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# Lipids in plant–microbe interactions☆

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ABSTRACT

Bacteria and fungi can undergo symbiotic or pathogenic interactions with plants. Membrane lipids and lipid-derived molecules from the plant or the microbial organism play important roles during the infection process. For example, lipids (phospholipids, glycolipids, sphingolipids, sterol lipids) are involved in establishing the membrane interface between the two organisms. Furthermore, lipid-derived molecules are crucial for intracellular signaling in the plant cell, and lipids serve as signals during plant–microbial communication. These signal lipids include phosphatidic acid, diacylglycerol, lysophospholipids, and free fatty acids derived from phospholipase activity, apocarotenoids, and sphingolipid breakdown products such as ceramide, ceramide-phosphate, long chain base, and long chain base-phosphate. Fatty acids are the precursors for oxylipins, including jasmonic acid, and for azelaic acid, which together with glycerol-3-phosphate are crucial for the regulation of systemic acquired resistance. This article is part of a Special Issue titled "Plant Lipid Biology," guest editors Kent Chapman and Ivo Feussner.



## 1. Introduction

Lipids are major constituents of prokaryotic and eukaryotic membranes. Besides serving as structural components of the plasma membrane and intracellular membranes, they provide diverse biological functions in energy and carbon storage, signal transduction, and stress responses [1]. Plants contain a diverse set of lipids, including fatty acids, phospholipids, glycolipids, sterol lipids, sphingolipids, and waxes. As plants are constantly exposed to soil, water, and air, they interact with a multitude of organisms, which can be mutualistic, parasitic, or pathogenic. When plant cells come into contact with microbes, molecular information between the host and the microbial invader is exchanged. Cuticular waxes establish a physical barrier on epidermal cell surfaces, modulate the communication between host and microbe, affect pathogen development, serve as signaling molecules, or provide elicitors for the recognition of pathogen attack. Elicitors are signal-inducing compounds recognized by the innate immune system, resulting in defense response induction. The elicitors can be derived from the plant or the microbe. The perception of chemical structures triggers immune responses induced by microbe or pathogen-associated molecular patterns (MAMPs/PAMPs). MAMPs describe common microbial molecules, including those originating from beneficial microbes. PAMPs are molecules specifically derived from pathogenic microbes. These molecules are often recognized by plasma membrane-resident pattern recognition receptors (PRR), and these responses must be suppressed for successful pathogen reproduction in the host plant [2].

Our knowledge on the role of lipids in plant–microbe interactions has strongly increased since the availability of highly sensitive analytical technologies, including gas chromatography and high-pressure liquid chromatography coupled to mass spectrometry [1,3]. The present review focuses on recent advances in our understanding of the numerous roles of lipids and lipid-modifying enzymes in plants during interactions with symbionts, pathogen perception, signal transduction, and downstream defense responses. As this area of research has seen a tremendous increase in the recent years, it was not possible to include all the contributions on this topic, and we would like to apologize to all authors whose manuscripts could not be cited due to restriction in space.

*Abbreviations:* ACP, acyl-carrier protein; AOC, allene oxide cyclase;; AOS, allene oxide synthase; ASG, acylated sterol glucoside; AzA, azelaic acid; CCD, carotenoid cleavage dioxygenase; Chol-P, choline-phosphate; CL, cardiolipin; DAG, diacylglycerol; ER, endoplasmic reticulum; ERM, extraradical mycelium; FA, fatty acid; FAS, fatty acid synthase; FS, free sterols; GIPC, glucosyl inositol phosphoceramide; GlcCer, glucosyl ceramide; Gro3P, glycerol-3-phosphate; IRM, intraradical mycelium; JA, jasmonic acid; JAZ, jasmonate ZIM domain; LCB, long chain base; LOX, lipoxygenase; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; MAMP, microbe-associated molecular pattern; OPDA, oxophytodienoic acid; PA, phosphatidic acid; PAMP, pathogen-associated molecular pattern; PC, phosphatidylcholine; PCD, programmed cell death; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PLA, phospholipase A; PLC, phospholipase C; PLD, phospholipase D; PR, pathogen related; PS, phosphatidylserine; ROS, reactive oxygen species; SA, salicylic acid; SAR, systemic acquired resistance; SE, sterol esters; SG, sterol glucoside; TAG, triacylglycerol; Fatty acids are abbreviated as X:Y, where X denotes the number of carbon atoms and Y the number of double bonds in the acyl chain. The position of the double bond is counted from the carboxy (Δ) or methyl (ω) end. Hydroxylated fatty acids are indicated as hX:Y., Long chain bases are depicted as d18:0, d18:1, t18:0, or t18:1 with d and t indicating the number of hydroxyl groups (2 or 3), 18 is the number of carbon atoms, and 0 or 1 the number of double bonds.

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## 2. Plant lipids

Membrane lipids are key players in plant cells during the response to microbial attack and during interactions with beneficial microbes. The expression of several genes encoding enzymes of lipid metabolism is upregulated after infection of plant cells, resulting in the synthesis, modification, or re-allocation of lipid-derived molecules. Lipid-modifying enzymes are essential regulators of the spatial and temporal production of lipid metabolites involved in signaling and membrane proliferation for the establishment of intracellular compartments or compositional changes of lipid bilayers [4].

### 2.1. Phospholipids and phospholipases

Phospholipids contain two fatty acids esterified to the *sn*-1 and *sn*-2 positions of a glycerol backbone, and a polar headgroup attached to the *sn*-3 position. Phospholipids of plants mainly comprise phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and phosphatidylinositol (PI). Each phospholipid class includes many molecular species due to a large number of fatty acids varying in chain length and degree of desaturation. Phospholipids are predominantly synthesized at the endoplasmic reticulum (ER). Glycerolipid biosynthesis will be discussed in more detail in [5]. Phospholipases and phospholipid-derived molecules are involved in signaling and plant immunity during plant–pathogen interactions [6,7]. Phospholipases catalyze the conversion of phospholipids into fatty acids and lysophospholipids, diacylglycerol (DAG), or PA, depending on their positional specificity (Fig. 1). Upon microbe infestation, phospholipid-hydrolyzing enzymes are activated, contributing to the establishment of an appropriate defense response by inducing the production of defense-signaling molecules such as oxylipins, including jasmonic acid (JA), and the potent second messenger PA. Changes in phospholipid content and phospholipase activities during host–pathogen interactions or in elicited plant cells have been the focus of numerous studies [4,6,8].

#### 2.1.1. Phospholipase D

Phospholipase D (PLD) cleaves the terminal phosphodiester bond of phospholipids, resulting in the formation of PA. PLDs have diverse functions in lipid metabolism and hormone signaling (abscisic acid, ABA; JA) and during responses to biotic and abiotic stress [8]. PLDs and PLD-derived PA play important roles in the plant defense response [9]. In *Arabidopsis thaliana*, *PLDβ1* is a positive regulator of the pathogen-induced JA production and plant resistance to the necrotrophic fungal pathogen *Botrytis cinerea* [9]. The levels of PA and JA were decreased in PLDβ1-deficient *Arabidopsis* plants after infection with *B. cinerea*, and JA-dependent defense gene expression was compromised. Simultaneously, *PLDβ1* downregulates the salicylic acid (SA)-dependent signaling pathway and is involved in plant tolerance to *Pseudomonas syringae* tomato pv DC3000 (*Ps*t DC3000). The infection of *PLDβ1*-deficient plants resulted in reduced bacterial growth accompanied by increased

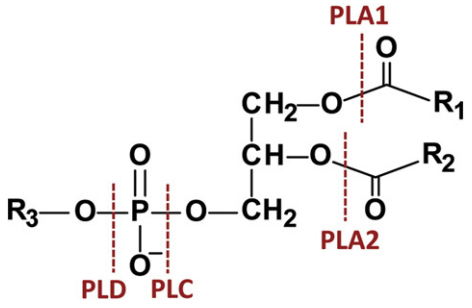


**Fig. 1.** Phospholipases. The phospholipases PLD, PLC, and PLA cleave phospholipids at different sites of the phospholipid molecule, as indicated by dashed lines. PLA1 and PLA2 activities are specific for the acyl groups at *sn*1 and *sn*2, respectively.

accumulation of reactive oxygen species (ROS) [4]. After incubation of tomato (*Solanum lycopersicum*) suspension cultures with the non-specific elicitor xylanase, the PLD pathway was activated within minutes [10], accompanied by an increased *PLDβ1* expression [11]. Furthermore, different lysophospholipids such as lysophosphatidylethanolamine (LPE), lysophosphatidylcholine (LPC), and lysophosphatidylglycerol (LPG) accumulated in *PLDβ1*-deficient mutants [4]. Lysophospholipids were recently identified as important signaling molecules mediating plant defense responses [12]. PLD-derived PA often directly binds to proteins, leading to alterations in protein localization or enzyme activity. PLD-derived PA targets more than 30 proteins involved in diverse physiological pathways, although the roles of PLD and PA in numerous stress responses remain elusive [13]. PA regulates a range of physiological processes such as the activity of kinases, phosphatases, phospholipases, and proteins involved in membrane trafficking, $Ca^{2+}$ signaling, or the oxidative burst [7,9,14]. Membrane-bound PA acts as a specific membrane-docking site for PA-binding proteins [7,8,15]. In addition, PA serves as the precursor for the lipid intermediates LPA, DAG, and free fatty acids, all of which can be involved in plant defense signaling [14]. PA levels were increased upon pathogen infection or elicitor treatment in rice (*Oryza sativa*) [16], tomato [10], tobacco (*Nicotiana tabacum*) [17], and *Arabidopsis* [18].

#### 2.1.2. Phospholipase C

The group of phospholipases C (PLC) in plants can be divided into three families according to substrate specificity and cellular function: (i) PC-PLCs or non-specific PLCs that hydrolyze PC and other phospholipids, (ii) phosphatidylinositol-4,5-bisphosphate ($PIP_2$)-PLCs (PI-PLC) that act on phosphoinositides, and (iii) glycosylphosphatidylinositol (GPI-PLCs) that hydrolyze GPI anchors on proteins. *Arabidopsis* contains six *PC-PLC* genes (non-specific phospholipases, NPC1–NPC6) with sequence similarities to bacterial non-specific PLCs. The expression of two genes (*NPC4*, *NPC5*) is strongly induced upon low Pi conditions, implicating an involvement in phospholipid turnover [19,20]. PI-PLCs cleave $PIP_2$, producing DAG and $IP_3$ (1,4,5-inositol trisphosphate) both acting as second messengers. PI-PLC activity is stimulated in plants in response to pathogenic infection [21]. PAMP recognition triggers the activation of the PLC/DAG kinase pathway, resulting in the accumulation of PA [10,16]. Thus, PA in part originates from DAG produced by PLC because DAG can be further phosphorylated by DAG kinase (DGK) [15]. In *Arabidopsis*, the recognition of the *P. syringae* avirulence proteins AvrRpm1 or AvrRpt2 leads to a biphasic accumulation of PA, with the first wave attributed to PLC/DGK activities whereas the majority of pathogen-induced PA is synthesized through PLD activity in a second wave [15]. Furthermore, the tomato PLC isoform SlPLC4 is required for the recognition of the pathogenic fungus *Cladosporium fulvum* (*Cf*) and for the expression of the corresponding avirulence gene (*AVR4*). PA rapidly accumulates in tomato suspension culture cells expressing the *Cf4* resistance gene after treatment with the pathogen effector Avr4. This increase is dependent on the PLC/DGK pathway [15]. Silencing of *SlPLC4* increased the susceptibility of *Cf4* plants to *C. fulvum* [21]. An additional tomato PLC isoform, SlPLC6, is involved in the establishment of general plant defense responses against different pathogens [22]. Furthermore, PLC does not only play a role in elicitor recognition processes, but also in downstream disease resistance signaling [23]. The expression of *PLC1* from *O. sativa* was highly induced by different chemical and biological inducers of plant defense pathways leading to disease resistance [24]. Nine PI-PLC sequences are known in *Arabidopsis*, and the expression of most *PI-PLC* genes is induced during plant defense responses [25]. Likewise, DGK expression is also induced during microbial elicitation, generating PA as a key factor in plant basal resistance [6].

#### 2.1.3. Phospholipase A

The phospholipase A (PLA) superfamily is divided into $PLA_1$ and $PLA_2$ families. PLA enzymes catalyze the hydrolysis of the acyl ester bonds of phospholipids at their *sn*-1 and *sn*-2 positions, respectively, yielding

free fatty acids and lysophospholipids. $PLA_2$-derived LPC and LPE are involved in systemic responses after wounding [13]. Lysophospholipids are further hydrolyzed by lyso-PLAs yielding glycerophosphodiesters. PLAs are believed to be involved in the regulation of plant growth, root and pollen development, stress responses, and defense signaling [26]. They have been mainly linked to plant immunity through their role in oxylipin and JA biosynthesis and the stimulation of downstream defense products [27]. Furthermore, PLA is important during the oxidative burst which is induced by certain biotic elicitors and protects the plant against pathogens. The oxidative burst is one of the earliest plant defense responses and leads to ROS production [28]. The link between oxidative burst and PLA activity was demonstrated by applying extracts of the pathogenic fungus *Verticillium dahliae*, thereby inducing PLA activity and ROS production in soybean cells [29].

#### 2.1.4. Glycerol-3-phosphate and azelaic acid in systemic acquired resistance

Systemic acquired resistance (SAR) represents a whole plant defense response and is based on an previous infection. SAR is directed against a wide spectrum of pathogens and leads to enhanced resistance during subsequent rounds of infections. The phytohormone salicylic acid (SA) is involved in regulating plant–pathogen interactions. SA is essential to trigger the systemic response of SAR, but it is not the initial signal of the effector-triggered response (ETI) at the site of infection that leads to SAR [30]. There has been a long debate about the question how the signal of the primary infection is transported to distal (systemic) plant organs. Small molecules, some derived from lipid metabolism, are believed to mediate SAR. While SA was ruled out as a mobile SAR signal, methyl-salicylic acid was implicated in being produced at the site of infection and moved to distal plant organs, where it could be hydrolyzed to SA and trigger SAR. However, *Arabidopsis* mutant plants deficient in SA methyltransferase activity still showed SAR, suggesting that the transfer of the mobile signal is more complex [30]. In addition to methyl-salicylic acid, glycerol-3-phosphate (Gro3P), azelaic acid (AzA), the isoprenoid-derived dehydroabietanal, nitric oxide (NO), and ROS were implicated in mediating SAR [31–33].

Besides pathogen infections, two further plant–microbe interactions can be described, i.e., "real" mutualistic symbiotic interactions (e.g., arbuscular mycorrhiza formation, nodulation with rhizobial bacteria; see below) and interactions with beneficial, growth-promoting bacteria. Growth-promoting bacteria mostly found in the rhizosphere can stimulate the plant immune system, resulting in induced systemic resistance (ISR), which is similar to SAR, mediating resistance to a broad spectrum of diseases [34]. SAR and ISR partly overlap and share common signaling components, but ISR action in distal organs is mainly based on the action of the phytohormone JA in contrast to SAR which is based on SA [34].

Gro3P contributes to basal resistance against the hemibiotrophic fungus *Colletotrichum higginsianum* and represents a key factor in SAR [35]. The enzymatic products of PLA and lyso-PLA activities are glycerophosphodiesters, which are further hydrolyzed by glycerophosphodiester phosphodiesterases yielding Gro3P. Gro3P accumulation is a highly conserved process in different organisms [36]. In addition to glycerophosphodiester phosphodiesterases, Gro3P can be synthesized via the glycerol kinase pathway, or via the reduction of dihydroxyacetone phosphate through Gro3P dehydrogenase. The genome of *Arabidopsis* harbors five Gro3P-dehydrogenase genes and one glycerol kinase gene. In plants, Gro3P is a proposed regulator of plant defense signaling, an important component of diverse energy producing reactions and the precursor for glycerolipid biosynthesis [35,36].

Pathogen infection results in the release of free unsaturated fatty acids, which serve as precursors for AzA. Oxidative cleavage of unsaturated fatty acids (18:1, 18:2, 18:3) carrying a double bond at position 9 results in AzA synthesis [37]. AzA is a C-9 dicarboxylic acid and a general oxidative stress signal which has been implicated in SAR [38,39] (Fig. 2). AzA-induced SAR depends on Gro3P, and accumulation of AzA in turn induces Gro3P synthesis [37]. Increased levels of AzA stimulate Gro3P synthesis even in the absence of pathogen infection, providing evidence for an interconnection between AzA- and Gro3P-induced SAR [37]. High Gro3P levels are required for the transcriptional stability of the transfer proteins defective in induced resistance 1 (DIR1) and DIR-like [40] as well as azelaic acid induced 1 (AZI1) [39], which are in turn required for the pathogen-induced biosynthesis of Gro3P. Furthermore, DIR1 and AZI1 might act in a positive feedback loop with Gro3P promoting SAR downstream of AzA [35,37,39,40] (Fig. 2).

### 2.2. Glycolipids

Glycolipids are abundant membrane components in chloroplasts of plants and algae and in cyanobacteria, and some bacterial phyla [41]. Galactolipids make up the major glycolipid fraction in plants because monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) constitute about 50% and 20% of total glycerolipids in chloroplasts, respectively. A minor but also important glycolipid is the sulfolipid sulfoquinovosyldiacylgylcerol. The biosynthesis and function of galactolipids and sulfolipids were summarized previously [41]. Here, the focus will be on the role of galactolipids in plant–microbe interactions.

Galactolipids play important roles in signal transduction, cell communication and pathogen responses. In the *Arabidopsis mgd1* mutant deficient in MGDG synthesis, SAR was compromised during a secondary infection with the virulent pathogen *P. syringae* pv. *maculicola* ES4326 (*Psm*) after the primary inoculation with the Avr pathogen *P. syringae* pv. *tomato* DC3000, suggesting that galactolipids are required for SAR [42]. Later, Gao et al. (2014) showed that SAR is compromised in the *Arabidopsis dgd1* mutant deficient in DGDG synthesis [33]. The two galactolipids apparently have different functions in SAR because MGDG regulates the biosynthesis of AzA and Gro3P, while DGDG affects the biosynthesis of NO and SA and is also required for AzA -induced SAR in *Arabidopsis* (Fig. 2). In addition to the *dgd1* mutant, a *dgd1* mutant line (*dgd1*-GlcT) complemented with a bacterial glucosyltransferase was employed for SAR assays. The *dgd1*-GlcT line grows like wild type, but accumulates a glucosylgalactosyl diacylglycerol lipid instead of DGDG. Interestingly, the replacement of the terminal galactose moiety by glucose is unable to restore the SAR in *Arabidopsis dgd1* mutants although it can rescue the morphological and fatty acid phenotype of the mutant suggesting that the digalactosyl moiety of DGDG plays a special role during SAR [33]. Besides SAR, galactolipids are also involved in additional plant–microbe interactions. For example, Gaude et al. (2004) detected DGDG in the peribacteroid membrane during root nodule symbiosis [43]. Presumably, the accumulation of DGDG in the peribacteroid membrane of nodules helps to save phosphate because of a reduced requirement for phospholipids.

### 2.3. Free fatty acids

Fatty acids are components of glycerolipids and thus are major structural constituents of the cell. In addition, fatty acids and fatty acid metabolites also function as modulators of many signal transduction pathways. Free fatty acid levels increase in response to different stresses and play a crucial role in plant–microbe interactions. Fatty acids can be produced by *de novo* synthesis or are released from glycerolipids by lipases and subsequently converted into bioactive lipid mediators. The fatty acid *de novo* synthesis in plants is localized to the chloroplasts. A key step in fatty acid biosynthesis is the desaturation of 18:0 (stearic acid) to 18:1 (oleic acid), the latter acting as a signal of biotic stress responses via NO [44]. This desaturation step is catalyzed by a stearoyl-acyl-carrier protein desaturase (SSI2) in *Arabidopsis*. Changes in 18:1 levels result in alterations of SA- and JA-mediated defense responses [45]. The *Arabidopsis ssi2* mutant (allelic to *fab2*) accumulates high levels of 18:0 and low levels of 18:1 [46]. In this mutant, the SA-mediated defense-signaling pathway is constitutively active, causing

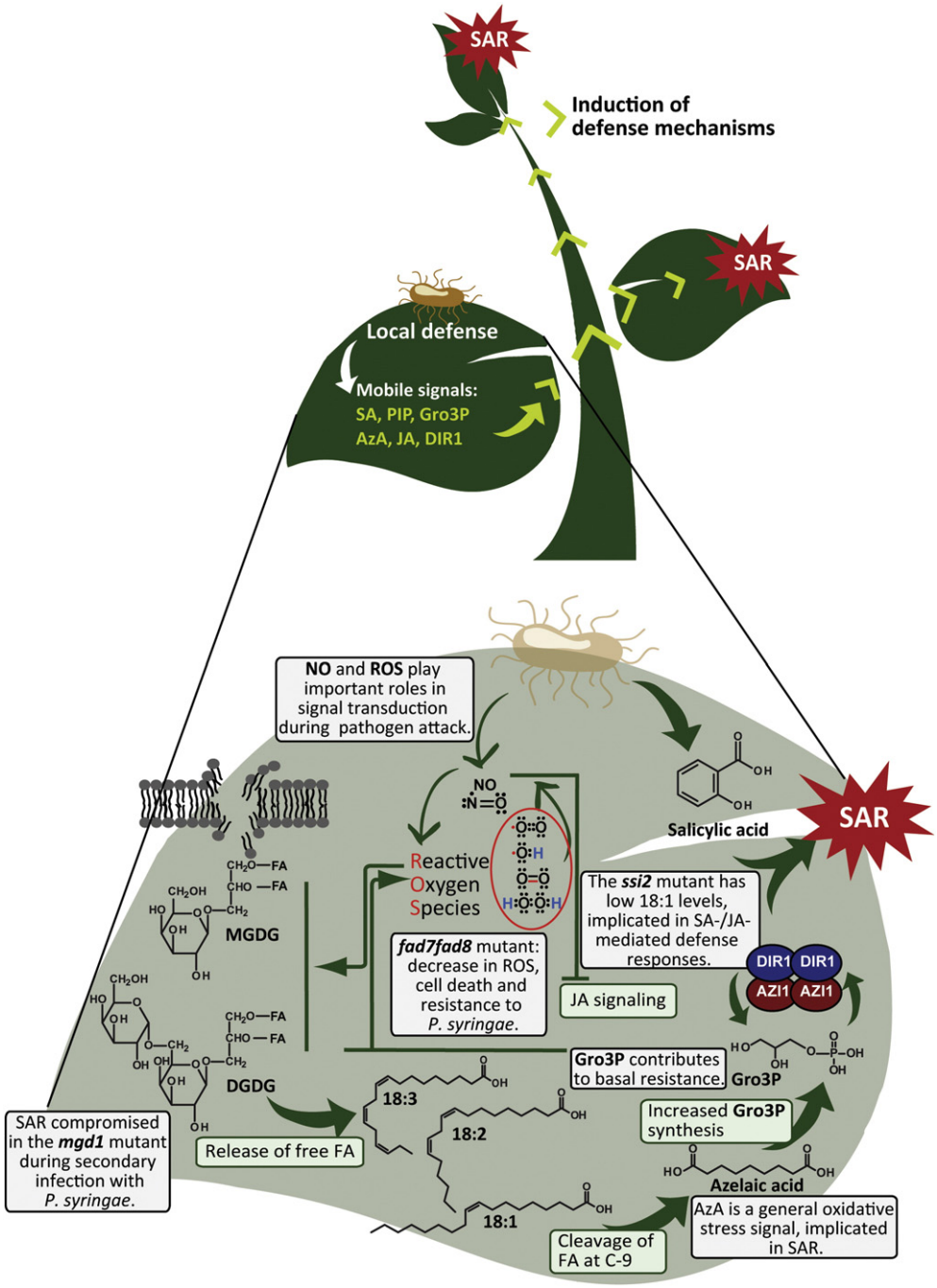


**Fig. 2.** Lipid-based long distance signaling for SAR. After pathogen attack, SA levels rise in the primary infected tissue and trigger a SAR response. The *mgd1* mutant deficient in MGDG synthesis shows reduced SAR during secondary infection with *P. syringae* [33]. MGDG can serve as a substrate for JA synthesis during defense responses [54]. Free fatty acids are released and cleaved at C-9 yielding AzA. AzA is a general oxidative stress signal implicated in SAR [38]. Increased synthesis of Gro3P contributes to basal resistance and to SAR [35]. NO and ROS are also implicated in mediating SAR [31]. ROS-mediated lipid peroxidation is induced by biotic stress. NO and ROS play important roles in signal transduction in response to pathogen attack [28]. The *fad7fad8* double mutant is deficient in trienoic fatty acids and show reduced ROS accumulation, cell death, and lower resistance to *P. syringae* [47]. The *ssi2* mutant accumulates low levels of 18:1. 18:1 is implicated in SA- and JA-mediated defense response [45,46]. AzA, azelaic acid; AZI1, azelaic acid induced 1; DGDG, digalactosyldiacylglycerol; DIR1, defective in induced resistance 1; FA, fatty acid; Gro3P, glycerol-3-phosphate; JA, jasmonic acid; MGDG, monogalactosyldiacylglycerol; NO, nitric oxide; OPDA, oxophytodienoic acid, PIP, phosphatidylinositol phosphates; ROS, reactive oxygen species; SA, salicylic acid; SAR, systemic acquired resistance.

lesion formation and an increased the expression of genes related to pathogenesis (*PR* genes) (Fig. 2). Besides 18:1, trienoic fatty acids (16:3, 18:3) play important roles in the regulation of plant defense responses. Trienoic fatty acids are the most abundant fatty acids in plant membranes, particularly in the galactolipids in *Arabidopsis*. 18:3 is also involved in defense responses against avirulent bacterial pathogens. Low levels of 18:3 in the *Arabidopsis fad7fad8* mutant deficient in two ω-3 desaturases (FADs) led to a decrease in ROS accumulation, cell death initiation, and resistance to avirulent strains of *P. syringae* [47].

### 2.4. Glycerolipid synthesis during arbuscular mycorrhiza formation

Arbuscular mycorrhiza formation is an ancient endomycorrhizal symbiosis between arbuscular mycorrhizal fungi and most land plants.

After infection, the fungi (Glomeromycota, e.g., *Rhizophagus irregularis*) produce hyphae which grow within the root and produce arbuscules inside infected root cells. The arbuscules are covered with a host-cell-derived periarbuscular membrane, the site of nutrient exchange between the plant and the fungus. Phosphate, acquired from the soil by the extraradical hyphae, is delivered to the plant root in exchange of organic compounds derived from photosynthesis.

A number of recent studies showed differential regulation of genes involved in plant lipid metabolism during mycorrhization [48–52] (Fig. 3). Among others, genes encoding enzymes of fatty acid synthesis export from the plastid and glycerolipid synthesis are strongly upregulated, indicating that increased amounts of lipids are produced by the root during mycorrhization. This lipid demand might be explained by the requirement of phospholipids to establish the large surface of the periarbuscular membrane and by the accumulation of triacylglycerol in the fungus.

Different lipid-derived signals were found to be involved in arbuscular mycorrhiza formation [49,53]. In the *Medicago truncatula* mutant *ram2*, mycorrhiza formation was severely impaired [53]. *RAM2* shows sequence similarities with the Gro3P acyltransferase genes *GPAT5* and *GPAT6* from *Arabidopsis* involved in suberin and cutin biosynthesis, respectively. Like *Arabidopsis* GPAT6, RAM2 has a functional phosphatase domain, and the *M. truncatula ram2* mutant could be complemented with *Arabidopsis GPAT6*. Furthermore, the overexpression of RAM2 in *Arabidopsis* led to the accumulation of ω-hydroxy-fatty acids (OHFAs) and α,ω-dicarboxylic acids (DCAs). The authors concluded that RAM2 is involved in the synthesis of ω-hydroxy and α,ω-dicarboxylic acids in *M. truncatula*, and that these fatty acids, usually associated with cutin and suberin, are important for arbuscular mycorrhiza formation. Because the application of C16 ω-hydroxy-fatty acids and 1,16-hexadecanediol rescued the reduced hyphopodia formation of *ram2* plants, a role for RAM2 in signaling was proposed.

### 2.5. Oxylipins

Lipid peroxidation can be induced by abiotic and biotic stresses and plays an important role in signal transduction and during programmed cell death (PCD). Lipid peroxidation can be mediated via enzymatic (lipoxygenase, LOX) or non-enzymatic (ROS) pathways. Besides galactolipids, free fatty acids or acyl groups bound to triacylglycerol can be subject to lipid peroxidation in plants. For example, in addition to free fatty acids, MGDG can serve as an alternative substrate to generate jasmonate (JA) during defense responses in soybean [54].

JA-related oxylipins are derived from the 13-lipoxygenase reaction of 18:3, 18:2, or 16:3. The products of these reactions (hydroperoxidienoic acid or hydroperoxitrienoic acid) can be used for the synthesis of *cis*-oxophytodienoic acid (OPDA) or dinor-OPDA following the allene oxide synthase (AOS) pathway. After export from the chloroplast, OPDA and dinor-OPDA can be converted into JA. Alternatively, the lipoxygenase products hydroperoxidienoic acid and hydroperoxitrienoic acid can be cleaved by the hydroperoxide lyase reaction, resulting in the release of short-chain oxylipin molecules such as *cis*-3-hexenal or *trans*-2-hexenal. In the cytosol, JA can be covalently linked to isoleucine (jasmonyl-Ile) or tryptophane (jasmonyl-Trp). Together, JA, jasmonyl-Ile, jasmonyl-Trp, OPDA, and dinor-OPDA constitute the class of JA-related molecules with signaling function in plants [55]. The biosynthesis and the function of JAs and related products are described in detail in Koo et al. (2016) in this issue [56]. Here, we will focus on the roles of JAs in plant–pathogen and symbiotic interactions with bacteria and fungi. JAs are produced in particular after wounding. Therefore, JAs accumulate after herbivore attack, but also after infections with microbes [57]. JAs are important for defense responses to different fungal and bacterial pathogens [58,59]. In *Arabidopsis* the lipoxygenase reaction is predominantly confined to the chloroplast, and precedes PCD after exposure to *P. syringae* [38].

The fungus *Aspergillus nidulans* produces a set of oxylipins related to plant oxylipins. These so-called psi factors, 8-hydroxyoleic acid, 8-hydroxylinoleic acid, 8-hydroxylinolenic acid, leukotrienes, and prostaglandins, are structurally similar to oxylipins derived from 18:2 or 18:3 fatty acids. Thus, it has been speculated that plants and fungi "communicate" via the oxylipin language [60]. In agreement with this hypothesis, the application of plant-derived fatty acid hydroperoxides affects development of *Aspergillus*, while fungal oxylipins modulate the plant's JA response [60]. Interestingly, *P. syringae* produces a phytotoxic compound, coronatine, which is a structural mimic of jasmonoyl-Ile [61]. Upon infection, coronatine binds to the coronatine insensitive 1 (COI1) protein, which is part of the co-receptor complex perceiving JA, thereby triggering chlorosis and senescence in the plant [62].

Exogenously applied JA negatively affects nodulation on *Medicago truncatula* roots by the inhibition of cell cycle and nodule primorida

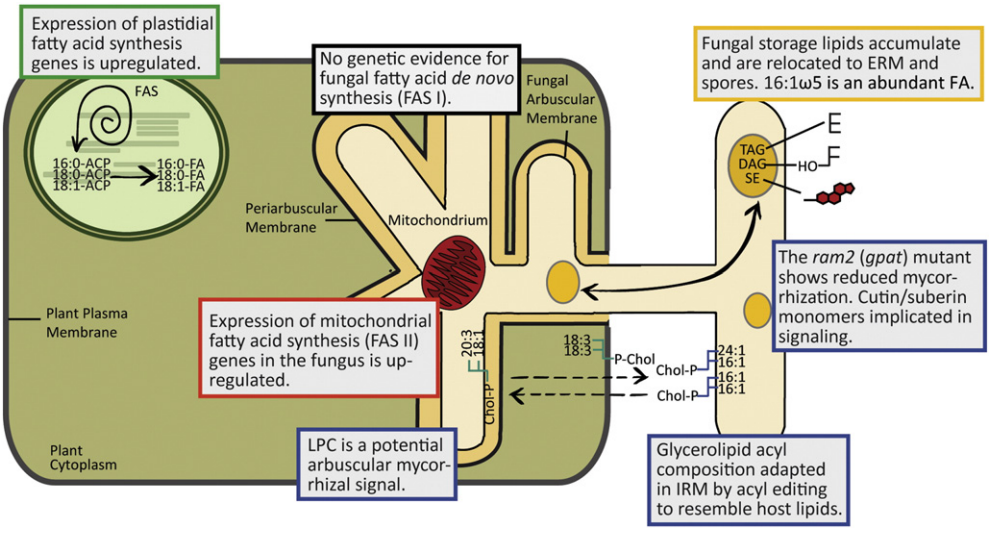


**Fig. 3.** The role of lipids during arbuscular mycorrhiza formation. Expression of plastidial fatty acid synthesis genes is upregulated [48–51]. There is no genetic evidence for fungal fatty acid *de novo* synthesis (FAS I). The fungus might be dependent on plant-derived FA. [50,113,114,153]. Fungal storage lipids accumulate and are relocated to ERM and spores. 16:1 ω5 is an abundant FA and can serve as mycorrhizal marker [112]. LPC is a potential arbuscular mycorrhizal signal [49]. Expression of mitochondrial fatty acid synthesis (FAS II) genes in the fungus is upregulated [49]. The *ram2* (*gpat*) mutant shows reduced mycorrhization. Cutin/suberin monomers are implicated in signaling [53]. Glycerolipid acyl composition is adapted in IRM by acyl editing to resemble the host lipids [50]. ACP, acyl-carrier protein; Chol-P, choline-phosphate; DAG, diacylglycerol; ERM, extraradical mycelium; FA, fatty acid; FAS, fatty acid synthase; IRM, intraradical mycelium; LPC, lysophosphatidylcholine; SE, sterol esters; TAG, triacylglycerol.

formation [63,64]. JA inhibits calcium spiking of root nodules at high concentrations and decreases the frequency of calcium spiking at lower concentrations [64]. Furthermore, some plants, including soybean and wheat secrete JAs from the roots, and JA application on rhizobial bacteria increases the expression of nod factors, a prerequisite for root colonization and nodule formation [63,65].

Colonization by arbuscular mycorrhizal fungi causes an accumulation of JA in barley roots [63,66]. In invaded cells of *Medicago truncatula* and barley, the expression of JA biosynthetic genes (allene oxide cyclase, AOC; allene oxide synthase, AOS) was upregulated [66,67]. In agreement with a role of JA during arbuscular mycorrhiza infection, the downregulation of AOC expression by RNAi resulted in a delay in mycorrhizal colonization and reduction in arbuscule numbers [63,67].

In tomato roots, mycorrhiza infection resulted in the upregulation of the expression of AOS1, methyl jasmonate esterase (JAME), and the jasmonate ZIM domain 2 (JAZ2) genes involved in the 13-lipoxygenase pathway (leading to 12-OPDA and JA production). Furthermore, the expression of lipoxygenase A (LOXA), AOS3, and divinylether synthase (DES), genes related to the 9-LOX pathway, was induced upon mycorrhization. The 9-lipoxygenase pathway results in the production of 10-OPDA, an isomer of the 13-lipoxygenase-derived "normal" 12-OPDA, and of colnelenic and colneleic acids (via the divinylether synthase DES). The 9-LOX pathway seems to be root specific and might be involved in arbuscular mycorrhiza formation [68,69].

Genes regulated by JA are upregulated during the colonization of *Populus* with the ectomycorrhizal fungus *Laccaria bicolor* and JA application to poplar roots affects fungal colonization [70]. The MiSSP7 protein (mycorrhiza-induced small secreted protein 7) produced by *Laccaria* blocks JA action in the host plant by interaction with JAZ6 protein of poplar. Binding of MiSSP7 to JAZ6 protects JAZ6 from JA-dependent degradation, thereby suppressing the negative impact of JA on colonization of poplar by *Laccaria* [71].

### 2.6. Sterol lipids

Sterol lipids are synthesized via the isoprenoid pathway in the cytosol of plant cells (Fig. 4). Sterols can occur in their free form (free sterols) or are derivatized at the C3 hydroxy group (conjugated sterols). Free sterols (FS) and the conjugated forms of sterol glucosides (SG) and acylated sterol glucosides (ASG) are constituents of extraplastidial membranes, while sterol esters (SE) are deposited in oil bodies in the cytosol. The major sterols in plants (phytosterols) are stigmasterol, β-sitosterol, and campesterol, while cholesterol is of low abundance. In *Arabidopsis* leaves, free sterols make up the largest fraction of total sterols, while sterol glucosides and acylated sterol glucosides are less abundant [3]. Sterol esters are also of low abundance but their content increases during abiotic and biotic stress

#### 2.6.1. Free sterols

Stigmasterol plays a vital role in *Arabidopsis* during resistance to virulent and avirulent *P. syringae* strains [72]. Infection with *P. syringae* stimulates stigmasterol synthesis in *Arabidopsis* via desaturation of β-sitosterol by the cytochrome P450 enzyme *CYP710A1*. Stigmasterol differs from β-sitosterol only by the presence of the C22 double bond. The stigmasterol to β-sitosterol ratio increases in membranes at sites of infection with *P. syringae*, rendering *Arabidopsis* plants more susceptible to primary bacterial infections than *CYP710A1*-deficient mutants. However, the induction of SAR in *Arabidopsis* wild type and *CYP710A1* mutant plants is not affected indicating that SAR is independent of stigmasterol. The sterol desaturation response is also induced with non-virulent bacteria and only occurs locally on inoculated leaves. The conversion of β-sitosterol to stigmasterol in *Arabidopsis* plants infected with *P. syringae* strains with mutated type III secretion systems is compromised but can be induced by typical microbial elicitors, like flagellin, and also by the fungus *B. cinerea*. Therefore, the conversion of β-sitosterol to stigmasterol is locally induced by a rather universal MAMP recognition system. The molecular basis for the relationship between phytopathogenic proliferation and increased stigmasterol content is unknown. Interestingly, the increase in resistance of the *Arabidopsis CYP710A1* mutant is attenuated after exogenous stigmasterol application, while β-sitosterol application had the opposite effect. These results show a correlation between an increased stigmasterol/β-sitosterol ratio and bacterial virulence, probably due to changed membrane integrity resulting from the shift in sterol lipids

A further study on sterol biosynthesis mutants confirmed the accumulation of stigmasterol in *Arabidopsis* leaves when challenged with different pathovars of *P. syringae* [73]. In this work, three mutants impaired in different steps in stigmasterol biosynthesis were used, squalene synthase (SQS), sterol methyltransferase 2 (SMT2), and β-sitosterol desaturase (CYP710A1). The decreased stigmasterol contents in the three mutants were, in contrast to the previous findings, correlated with increased bacterial virulence, while the overexpression of CYP710A1 led to decreased proliferation of bacteria. Silencing of SQS in *Nicotiana benthamiana* also led to increased electric conductivity of apoplastic fluid and increased apoplastic colonization with *P. syringae*. Therefore, it was concluded that the *P. syringae* induced accumulation of stigmasterol in membranes represents a defense response of plants to prevent unwanted nutrient efflux and thus bacterial proliferation in the apoplast. While the two studies revealed that stigmasterol biosynthesis is induced upon microbial contact, the precise role in the interplay between plants and bacteria remains to be resolved.

#### 2.6.2. Sterol esters

The enzyme phospholipid:sterol acyltransferase 1 (PSAT1) catalyzes the synthesis of sterol esters from free sterols using a phospholipid as acyl donor (Fig. 4). In *Arabidopsis psat1* mutant plants, *Phytophtora infestans*, *Blumeria graminis,* and *Golovinomyces cichoracearum* inoculation led to hyperaccumulation of callose and enhanced cell death in mesophyll cells, a response that was absent when challenged with *P. syringae* [74]. However, the *psat1* mutant did not show increased pathogen proliferation that could be correlated with the increased callose deposition. These results suggest that *Arabidopsis* employs a specific PAMP recognizing signaling response to filamentous pathogens that involves sterol ester homeostasis.

Bhat et al. (2005) used the sterol-specific fluorochrome filipin to visualize the aggregation of sterol lipids in the tip of appressorial germ tubes and the septum of *B. graminis* after germination on barley leaves [75]. Most importantly, filipin staining showed circular enrichment in epidermis cells beneath appressoria at 12–15 h post inoculation. The authors concluded that the enhanced filipin signal is the result of aggregation of sterol lipids in the plasma membrane, forming microdomains (lipid rafts) enriched in sterol lipids and specific plasma membrane-resident proteins. Proteins that are required for penetration of *B. graminis*, encoded by the genes *mildew resistance locus o* (*Mlo*) and ROR2 syntaxin, were recruited to these microdomains beneath fungal entry points and this was independent of actin-mediated vesicle trafficking and was thus likely due to sterol clustering rather than targeted transport of vesicles.

### 2.7. Carotenoids and apocarotenoids

Carotenoids are C-40 polyenes present in all photosynthetic organisms and serve as accessory pigments in the light harvesting complex, as photoprotectants and antioxidants. Some non-photosynthetic pathogens such as *Erwinia herbicola* are able to synthesize carotenoids to protect their metabolism against ROS during plant colonization. In the sweet corn pathogen *Pantoea stewartii* subsp. *Stewartii*, the disruption of a gene homologous to phytoene synthase, *crtB*, which catalyzes the first step in carotenoid biosynthesis, results in decreased virulence. The *P. stewartii crtB* mutant was devoid of β-carotene and more sensitive to $H_2O_2$ and UV light, and it was postulated that β-carotene is

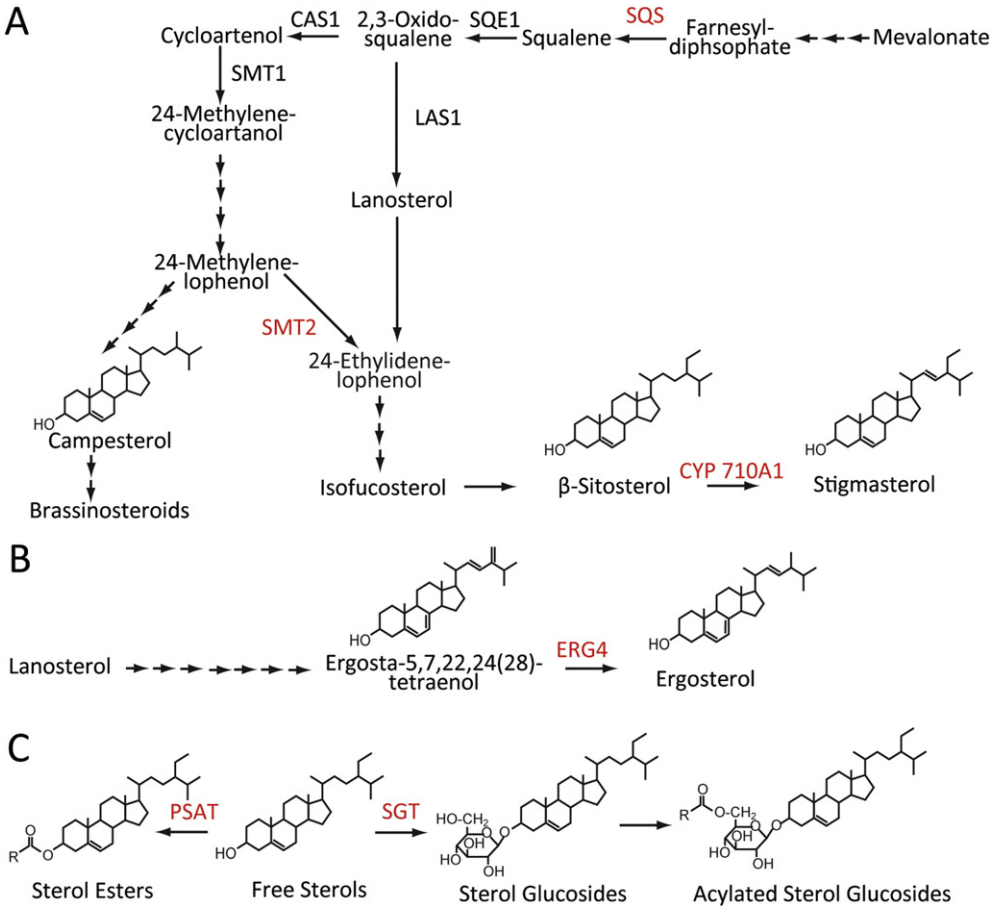


**Fig. 4.** Sterol biosynthesis in plants and fungi. (A) Plants synthesize the sterol lipids campesterol, β-sitosterol, and stigmasterol from squalene derived from the mevalonate pathway. (B) Fungal ergosterol is synthesized from lanosterol. (C) Sterol esters are synthesized from free sterols catalyzed by PSAT. Sterol glucosides are produced from free sterols by sterol glucosyltransferases. Sterol glucosides can be acylated at the glucose C6 position to produce acylated sterol glucosides (ASG). CAS1, cycloartenol synthase; PSAT, phospholipid:sterol acyltransferase; SGT, sterol glucosyltransferase; SMT, sterol methyltransferase; SQE1, squalene epoxidase; SQS, squalene synthase.

incorporated into membranes and acts as antioxidant during initial xylem and subsequent leaf colonization of sweet corn [76].

Apocarotenoids are isoprenoids derived by cleavage from C40 carotenoid precursors catalyzed by carotenoid cleavage dioxygenases (CCDs) [77] (Fig. 5). Apocarotenoids comprise the plant hormones abscisic acid (ABA), strigolactones, the acyclic $C_{14}$ polyene mycorradicin, the cyclic $C_{13}$ cyclohexenone, as well as hydroxylated and glycosylated derivatives of the latter [78]. Strigolactones are heterocyclic sesquiterpenes ($C_{15}$) that together with ABA belong to the group of apocarotenoid-derived phytohormones. In *Pisum sativum*, *all-trans*-β-carotene is isomerized to 9-*cis*-β-carotene and subsequently cleaved by CCD enzymes (CCD7, CCD8), producing carlactone, which is used by a cytochrome P450 to produce 5-deoxystrigol [77]. The main function of strigolactones is growth regulation. Stigolactones are present in root exudates where they attract symbiotic endomycorrhizal fungi and are required for seed germination of parasitic *Striga* plants, hence their name.

The strigolactones 5-deoxystrigol, sorgolactone, and strigol were isolated from *Lotus japonicus* root exudates and shown to induce hyphal branching of the arbuscular mycorrhizal fungus *Gigaspora margarita* [79]. How arbuscular mycorrhizal fungi perceive the strigolactone signal is unclear because fungal genomes lack genes coding for the D14 protein. The D14 (or DAD2) protein from *Arabidopsis*, rice, and petunia is a member of the α/β hydrolase family and was implicated in the perception of the strigolactone signal in plants [80]. Treatment of *Gigaspora rosea* cultures with the synthetic strigolactone GR24 led to upregulation of mitochondrial gene expression and increased NADH and ATP concentrations, as well as NADPH dehydrogenase activity, and hyphal tips, and spores exhibited increased numbers of nuclei. This stimulating effect on the oxidative metabolism and mitotic activity was defined as presymbiotic stage presumably required for host colonization [81]. Furthermore, GR24 treatment led to secretion of chitin oligomers by *R. irregularis*. Chitin oligomers act as plant perceived diffusible signal and are termed "myc" factors [82].

In tomato, the main strigolactones are solanacol, orobanchol, and didehydro-orobanchol [83]. Plants carrying silencing constructs for the MEP pathway gene 1-deoxy-D-xylulose 5-phosphate synthase 2 (*DXS2*), or for the strigolactone biosynthesis genes *CCD1* and *CCD8*, exhibited decreased biosynthesis of apocarotenoids. However, mycorrhization rate was mildly affected only in *CCD8* silenced plants, while *CCD1* and *DXS2* knockdown led to the appearance of more degenerating arbuscules [78,84,85]. However, the colonization rate of roots by an arbuscular mycorrhial fungus was affected in plants carrying a mutation in the *PDR1* gene, encoding an ABC-transporter required for strigolactone root exudation [86]. Strigolactone production is enhanced in response to phosphate limitation, which results in repressed shoot branching while enhancing lateral root growth and strigolactone exudation to increase the chance for arbuscular mycorrhizal symbiosis and to reach nutrient rich areas of the soil [87].

The strigolactone biosynthesis genes *CCD7* and *CCD8* are constitutively expressed in arbuscular mycorrhizal symbiotic and non-inoculated tomato roots, with *CCD7* being moderately induced by arbuscular mycorrhizal colonization. Therefore, on the transcriptional level, these strigolactone biosynthetic genes are not strongly regulated by the colonization stage of the root, contrary to the mycorrhiza-specific gene *CCD1*. The induction of CCD1 expression correlates with the accumulation of C13/C14 apocarotenoids (both cycloxhexenone and

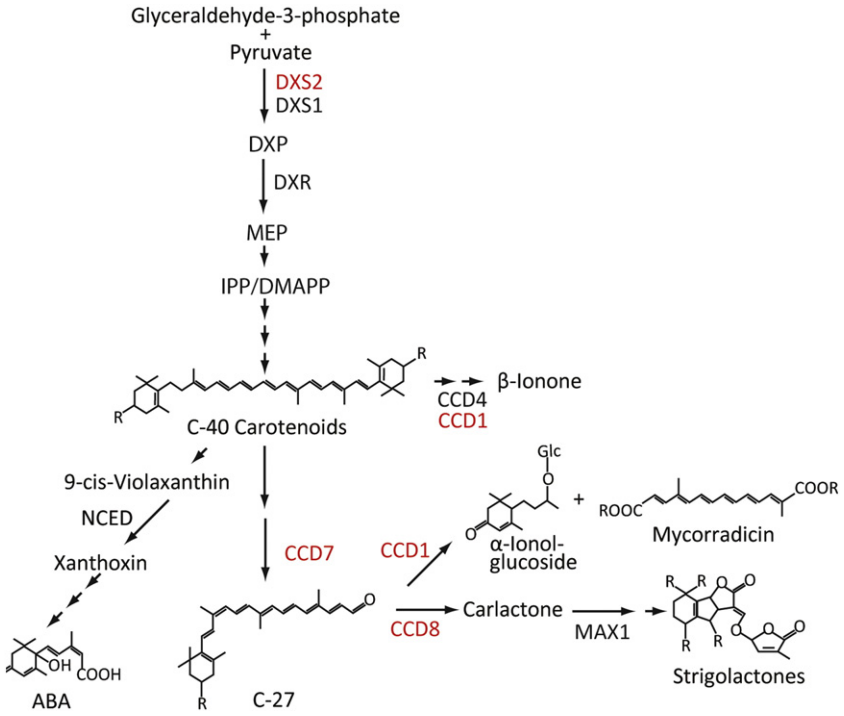


**Fig. 5.** Apocarotenoid biosynthesis in plants. Apocarotenoids are derived from the plastidial non-mevalonate (MEP) pathway by cleavage of C-40 carotenoid precursors via carotenoid cleavage dioxygenases. ABA, abscisic acid; CCD, carotenoid cleavage dioxygenase; DMAPP, dimethylallyl diphosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; IPP, isopentenyl diphosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; MAX1, more axillary growth 1; NCED, 9-*cis*-epoxycarotenoid dioxygenase; R, alkyl rest of fatty acid. Mutants important for plant–microbe interactions that are discussed in the text are highlighted in red.

mycorradicin) in the late phase after infection with arbuscular mycorrhizal fungi [88]. A specific isoform, *CCD1a* is induced late during symbiosis and is expressed in arbuscule-containing cells only. *CCD1* catalyzes the final step in $C_{13}/C_{14}$ apocarotenoid synthesis [89]. The arbuscular mycorrhiza-specific induction of *CCD1a* might prevent over-colonization by channeling the intermediates for strigolactones into C13/C14 apocarotenoids, which can be detected once the symbiosis is fully established (about 6 weeks post infection) [88]. C13/C14 apocarotenoids occur in higher concentrations than strigolactones, suggesting a distinct function. During symbiosis, arbuscules undergo constant turnover, involving rounds of generation, degeneration, and re-colonization of the same cortex cell with newly formed arbuscules. C13/C14 apocarotenoids accumulate in the cytosol of cells that contain degenerating arbuscules, and it was proposed that they are involved in plant-mediated arbuscule degeneration required for successive re-colonization [78]. Consistent with the role as degeneration accelerator, *CCD1* RNAi knockdown let to a moderate enrichment of degenerating arbuscules [85]. How C13/C14 apocarotenoids control the arbuscule population is unknown, but these apocarotenoids might contribute to control the efficiency of symbiosis by the host [78].

Strigolactones also play a role in root nodule symbiosis, although not as attractant signal [90]. Analogous to the stimulating effect of phosphate deprivation on arbuscular mycorrhiza formation, nitrogen deficiency induces formation of nodules. Under low N conditions in pea, strigolactone production is increased [91]. The application of low concentrations of GR24 stimulated nodule formation, while higher concentrations had the adverse effect [92]. The pea *CCD8* mutant *rms1* (*ramosus1*) showed decreased nodule numbers, and the wild-type phenotype was partially restored by GR24 application [91].

In view of the emerging function of strigolactones during regulation of plant growth and arbuscular mycorrhizal and root nodule symbiosis, first studies on their role in plant–pathogen resistance have been performed [93]. Leaves of tomato *ccd8* RNAi plants showed an increased susceptibility toward *A. alternata* and *B. cinerea* infections [84]. The increased susceptibility was attributed to hormonal cross talk specifically with the JA-defense pathway. GR24 application did not affect fungal proliferation of root or foliar pathogens nor ectomycorrhizal fungi *in vitro*, suggesting that strigolactones are not involved in regulation of virulence of these fungi [93].

The phytohormone ABA is produced by specialized CCDs termed NCED (9-*cis*-epoxycarotenoid dioxygenase) from C40 carotenoids and therefore represents another member of the class of apocarotenoids (Fig. 5). The different functions of ABA during plant–pathogen interactions were recently summarized [94]. A direct correlation between ABA with plant resistance was deduced from the finding that ABA promotes stomata closure and callose deposition at the cell wall, thereby preventing the entry of penetrating pathogens [95]. Other studies suggested a link between elevated ABA levels and decreased resistance [96,97]. This negative effect of ABA on plant resistance was proposed to be due to a suppression of SA-mediated SAR [94]. The inhibition of early ABA signaling led to decreased susceptibility to *Magnaporthe oryzae* infection in rice [97], while ABA accumulated at *Cercospora beticola* infection sites on sugar beet as late as 15 days post infection, suggesting a temporal regulation of ABA signaling in plants [98]. The pathway of ABA biosynthesis is conserved among plants and phytopathogenic fungi, with some variance in ABA intermediates. It has been speculated that pathogenic fungi produce ABA to disturb plant hormonal signaling leading to the inhibition of SAR [94]. The inhibition of ABA synthesis in tomato by the application of the ABA biosynthetic inhibitor sodium tungstate decreased the rate of mycorrhization [99]. Similarly, mycorrhization in the tomato *sitiens* mutant which is affected in ABA synthesis is compromised, while mycorrhization recovers in *sitiens* plants after exogenous addition of ABA [99]. Therefore, ABA is a positive regulator of mycorrization in plants [100]. In agreement with this finding, low concentrations of ABA have a stimulating effect on mycorrhization in *Medicago truncatula*, and mycorrhization is

suppressed in an ABA insensitive *Medicago* line [101]. The regulation of mycorrhiza infection via ABA depends on protein phosphatase 2 A (PP2A) [101].

### 2.8. Sphingolipids

Sphingolipids are a diverse lipid class comprising small molecules implicated in signaling as well as large glycosylated lipids, which are crucial for membrane integrity and membrane raft formation. Sphingolipids lack a glycerol backbone but contain an amino alcohol (long chain base, LCB) (Fig. 6). In plants, this LCB is usually a C18 carbon chain hydroxylated at C-1 and C-4 (dihydroxy LCB, d18:0) and carrying an amino group at C-2. The LCB can be further hydroxylated at C-3 (trihydroxy LCB, t18:0) or desaturated at C-4 or C-8 (major species: d18:1, d18:2, t18:1) in *cis* or *trans* configuration leading to a variety of molecular species. The pattern of molecular sphingolipid species is highly complex due to combinations of the different LCBs with fatty acid residues to form ceramides. The chain lengths of these fatty acids, which are often hydroxylated at the C-2 position (e.g., h16:0), range from C16 to C20–C26 (very long chain fatty acids, VLCFAs). Ceramides are the substrates for the synthesis of the two major sphingolipid classes in plants, glucosylceramides (GlcCer) and glycosylinositol phosphoceramides (GIPC). GlcCer and GIPC are abundant components of the plasma membrane, tonoplast, and ER membrane in plant cells and together with sterols, they are involved in membrane raft formation.

The nature of the molecular species also determines sphingolipid function, which has recently been demonstrated by the analysis of mutants and overexpression lines of *Arabidopsis* of ceramide synthases LOH1, LOH2, and LOH3 [102,103]. *Arabidopsis* ceramide synthases display substrate specificity toward specific LCBs and fatty acyl-CoAs when heterologously expressed in yeast [102]. LOH2 is specific for the synthesis of ceramides containing a dihydroxy LCB and C16 fatty acid (e.g., d18:1-h16:0), whereas LOH1 and LOH3 synthesize ceramides with a trihydroxy LCB and VLCFAs (e.g., t18:1-h24:0). Apparently, the resulting ceramides have very distinct functions in plant metabolism. The *loh1* mutant displayed spontaneous cell death when grown under short-day conditions. The expression of the pathogenesis-related gene *PR-1* was induced in *loh1* under short-day conditions, while the senescence-associated gene *SAG12* was not, indicating a hypersensitive response (HR)-like reaction [102]. This phenotype was not observed in the *loh3* mutant, although the two enzymes showed the same substrate specificity. Possibly LOH1, which is higher expressed than LOH3 in *Arabidopsis*, can compensate for the loss of LOH3 [102]. In contrast, the *loh1 loh3* double mutant, which shows an accumulation of C16 fatty acid containing sphingolipids and an absence of VLCFA-containing sphingolipids, is severely impaired in growth and development at the very early seedling stage [104]. The *loh2* mutant displays no visible growth defect [102,104].

Several studies showed that sphingolipids are involved in PCD [103,105–107], which is a defense reaction against microbial pathogens [108]. While the overexpression of LOH1 and LOH3 leads to increased plant growth in *Arabidopsis*, the overexpression of LOH2 results in dwarfed growth and the constitutive expression of HR genes and PCD [103]. The PCD occurring in LOH2 overexpression plants may be linked to the accumulation of C16 fatty acid containing dihydroxy ceramides [103]. Sphingolipids containing trihydroxy LCBs are crucial for maintaining sphingolipid homeostasis, a function which cannot be fulfilled by sphingolipids containing C16 fatty acids and dihydroxy LCBs alone [109]. The *Arabidopsis* double mutant *sbh1 sbh2* deficient in the two LCB C4 hydroxylase genes was devoid of sphingolipids containing trihydroxy LCB, which resulted in a phenotype similar to that of the LOH2 overexpression lines. The plants showed an accumulation of C16 fatty acid containing dihydroxy sphingolipids, were reduced in growth and showed a constitutive expression of genes involved in PCD. The total sphingolipid content in the *sbh1 sbh2* mutant was increased, which was also the case in the LOH2 overexpression line as well as in the *loh1 loh3* double mutant. The expression of PCD-related genes appears to be induced by the accumulation of sphingolipids containing a C16 fatty acid and a dihydroxy LCB [103,109]. However, in these two studies the accumulation of C16-dihydroxy LCB sphingolipids was also linked to

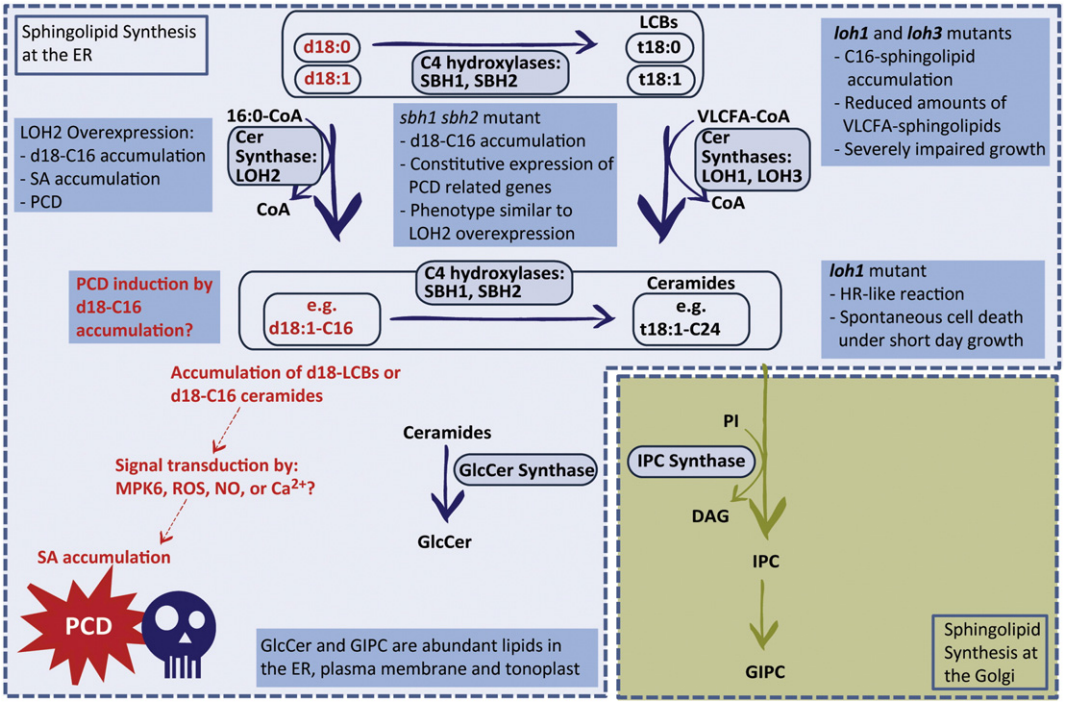


**Fig. 6.** Sphingolipid metabolism and programmed cell death. Sphingolipids are synthesized at the ER and at the Golgi apparatus [152]. Different mutants and transgenic plants affected in sphingolipid metabolism show programmed cell death, most notably the C4 hydroxylase double mutant *sbh1sbh2* [109], the ceramide synthase mutant *loh1* [102], and overexpression lines of the ceramide synthase LOH2 [103]. Cer, ceramide; DAG, diacylglycerol; GIPC, glycosylinositol phosphocholine; GlcCer, glucosylceramide; HR, hypersensitive response; IPC, inositolphosphocholine; MPK6, mitogen-activated protein kinase 6; PCD, programmed cell death; ROS, reactive oxygen species; SA, salicylic acid; VLCFA, very long chain fatty acids.

a disturbed sphingolipid homeostasis. Therefore, the induction of PCD genes could also be an indirect effect, triggered by alterations in any of the other sphingolipid classes. Exactly how PCD is induced in sphingolipid mutants is unclear. One aspect addressed in many studies is the accumulation of SA in the sphingolipid mutants. A putative interaction between sphingolipid metabolism and SA signaling during PCD and HR has recently been proposed [110]. Two major questions were discussed: Firstly, which sphingolipid molecules influence SA levels, and secondly, which transduceable signal provides the link between sphingolipid metabolism and SA biosynthesis. Several studies showed increased SA levels in sphingolipid mutants [105–107,110], and in only one study SA levels were unaffected [102]. Another recent study also reported increased SA levels associated with PCD after the overexpression of ceramide synthases [103]. The proposed sphingolipid molecules most likely responsible for the activation of SA biosynthesis are LCBs or ceramides [102]. Four possible candidates for signal transduction from alterations in sphingolipid content to SA biosynthesis were discussed, including MPK6 (mitogen-activated protein kinase 6) ROS, $Ca^{2+}$, and NO [110,111].

## 3. Fungal lipids

Fungal membranes contain large amounts of phospholipids typically found in eukaryotic cells (PC, PE, PG, PS, PA, CL). On the other hand, the sterol composition of most fungi is different from plants because they accumulate ergosterol instead of the phytosterols stigmasterol, β-sitosterol, and campesterol, and this difference has been shown to be important during plant–fungal interactions. Furthermore, fungi accumulate considerable amounts of sphingolipids in their membranes, but with a different set of LCBs as found in plants. The focus of this review is on the roles of glycerolipids and glycerolipid-derived metabolites during arbuscular mycorrhiza symbiosis, fungal sterol lipids (ergosterol and ergosterol glucoside), and fungal sphingolipids in interactions with plants.

### 3.1. Fungal lipid metabolism during arbuscular mycorrhizal symbiosis

Long before the lipidome of the arbuscular mycorrhiza interaction was studied in detail, mycorrhizal fungi had been well known for their accumulation of large amounts of fatty acids in the form of triacylglycerol and were thus termed “oleaginous fungi” [112]. The abundant fungal fatty acid 16:1 ω5 (palmitvaccenic acid), which accumulates in triacylglycerol, can be employed as a marker for the evaluation of mycorrhizal colonization. It has been a matter of debate whether arbuscular mycorrhizal fungi actively synthesize fatty acids using carbon derived from hexoses from the plant host, or whether fatty acids are supplied by the plant [48–50,112,113].

The supplementation of mycorrhizal fungi in axenic root cultures with radioactive sugars indicated that the fungus is capable of elongating and desaturating fatty acids in its extraradical hyphae but cannot produce fatty acids *de novo* outside of the plant roots [113]. The authors discussed that fungal *de novo* of fatty acid synthesis might be restricted to intraradical mycelium, possibly due to favorable growth conditions and nutrient supply. While direct proof for the transport of plant-derived fatty acids to the mycorrhizal fungi is missing, the apparent lack of a gene encoding type I fatty acid synthase (FAS I) in the mycorrhizal fungus *R. irregularis* (syn. *Glomus intraradices*) might suggest that fatty acids are delivered by the plant [50,114]. On the other hand, sequences predicted to encode mitochondrial FAS (type II) were found in *R. irregularis*, and the expression of the corresponding genes was upregulated during mycorrhization [49,50,114]. It has been suggested that mitochondrial FAS might be sufficient for fungal fatty acid *de novo* synthesis, although this would represent a rare exception [49].

Another interesting aspect is the close resemblance of fungal membrane lipids with the membrane lipids of the plant host. As described below, arbuscular mycorrhizal fungi, in contrast to other fungi, do not produce ergosterol but sterols, which closely resemble phytosterols such as campesterol and β-sitosterol [115]. Furthermore, the glycerolipid molecular species composition of the extraradical mycelium of arbuscular mycorrhizal fungi differs from that of intraradical mycelium [50]. One possible explanation could be fatty acyl editing by the fungus to adapt the acyl chain length in PC and PE to the chain lengths of the plant host phospholipids, possibly to improve compatibility of the fungal arbuscular membrane with the plant-derived periarbuscular membrane.

Another lipid with a presumed signaling function in arbuscular mycorrhizal symbiosis is LPC [49]. LPC elicited rapid alkalinization of the medium in suspension-cultured cells of *Lotus japonicus*, a response that was also triggered by the bacterial elicitor flagellin. Furthermore, *L. japonicus* root tips infiltrated with LPC showed an upregulated expression of the plant phosphate transporter *PT4* known to be induced during mycorrhization. The LPC contents showed strong differences between different host plants colonized by the same arbuscular mycorrhizal fungus [49]. LPC molecular species carrying C20 fatty acids are presumably derived from the fungus, while LPCs with C16 or C18 acyl groups can be produced in the fungus or in the plant.

### 3.2. Free sterols and sterol esters in fungi

Ergosterol is the most abundant sterol in most fungi, and it is therefore employed as fungal lipid marker. It is one of the MAMPs that acts as elicitor of microbe-triggered immunity (MTI) upon contact with the plant. This MTI response is characterized by the production of ROS, changes in plasma membrane potential, changes in proton fluxes across the plasma membrane, activation of typical defense genes such as *PAL* (phenylalanine ammonia lyase), and activation of isoprenoid synthesis leading to production of phytoalexins, ABA, and phytosterols as well as phenylpropanoid precursors [116,117].

Lanosterol derived from oxido-squalene is an important intermediate in the synthesis of sterols in plants and fungi (Fig. 4). However, the pathway of sterol synthesis from lanosterol differs between plants and fungi. The final step of ergosterol synthesis in fungi is the conversion of ergosta-5,7,22,24(28)-tetraenol to ergosterol catalyzed by the C-24 reductase *ERG4*. Fungal *Δerg4* mutants of *Fusarium graminearum* are devoid of ergosterol. The *Δerg4* mutants were capable of plant colonization but showed reduced virulence, which was attributed to reduced mycelia growth, increased sensitivity to ROS due to impaired membrane integrity and a decrease in deoxynivalenol content, a toxin essential for virulence [118].

The array of plant responses to ergosterol is similar to the one toward other receptor-mediated defense responses, including flagellin. Therefore, it is possible that plants express a membrane receptor-like kinase that binds ergosterol and transduces the defense signal, analogous to the brassinosteroid binding receptor BRI1. The putative ergosterol receptor and the interacting proteins remain yet to be identified. The interaction with oxylipin binding proteins was hypothesized, based on the findings that oxysterols can be generated from sterols in the present of ROS and that an oxysterol-binding protein (OSBP) is induced upon *P. infestans* inocualtion of potato [119].

The host-penetrating arbuscular mycorrhizal fungi are devoid of ergosterol [115], probably to escape the host defense response, but ergosterol is present in pathogenic fungi and in ectomycorrhizal fungi (EMF), which do not rely on penetration of the plant cell wall for symbiosis. In the extraradical mycelium of the arbuscular mycorrhizal fungus *R. irregularis*, sterol esters and free sterols are the most abundant sterols [50]. Furthermore, low amounts of glycosylated sterols are present. The most prominent sterols of extraradical mycelium are 24-methylcholesterol and 24-ethylcholesterol. Furthermore, lanosterol, ergosta-7,24(28)-dienol and 24-ethylcholesta-5,22-dienol were also identified in extraradical mycelium and infected roots [50,116,117].

### 3.3. Fungal sterol glucosides

Sterol glycosyltransferases (SGT) catalyze the addition of a glucose moiety to the C3 hydroxy group of sterols (Fig. 4). SGTs were first described in plants. Fungi of the ascomycetes and basidiomycetes contain sequences homologous to plant SGT [120]. In yeast, glucosyltransferase activity was described as being essential for the development of the micropexophagic apparatus (MIPA) required for pexophagy (peroxisome degradation). The exact mechanism is unknown, but SGs were suggested to be incorporated into membranes during MIPA-precursor formation. The gene *Atg26* (*autophagy-related 26*) encoding a sterol glucosyltransferase of the plant–pathogenic fungus *Colletotrichum orbiculare* was mutagenized, resulting in reduced virulence during infection of cucumber [121]. The appressorium formation of the *C. orbiculare Δatg26* mutant was not compromised, but peroxisome degradation was affected in the appressoria. Another study showed that a mutation in the *Agt26* gene of *M. oryzae* reduced the capacity to cause rice blast [122]. Contrasting results were obtained in another study because targeted disruption of *Agt26* in *M. oryzae* did not affect peroxisomal degradation in appressoria or virulence in rice [123]. Therefore, further studies are required to corroborate these results, which suggest that ergosterol glucoside formation is essential for maturation of autophagosomal structures in appressoria, finally leading to peroxisome degradation, a prerequisite for penetration of the host epidermis.

### 3.4. Fungal sphingolipids

Similar to plants, fungi contain two classes of complex sphingolipids, phosphoinositol sphingolipids and glucosylceramides (GlcCer). However, a characteristic and distinguishing feature of fungal sphingolipids is the presence of a C-9 methyl group on the LCB [124]. This C-9 methyl group is introduced by a sphingolipid C-9 methyltransferase [125]. C-9 methylated sphingolipids and especially fungal GlcCer are important for pathogenesis and induce different plant defense mechanisms. For example, fungal GclCers sprayed on rice leaves function as elicitors to induce phytoalexin and PR protein synthesis. Furthermore, they confer resistance to different phytopathogens [126]. Non-fungal GlcCers do not show elicitor activity. The virulence of *Fusarium graminearum* sphingolipid mutants is host dependent. GlcCers-deficient mutants show reduced virulence on wheat but not on *Arabidopsis* leaves and flowers [125]. Surprisingly, the reduction of methylated GlcCer to 25–35% in mutants disrupted in the C9-methyltransferase *FgMT2* leads to a more severe decrease of pathogenicity than the complete loss of GlcCer. These *Fusarium* mutants show strongly reduced virulence on wheat and delayed symptom formation on *Arabidopsis*. Fungal GlcCers are also often the target of defensins, small peptides from plants or insects, which possess antifungal/antibacterial activity and mediate membrane permeabilization. The defensins MsDef1 from *Medicago sativa* or RsAFP2 from radish seed (*Raphanus sativus*) inhibit growth of *F. graminearum* and of the two yeasts *Pichia pastoris* and *Candida albicans*, respectively, while GlcCers-deficient mutants are resistant to these defensins [125,127]. No resistance against MsDef1 or RsAFP2 was observed in *FgMT2* disrupted *F. graminearum* mutants with reduced amounts of methylated GlcCers. A possible explanation was that the antifungal activity of different defensins may depend on the Δ3 double bond in the fatty acid of fungal GlcCers [128]. Antifungal activity against *C. albicans* was reported for the defensin AFP1 from *Brassica juncea* interacting with the C9-methyl group of GlcCers but not with the Δ8 double bond of the LCB [129].

## 4. Bacterial lipids

This section focuses mainly on bacterial membrane lipids comprising phospholipids and different phosphorus-free glycerolipids (non-phospholipids) and their role in plant–microbe interactions. Complex lipids such as lipoteichoic acid or lipid A are not included here. Moreover, different bacteria use small signal molecules like *N*-acyl homoserine lactones for inter-kingdom communication (including communication with plants, quorum sensing). Although derived from fatty acids, these compounds are also not discussed in this review.

### 4.1. Bacterial phospholipids

Phospholipids are the main building blocks of most bacterial membranes, with PE, PG and CL as the most common lipids. Their synthesis starts with the conversion of PA to cytidine diphosphate-diacylglycerol (CDP-DAG) by the CDP-DAG synthase CdsA with CTP as co-substrate (Fig. 7) [130]. The zwitterionic lipid PE results from the decarboxylation of PS by the PS decarboxylase Psd. PS is formed by condensation of the phosphatidyl moiety from CDP-DAG with L-serine catalyzed by the PS synthase PssA. The synthesis of the acidic phospholipid PG starts with the condensation reaction of the phosphatidyl moiety from CDP-DAG with Gro3P, leading to the intermediate PG-phosphate (PGP) catalyzed by PgsA. Subsequent dephosporylation by the PG-phosphate phosphatase Pgp leads to the end product PG. Different pathways have been described for the synthesis of CL with multiple CL synthases (Cls) present in bacteria. The main route follows the condensation of two PG molecules with release of glycerol, while a minor pathway is the condensation of PG with PE accompanied with the release of ethanolamine. The zwitterionic lipid PC is a typical eukaryotic lipid, but about 15% of bacteria are able to produce PC [131]. PC-producing bacteria are often symbionts or pathogens of plants. The two most common PC synthesis pathways in bacteria are the PE methylation and the PC synthase pathways (Fig. 7). In the more frequent PE methylation route, PE is methylated in three consecutive steps by one or several phospholipid methyltransferases (Pmt) to produce monomethyl-PE (MMPE), dimethyl-PE (DMPE), and finally PC. The methyl donor is *S*-adenosyl methionine. In the PC synthase pathway which is specific for bacteria, choline is condensed with the phosphatidyl moiety of CDP-DAG by the unique bacterial PC synthase Pcs.

PC is required for the virulence of different bacteria. The plant–pathogenic bacterium *Agrobacterium fabrum* (formerly: *A. tumefaciens* C58) uses both the major PE methylation (PmtA) and the minor PC synthesis

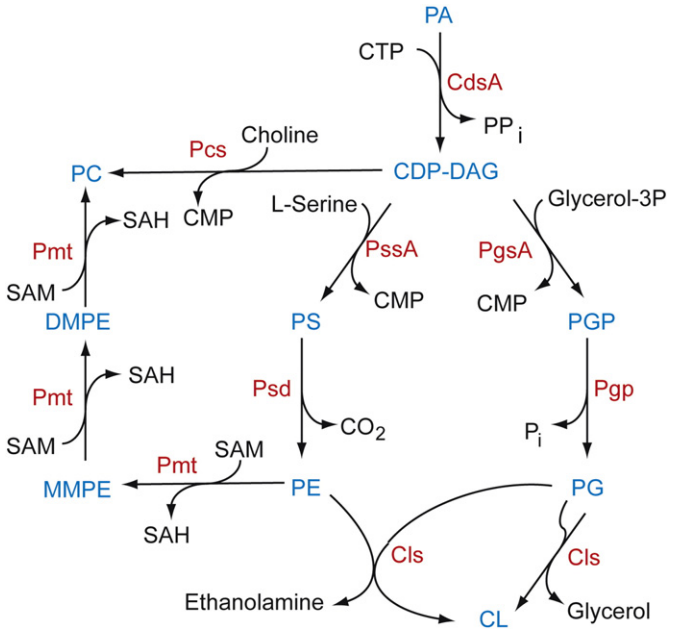


**Fig. 7.** Phospholipid synthesis in bacteria. CdsA, CDP-DAG synthase; PssA, PS synthase; Psd, PS decarboxylase; PgsA, PGP synthase; Pgp, PGP phosphatase; Cls, different CL synthases; Pmt, one, or several phospholipid methyltransferase(s); Pcs, PC synthase; CTP, cytidine triphosphate; CMP, cytidine monophosphate; SAM, *S*-adenosyl methionine; SAH, *S*-adenosyl homocysteine.

(Pcs) pathway (Fig. 7) to form PC, which accounts for 23% of total phospholipids [132,133]. *Agrobacterium* belongs to the family of Rhizobiaceae and causes crown gall disease in many dicotyledonous plants. Its pathogenicity is based on the ability to transfer oncogenic DNA to the host cell, triggering the tumor formation. The transfer of DNA is mediated by a membrane spanning type IV secretion system (T4SS) [133]. The virulence of *Agrobacterium* strictly depends on the presence of PC in the bacterial membranes. Infection of *Kalanchoë* leaves with the Pmt-deficient *Agrobacterium* mutant *ΔpmtA* containing reduced amounts of PC showed delayed tumor formation and a markedly reduced tumor size, while tumor formation was completely abolished on leaves infected with the PC-free double mutant *ΔpmtA Δpcs*. The reason for this defect in virulence is the reduced amount of the proteins of the T4SS secretion system in the PC-reduced single mutant and their complete loss in the PC-free double mutant [133]. It remains an open question whether the defect in virulence is PC specific or can be explained by altered physicochemical characteristics of the PC-deficient membrane. A further important plant–pathogen is *P. syringae pv. syringae* van Hall. During infection, this bacterium secretes the HrpZ protein via a type III secretion system (T3SS) into the extracellular space of plant cells. HrpZ suppresses the defense response of the infected cell. This protein is also able to induce a HR on non-host plants. *P. syringae pv. syringae* van Hall uses only the Pcs pathway (Fig. 7) and contains about 8% of PC [134]. The deletion of the *pcs* gene results in the complete loss of PC and of the ability to elicit the typical HR in non-host soybean plants. The *P. syringae Δpcs* mutant is impaired in the secretion of HrpZ, although this protein is expressed in wild-type levels. Further confirmation for the requirement of PC for virulence comes from experiments with a non-pathogenic *Pseudomonas* sp. strain, where the heterologous *hrpZ* gene was introduced and expressed in the wild type and in a *Δpcs* deletion mutant [134]. Both strains produced the HrpZ protein; however, only the wild type but not the PC lacking *Δpcs* mutant was able to secrete HrpZ and elicit HR in tobacco or soybean leaf cells. Presumably, altered membrane properties originating from PC deficiency cause misfolding of T3SS proteins or HrpZ. PC is furthermore essential for symbiotic interactions of rhizobia with legumes. For example, root nodule formation on soybean with efficient nitrogen fixation after infection with *Bradyrhizobium diazoefficiens* (*formerly B. japonicum*) depends on the presence of PC in the bacterium. PC in *B. diazoefficiens* is mainly synthesized via the PE methylation (PmtA) pathway (Fig. 5) and to a minor extent by PC synthase (Pcs). Besides PmtA three different functional isoenzymes, PmtX1, PmtX3, and PmtX4 are expressed in *B. japonicum*, with PmtA catalyzing the first, and PmtX1 the next two methylation steps [135]. The expression of PmtX3 and PmtX4 is very low. *B. diazoefficiens* contains about 52% PC of total phospholipids [136]. The disruption of PmtA leads to a strong reduction of PC to 6% in the *ΔpmtA* mutant. The residual amount of PC might be synthesized by the Pcs pathway or by the upregulation of PmtX4 [135]. Infection of soybean with the *ΔpmtA* mutant led to the formation of nodules with impaired nitrogen fixation, which was reduced to 18% compared to wild type [136]. Both the *B. diazoefficiens ΔpmtA* mutant and the wild type produced the same number of nodules on soybean roots, indicating that the initial plant–microbe interactions were not impaired by PC deficiency in the bacterium. However, the nodules containing *ΔpmtA* rhizobia were smaller with a beige color indicating reduced amounts of leghemoglobin, compared to the reddish color of wild-type nodules. Besides, the infected plant cells contained less bacteroids, the symbiotic form of rhizobia. The plants infected with the *B. japonicum ΔpmtA* mutant were pale green as a consequence of nitrogen deprivation. The loss of PC affects the expression of a confined set of 17 genes, most of them were upregulated in the *ΔpmtA* mutant, including *pmtX3* and *pmtx4* and several transcriptional regulator genes [135]. These regulators are involved in fine-tuning of a putative resistance nodulation cell division (RND)-type efflux system and are only weakly expressed under standard conditions in the wild type. RND-type transporters presumably play a role in host–microbe interactions. Components of this transporter were highly upregulated in the *ΔpmtA* mutant. Another *Bradyrhizobium* strain (sp. SEMIA 6144), which infects peanut, showed a decrease of 50% of PC after disruption of the *pmtA* gene [137]. The *ΔpmtA* mutant formed wild-type-like nodules on peanut roots with a nitrogen fixation activity comparable to wild type, but *ΔpmtA* mutant bacteria were less competitive. The reduced competitiveness was explained by a decreased motility or chemotaxis of *ΔpmtA* mutant cells. This may be caused by the changes in membrane-associated functions caused by the PC reduction. The importance of PC for nodulation was also shown for another nodule-forming bacterium, *Sinorhizobium meliloti*. The *S. meliloti ΔpmtA Δpcs* double mutant completely lacks PC and is unable to form nitrogen-fixing nodules on *M. sativa* [138]. Further *S. meliloti* mutants were generated to investigate the role of other phospholipids during symbiosis [139]. The *ΔpssA* mutant unable to synthesize the PE precursor PS and therefore lacking PE was still able to induce nodule formation with comparable kinetics, but nodule numbers were reduced by 30%. Therefore, PE in *S. meliloti* plays a minor role in plant–microbe interactions. A more severe nodulation phenotype was observed after inoculation of *M. sativa* with the *S. meliloti Δpsd* mutant incapable of PS decarboxylation [139]. The *Δpsd* mutant lacks PE but accumulates about 18% of PS compared to less than 0.1% in the wild type. Nodulation with this mutant was delayed and the nodule numbers were reduced by 90% compared to wild type. Furthermore, the nodules were almost devoid of bacteroids and unable to fix nitrogen. The reason why the accumulation of PS causes such a drastic reduction in nodulation remains unknown.

### 4.2. Bacterial non-phospholipids

Bacteria produce a high variety of phosphate-free membrane lipids, including glycolipids, betaine lipids, ornithine lipids (OLs), and hopanoids. Glycolipids are characteristic for cyanobacteria, and for Gram-positive bacteria where they serve as membrane anchors for lipoteichoic acids. They are furthermore found in nodule-forming bacteria or in the plant–pathogen *A. fabrum* [41,140]. The betaine lipid diacylglyceryl trimethylhomoserine (DGTS) is restricted to α-proteobacteria, but homologs of enzymes involved in DGTS synthesis are encoded in the genomes of some other bacterial groups [131]. DGTS and glycolipids in *Agrobacterium* and nodule-forming bacteria are mainly synthesized under phosphate deprivation and serve as surrogate for phospholipids [141,142]. A function of these lipids in plant–microbe interactions could not been shown.

Ornithine lipids (OLs) are widespread in bacteria, but absent from eukaryotic organisms [131]. Based on phylogenetic analyses, it has been estimated that about 50% of the sequenced bacteria have the capacity to form OLs [143]. OLs are free of glycerol. The amino acid ornithine is directly linked via an amide bond to a 3-hydroxy-fatty acid (Fig. 8). OL synthesis involves two acyltransferases using acyl-ACP as substrate. The *N*-acyltransferase OlsB catalyzes the acylation of ornithine, while the *O*-acyltransferase OlsA transfers a second fatty acid to the 3-hydroxy group of the amide-linked fatty acid to form an ester bond. OLs can be further modified for example by hydroxylation at different positions in the molecule (Fig. 8). So far, three hydroxylases are known from different bacteria [140,144,145]. OlsE introduces a hydroxy group at an unknown position of the ornithine head group, while OlsC and OlsD hydroxylate the ester and the amide-linked fatty acids, respectively, both at C2 positions. Hydroxylated OLs often accumulate in bacteria grown under stress conditions. Moreover, OLs and their hydroxylated forms are important for plant–microbe interactions. *A. fabrum* contains OL and an OlsE-dependent hydroxy-OL. Two *A. fabrum* mutants were created lacking hydroxy-OL (*ΔolsE*) or all OLs (*ΔolsB*) to study the role of these lipids for virulence [146]. Interestingly, infection experiments with the two mutants resulted in earlier formation and larger sizes of tumors than with the wild type. It was hypothesized that the plants recognize OL or hydroxy-OL leading to a plant defense response. In the absence of bacterial OL or hydroxy-OL, the

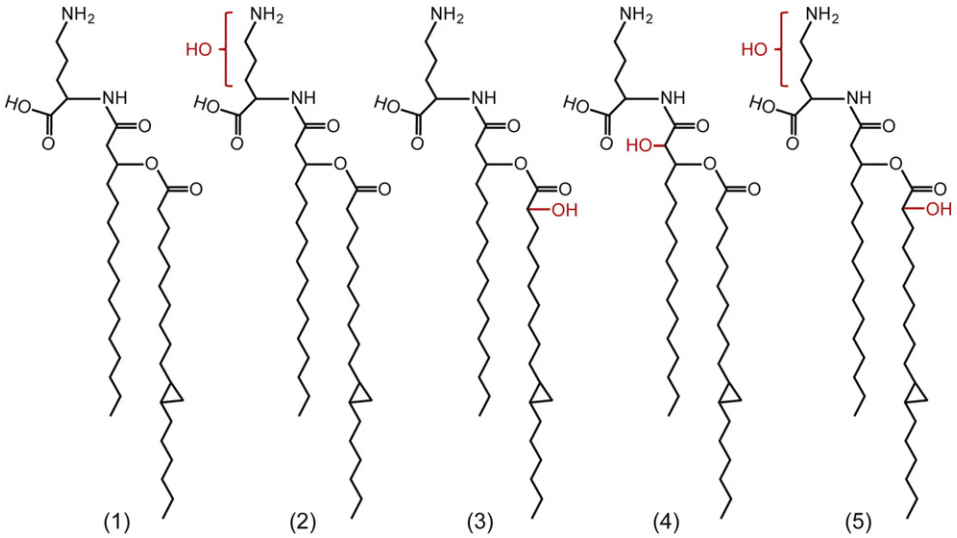


**Fig. 8.** Structures of unmodified ornithine lipid (OL) (1) and of different hydroxylated ornithine lipids (2–5). The hydroxy group of hydroxyl-OL can be introduced by OlsE into the ornitihine head group (2) at an unknown position, or by OlsC (3) or OlsD (4) at the C2 position of one of the two fatty acids. Hydroxylation at two positions of OL by OlsE and OlsC is also possible (5).

plant response might be abolished, leading to an accelerated infection process. OLs also play a role during the symbiosis of *Rhizobium tropici* with common bean (*Phaseolus vulgaris*) [145]. *R. tropici* contains three forms of hydroxy-OLs synthesized by OlsE and OlsC (Fig. 8, structures 2, 3, and 5). Different mutants, *ΔolsE*, *ΔolsC*, and a double mutant *ΔolsE ΔolsC*, were created, lacking the corresponding hydroxy-OLs. These mutations affect stress tolerance and lead to severe nodulation phenotypes. *R. tropici* can grow in media with very low pH (4.0) and therefore, under acidic conditions, benefits for the competition for host infection. Hydroxy-OLs were shown to be involved in this acid tolerance and thus might confer this competitive advantage. Growth of OlsC-deficient single (*ΔolsC*) and double (*ΔolsC ΔolsE*) mutants of *R. tropici* is retarded under acidic conditions as compared to *ΔolsE* or wild type. OLs and hydroxy-OLs are enriched in the outer bacterial membrane. Presumably, the extra hydroxy groups on the fatty acids stabilize the bilayer and decrease membrane permeability because of increased hydrogen bonding between neighboring hydroxyl-OL molecules. This mechanism could explain the decrease in acid resistance of OlsC-deficient mutants. Hydroxy-OLs are furthermore required to develop functional nodules for symbiosis of *R. tropici* with common bean. Although *ΔolsE*, *ΔolsC*, *ΔolsE ΔolsC*, and wild type produced similar numbers of nodules on bean roots, many of the nodules formed by the mutants were smaller and whitish indicating the lack of leghemoglobin. Besides, the nitrogen fixation rate of nodules from the three mutants was affected. Therefore, the non-hydroxylated OL still present in these mutants cannot substitute for the function of hydroxy-OL during symbiosis. In *S. meliloti*, which contains only the non-hydroxylated form, OL is not required for symbiosis with *M. sativa* [141].

Heterocyst glycolipids in cyanobacteria and hopanoids in the Gram-positive bacterium *Frankia* sp. and in different *Bradyrhizobium* species are specific lipids which play a role during nitrogen fixation [147–149]. *Frankia* and different cyanobacteria (e.g., *Nostoc*) are able to undergo symbiotic interactions with non-legume plants [150]. Heterocysts from cyanobacteria are specialized cells for nitrogen fixation surrounded by a thick cell wall. This cell wall consists of different layers containing heterocyst glycolipids protecting the nitrogenase against oxygen. Heterocyst glycolipids are glycerol free and consist of a long chain polyhydroxy alcohol in direct glycosidic linkage with a sugar head group [147]. Endosymbiotic cyanobacteria differ in their heterocyst glycolipid composition compared to free-living species because the endosymbiotic cyanobacteria contain longer chain alcohols and C5 sugars instead of C6 sugars [151]. It has been speculated that accumulation of these unusual heterocyst glycolipids represents an important adaptation process to the higher intracellular $O_2$ concentrations in the host. The nitrogen-fixing vesicles of *Frankia* are mainly composed of hopanoids, which are pentacyclic isoprenoids resembling the sterols of eukaryotes, and protect the nitrogenase from oxygen inactivation (Fig. 9). A specific function of hopanoids during *Frankia* symbiosis is unknown. Hopanoids are dispensable during plant–microbe interactions of *B. diazoefficiens* with its native host soybean, but are required for symbiosis of *B. diazoefficiens* and the photosynthetic *Bradyrhizobium* strain BTAi1 with different species of the tropical legume *Aeschynomene* [148,149]. Hopanoids can be present in the membranes of *Bradyrhizobium* as free molecules or covalently linked to lipid A. A key step in hopanoid synthesis is the formation of hopene by the squalene hopene cyclase Shc (Fig. 9). A further important enzyme in the hopanoid pathway is HpnH catalyzing the first step in the synthesis of C35 hopanoids, with bacteriohopanetetrol as the most abundant hopanoid in living organisms. Shc is not essential for *Bradyrhizobium* BTAi1, but for *B. diazoefficiens*, and the deletion of HpnH in *B. diazoefficiens* led to the loss of free and lipd A-linked C35 hopanoids in the mutants with reduced nitrogen fixation activity in the nodules from *Aeschynomene afraspera* [149].

## 5. Conclusions and perspectives

Lipids play diverse functions in plant–microbe interactions, i.e., during pathogen infections, true mutualistic symbioses, and during interactions with beneficial microorganisms. Lipids are involved in

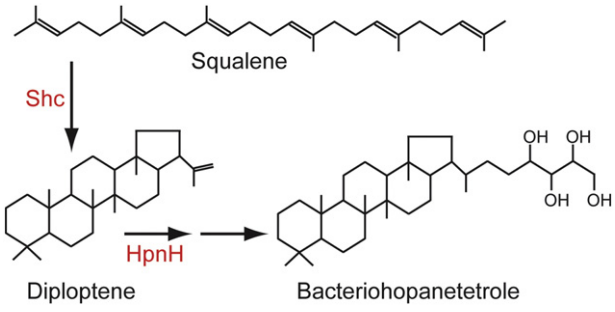


**Fig. 9.** Initial steps of hopanoid synthesis. The synthesis of bacteriohopanetetrol is shown as an example. Shc (squalene hopene cyclase) is responsible for the cyclization of squalene, while HpnH (adenosylhopane synthesizing enzyme) catalyzes the first step in the elongation of C30 to C35 hopanoids.

pathogen recognition by the host (e.g., ergosterol), and lipids or lipid breakdown products are involved in signaling in cells at the site of infections (free fatty acids, oxylipins, glycerol-3-phosphate). Furthermore, some lipids (azelaic acid) mediate the transfer of the signal of infection to distal plant organs during SAR. Lipids might play a role during the signaling after infection with mycorrhizal fungi (lysophospholipid, Ω-hydroxy-fatty acids). Furthermore, bulk membrane lipid synthesis is required for establishing the membrane structures of the host cell and the microbe to provide the large surface area for metabolite exchange during colonization. In fact, the two membranes establish a barrier for signaling events and for the exchange of nutrients and metabolites. This might be the reason why lipids were chosen as signal molecules for communication between host and microbe, or as metabolites during nutrient exchange. Further, "lipidomic" studies are required to identify additional lipid molecules involved in plant–microbe interactions, and to reveal lipid patterns that are common or distinct among infections with pathogenic, symbiotic, or beneficial microbes.

## Transparency document

The Transparency document associated with this article can be found, in the online version.

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