthesis of brassinosteroids, a group of hormones that are essential for the regulation of plant development and morphogenesis [4]. Besides these functions, sterols are also involved in plant growth and development [5–13] as well as in plant responses to biotic and abiotic stresses [14–22].

The common structure of sterols is based on the cyclopentane-perhydrophenanthrene ring system (formed by four rigid rings) with a hydroxyl group at position C3 and a lateral chain of variable length (8–10 carbons) attached to carbon 17 (Fig. 1). In contrast to animals and fungi, plants produce a complex mixture of sterols that mainly differ in the nature of the side chain at position C17 and the number

and position of double bonds in the rings or the lateral chain. β -Sitosterol, stigmasterol and campesterol (Fig. 1) are the most abundant sterols found in plants. Whereas β -sitosterol and stigmasterol have a major role in maintaining the structure and function of cell membranes, campesterol acts as the precursor of brassinosteroids [6,7]. Aspects related with the chemistry and biosynthesis of plant sterols have extensively been reviewed in recent years [23–26].

In plants, sterols are present not only as free sterols (FS) but also conjugated in form of steryl esters (SEs), steryl glycosides (SGs) and acyl steryl glycosides (ASGs) (Fig. 2). In SEs the hydroxyl group at C3 position is esterified with a fatty acid. SGs are characterized by having a sugar linked to the C3 hydroxyl group of the sterol moiety through a β -glycosidic bond. ASGs are derivatives of SGs in which the hydroxyl group of the C6 position of the sugar moiety is esterified with a fatty acid. The structural diversity of SEs, SGs and ASGs has extensively been reviewed [23,27–29]. In a restricted number of plants (mainly cereals) sterols are also found conjugated in form of steryl ferulates [30,31]. Ferulic acid is a hydroxylated derivative of cinnamic acid, a metabolic intermediate in the phenylpropanoid pathway. Because steryl ferulates seem to be present only in a limited number of plant species, studies

Abbreviations: ASG, acyl steryl glycoside; DIM, detergent-insoluble membrane; ER, endoplasmic reticulum; FS, free sterol; LD, lipid droplet; SE, steryl ester; SG, steryl glycoside; PM, plasma membrane

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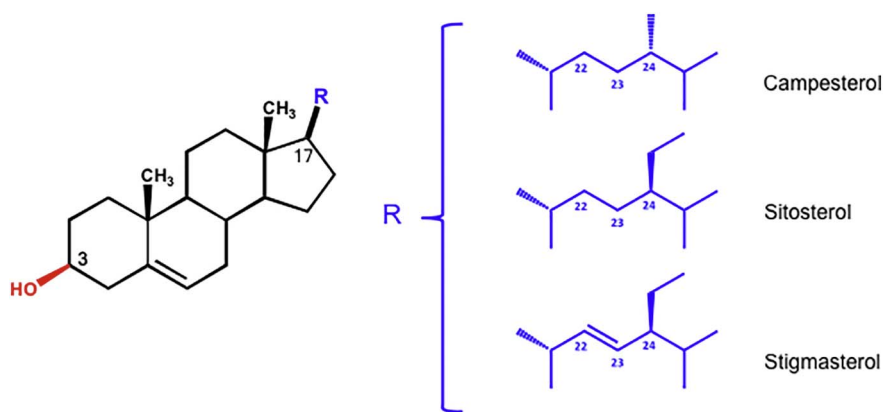


Fig. 1. Chemical structure of the major plant free sterols.

related with this type of conjugated sterols are very scarce.

In contrast to the increasing evidences supporting a relevant role of SEs, SGs and ASGs in a variety of cellular and biological processes in plants very little is known about their metabolism. This review provides an overview of the tissue and cellular distribution of these conjugated sterols and summarizes the knowledge about the genes and the corresponding enzyme activities currently identified in plants for the biosynthesis of SEs and SGs. New information about the function of conjugated sterols derived from functional genomic studies targeting sterol acyltransferases and sterol glycosyltransferases is also discussed.

2. Steryl esters

2.1. Tissue and cellular distribution of plant steryl esters

SEs are found in all plant tissues. However, their relative content usually differ among organs and tissues and may change in response to developmental and environmental factors. The sterol moiety of SEs usually corresponds to the same sterols present in the FS fraction, but in some tissues they may contain unusual sterols as well as sterol biosynthetic intermediates. The fatty acids found in SEs cover a wide range of lengths (from C12 to C22), being palmitic, stearic, oleic, linoleic and linolenic acids the most common species [28]. The high structural diversity of the SEs present in plants highlights the relevance of the sterol esterification process regarding their specialized function in different plant tissues.

SEs are remarkably abundant in particular plant tissues such as the tapetal cells of anthers, pollen grains, seeds, and senescent leaves [28,32,33]. High levels of SEs have also been reported in mutant and transgenic plants overproducing sterols [34,35]. It has recently been reported that SEs are also present at relatively high levels in the phloem sap, being cholesterol the most abundant phytosterol in this fraction [36].

It is widely accepted that SEs perform a primary role in cell membrane sterol homeostasis [6]. However, in some plant tissues SEs also

represent a storage pool of sterols that can be used during growth and development. For instance, seeds store high amounts of SEs that, together with triacylglycerols, are mobilized during germination to support seedling growth during the early stages of development [37]. This is in agreement with the increase of SE content during seed development [38–40], which in developing tobacco and rape seeds occurs in parallel with the synthesis of triacylglycerols [40].

Early cell fractionation studies in different plant tissues indicated that SEs were mainly enriched in mitochondrial, nuclear and microsomal fractions [28]. However, more recent reports have shown that, like in other eukaryotic organisms, plants accumulate SEs in cytoplasmic particles known as lipid droplets (LDs) [41]. LDs (also known in plants as oleosomes, lipid bodies and oil bodies) are evolutionary conserved organelles present in almost all organisms and cell types [42]. Like in yeast and mammals, plant LDs consist of a neutral lipid core surrounded by a membrane monolayer derived from the endoplasmic reticulum (ER) and are stabilized by proteins bound to the LD surface [41]. Evidences available so far support the view that LDs are generated at specific subdomains of the ER in which triacylglycerols and possibly SEs are actively synthesized [41,43]. The induction of LD formation in mutant and transgenic plants overproducing sterols [6] supports the role of SEs in promoting LD biogenesis.

The proteomic analysis of LDs from mammals and yeast has led to the identification of proteins involved in a variety of cellular processes, indicating that these subcellular structures may have multiple functions in addition to their role as storage of lipids [44]. In contrast, only a few LD associated proteins have been identified in plants [45]. The best characterized ones are oleosins, caleosins and steroleosins, which correspond to the most abundant proteins present in LDs isolated from seeds [41]. Despite the widespread occurrence of LDs in plants their associated proteins in vegetative tissues remain largely unknown. Recently, a new class of LD associated proteins (LDAPs) required for the regulation of neutral lipid compartmentation has been identified in non-seed cell types [46,47].

The observation that many LD proteins are related with lipid and

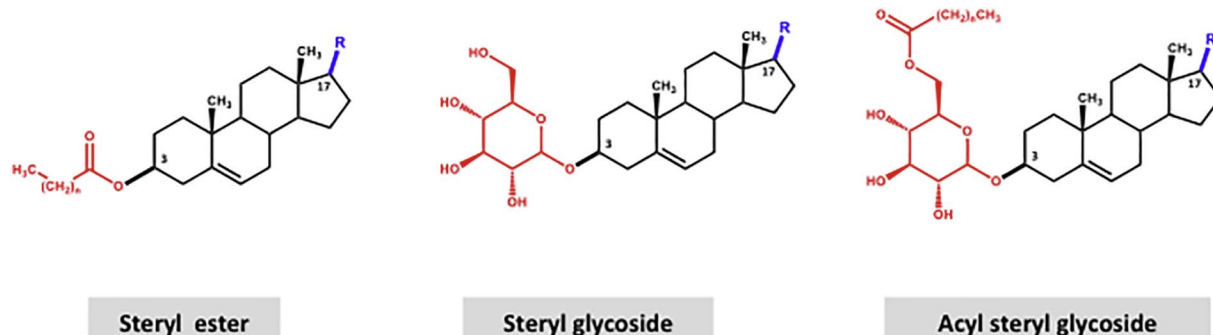


Fig. 2. Chemical structure of conjugated sterols.

sterol metabolism in mammals and yeast has reinforced the view that LDs may also have a relevant function in neutral lipid metabolism and membrane lipid homeostasis in plants [41,48]. In contrast, only two LD associated proteins related with sterol metabolism have been identified in plants: steroleosins, which have hydroxysteroid dehydrogenase activity and are likely involved in brassinosteroid metabolism [49,50] and the sterol biosynthetic enzyme $\Delta 7$ -sterol-C5-desaturase, which is targeted to leaf lipid particles in addition to the ER [51].

SEs have been identified as a main structural component of the pollen coat of some *Brassica* species and are synthesized in the elaioplasts present in the tapetal cells of the anthers [32,33,52]. Nevertheless, a significant amount of SEs is also found in the intracellular domain of the pollen grain [32], suggesting that this SE pool could provide sterols for the synthesis of new membranes or to maintain sterol homeostasis during the active growth of the pollen tube. It has recently been reported that pollen from a variety of plant species show great differences in the composition of the SE fraction [53]. Moreover, the sterol composition of the SE fractions differs from that of the corresponding FS fraction. Thus, whereas $\Delta 5$ -sterols (sterol end products) are predominant in the FS fraction, SEs are usually enriched in $9\beta,19$ -cyclopropyl sterol precursors [53]. The biological significance of the SE diversity found in pollen is currently unknown but has been related with specific plant-insect interactions or the optimization of the contact between the pollen grain and the stigma to promote germination [53,54].

In senescing tissues SEs have been proposed to participate in the cellular recycling and transport of sterols when FSs are released from the degenerating cell membranes [28,55]. In agreement with this tobacco leaves show a clear increase in SE levels which parallels a decrease in the FSs and ASGs levels during senescence [56]. The role of SEs in leaf senescence is also supported by the characterization of Arabidopsis mutant plants defective in SE biosynthesis [57].

2.2. Biosynthesis of steryl esters

Sterol acyltransferase activity (Fig. 3) has been detected in a variety of plant tissues and early studies suggested that the plant enzyme(s) were using acyl donors different from those previously described in mammals and yeast. In mammals, cellular cholesterol is esterified by the action of acyl CoA cholesterol acyltransferase (ACAT), that uses acyl-CoA as fatty acid donor [58], but blood cholesterol is esterified by a lecithin cholesterol acyltransferase (LCAT), which uses lecithin as acyl donor [59]. In *S. cerevisiae*, ergosterol is esterified by two isoforms of ACAT, ARE1 and ARE2 [60]. ACAT and LCAT are completely different proteins and their amino acid sequences have no significant similarity.

Early studies in plants showed that phosphatidylcholine and phosphatidylethanolamine were efficiently used as acyl donors in enzyme assays using microsomal preparations obtained from spinach leaves [61]. However, it was subsequently found that these phospholipids were rapidly hydrolyzed under the assay conditions and that 1,2-diacylglycerol was the true acyl donor in the reaction [62]. Sterol acyltransferase activity was also measured in tobacco leaf microsomal fractions using dipalmitoylglycerol as acyl donor [63]. Enzyme assays using cell-free extracts prepared from white mustard (*Sinapis alba*) roots showed that triacylglycerols were used as a source of fatty acids for sterol esterification [64,65]. Sterol acyltransferase activity using both triacylglycerols and diacylglycerols as acyl donors was also measured in *Zea mays* root extracts [66]. An alternative mechanism for the synthesis of SEs has been described in cell-free extracts of white mustard roots where a reversible trans-esterification process involving wax esters and FSs has been described in addition to the reported synthesis of SEs using triacylglycerols as acyl donors [67] (Fig. 4).

In contrast with all these biochemical data, the sterol acyltransferases currently characterized in plants after the cloning of the corresponding genes in Arabidopsis are a phospholipid:sterol acyltransferase (PSAT) and an acyl CoA:sterol acyltransferase (ASAT)

(Fig. 4).

In Arabidopsis PSAT is encoded by the gene *PSAT1* (*At1g04010*) and was first characterized using microsomal fractions obtained from leaves of transgenic Arabidopsis plants constitutively overexpressing the *PSAT1* transcript [68]. The highest level of PSAT activity was found when phosphatidylethanolamine was used as acyl donor. The enzyme showed high preference for *sn*-2 fatty acids (both saturated and unsaturated) and was able to acylate several sterols and sterol intermediates. The observation that PSAT1 did not use neutral lipids as acyl donor suggested that the polar head of phospholipids is a relevant factor in substrate recognition [68]. Although sterol intermediates were poor substrates for PSAT1, they were preferentially used when sterol end products (mainly sitosterol) were present in the enzyme reaction. This observation led to suggest the allosteric regulation of PSAT activity by sterol end products. Thus PSAT activity could modulate the level of FSs in the membrane through a mechanism involving the sequestration of biosynthetic intermediates in form of SEs. This is in agreement with the high enrichment of sterol precursors (mainly cycloartenol) in the SE fraction of mutant and transgenic plants overaccumulating sterols [34,35]. PSAT1 fused to GFP localizes in spherical structures of unknown identity that do not seem to correspond to LDs [69].

The Arabidopsis gene encoding ASAT (*At3g51970*) was cloned by Chen et al. [70] and the corresponding enzyme was characterized by expression of the *ASAT1* transcript in a *S. cerevisiae* mutant strain in which the two endogenous genes encoding ASAT (*ARE1* and *ARE2*) were disrupted. The characterization of the enzyme activity in cell extracts of the transformed yeast cells indicated that cycloartenol was the preferred acyl acceptor. Seeds from transgenic Arabidopsis overexpressing ASAT under the control of a seed-specific promoter showed an increase in total sterol content associated to an increase in the SE levels and a small reduction of FSs. Cycloartenol esters were highly increased at expenses of a significant decrease of campesterol and sitosterol esters. The SE fraction extracted from the transgenic seeds was enriched in saturated and long-chain fatty acids [70].

2.3. Genetic analysis of steryl ester function

The biological significance of the occurrence of two alternative enzymatic systems for the synthesis of SEs in plants has been investigated by Bouvier-Navé et al. [57] using Arabidopsis T-DNA insertion mutants defective in the expression of *PSAT1* and *ASAT1* genes. It was found that SE levels were strongly reduced (5- to 10-fold) in seeds of the *psat1-1* and *psat1-2* mutants but not in the *asat1-1* mutant, thus suggesting a major role of PSAT1 in the synthesis of SEs in seeds. However, *psat1-1*, *psat1-2* and *asat1-1* mutant showed a decrease in the level of SEs in leaves. In contrast, the SE levels in the flowers of all mutant lines were similar to those of wild type plants. These results point towards the existence of at least another sterol acyltransferase that remains to be identified in Arabidopsis. One possibility could be an enzyme phylogenetically related with PSAT or ASAT. The *PSAT1* gene is a member of the Arabidopsis PDAT/LCAT-like gene family composed by six genes (*At1g04010*, *At5g13640*, *At3g03310*, *At4g19860*, *At3g44830* and *At1g27480*) encoding proteins with similarity to human LCAT and lysosomal phospholipase A2 [71]. The genes *At5g13640*, *At3g03310* and *At4g19860* encode a phospholipid:diacylglycerol acyltransferase (PDAT) [71], a phospholipase A1 [72] and a phospholipase A2 [73], respectively. The protein encoded by *At3g44830* shows high similarity with PDAT and therefore it is unlikely to encode an enzyme with sterol acyltransferase activity. The remaining member of the PDAT/LCAT-like gene family corresponds to *At1g27480*, which encodes a protein showing high similarity to human LCAT. Surprisingly, no function for this protein could be determined after its expression in yeast [72].

Arabidopsis ASAT1 belongs to the superfamily of membrane-bound O-acyltransferases (MBOATs) [74]. When the fifteen members of the Arabidopsis MBOAT gene family were tested for complementation of a

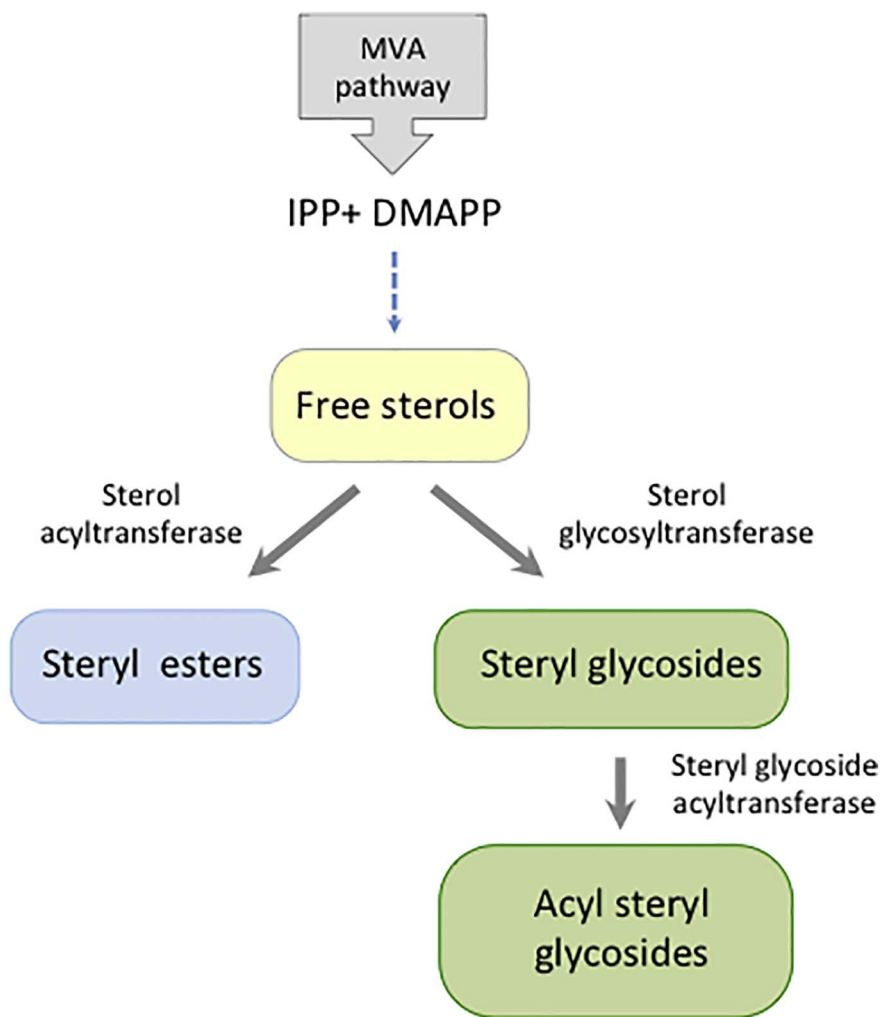


Fig. 3. Schematic representation of the biosynthesis of conjugated sterols showing the enzymatic activities involved. Free sterols derive from isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) produced by the mevalonic acid (MVA) pathway [26]. The dashed arrow indicates multiple steps.

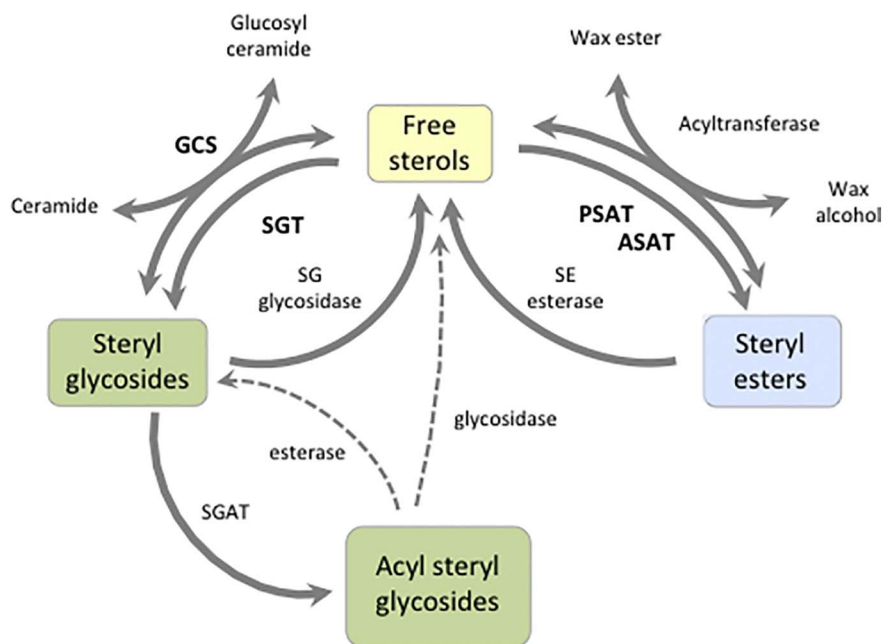


Fig. 4. Schematic overview of conjugated sterol metabolism in plants. Those enzymes for which the corresponding genes have been cloned are indicated in bold: ASAT, acyl-CoA:sterol acyltransferase; PSAT, phospholipid:sterol acyltransferase; SGT, UDP-glucose:sterol glycosyltransferase; SGAT, steryl glycoside acyltransferase; GCS, glucosylceramide synthase. Dashed arrows indicate enzymatic conversions not confirmed at experimental level.

yeast strain defective in SE biosynthesis only the expression of the protein encoded by *At3g51970* (ASAT1) was able to restore SE biosynthesis [70]. These results indicate that ASAT1 is the only member of the Arabidopsis MBOAT gene family having ASAT activity.

As mentioned above, several reports have shown sterol acyltransferases activity in plants using triacylglycerols and diacylglycerols as acyl donors. However, neither ASAT1 nor PSAT1 can efficiently use these compounds as fatty acid donors [68,70]. Therefore it is likely that plants could contain other genes encoding at least one sterol acyltransferase different from PSAT and ASAT.

The involvement of PSAT1 and ASAT1 in sterol homeostasis has been evaluated in the Arabidopsis *psat1-1*, *psat1-2* and *asat1-1* mutant lines. Feeding experiments using mevalonolactone and squalene (metabolic precursors known to enhance the metabolic flux towards sterols) revealed that *psat1-1* and *psat1-2* plants were highly sensitive to these compounds, whereas only minor effects were observed in the *asat1-1* plants [57]. This was explained by a limitation of the PSAT1 defective lines to transform the overproduced sterols into SEs. These results are in agreement with a major contribution of PSAT1 in maintaining FS homeostasis in plant cell membranes [57]. Furthermore, the observation that PSAT1 is activated by sterol end products [68] has led to suggest that this enzyme could also act as a cellular sensor for FS levels in the membrane [57].

The only visible phenotype associated with reduced levels of SEs was observed in the *PSAT1* defective mutants, which showed early leaf senescence [57]. Whereas SE levels increased about three-fold in detached leaves of wild type plants only minor changes were observed in the leaves of *psat1-1* and *psat1-2* plants. This is in accordance with the increase of PSAT1 transcript levels detected in the detached leaves of wild type plants. However, no changes were observed in the level of the *HMGR1* and *ASAT1* transcripts. The lack of induction of the *HMGR1* gene (which encodes HMG-CoA reductase, a key regulatory enzyme in sterol biosynthesis [75]) during the aging period suggests that the increase of SE levels in the senescent leaves derives from the endogenous recycling of membrane sterols.

Taken together, current data derived from the characterization of sterol acyltransferase defective mutants in Arabidopsis highlight a major involvement of PSAT1 in the synthesis of SEs and FS homeostasis and raise the question about the real contribution of ASAT1 in SE metabolism.

3. Glycosylated sterols

3.1. Tissue and cellular distribution of steryl glycosides and acyl steryl glycosides

As described above for SEs, glycosylated sterols (SGs and ASGs) are also widely distributed in plants and their relative levels differ among tissues and may change in response to developmental and environmental cues. SGs and ASGs usually have a sterol composition similar to that of the corresponding FS fraction [27]. Glucose is the predominant monosaccharide found in plant glycosylated sterols, but conjugation with other monosaccharides such as galactose, xylose and mannose have also been described [27,29,76]. Unusual SGs containing up to four sugar residues have been reported [77]. ASGs usually contain saturated and unsaturated C16 and C18 fatty acids, but some plants accumulate ASGs with atypical fatty acid chains [27].

SGs and ASGs are usually minor components of the total sterol fraction present in most plant species [27,78]. However, a remarkable exception is provided by plants of the genus *Solanum*, which show a very high content of glycosylated sterols [78,79]. Thus, whereas in most plant tissues the amount of SGs and ASGs is relatively low (usually less than 20% of total sterols), the SG + ASG fraction of tomato fruit may account for more than 85% of total sterols [80]. Although the evolutionary and biological significance of the high content of glycosylated sterols in a restricted number of plant species is not known with

certainty, such a high content of glycosylated sterols in *Solanum* species has been suggested to play a role in protecting cell membrane integrity against the disruptive effect of the high levels of steroidal glycoalkaloids present in these plant species [81–83].

Along with phospholipids and sphingolipids, sterols represent a main component of the lipids present in the PM (20–50% of total lipids). In contrast to mammals and yeasts, the plant PM contains SGs and ASGs in addition to FSs. The analysis of the sterol composition of PM fractions from different plant tissues and growth conditions has revealed striking differences in the relative amount of free and glycosylated sterols [84]. Thus, while FSs represent the most abundant sterol in the PM of most plant species (with values higher than 90% in some cases), in oat and some *Solanum* species, the major sterols found in the PM correspond to ASGs (up to 78% in potato leaves) [84].

It is known that the presence of a sugar moiety attached to the sterol backbone alters its physicochemical properties. Because of this, the presence of SGs and ASGs in plant cell membranes raises the question about how are they organized in the lipid bilayer and the way in which they interact with other membrane lipids to modulate membrane function. SGs are likely oriented with the sterol moiety buried in the hydrophobic core of the lipid bilayer and the sugar located in the plane of the polar head groups of the membrane. In the case of ASGs, both the sterol moiety and the fatty acid chain are probably embedded in the hydrophobic phase of the membrane with the sugar oriented to the hydrophilic surface [84].

The classical model of PM organization suggests that sphingolipids associate among them through interactions between the polar heads and their long and saturated acyl chains, whereas sterols fill voids between the fatty acid chains of phospholipids and sphingolipids. Studies on the effect of plant sterols in modulating membrane properties have shown that β -sitosterol and stigmasterol, which only differ by a double bond at position C22 in stigmasterol (Fig. 1), behave in a very different way [85]. Thus it is likely that changes in the level and relative ratios of FSs, together with the presence of glycosylated sterols, may have a strong influence on the physicochemical properties and function of the cell membranes. Recent studies have shown that glycosylated sterols, alone and in synergy with FSs, have a major role in modulating plant PM organization [86]. Taken together, these observations suggest that specific changes in the relative level and profile of free and glycosylated sterols in the PM may be relevant in the adaptive responses of plants to environmental changes.

Over the last decades the classical view of cell membrane organization, in which the inserted proteins and lipids could diffuse freely (fluid mosaic model), has changed to a model based on a dynamic segregation of membrane components (lipids and proteins) to form microdomains which exhibit a composition, structure and biological function different from the surrounding membrane [2,87]. These membrane microdomains (also known as membrane or lipid rafts) have been involved in a variety of biological processes such as cell-to-cell interactions, membrane transport, protein trafficking, signal transduction, stress responses and polarized growth [2,3].

Lipid rafts are mainly composed by sphingolipids, sterols and proteins and their formation relies on the physicochemical properties of sphingolipids and sterols that allow the formation of liquid-ordered domains that promote the clustering of specific proteins at defined regions of the cell membrane [3]. PM microdomains have been characterized at biochemical level because of their insolubility in non-ionic detergents at cold temperatures. This feature allows the isolation of membrane fractions called detergent-insoluble membranes (DIMs) or detergent-resistant membranes (DRMs). Plant DIMs are enriched in sterols and sphingolipids. The sterol composition of PM and DIM samples isolated from different tissues of a variety of plant species has revealed a general enrichment of free and glycosylated sterols [88,89], thus highlighting the contribution of SGs and ASGs in the organization of plant PM microdomains.

In addition to lipids, DIMs also contain proteins. The proteomic

analysis of plant DIMs has shown that their protein profiles are different from that of the whole PM. The DIM associated proteins identified so far suggest their involvement in a variety of cellular processes [3,87,90,91]. It has also been reported that the DIM proteome of some plants dynamically respond to external stimuli [90]. Thus, it is likely that the enrichment of free and glycosylated sterols in DIMs represents an important factor in the recruitment of specific proteins into particular membrane microdomains during plant development and in response to environmental challenges.

Like in animal cells, the lipids of the plant PM also show an asymmetrical distribution between the cytoplasmic and the apoplasmic leaflets [84]. The asymmetric distribution of lipids in the membrane bilayer affects the biophysical properties of cell membranes and influence numerous cell functions [84]. Free and glycosylated sterols are among the membrane lipids having an asymmetrical distribution in the plant PM [92,93]. Labeling studies using filipin (a fluorophore that specifically reacts with sterols) has revealed an enrichment of sterols (about 3-fold) in the apoplasmic leaflet of the PM [92]. However, the relative distribution of FSs, SGs and ASGs between the cytoplasmic and the apoplasmic leaflets has not yet been established. The mechanisms underlying the asymmetrical distribution of free and glycosylated sterols in the PM is not clear but could be associated to the interconnection of SG and glucosylceramide metabolism in the membrane [94] (Fig. 4).

In addition to their relevance in cell membranes, glycosylated sterols have been described in other plant tissues and cell compartments playing different roles. Glycosylated sterols are present in the pollen grain of Arabidopsis and have shown to be critical for pollen fitness by supporting pollen coat maturation [95].

Glycosylated sterols also represent the major sterol fraction found in the phloem sap, being cholesterol the dominant sterol form [36]. The biological significance of the sterols present in the phloem sap remains to be elucidated but may be relevant in physiological and ecological aspects related with phloem-feeding insects.

SGs have since long been proposed to act as primers for the synthesis of cellulose [96]. However the participation of SGs in cellulose biosynthesis is still a controversial matter [97].

3.2. Biosynthesis of glycosylated sterols

The synthesis of SGs is catalyzed by sterol glycosyltransferases (Fig. 3). In this reaction a sugar, mostly glucose, is transferred to the C3-hydroxyl group of the sterol backbone [76,98]. Sterol glycosyltransferase activity has been determined in different plant species and found to be associated to cell membranes [99–103]. However, cytosolic sterol glycosyltransferases have been identified in *Withania somnifera* [104] and cotton [105]. Biochemical studies using partially purified enzyme preparations revealed that sterol glycosyltransferase activity was enhanced by negatively charged phospholipids [100,103,106]. Although the mechanism underlying this activation was not elucidated this observation suggests that sterol glycosyltransferase activity could be modulated *in vivo* by changes in the lipid environment of the cell membrane. It has been proposed that plants can also synthesize SGs by the action of the enzyme UDP-glucose-dependent glucosylceramide synthase [107] (Fig. 4).

The first plant sterol glycosyltransferase cDNAs were cloned in oat and Arabidopsis by Warnecke et al. [108]. In recent years, other sterol glycosyltransferase cDNAs have been cloned in *Withania somnifera* [109,110], *Gymnema sylvestris* [111] and cotton [105]. Functional studies using the recombinant enzymes expressed in *E. coli* demonstrated that a general feature of the plant sterol glycosyltransferases characterized so far is the highest preference for UDP-glucose as a sugar donor and the capacity to use a wide spectrum of sterols as sugar acceptors [76,105,108–111].

Two genes encoding sterol glycosyltransferases (*At3g07020* and *At1g43620*) have been identified in Arabidopsis. They encode sterol 3- β -glucosyltransferases UGT80A2 and UGT80B1 respectively ([http://](http://www.p450.kvl.dk/At_ugts/family.shtml)

www.p450.kvl.dk/At_ugts/family.shtml) and have been designated as UGT80A2 and UGT80B1 [112]. A recent report has shown that the related protein encoded by the gene *UGT713B* (*At5g24750*) does not have sterol glycosyltransferase activity [113]. UGT80A2 and UGT80B1 are differentially expressed in distinct plant organs and tissues [112] and their transcript levels are coordinately down-regulated short after seed imbibition [113].

Concerning ASG biosynthesis, steryl glycoside acyltransferase activity (Fig. 3) has been measured in cell free extracts from a variety of plant tissues [114–122]. In addition, partially purified enzyme preparations were obtained and characterized from carrot roots [123] and eggplant leaves [124]. These studies unveiled a complex scenario concerning the properties of the enzymes involved in ASG biosynthesis as well as the nature of the acyl-donors used by these enzymes. At present, no genes encoding steryl glycoside acyltransferases have yet been identified in any organism producing ASG, including animals, algae, fungi and bacteria. Therefore, the understanding of plant ASG biosynthesis has to wait until the identification and cloning of the genes encoding the corresponding enzymes.

3.3. Genetic analysis of steryl glycoside function

The characterization of Arabidopsis T-DNA insertion mutants defective in the UGT80A2 and UGT80B1 genes has provided interesting clues about the role of steryl glycosides in plants. Arabidopsis mutants *ugt80A2*, *ugt80B1* and the *ugt80A2,B1* double mutant are viable and fertile [112,113]. However, the *ugt80B1* mutant displays an array of phenotypes that are particularly pronounced in the embryo and seed [112]. The most remarkable feature of UGT80B1 defective plants is the transparent testa phenotype and the reduction of seed size. Actually, the mutation *ugt80B1* was shown to be allelic to *transparent testa 15* (*tt15*) [112]. In the *ugt80A2,B1* double mutant background, the UGT80B1 deficiency is also associated to other seed related defects such as small embryo size, defects in flavonoid deposition and loss of suberization [112]. In contrast, UGT80A2 defective plants show a small reduction of seed size but not the transparent testa phenotype. At the seedling stage, *ugt80A2* and *ugt80B1* mutants show a reduced elongation of the root, a phenotype that is clearly enhanced in the *ugt80A2,B1* double mutant [112]. The *ugt80A2,B1* double mutant is also affected in pollen coat maturation [95]. Neither the single *ugt80A2* and *ugt80B1* mutants nor the *ugt80A2,B1* double mutant show differences with respect to wild type plants in cold acclimation experiments [112].

The sterol analysis of UGT80A2 and UGT80B1 defective plants has provided valuable information about the biological significance of the functional redundancy of plant sterol glycosyltransferases. Leaf, stem and inflorescence/silique samples show a strong reduction of the SG + ASG contents in all the defective lines, although the largest effect is found in the *ugt80A2,B1* double mutant [112]. Unexpectedly, the FS + SE content was significantly increased in the inflorescence/silique samples but not in leaves and stems, thus suggesting the operation of a regulatory network in particular tissues affecting the homeostasis of free and conjugated sterols. In all cases, sitosterol and campesterol were the most abundant sterols found in the SG + ASG fraction [112]. Altogether, these results indicate that UGT80A2 and UGT80B1 have a similar contribution to the production of glycosylated sterols in vegetative and reproductive tissues. However, a more complex scenario was revealed when sterol profiling was focused on seeds [113]. It was found that the total SG content was strongly reduced in the seeds of *ugt80A2* and *ugt80A2,B1* plants but not in *ugt80B1* plants, thus suggesting a major role of UGT80A2 in the synthesis of SGs in seeds. Surprisingly, ASG levels were not significantly affected in the seeds of *ugt80A2* and *ugt80B1* plants but were decreased in the *ugt80A2,B1* double mutant. These results unveil an unexpected combined role of UGT80A2 and UGT80B1 in the biosynthesis of ASGs, at least in seeds.

A detailed analysis of the sterol composition of SG and ASG fractions of seeds from *ugt80A2* and *ugt80B1* mutants, together with the

biochemical characterization of the recombinant UGT80A2 and UGT80B1 proteins, uncovered differences in the substrate specificity of these enzymes towards the major plant sterols [113]. Thus, whereas UGT80A2 activity seems to account for most of the β -sitosterol and stigmasteryl glycoside production in seeds, UGT80B1 preferentially forms brassicasterol esters. These differences in substrate specificity have been related with the fact that UGT80A2 represents the more ancient UGT80 enzyme in plants, whereas UGT80B1 may have arisen during the evolution of vascular plants to play a more specialized role in the synthesis of SGs [113].

It is surprising that the phenotypes observed in the seeds of the *ugt80B1* mutant occur in the absence of significant changes in SG and ASG levels [113]. Thus it is likely that the seed phenotypes associated to UGT80B1 deficiency may derive from minor changes affecting the composition of SG and/or ASG rather than the overall ratios of SG and/or ASG in the total sterol fraction. Since SG and ASG are components of lipid rafts it is likely that alterations in the sterol profile of the PM could modify its function in specific cell types. Alternatively, UGT80B1 could be implicated in the synthesis of an as yet unidentified sterol glycoside relevant for seed development.

It is worth noting that the *ugt80A2, B1* double mutant has residual levels of SG and ASG [112,113,125]. This suggests the presence of additional enzymes involved in the synthesis of glycosylated sterols in Arabidopsis. Such enzyme activity could be provided, at least in part, by glucosylceramide synthase, which has been shown to glycosylate sterols *in vitro* [107]. The characterization of Arabidopsis mutants defective in *At2g19880*, the only gene encoding glucosylceramide synthase in Arabidopsis, has revealed an essential role for this enzyme in cell-type differentiation and organogenesis [126]. The fact that *At2g19880* null mutants show seedling lethality makes difficult to evaluate the hypothetical contribution of glucosylceramide synthase in the synthesis of sterol glycosides *in vivo*. The existence of other enzymes having sterol glycosyltransferase activity cannot be ruled out.

4. Conjugated sterols and plant stress responses

As indicated in Section 3.1, changes in the relative composition of sterols in cell membranes affect their biophysical properties and hence their biological functions. Because of this, it has been assumed that sterols play a prominent role in plant stress responses. Concerning conjugated sterols, there are many reports correlating changes in their profile with specific responses to different types of stress [69,105,127–141]. However, the biological basis underlying the role of conjugated sterols in plant stress is just starting to be uncovered.

The biochemical characterization of the Arabidopsis chilling-sensitive mutant *chs1* correlated this phenotype to an increase in SE levels [129]. However, it could not be established whether the increase in SE levels was a direct consequence of the *chs1* mutation or a secondary response associated to chilling injury. It has recently been reported that the *chs1* mutant is affected in the CHS1 gene (*At1g17610*), which encodes a TIR-NB type protein that induces different defense responses under chilling stress [142]. It has also recently been described that impaired SE synthesis in Arabidopsis is associated with the resistance to *Phytophthora infestans* [69]. However, the fact that the decrease in the SE levels, derived from a loss of function of PSAT1, is associated to unexpected overall changes in glycosylated sterol levels (increase of SGs and decrease of ASGs) does not allow to conclude whether the resistance phenotype is due to a decrease in SE levels or to changes in the glycosylated sterol levels.

With regard to glycosylated sterols, different reports have shown the induction of genes encoding sterol glycosyltransferases in *W. somnifera* and cotton in response to high and low temperatures [104,105,110]. The differential expression of sterol glycosyltransferase isoforms in *W. somnifera* suggested their specific functional recruitment in response to particular environmental challenges [110]. Furthermore, the rapid increase in the transcript level of some members of the sterol

glycosyltransferase gene family in response to MeJA or SA treatments has led to propose their role in both biotic and abiotic stresses [110].

The role of SGs in stress responses has been further established using forward- and reverse-genetic approaches in different plant species. In Arabidopsis it has been reported that a T-DNA insertion mutant defective in the UGT80B1 gene shows increased sensitivity to cold and heat stress [143]. Furthermore, transgenic Arabidopsis plants overexpressing WsSGTL1, a sterol glycosyltransferase from *W. somnifera* [109], show increased salt, heat and cold tolerance [144]. Salt stress tolerance was also observed in transgenic tobacco plants overexpressing WsSGTL1 [145], while the overexpression of this gene in *W. somnifera* led to enhanced tolerance to cold [146]. In both cases, the plants overexpressing WsSGTL1 also presented enhanced resistance against the insect *Spodoptera litura* [146]. It has also been reported that down-regulation of sterol glycosyltransferase in *W. somnifera* results in increased susceptibility to the fungal pathogen *Alternaria alternata* [147] and heat stress [148]. However, it is possible that the changes in biotic stress tolerance reported in *W. somnifera* could be due not only to changes in SG and ASG levels but rather to changes in the level of whitanosides, a type of glycosylated sterol-related secondary metabolite present in this plant species [146,147].

5. Hydrolysis of conjugated sterols

Sterol homeostasis in eukaryotic cells relies on the availability of enzymatic systems catalyzing the interconversion of free and conjugated sterols. This is well documented in *S. cerevisiae* where sterol homeostasis is achieved through the operation of a complex regulatory network involving the sterol acyltransferases ARE1 and ARE2 acting in concert with the sterol ester hydrolases Yeh1p, Yeh2p and Tgl1p [149]. At present, little is known about SE hydrolases in plants. SE hydrolase (esterase) activity has been measured in microsomal fractions of tobacco plants [63] and the enzyme has been partially purified from roots of *S. alba* [150]. However, no genes encoding SE esterases have yet been identified in plants. Searches of plant genome databases using yeast SE hydrolases Yeh1p, Yeh2p and Tgl1p as a bait retrieve proteins with significant similarity to the yeast enzymes, thus suggesting the existence of genes encoding similar enzymes in plants (Fig. 4).

Glycosidase activities able to hydrolyze SGs have been reported in plants (Fig. 4). A membrane bound sterol β -glycoside hydrolase was purified from *S. alba* seedlings by Kalinowska and Wojciechowski [151]. This enzyme activity was later shown to be enhanced by zwitterionic phospholipids (such as phosphatidyl choline and phosphatidyl ethanolamine), suggesting that sterol β -D-glycoside hydrolase activity may be influenced by the membrane lipid environment [152]. Genes encoding sterol β -glucoside hydrolases have recently been identified in the yeasts *Cryptococcus neoformans* and *Saccharomyces cerevisiae* [153,154]. However, in contrast to SE hydrolases, no orthologs of the *S. cerevisiae* EGH1 gene (encoding ergosterol- β -glucosidase Egh1) seem to be present in plant genomes.

To the best of our knowledge no hydrolase activities (either esterases or glycosidases) acting on ASGs have yet been reported in plants (Fig. 4). However, it has been shown that mammalian digestive enzymes such as cholesteryl ester hydrolase (also known as lysosomal acid lipase) and pancreatin (a mixture of pancreatic enzymes) are able to hydrolyze the fatty acyl moiety of ASGs [155]. No hydrolase activity acting on SGs was detected in these enzyme preparations. Interestingly, the gene encoding mammalian cholesteryl ester hydrolase (LIPA) is conserved in many eukaryotic organisms, including plants. Thus, it is likely that the plant orthologs of the mammalian LIPA gene could encode enzymes involved in ASGs deacylation. The SGs formed from the deacylation of ASGs could eventually be converted to FSs by the action of SG glycosidases (Fig. 4).

6. Conclusions and future directions

Conjugated sterols are ubiquitously present in plants and are known to be involved in a variety of biological processes. However, the biochemical, molecular and cellular bases underlying the role of conjugated sterols in plants have just started to emerge in recent years. The cloning of genes encoding sterol acyltransferases and sterol glycosyltransferases in *Arabidopsis* has been a major achievement to identify the nature of the enzymes involved in the biosynthesis of SEs and SGs and allow their biochemical characterization. Furthermore, forward- and reverse-genetic approaches targeting these genes in different plant species has also provided new insights into the role of conjugated sterols. However, current data support the existence of additional enzymes involved in the biosynthesis of SEs and SGs that remain to be identified. In addition, no genes encoding enzymes participating in ASG biosynthesis or implicated in the hydrolysis of conjugated sterols in plants have yet been described. Therefore, the identification of the full set of genes/enzymes participating in conjugated sterol metabolism in model plant species represents a major milestone to undertake functional genomics approaches to fully elucidate the role of conjugated sterols in plants. Another challenging aspect is to define the subcellular compartments involved in the metabolism of conjugated sterols and the mechanisms involved in their intracellular trafficking during plant growth and development and in response to environmental challenges.

Conflict of interest statement

The authors declare that they have no conflicts of interest with the contents of this review.

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References

- [1] M.-A. Hartmann, Plant sterols and the membrane environment, *Trends Plant Sci* 3 (1998) 170–175.
- [2] J. Malinsky, M. Opekarová, G. Grossmann, W. Tanner, Membrane microdomains, rafts, and detergent-resistant membranes in plants and fungi, *Annu Rev Plant Biol* 64 (2013) 501–529, <http://dx.doi.org/10.1146/annurev-arplant-050312-120103>.
- [3] W. Tapken, A.S. Murphy, Membrane nanodomains in plants: capturing form, function, and movement, *J Exp Bot* 66 (2015) 1573–1586, <http://dx.doi.org/10.1093/jxb/erv054>.
- [4] C. Vriet, K. Lemmens, K. Vandepoel, C. Reuzeau, E. Russinova, Evolutionary trails of plant steroid genes, *Trends Plant Sci* 20 (2015) 301–308, <http://dx.doi.org/10.1016/j.tplants.2015.03.006>.
- [5] H. Schaller, The role of sterols in plant growth and development, *Prog Lipid Res* 42 (2003) 163–175 [<http://www.ncbi.nlm.nih.gov/pubmed/12689617> (accessed March 14, 2017)].
- [6] H. Schaller, New aspects of sterol biosynthesis in growth and development of higher plants, *Plant Physiol Biochem* 42 (2004) 465–476, <http://dx.doi.org/10.1016/j.plaphy.2004.05.012>.
- [7] Y. Boutté, M. Grebe, Cellular processes relying on sterol function in plants, *Curr Opin Plant Biol* 12 (2009) 705–713, <http://dx.doi.org/10.1016/j.cpb.2009.09.013>.
- [8] H.B. Kim, *Arabidopsis* cyp51 mutant shows postembryonic seedling lethality associated with lack of membrane integrity, *Plant Physiol* 138 (2005) 2033–2047, <http://dx.doi.org/10.1104/pp.105.061598>.
- [9] S. Men, Y. Boutté, Y. Ikeda, X. Li, K. Palme, Y.-D. Stierhof, et al., Sterol-dependent endocytosis mediates post-cytokinetic acquisition of PIN2 auxin efflux carrier polarity, *Nat Cell Biol* 10 (2008) 237–244, <http://dx.doi.org/10.1038/ncb1686>.
- [10] M. Ovečka, T. Berson, M. Beck, J. Derksen, J. Šamaj, F. Baluška, et al., Structural sterols are involved in both the initiation and tip growth of root hairs in *Arabidopsis thaliana*, *Plant Cell* 22 (2010) 2999–3019, <http://dx.doi.org/10.1105/tpc.109.069880>.
- [11] X. Zhang, S. Sun, X. Nie, Y. Boutté, M. Grison, P. Li, et al., Sterol Methyl Oxidases Affect Embryo Development via Auxin-Associated Mechanisms, *Plant Physiol* 171 (2016) 468–482, <http://dx.doi.org/10.1104/pp.15.01814>.
- [12] S. Honkanen, L. Dolan, Growth regulation in tip-growing cells that develop on the epidermis, *Curr Opin Plant Biol* 34 (2016) 77–83, <http://dx.doi.org/10.1016/j.cpb.2016.10.006>.
- [13] J. Dalal, D.R. Lewis, O. Tietz, E.M. Brown, C.S. Brown, K. Palme, et al., ROSY1, a novel regulator of gravitropic response is a stigmasterol binding protein, *J Plant Physiol* 196–197 (2016) 28–40, <http://dx.doi.org/10.1016/j.jplph.2016.03.011>.
- [14] T. Griebel, J. Zeier, A role for beta-sitosterol to stigmasterol conversion in plant-pathogen interactions, *Plant J* 63 (2010) 254–268, <http://dx.doi.org/10.1111/j.1365-3113.2010.04235.x>.
- [15] M. Sharma, Z. Sasvari, P.D. Nagy, Inhibition of sterol biosynthesis reduces tobamovirus replication in yeast and plants, *J Virol* 84 (2010) 2270–2281, <http://dx.doi.org/10.1128/JVI.02003-09>.
- [16] D. Posé, I. Castanedo, O. Borsani, B. Nieto, A. Rosado, L. Taconnat, et al., Identification of the *Arabidopsis* dry2/seq1-5 mutant reveals a central role for sterols in drought tolerance and regulation of reactive oxygen species, *Plant J* 59 (2009) 63–76, <http://dx.doi.org/10.1111/j.1365-3113.2009.03849.x>.
- [17] K. Wang, M. Senthil-Kumar, C.-M. Ryu, L. Kang, K.S. Mysore, Phytosterols play a key role in plant innate immunity against bacterial pathogens by regulating nutrient efflux into the apoplast, *Plant Physiol* 158 (2012) 1789–1802, <http://dx.doi.org/10.1104/pp.111.189217>.
- [18] H. Wang, D.A. Nagegowda, R. Rawat, P. Bouvier-Navé, D. Guo, T.J. Bach, et al., Overexpression of *Brassica juncea* wild-type and mutant HMG-CoA synthase 1 in *Arabidopsis* up-regulates genes in sterol biosynthesis and enhances sterol production and stress tolerance, *Plant Biotechnol J* 10 (2012) 31–42, <http://dx.doi.org/10.1111/j.1467-7652.2011.00631.x>.
- [19] M. Sharfman, M. Bar, S. Schuster, M. Leibman, A. Avni, Sterol-dependent induction of plant defense responses by a microbe-associated molecular pattern from *Trichoderma Viride*, *Plant Physiol* 164 (2013) 819–827, <http://dx.doi.org/10.1104/pp.113.230136>.
- [20] T. Wagatsuma, M.S.H. Khan, T. Watanabe, E. Maejima, H. Sekimoto, T. Yokota, et al., Higher sterol content regulated by CYP51 with concomitant lower phospholipid content in membranes is a common strategy for aluminium tolerance in several plant species, *J Exp Bot* 66 (2015) 907–918, <http://dx.doi.org/10.1093/jxb/eru455>.
- [21] D. Manzano, P. Andrade, D. Caudepón, T. Altabella, M. Arró, A. Ferrer, Suppressing farnesyl diphosphate synthase alters chloroplast development and triggers sterol-dependent induction of Jasmonate- and Fe-related responses, *Plant Physiol* 172 (2016) 93–117, <http://dx.doi.org/10.1104/pp.16.00431>.
- [22] J. Gamir, R. Darwiche, P. Van't Hof, V. Choudhary, M. Stumpe, R. Schweiter, et al., The sterol-binding activity of PATHOGENESIS-RELATED PROTEIN 1 reveals the mode of action of an antimicrobial protein, *Plant J* (2016) 502–509, <http://dx.doi.org/10.1111/tbj.13398>.
- [23] R.A. Moreau, B.D. Whitaker, K.B. Hicks, Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses, *Prog Lipid Res* 41 (2002) 457–500, [http://dx.doi.org/10.1016/S0163-7827\(02\)00006-1](http://dx.doi.org/10.1016/S0163-7827(02)00006-1).
- [24] P. Benveniste, Biosynthesis and accumulation of sterols, *Annu Rev Plant Biol* 55 (2004) 429–457, <http://dx.doi.org/10.1146/annurev.arplant.55.031903.141616>.
- [25] M. Suzuki, T. Muranaka, Molecular genetics of plant sterol backbone synthesis, *Lipids* 42 (2007) 47–54, <http://dx.doi.org/10.1007/s11745-006-1000-5>.
- [26] A. Hemmerlin, J.L. Harwood, T.J. Bach, A raison d'être for two distinct pathways in the early steps of plant isoprenoid biosynthesis? *Prog Lipid Res* 51 (2012) 95–148, <http://dx.doi.org/10.1016/j.plipres.2011.12.001>.
- [27] Z. Wojciechowski, Biochemistry of phytosterol conjugates, in: G.W. Patterson, W.D. Nes (Eds.), *Physiol. Biochem. Sterols*, American Oil Chemists' Society, 1991, pp. 361–394.
- [28] L. Dyas, L.J. Goad, Steryl fatty acyl esters in plants, *Phytochemistry* 34 (1993) 17–29, [http://dx.doi.org/10.1016/S0031-9422\(00\)90777-5](http://dx.doi.org/10.1016/S0031-9422(00)90777-5).
- [29] N.V. Kovganko, Z.N. Kashkan, Sterol glycosides and acylglycosides, *Chem Nat Compd* 35 (1999) 479–497.
- [30] R.A. Moreau, M.J. Powell, K.B. Hicks, Extraction and quantitative analysis of oil from commercial corn fiber, *J Agric Food Chem* 44 (1996) 2149–2154, <http://dx.doi.org/10.1021/jf950743h>.
- [31] E. Mandak, L. Nyström, Steryl ferulates, bioactive compounds in cereal grains, *Lipid Technol* 24 (2012) 80–82, <http://dx.doi.org/10.1002/lite.201200179>.
- [32] I. Hernández-Pinzón, J.H.E. Ross, K.A. Barnes, A.P. Damant, D.J. Murphy, Composition and role of tapetal lipid bodies in the biogenesis of the pollen coat of *Brassica napus*, *Planta* 208 (1999) 588–598.
- [33] S.S.H. Wu, R.A. Moreau, B.D. Whitaker, A.H.C. Huang, Steryl esters in the elaioplasts of the tapetum in developing *Brassica* anthers and their recovery on the pollen surface, *Lipids* 34 (1999) 517–523, <http://dx.doi.org/10.1007/s11745-999-0393-5>.
- [34] L. Gondet, R. Bronner, P. Benveniste, Regulation of sterol content in membranes by subcellular compartmentation of Steryl-esters accumulating in a sterol-overproducing tobacco mutant, *Plant Physiol* 105 (1994) 509–518, <http://dx.doi.org/10.1104/pp.105.2.509>.
- [35] H. Schaller, B. Crausem, P. Benveniste, M. Chye, C. Tan, Y. Song, et al., Expression of the *Hevea brasiliensis* (H. B. K.) Müll. Arg. tobacco results in sterol overproduction, *Plant Physiol* 109 (1995) 761–770, <http://dx.doi.org/10.1104/pp.109.3.761>.
- [36] S.T. Behmer, N. Olszewski, J. Sebastiani, S. Palka, G. Sparacino, E. Sciarro, et al., Plant phloem sterol content: forms, putative functions, and implications for phloem-feeding insects, *Front Plant Sci* 4 (2013) 370, <http://dx.doi.org/10.3389/fpls.2013.00370>.
- [37] K. Athenstaedt, G. Daum, The life cycle of neutral lipids: synthesis, storage and

- degradation, *Cell Mol Life Sci* 63 (2006) 1355–1369, <http://dx.doi.org/10.1007/s00018-006-6016-8>.
- [38] D.L. Davis, C.G. Poneleit, Sterol accumulation and composition in developing *Zea mays* L. Kernels, *Plant Physiol* 54 (1974) 794–796 [<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=366606&tool=pmcentrez&rendertype=abstract>].
- [39] M. Katayama, K. Katoh, Soybean sterols during maturation of seeds I. Accumulation of sterols in the free form, fatty acid esters, acylated glucosides and non-acylated glucosides, *Plant Cell Physiol* 14 (1973) 681–688.
- [40] M. Harker, A. Hellyer, J.C. Clayton, A. Duvoix, A. Lanot, R. Safford, Co-ordinate regulation of sterol biosynthesis enzyme activity during accumulation of sterols in developing rape and tobacco seed, *Planta* 216 (2003) 707–715, <http://dx.doi.org/10.1007/s00425-002-0913-3>.
- [41] K.D. Chapman, J.M. Dyer, R.T. Mullen, Biogenesis and functions of lipid droplets in plants: thematic review series: lipid droplet synthesis and metabolism: from yeast to man, *J Lipid Res* 53 (2012) 215–226, <http://dx.doi.org/10.1194/jlr.R021436>.
- [42] D.J. Murphy, The dynamic roles of intracellular lipid droplets: from archaea to mammals, *Protoplasma* 249 (2012) 541–585, <http://dx.doi.org/10.1007/s00709-011-0329-7>.
- [43] F. Wilfling, J.T. Haas, T.C. Walther, R.V.F. Jr, Lipid droplet biogenesis, *Curr Opin Cell Biol* 29 (2014) 39–45, <http://dx.doi.org/10.1016/j.cob.2014.03.008>.
- [44] J.K. Zehmer, Y. Huang, G. Peng, J. Pu, R.G.W. Anderson, P. Liu, A role for lipid droplets in inter-membrane lipid traffic, *Proteomics* 9 (2009) 914–921, <http://dx.doi.org/10.1002/prot.200800584>.
- [45] L. Yang, Y. Ding, Y. Chen, S. Zhang, C. Huo, Y. Wang, et al., The proteomics of lipid droplets: structure, dynamics, and functions of the organelle conserved from bacteria to humans, *J Lipid Res* 53 (2012) 1245–1253, <http://dx.doi.org/10.1194/jlr.R024117>.
- [46] P.J. Horn, C.N. James, S.K. Gidda, A. Kilaru, J.M. Dyer, R.T. Mullen, et al., Identification of a new class of lipid droplet-associated proteins in plants, *Plant Physiol* 162 (2013) 1926–1936, <http://dx.doi.org/10.1104/pp.113.222455>.
- [47] S.K. Gidda, S. Park, M. Pyc, O. Yurchenko, Y. Cai, P. Wu, et al., Lipid droplet-associated proteins (LDAPs) are required for the dynamic regulation of neutral lipid compartmentation in plant cells, *Plant Physiol* 170 (2016) 01977.2015, <http://dx.doi.org/10.1104/pp.15.01977>.
- [48] S. D'Andrea, Lipid droplet mobilization: the different ways to loosen the purse strings, *Biochimie* 120 (2016) 17–27, <http://dx.doi.org/10.1016/j.biochi.2015.07.010>.
- [49] L.-J. Lin, S.S.K. Tai, C.-C. Peng, J.T.C. Tzen, Steroleosin, a sterol-binding dehydrogenase in seed oil bodies, *Plant Physiol* 128 (2002) 1200–1211, <http://dx.doi.org/10.1104/pp.010928>.
- [50] C. van der Schoot, L.K. Paul, S.B. Paul, P.L.H.H. Rinne, Plant lipid bodies and cell-cell signaling: a new role for an old organelle? *Plant Signal Behav* 6 (2011) 1732–1738, <http://dx.doi.org/10.4161/psb.6.11.17639>.
- [51] D. Silvestro, T.G. Andersen, H. Schaller, P.E. Jensen, Plant sterol metabolism. Δ^7 -Sterol-C5-Desaturase (STE1/DWARF7), $\Delta^5,7$ -Sterol- Δ^7 -Reductase (DWARF5) and Δ^24 -Sterol- Δ^24 -Reductase (DIMINUTO/DWARF1) show multiple subcellular localizations in *Arabidopsis thaliana* (Heynh) L, *PLoS One* 8 (2013), <http://dx.doi.org/10.1371/journal.pone.0056429>.
- [52] J.T.L. Ting, S.S.H. Wu, C. Ratnayake, A.H.C. Huang, Constituents of the tapetosomes and elaioplasts in *Brassica campestris* Tapetum and their degradation and retention during microsporogenesis, *Plant J* 16 (1998) 541–551, <http://dx.doi.org/10.1046/j.1365-313X.1998.00325.x>.
- [53] C. Villette, A. Berna, V. Compagnon, H. Schaller, Plant sterol diversity in pollen from angiosperms, *Lipids* 50 (2015) 749–760, <http://dx.doi.org/10.1007/s11745-015-4008-x>.
- [54] T. Ischebeck, Lipids in pollen -they are different, *Biochim Biophys Acta Mol Cell Biol Lipids* 1861 (2016) 1315–1328, <http://dx.doi.org/10.1016/j.bbalip.2016.03.023>.
- [55] G. Mckegney, K. Yao, S. Ghosh, A.N.N. Huff, S. Mayak, J.E. Thompson, The lipid composition of cytosolic particles isolated from senescing bean cotyledons, *Phytochemistry* 39 (1995) 1335–1345.
- [56] L. Li, J. Zhao, Y. Zhao, X. Lu, Z. Zhou, C. Zhao, et al., Comprehensive investigation of tobacco leaves during natural early senescence via multi-platform metabolomics analyses, *Sci Rep* 6 (2016) 37976, <http://dx.doi.org/10.1038/srep37976>.
- [57] P. Bouvier-Navé, A. Berna, A. Noiriél, V. Compagnon, A.S. Carlsson, A. Banas, et al., Involvement of the phospholipid sterol acyltransferase1 in plant sterol homeostasis and leaf senescence, *Plant Physiol* 152 (2010) 107–119, <http://dx.doi.org/10.1104/pp.109.145672>.
- [58] K.F. Buhman, M. Accad, R.V. Farese Jr., Mammalian acyl-CoA: cholesterol acyltransferases, *Biochim Biophys Acta Mol Cell Biol Lipids* 1529 (2000) 142–154 [<http://linkinghub.elsevier.com/retrieve/pii/S138819810000144X>].
- [59] A. Jonas, Lecithin cholesterol acyltransferase, *Biochim Biophys Acta* 1529 (2000) 245–256 [doi:S1388-1981(00)00153-0 [pii]].
- [60] H. Yang, M. Bard, D.A. Bruner, A. Gleeson, R.J. Deckerbaum, G. Aljinovic, et al., Sterol esterification in yeast: a two-gene process, *Science* 272 (1996) 1353–1356 [80-].
- [61] R.E. Garcia, J.B. Mudd, Fatty acid and sterol specificity in the biosynthesis of steryl esters by enzyme preparations from spinach leaves, *Arch Biochem Biophys* 190 (1978) 315–321.
- [62] R.E. Garcia, J.B. Mudd, Identification of an acyl donor in steryl ester biosynthesis by enzyme preparations from spinach leaves, *Plant Physiol* 62 (1978) 348–353.
- [63] P. Bouvier-Navé, P. Benveniste, Sterol acyl transferase and steryl ester hydrolase activities in a tobacco mutant which overproduces sterols, *Plant Sci* 110 (1995) 11–19, [http://dx.doi.org/10.1016/0168-9452\(95\)04181-S](http://dx.doi.org/10.1016/0168-9452(95)04181-S).
- [64] J. Zimowski, Z.A. Wojciechowski, Acyl donors for sterol esterification by cell-free preparations from *Sinapis alba* roots, *Phytochemistry* 20 (1981) 1795–1798, [http://dx.doi.org/10.1016/0031-9422\(81\)84006-X](http://dx.doi.org/10.1016/0031-9422(81)84006-X).
- [65] J. Zimowski, Z.A. Wojciechowski, Partial purification and specificity of triacylglycerol: sterol acyltransferase from *Sinapis alba*, *Phytochemistry* 20 (1981) 1799–1803, [http://dx.doi.org/10.1016/0031-9422\(81\)84007-1](http://dx.doi.org/10.1016/0031-9422(81)84007-1).
- [66] L.J. Goad, J. Zimowski, R.P. Evershed, V.L. Male, The steryl esters of higher plants, in: P.K. Stumpf, J.B. Mudd, W.D. Nes (Eds.), *Metab. Struct. Funct. Plant Lipids*, Springer, 1987, pp. 95–102.
- [67] M. Kalinowska, J. Zimowski, M. Bucholc, Z.A. Wojciechowski, Reversible enzymatic transesterification between wax esters and sterols in *Sinapis alba* roots, *Phytochemistry* 28 (1989) 2931–2935, [http://dx.doi.org/10.1016/0031-9422\(89\)80256-0](http://dx.doi.org/10.1016/0031-9422(89)80256-0).
- [68] A. Banas, A.S. Carlsson, B. Huang, M. Lenman, W. Banas, M. Lee, et al., Cellular sterol ester synthesis in plants is performed by an enzyme (phospholipid:sterol acyltransferase) different from the yeast and mammalian acyl-CoA:sterol acyltransferases, *J Biol Chem* 280 (2005) 34626–34634, <http://dx.doi.org/10.1074/jbc.M504459200>.
- [69] M. Kopsischke, L. Westphal, K. Schneeberger, R. Clark, S. Ossowski, V. Wewer, et al., Impaired sterol ester synthesis alters the response of *Arabidopsis thaliana* to *Phytophthora infestans*, *Plant J* 73 (2013) 456–468, <http://dx.doi.org/10.1111/tpj.12046>.
- [70] Q. Chen, L. Steinhauer, J. Hammerlindl, W. Keller, J. Zou, Biosynthesis of phytosterol esters: identification of a sterol O-acyltransferase in *Arabidopsis*, *Plant Physiol* 145 (2007) 974–984, <http://dx.doi.org/10.1104/pp.107.106278>.
- [71] U. Stahl, A. Carlsson, M. Lenman, A. Dahlqvist, B. Huang, W. Banas, et al., Cloning and functional characterization of a phospholipid: diacylglycerol acyltransferase from *Arabidopsis*, *Am Soc Plant Biol* 135 (2004) 1324–1335, <http://dx.doi.org/10.1104/pp.104.044354.cerevisiae>.
- [72] A. Noiriél, P. Benveniste, A. Banas, S. Stymne, P. Bouvier-Navé, Expression in yeast of a novel phospholipase A1 cDNA from *Arabidopsis thaliana*, *Eur J Biochem* 271 (2004) 3752–3764, <http://dx.doi.org/10.1111/j.1432-1033.2004.04317.x>.
- [73] G. Chen, M.S. Greer, I. Lager, J. Lindberg Yilmaz, E. Mietkiewska, A.S. Carlsson, et al., Identification and characterization of an LCAT-like *Arabidopsis thaliana* gene encoding a novel phospholipase A, *FEBS Lett* 586 (2012) 373–377, <http://dx.doi.org/10.1016/j.febslet.2011.12.034>.
- [74] P. Wang, Z. Wang, Y. Dou, X. Zhang, M. Wang, X. Tian, Genome-wide identification and analysis of membrane-bound O-acyltransferase (MBOAT) gene family in plants, *Planta* 238 (2013) 907–922, <http://dx.doi.org/10.1007/s00425-013-1939-4>.
- [75] M. Rodríguez-Concepción, A. Boronat, Breaking new ground in the regulation of the early steps of plant isoprenoid biosynthesis, *Curr Opin Plant Biol* 25 (2015) 17–22, <http://dx.doi.org/10.1016/j.copbi.2015.04.001>.
- [76] S. Grille, A. Zaslowski, S. Thiele, J. Plat, D. Warnecke, The functions of steryl glycosides come to those who wait: recent advances in plants, fungi, bacteria and animals, *Prog Lipid Res* 49 (2010) 262–288, <http://dx.doi.org/10.1016/j.plipres.2010.02.001>.
- [77] M. Kojima, M. Ohnishi, S. Ito, Y. Fujino, Characterization of acylmono-, mono-, di-, tri- and tetraglycosylsterol and saponin in adzuki bean (*Vigna angularis*) seeds, *Lipids* 24 (1989) 849–853.
- [78] L. Nyström, A. Schär, A.M. Lampi, Steryl glycosides and acylated steryl glycosides in plant foods reflect unique sterol patterns, *Eur J Lipid Sci Technol* 114 (2012) 656–669, <http://dx.doi.org/10.1002/ejlt.201200033>.
- [79] R. Dupéron, M. Thiersault, P. Dupéron, High level of glycosylated sterols in species of solanum and sterol changes during the development of the tomato, *Phytochemistry* 23 (1984) 743–746, [http://dx.doi.org/10.1016/S0031-9422\(00\)85016-5](http://dx.doi.org/10.1016/S0031-9422(00)85016-5).
- [80] B.D. Whitaker, Changes in the steryl lipid content and composition of tomato fruit during ripening, *Phytochemistry* 27 (1988) 3411–3416.
- [81] C.C. Steel, R.B. Drysdale, Electrolyte leakage from plant and fungal tissues and disruption of liposome membranes by α -tomatine, *Phytochemistry* 27 (1988) 1025–1030, [http://dx.doi.org/10.1016/0031-9422\(88\)80266-8](http://dx.doi.org/10.1016/0031-9422(88)80266-8).
- [82] E.A.J. Keukens, T. de Vrije, C. van den Boom, P. de Waard, H.H. Plasman, F. Thiel, et al., Molecular basis of glycoalkaloid induced membrane disruption, *BBA-Biomembranes* 1240 (1995) 216–228, [http://dx.doi.org/10.1016/0005-2736\(95\)00186-7](http://dx.doi.org/10.1016/0005-2736(95)00186-7).
- [83] J.T. Blankemeyer, J.B. White, B.K. Stringer, M. Friedman, Effect of α -tomatine and tomatidine on membrane potential of frog embryos and active transport of ions in frog skin, *Food Chem Toxicol* 35 (1997) 639–646, [http://dx.doi.org/10.1016/S0278-6915\(97\)00038-0](http://dx.doi.org/10.1016/S0278-6915(97)00038-0).
- [84] F. Furt, F. Simon-Plas, S. Mongrand, Lipids of the plant plasma membrane, in: A.S. Murphy, B. Schulz, W. Peer (Eds.), *Plant Plasma Membr*, Springer, 2011, pp. 3–30.
- [85] E.J. Dufourc, Sterols and membrane dynamics, *J Chem Biol* 1 (2008) 63–77, <http://dx.doi.org/10.1007/s12154-008-0010-6>.
- [86] K. Grosjean, S. Mongrand, L. Beney, F. Simon-Plas, P. Gerbeau-Pissot, Differential effect of plant lipids on membrane organization specificities of phytosphingolipids and phytosterols, *J Biol Chem* 290 (2015) 5810–5825, <http://dx.doi.org/10.1074/jbc.M114.598805>.
- [87] F. Simon-Plas, A. Perraki, E. Bayer, P. Gerbeau-Pissot, S. Mongrand, An update on plant membrane rafts, *Curr Opin Plant Biol* 14 (2011) 642–649, <http://dx.doi.org/10.1016/j.copbi.2011.08.003>.
- [88] M. Laloi, A.-M. Perret, L. Chatre, S. Melsler, C. Cantrel, M.-N. Vaultier, et al., Insights into the role of specific lipids in the formation and delivery of lipid microdomains to the plasma membrane of plant cells, *Plant Physiol* 143 (2007) 461–472, <http://dx.doi.org/10.1104/pp.106.091496>.

- [89] J.L. Cacas, F. Furt, M. Le Guédard, J.M. Schmitter, C. Buré, P. Gerbeau-Pissot, et al., Lipids of plant membrane rafts, *Prog Lipid Res* 51 (2012) 272–299, <http://dx.doi.org/10.1016/j.plipres.2012.04.001>.
- [90] D. Takahashi, Y. Kawamura, M. Uemura, Changes of detergent-resistant plasma membrane proteins in oat and rye during cold acclimation: association with differential freezing tolerance, *J Proteome Res* 12 (2013) 4998–5011.
- [91] H. Zauber, A. Burgos, P. Garapati, W.X. Schulze, Plasma membrane lipid-protein interactions affect signaling processes in sterol-biosynthesis mutants in *Arabidopsis thaliana*, *Front Plant Sci* 5 (2014) 78, <http://dx.doi.org/10.3389/fpls.2014.00078>.
- [92] H. Tjellström, L.I. Hellgren, A. Wieslander, A.S. Sandelius, Lipid asymmetry in plant plasma membranes: phosphate deficiency-induced phospholipid replacement is restricted to the cytosolic leaflet, *FASEB J* 24 (2010) 1128–1138, <http://dx.doi.org/10.1096/fj.09-139410>.
- [93] J.-L. Cacas, C. Buré, K. Grosjean, P. Gerbeau-Pissot, J. Lherminier, Y. Rombouts, et al., Re-visiting plant plasma membrane lipids in tobacco: a focus on sphingolipids, *Plant Physiol* 170 (2016) 00564.2015, <http://dx.doi.org/10.1104/pp.15.00564>.
- [94] J.L. Cantatore, S.M. Murphy, D.V. Lynch, Compartmentation and topology of glucosylceramide synthesis, *Biochem Soc Trans* 28 (2000) 748–750, <http://dx.doi.org/10.1042/0300-5127-0280748>.
- [95] H. Choi, K. Ohya, Y.-Y. Kim, J.-Y. Jin, S.B. Lee, Y. Yamaoka, et al., The role of Arabidopsis ABCG9 and ABCG31 ATP binding cassette transporters in pollen fitness and the deposition of sterol glycosides on the pollen coat, *Plant Cell* 26 (2014) 310–324, <http://dx.doi.org/10.1105/tpc.113.118935>.
- [96] L. Peng, Y. Kawagoe, P. Hogan, D. Delmer, Sitosterol- β -glucoside as primer for cellulose synthesis in plants, *Science* 295 (2002) 147–150 [80-].
- [97] K. Schrick, S. Debolt, V. Bulone, Deciphering the molecular functions of sterols in cellulose biosynthesis, *Front Plant Sci* 3 (2012) 84, <http://dx.doi.org/10.3389/fpls.2012.00084>.
- [98] P. Chaturvedi, P. Misra, R. Tuli, Sterol glucosyltransferases—the enzymes that modify sterols, *Appl Biochem Biotechnol* 165 (2011) 47–68, <http://dx.doi.org/10.1007/s12010-011-9232-0>.
- [99] M.-A. Hartmann-Bouillon, P. Benveniste, Sterol biosynthetic capability of purified membrane fractions from maize coleoptiles, *Phytochemistry* 17 (1978) 1037–1042, [http://dx.doi.org/10.1016/S0031-9422\(00\)94275-4](http://dx.doi.org/10.1016/S0031-9422(00)94275-4).
- [100] P. Ullmann, D. Rimmelé, P. Benveniste, P. Bouvier-Navé, Phospholipid-dependence of plant UDP-glucose-sterol- β -D-glucosyltransferase. II. Acetone-mediated delipidation and kinetic studies, *Plant Sci Lett* 36 (1984) 29–36.
- [101] P. Ullmann, A. Ury, D. Rimmelé, P. Benveniste, P. Bouvier-Navé, UDP-glucose sterol β -D-glucosyltransferase, a plasma membrane-bound enzyme of plants: enzymatic properties and lipid dependence, *Biochimie* 75 (1993) 713–723.
- [102] D.C. Warnecke, E. Heinz, Purification of a membrane-bound UDP-glucose:sterol [β]-D-glucosyltransferase based on its solubility in diethyl ether, *Plant Physiol* 105 (1994) 1067–1073.
- [103] A. Potocka, J. Zimowski, Metabolism of conjugated sterols in eggplant. Part 1. UDP-glucose: sterol glucosyltransferase, *Acta Biochim Pol* 55 (2008) 127–134.
- [104] B.R. Madina, L.K. Sharma, P. Chaturvedi, R.S. Sangwan, R. Tuli, Purification and physico-kinetic characterization of β 3-hydroxy specific sterol glucosyltransferase from *Withania somnifera* (L) and its stress response, *Biochim Biophys Acta Proteomics* 1774 (2007) 392–402, <http://dx.doi.org/10.1016/j.bbapap.2006.12.009>.
- [105] X. Li, T. Xia, J. Huang, K. Guo, X. Liu, T. Chen, et al., Distinct biochemical activities and heat shock responses of two UDP-glucose sterol glucosyltransferases in cotton, *Plant Sci* 219–220 (2014) 1–8, <http://dx.doi.org/10.1016/j.plantsci.2013.12.013>.
- [106] P. Bouvier-Navé, P. Ullmann, D. Rimmelé, P. Benveniste, Phospholipid-dependence of plant UDP-glucose-sterol- β -D-glucosyltransferase. I. Detergent-mediated delipidation by selective solubilization, *Plant Sci Lett* 36 (1984) 19–27, [http://dx.doi.org/10.1016/0304-4211\(84\)90271-2](http://dx.doi.org/10.1016/0304-4211(84)90271-2).
- [107] I. Hillig, M. Leipelt, C. Ott, U. Zähringer, D. Warnecke, E. Heinz, Formation of glucosylceramide and sterol glucoside by a UDP-glucose-dependent glucosylceramide synthase from cotton expressed in *Pichia Pastoris*, *FEBS Lett* 553 (2003) 365–369, [http://dx.doi.org/10.1016/S0014-5793\(03\)01058-5](http://dx.doi.org/10.1016/S0014-5793(03)01058-5).
- [108] D.C. Warnecke, M. Baltrusch, F. Buck, F.P. Wolter, E. Heinz, UDP-glucose:sterol glucosyltransferase: cloning and functional expression in *Escherichia coli*, *Plant Mol Biol* 35 (1997) 597–603, <http://dx.doi.org/10.1023/A:1005806119807>.
- [109] L.K. Sharma, B.R. Madina, P. Chaturvedi, R.S. Sangwan, R. Tuli, Molecular cloning and characterization of one member of β 3-hydroxy sterol glucosyltransferase gene family in *Withania somnifera*, *Arch Biochem Biophys* 460 (2007) 48–55, <http://dx.doi.org/10.1016/j.abb.2007.01.024>.
- [110] P. Chaturvedi, M. Mishra, N. Akhtar, P. Gupta, P. Mishra, R. Tuli, Sterol glucosyltransferases-identification of members of gene family and their role in stress in *Withania somnifera*, *Mol Biol Rep* 39 (2012) 9755–9764, <http://dx.doi.org/10.1007/s11033-012-1841-3>.
- [111] P. Tiwari, R.S. Sangwan, B.N. Mishra Asha, F. Sabir, N.S. Sangwan, Molecular cloning and biochemical characterization of a recombinant sterol 3-O-glucosyltransferase from *Gymnema sylvestre* R.Br. Catalyzing biosynthesis of sterol glucosides, *Biomed Res Int* 2014 (2014) 934351, <http://dx.doi.org/10.1155/2014/934351>.
- [112] S. DeBolt, W.R. Scheible, K. Schrick, M. Auer, F. Beisson, V. Bischoff, et al., Mutations in UDP-glucose:sterol glucosyltransferase in Arabidopsis cause transparent testa phenotype and suberization defect in seeds, *Plant Physiol* 151 (2009) 78–87, <http://dx.doi.org/10.1104/pp.109.140582>.
- [113] D.F. Stucky, J.C. Arpin, K. Schrick, Functional diversification of two UGT80 enzymes required for sterol glucoside synthesis in Arabidopsis, *J Exp Bot* 66 (2015) 189–201, <http://dx.doi.org/10.1093/jxb/eru410>.
- [114] D.J. Baisted, Steryl glucoside and acyl glucoside biosynthesis in maturing pea seeds, *Phytochemistry* 17 (1978) 435–438.
- [115] D.S. Catz, J.S. Tandecarz, C.E. Cardini, Steryl glucoside and acyl sterol glucoside formation in the amyloplast membrane during the development of potato tuber, *Plant Sci* 38 (1985) 179–184, [http://dx.doi.org/10.1016/0168-9452\(85\)90036-6](http://dx.doi.org/10.1016/0168-9452(85)90036-6).
- [116] D.S. Catz, J.S. Tandecarz, C.E. Cardini, UDP-glucose: sterol glucosyltransferase and a sterol glucoside acyltransferase activity in Amyloplast membranes from potato tubers, *J Exp Bot* 36 (1985) 602–609.
- [117] A.D. Elbein, W.T. Forsee, Biosynthesis and structure of glycosyl diglycerides, sterol glucosides, and acylated sterol glucosides, *Lipids* 10 (1975) 427–436.
- [118] W.T. Forsee, G. Valkovich, A.D. Elbein, Acylation of sterol glucosides by phospholipids: Solubilization and properties of the acyl transferase, *Arch Biochem Biophys* 172 (1976) 410–418.
- [119] W. Frasch, C. Grunwald, Acylated sterol glycoside synthesis in seedlings of *Nicotiana tabacum* L. *Plant Physiol* 58 (1976) 744–748 [<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=542300&tool=pmcentrez&rendertype=abstract>].
- [120] M. Misiak, M. Kalinowska, Z. Wojciechowski, Characterization of acyl lipid: sterol glucoside acyltransferase from oat (*Avena sativa* L.) seedlings, *Acta Biochim Pol* 38 (1991) 43–45.
- [121] Z.A. Wojciechowski, J. Zimowski, Acyl composition and biosynthesis of acylated sterol glucosides in *Calendula officinalis*, *Biochim Biophys Acta* 398 (1975) 111–117.
- [122] J.G. Zimowski, Z.A. Wojciechowski, Enzymatic synthesis of sterol 6'-o-acyl- β -D-glucosides in *Sinapis alba* and some other plants, *Phytochemistry* 22 (1983) 2175–2178, [http://dx.doi.org/10.1016/S0031-9422\(00\)80140-5](http://dx.doi.org/10.1016/S0031-9422(00)80140-5).
- [123] W. Eichenberger, H.P. Siegrist, Steryl glucoside acyltransferase from carrots, *FEBS Lett* 52 (1975) 153–156, [http://dx.doi.org/10.1016/0014-5793\(75\)80660-0](http://dx.doi.org/10.1016/0014-5793(75)80660-0).
- [124] A. Potocka, J. Zimowski, Metabolism of conjugated sterols in eggplant. Part 2. Phospholipid: sterol glucoside acyltransferase, *Acta Biochim Pol* 55 (2008) 135–140.
- [125] K. Schrick, S. Shiva, J.C. Arpin, N. Delimont, G. Isaac, P. Tamura, et al., Steryl glucoside and acyl sterol glucoside analysis of Arabidopsis seeds by electrospray ionization tandem mass spectrometry, *Lipids* 47 (2012), <http://dx.doi.org/10.1038/jid.2014.371>.
- [126] J. Msanne, M. Chen, K.D. Luttgearm, A.M. Bradley, E.S. Mays, J.M. Paper, et al., Glucosylceramide is critical for cell-type differentiation and organogenesis, but not for cell viability in Arabidopsis, *Plant J* 84 (2015) 188–201.
- [127] D.V. Lynch, P.L. Steponkus, Plasma membrane lipid alterations associated with cold acclimation of winter rye seedlings (*Secale cereale* L. cv Puma), *Plant Physiol* (1987) 761–767.
- [128] B.D. Whitaker, Changes in lipids of tomato fruit stored at chilling and non-chilling temperatures, *Phytochemistry* 30 (1991) 757–761.
- [129] S. Hugly, P. McCourt, J. Browse, G.W. Patterson, C. Somerville, A chilling sensitive mutant of Arabidopsis with altered sterol-ester metabolism, *Plant Physiol* 93 (1990) 1053–1062, <http://dx.doi.org/10.1104/pp.93.3.1053>.
- [130] B.D. Whitaker, Lipid changes in mature-green tomatoes during ripening, during chilling, and after rewarming subsequent to chilling, *J Am Soc Hortic Sci* 119 (1994) 994–999 [<http://journal.ashspubs.org/content/119/5/994.abstract>].
- [131] J.P. Palta, B.D. Whitaker, L.S. Weiss, Plasma membrane lipids associated with genetic variability in freezing tolerance and cold acclimation of solanum species, *Plant Physiol* 103 (1993) 793–803, <http://dx.doi.org/10.1104/pp.103.3.793>.
- [132] M. Uemura, S. Peter, A contrast of the plasma membrane lipid composition of oat and rye leaves in relation to freezing tolerance, *Plant Physiol* 104 (1994) 479–496.
- [133] A. Minami, M. Fujiwara, A. Furuto, Y. Fukao, T. Yamashita, M. Kamo, et al., Alterations in detergent-resistant plasma membrane microdomains in *Arabidopsis thaliana* during cold acclimation, *Plant Cell Physiol* 50 (2009) 341–359, <http://dx.doi.org/10.1093/pcp/pcn202>.
- [134] A. Minami, A. Furuto, M. Uemura, Dynamic compositional changes of detergent-resistant plasma membrane microdomains during plant cold acclimation, *Plant Signal Behav* 5 (2010) 1115–1118, <http://dx.doi.org/10.4161/psb.5.9.12478>.
- [135] D.R. Rudell, D.A. Buchanan, R.S. Leisso, B.D. Whitaker, J.P. Mattheis, Y. Zhu, et al., Ripening, storage temperature, ethylene action, and oxidative stress alter apple peel phytosterol metabolism, *Phytochemistry* 72 (2011) 1328–1340, <http://dx.doi.org/10.1016/j.phytochem.2011.04.018>.
- [136] H.S. Vu, S. Shiva, M.R. Roth, P. Tamura, L. Zheng, M. Li, et al., Lipid changes after leaf wounding in *Arabidopsis thaliana*: expanded lipidomic data form the basis for lipid co-occurrence analysis, *Plant J* 80 (2014) 728–743, <http://dx.doi.org/10.1111/tpj.12659>.
- [137] P. Tarazona, K. Feussner, I. Feussner, An enhanced plant lipidomics method based on multiplexed liquid chromatography-mass spectrometry reveals additional insights into cold- and drought-induced membrane remodeling, *Plant J* 84 (2015) 621–633, <http://dx.doi.org/10.1111/tpj.13013>.
- [138] M.S.S. Kumar, K. Ali, A. Dahuja, A. Tyagi, Role of phytosterols in drought stress tolerance in rice, *Plant Physiol Biochem* 96 (2015) 83–89, <http://dx.doi.org/10.1016/j.plaphy.2015.07.014>.
- [139] S. Narayanan, P.J. Tamura, M.R. Roth, P.V.V. Prasad, R. Welty, Wheat leaf lipids during heat stress: I. High day and night temperatures result in major lipid alterations, *Plant Cell Environ* 39 (2016) 787–803, <http://dx.doi.org/10.1111/pce.12649>.
- [140] S. Narayanan, P.V.V. Prasad, R. Welty, Wheat leaf lipids during heat stress: II. Lipids experiencing coordinated metabolism are detected by analysis of lipid co-occurrence, *Plant Cell Environ* 39 (2016) 608–617, <http://dx.doi.org/10.1111/pce.12648>.
- [141] D. Takahashi, H. Imai, Y. Kawamura, M. Uemura, Lipid profiles of detergent

- resistant fractions of the plasma membrane in oat and rye in association with cold acclimation and freezing tolerance, *Cryobiology* 72 (2016) 123–134, <http://dx.doi.org/10.1016/j.cryobiol.2016.02.003>.
- [142] Y. Wang, Y. Zhang, Z. Wang, X. Zhang, S. Yang, A missense mutation in CHS1, a TIR-NB protein, induces chilling sensitivity in *Arabidopsis*, *Plant J* 75 (2013) 553–565, <http://dx.doi.org/10.1111/tpj.12232>.
- [143] M.K. Mishra, G. Singh, S. Tiwari, R. Singh, N. Kumari, P. Misra, Characterization of *Arabidopsis* sterol glucosyltransferase TTG15/UGT80B1 role during freeze and heat stress, *Plant Signal Behav* (2015) 1–9, <http://dx.doi.org/10.1080/15592324.2015.1075682>.
- [144] M.K. Mishra, P. Chaturvedi, R. Singh, G. Singh, L.K. Sharma, V. Pandey, et al., Overexpression of WsSGTL1 gene of *Withania somnifera* enhances salt tolerance, heat tolerance and cold acclimation ability in transgenic *Arabidopsis* plants, *PLoS One* 8 (2013), <http://dx.doi.org/10.1371/journal.pone.0063064>.
- [145] V. Pandey, A. Niranjana, N. Atri, K. Chandrashekar, M.K. Mishra, P.K. Trivedi, et al., WsSGTL1 gene from *Withania somnifera*, modulates glycosylation profile, antioxidant system and confers biotic and salt stress tolerance in transgenic tobacco, *Planta* 239 (2014) 1217–1231, <http://dx.doi.org/10.1007/s00425-014-2046-x>.
- [146] S. Saema, L. ur Rahman, R. Singh, A. Niranjana, I.Z. Ahmad, P. Misra, Ectopic overexpression of WsSGTL1, a sterol glucosyltransferase gene in *Withania somnifera*, promotes growth, enhances glycowithanolide and provides tolerance to abiotic and biotic stresses, *Plant Cell Rep* 35 (2016) 195–211, <http://dx.doi.org/10.1007/s00299-015-1879-5>.
- [147] G. Singh, M. Tiwari, S.P. Singh, S. Singh, P.K. Trivedi, P. Misra, Silencing of sterol glucosyltransferases modulates the withanolide biosynthesis and leads to compromised basal immunity of *Withania somnifera*, *Sci Rep* 6 (2016) 25562, <http://dx.doi.org/10.1038/srep25562>.
- [148] G. Singh, M. Tiwari, S.P. Singh, R. Singh, S. Singh, P.A. Shirke, P.K. Trivedi, P. Misra, Sterol glucosyltransferases required for adaptation of *Withania somnifera* at high temperature, *Physiol. Plant.* 160 (2017) 297–311, <http://dx.doi.org/10.1111/ppl.12563>.
- [149] B. Ploier, M. Korber, C. Schmidt, B. Koch, E. Leitner, G. Daum, Regulatory link between sterol ester formation and hydrolysis in the yeast *Saccharomyces cerevisiae*, *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1851 (2015) 977–986, <http://dx.doi.org/10.1016/j.bbalip.2015.02.011>.
- [150] M. Kalinowska, Z.A. Wojciechowski, The occurrence of sterol-ester hydrolase activity in roots of white mustard seedlings, *Phytochemistry* 22 (1983) 59–63.
- [151] M. Kalinowska, Z.A. Wojciechowski, Purification and some properties of sterol-β-D-glucoside hydrolase from *Sinapis alba* seedlings, *Phytochemistry* 17 (1978) 1533–1537.
- [152] M. Kalinowska, Z.A. Wojciechowski, Modulation of activities of sterol glucoside hydrolase and UDPG: sterol glucosyltransferase from *Sinapis alba* by detergents and lipids, *Phytochemistry* 25 (1985) 45–49, [http://dx.doi.org/10.1016/S0031-9422\(00\)94498-4](http://dx.doi.org/10.1016/S0031-9422(00)94498-4).
- [153] T. Watanabe, T. Ito, H.M. Goda, Y. Ishibashi, T. Miyamoto, K. Ikeda, et al., Sterylglucoside catabolism in *Cryptococcus neoformans* with endoglycoceramidase-related protein 2 (EGCrP2), the first sterol-β-glucosidase identified in fungi, *J Biol Chem* 290 (2015) 1005–1019, <http://dx.doi.org/10.1074/jbc.M114.616300>.
- [154] T. Watanabe, M. Tani, Y. Ishibashi, I. Endo, N. Okino, M. Ito, Ergosterol-β-glucosidase (Egh1) involved in sterolglucoside catabolism and vacuole formation in *Saccharomyces cerevisiae*, *Glycobiology* 25 (2015) 1079–1089, <http://dx.doi.org/10.1093/glycob/cwv045>.
- [155] R.A. Moreau, K.B. Hicks, The in vitro hydrolysis of phytosterol conjugates in food matrices by mammalian digestive enzymes, *Lipids* 39 (2004) 769–776, <http://dx.doi.org/10.1007/S11745-004-1294-3>.