Phytopathology Research

REVIEW

Open Access

Sphingolipids in plant immunity

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Abstract

Sphingolipids (lipids with a sphingoid base backbone) are important components of eukaryotic membrane systems and key signaling molecules that are essential for controlling cellular homeostasis, acclimating to stress, and regulating plant immunity. Studies using sphingolipid treatments, measuring sphingolipids in infected plants, and functionally studying sphingolipid biosynthetic mutants demonstrated that sphingolipids participate in plant cell death and defense responses. In this review, we present an updated map of sphingolipid signaling and review recent progress in understanding the functions of sphingolipids in plant immunity as structural components of biological membranes, and as mediators of defense signaling. Moreover, several pressing questions, such as how sphingolipids in the plasma membrane, particularly microdomains, act to perceive pathogens and transduce signals during plant–pathogen interactions, remain to be further explored in future research.

Keywords: Sphingolipids, Immunity, Cell death, Defense signaling

Background

Sphingolipids include free long-chain bases (LCBs), ceramides (Cers), hydroxyceramides (hCers), glycosylceramides (GlcCers), and glycosyl inositol phosphoceramides (GIPCs). These lipids participate in various cellular, developmental, and stress-response processes. Sphingolipid metabolism mainly occurs in the endoplasmic reticulum (ER) and Golgi apparatus, and once synthesized, sphingolipids may be translocated, sorted, transported, and then finally localized to membranes (Luttgeharm et al. 2016) (Fig. 1).

Sphingolipid metabolism is generally conserved across yeast, animals, and plants, but plant sphingolipids have unique characteristics and functions (Markham et al. 2013). Plant sphingolipids are involved in development and the responses to various abiotic and biotic stresses, as detailed in several recent reviews (Markham et al. 2013; Luttgeharm et al. 2016; Ali et al. 2018; Huby et al. 2020; Mamode Cassim et al. 2020; Liu et al. 2021). In this review, we focus on the roles of sphingolipids in plant immunity.

During evolution, plants have developed multi-layered strategies to fight infection from a broad range of pathogens, including bacteria, fungi, oomycetes, and viruses. To defeat these enemies, plants have evolved a two-layered immune system in which surface-localized or intracellular immune receptors recognize microbe- or damage-associated molecules (Wang et al. 2020). In the first layer of plant immunity, membrane pattern recognition receptors (PRRs) perceive conserved pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) and activate PAMP/DAMP-triggered immunity (PTI) (Zipfel 2014). PAMP recognition causes immediate responses, including generation of reactive oxygen species (ROS), an intracellular calcium influx, transient activation of mitogen-activated protein kinases (MAPKs), and production of the defense hormone salicylic acid (SA) (Seyfferth and Tsuda 2014). Some adapted pathogens counteract PTI by secreting effector proteins to interrupt plant immunity, resulting in effector-triggered susceptibility (ETS). However, in the second layer of defense, called effector-triggered immunity (ETI), plants recognize these effectors via nucleotide

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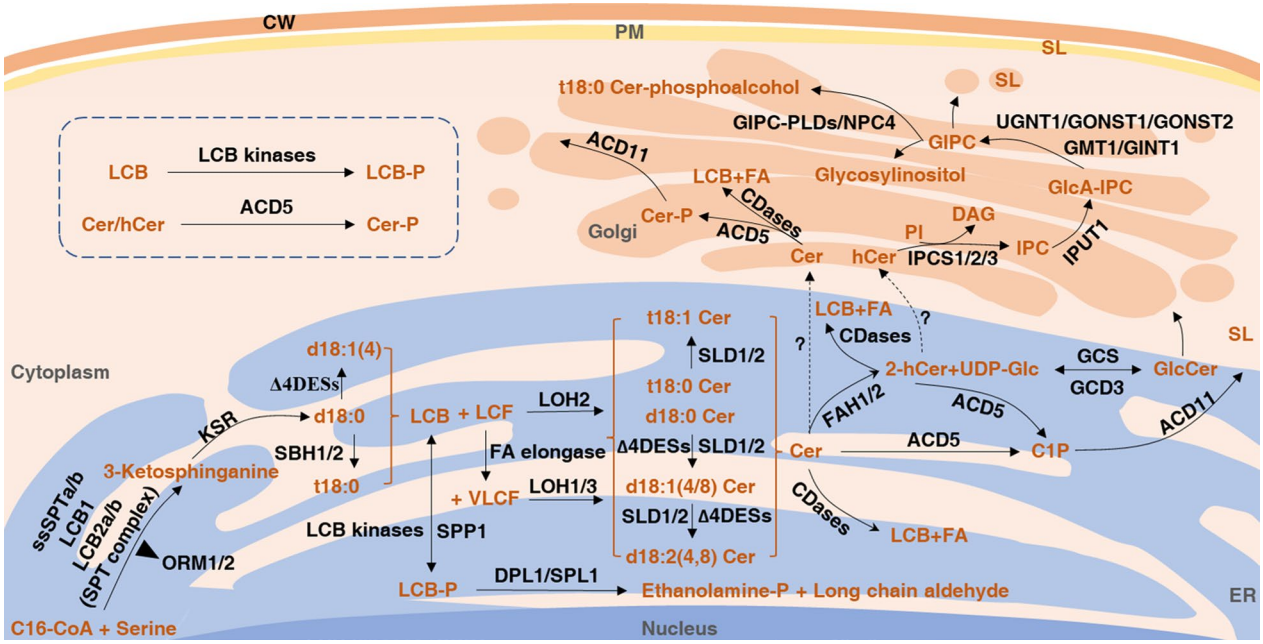

Fig. 1 Sphingolipid metabolism pathway. Enzymes or proteins involved in sphingolipid metabolism pathways are in black type. A dotted line with a question mark means that the details are unknown. A dashed box means that the enzymes inside are not in the ER or Golgi. ER, endoplasmic reticulum; SL, sphingolipid; FA, fatty acid; LCF, long-chain fatty acid; VLCF, very-long-chain fatty acid, LCB, long-chain base; d18:0, sphinganine or dihydrosphingosine; d18:1, sphingosine; d18:2, sphingadiene; t18:0, phytosphingosine or 4-hydroxysphinganine; t18:1, 4-hydroxysphingenine; Cer, ceramide; hCer, hydroxyceramide; GlcCer, glucosylceramide; GIPC, glycosyl inositol phosphoceramide; PI, phosphatidylinositol; DAG, diacylglycerol; SPT, serine palmitoyltransferase; SPT, serine palmitoyltransferase; LCB1 and LCB2a/b, subunits of SPT; ssSPTa/b, small subunits of SPT; ORM1/2, orosomucoid-like proteins; 3-KSR, a 3-ketodihydrosphingosine reductase; SBH1/2, sphingoid base hydroxylases; Δ4-DESs, Δ4-desaturases; LOH1/2/3, ceramide synthases named LAG ONE HOMOLOGUES; ACD5, a ceramide kinase named ACCELERATED CELL DEATH 5; ACD11, a Cer-P transporter named ACCELERATED CELL DEATH 11; PLM, phloem unloading modulator; FAHs, fatty acid alphahydroxylase; CDases, ceramidases; GCS, a glucosylceramide synthase; DPL1/SPL1, a LCB-P lyase; SPP1, a phyto-S1P phosphatase; IPCS1/2/3, inositolphosphorylceramide synthases; IPUT1, an inositol phosphorylceramide glucuronosyltransferase; GMT1, a GIPC mannosyl-transferase; GINT1, a glucosamine inositol phosphorylceramide transferase; GONST1/2, Golgi-localized nucleotide sugar transporters; UGNT1, an UDP-*N*-acetyl-D-glucosamine transporter; GCD1/2/3/4, glucosylceramidases; GIPCPLD, a GIPC-specific phospholipase D; NPC4, a nonspecific phospholipase C4

binding-leucine rich repeat (NLR) immune receptors, also known as resistance (R) proteins (Wang et al. 2020).

SA activates resistance that is effective against biotrophs; the lipid-derived phytohormone jasmonic acid (JA) and the gaseous phytohormone ethylene activate resistance that is effective against necrotrophs and herbivorous insects (Ngou et al. 2022). Multiple plant hormones can be induced in hosts after infection and the crosstalk among these signaling pathways fine-tunes the plant's response to external stresses. Sphingolipids activate multiple phytohormone signaling pathways (Asai et al. 2000; Magnin-Robert et al. 2015; Wu et al. 2015a; Zienkiewicz et al. 2019; Huang et al. 2021); therefore, one emerging question is how sphingolipids affect the crosstalk among these signaling pathways.

Systemic defense responses rely on the spread of signals between neighboring cells. Plasmodesmata (PD) are plant-special intercellular channels that allow fast and efficient communication between a plant cell and its immediate neighbors. PD contain complex membrane structures formed by the extension of the plasma membrane (PM) of two adjacent cells. These channels contain a central cylinder formed by elongation of the ER of the interconnected cells and sphingolipid- and sterol-rich membrane microdomains (Grison et al. 2015). When plant cells perceive attacking fungal, bacterial or viral pathogens, the cells close their PD by depositing the polysaccharide callose (Cheval and Faulkner 2018; Huang et al. 2019). Callose deposition appears to be associated with sphingolipids (Iswanto et al. 2020; Liu et al. 2020b).

Studies on sphingolipid metabolism mutants have shown that plant sphingolipids participate in various biotic stress responses and affect plant defense responses to microbial pathogens with different infection strategies. In this review, we discuss the multidimensional roles of sphingolipids in plant immunity and delve into the mechanisms of signal transduction mediated by sphingolipids.

Sphingolipid metabolism

Like in yeasts and animals, de novo biosynthesis of sphingolipids in plants begins with the condensation of serine and palmitoyl-CoA, a reaction catalyzed by serine palmitoyl transferase (SPT) complexes in the ER to generate 3-ketosphinganine (Chen et al. 2006) (Fig. 1). *Arabidopsis* SPT contains three subunits, LCB1, LCB2a, and LCB2b (Chen et al. 2006; Dietrich et al. 2008). In addition, small subunits of SPT (ssSPTa and b) promote SPT activity, while OROSOMUCOID-LIKE PROTEINs (ORM1 and ORM2) inhibit SPT activity (Markham et al. 2013; Kimberlin et al. 2016; Li et al. 2016).

Release of 3-ketodihydrosphingosine reductase (KSR) from the intermediate 3-ketosphinganine (Chao et al. 2011) forms the first long-chain base (LCB), namely dihydrosphingosine or sphinganine (d18:0) (Chao et al. 2011). Sphingoid base hydroxylases (SBH1 and SBH2) generate phytosphingosine (t18:0) by adding a third hydroxyl group at C-4 position of d18:0 (Sperling et al. 2001; Chen et al. 2008). Sphingolipid Δ4-desaturase (Δ4-DES) desaturates d18:0 at C-4 to generate sphingosine (d18:1) (Michaelson et al. 2009). SBH and Δ4-DES use d18:0 as substrates; therefore, C-4 hydroxylation of d18:1 precludes Δ4 desaturation, and conversely, Δ4 desaturation of d18:1 prevents C-4 hydroxylation (Luttgeharm et al. 2016). Several LCB kinases (LCBK1, LCBK2, SPHK1, and SPHK2) phosphorylate LCBs at the C-1 OH position to form LCB-1-phosphates (LCB-Ps) (Imai and Nishiura 2005; Worrall et al. 2008). These LCB-Ps can be degraded by PHYTO-S1P PHOSPHATASE (SPP1) or PHYTO-S1P LYASE (DPL1) (Nishikawa et al. 2008; Worrall et al. 2008; Nakagawa et al. 2012).

Cer, the sphingolipid core, is produced from a free LCB and a fatty acid chain (usually 16 to 26 carbons in length) via an amide bond catalyzed by the LAG ONE HOMOLOGUE (LOH) Cer synthases LOH1, LOH2, and LOH3 in *Arabidopsis* (Markham et al. 2011; Ternes et al. 2011). The class II Cer synthases LOH1 and LOH3 prefer to use very-long-chain fatty acids (VLCFAs, more than 18 carbons in length) and LCBs as substrates, whereas the class I Cer synthase LOH2 uses long-chain fatty acids (LCFAs, less than 20 carbons in length) and LCBs as substrates (Markham et al. 2011; Ternes et al. 2011).

The saturated LCB chains of Cers can be desaturated via sphingoid LCB desaturases (SLD1 and SLD2) at C-8 or via Δ4-DES at C-4 to generate d18:1-based, 4-hydroxysphingenine (t18:1)-based, or sphingadiene (d18:2)-based Cers (Michaelson et al. 2009; Chen et al. 2012). The Δ8 unsaturation of LCB chain can be *cis* or *trans* in *Arabidopsis* (Chen et al. 2012). In contrast to the LCB Δ8 desaturase, which largely uses LCBs bound in Cers as substrates, Δ4-DES most likely uses free LCBs as substrates and introduces double bonds exclusively in the *trans* configuration (Ternes et al. 2002). The hCers are generated by fatty acid C-2 hydroxylases (FAH1 and FAH2) which catalyze α-hydroxylation in the fatty acyl chain of Cers (Konig et al. 2012; Nagano et al. 2012a, b). In addition to FAH1 and FAH2, other enzymes catalyze the hydroxylation of sphingolipid fatty acids in *Arabidopsis* since 2-hydroxy sphingolipids are not completely absent in the *fah1 fah2* double null mutants (Ukawa et al. 2022). Cer phosphorylation can occur at the C-1 OH position of Cers, via Cer kinase (CERK), which is encoded by *ACCELERATED CELL DEATH5* (*ACD5*) in *Arabidopsis* (Liang et al. 2003). The product, Cer-1-phosphate (C1P), can be transferred between membranes by ACCELERATED CELL DEATH 11 (ACD11) (Simanshu et al. 2014).

Ceramidases convert Cers into LCBs. Human ceramidases can be divided into acidic, neutral, and alkaline ceramidases according to their pH optima (Mao and Obeid 2008). We previously reported an *Arabidopsis* alkaline ceramidase (AtACER) that possibly hydrolyzes t18:0-Cers, and several neutral ceramidases (AtNCER1–3) that may degrade hCers into sphingosine and fatty acids (Li et al. 2015; Wu et al. 2015b). In addition to ACER, the alkaline ceramidase TURGOR REGULATION DEFECT 1 (TOD1) has been identified in *Arabidopsis* and rice (*Oryza sativa*) (Chen et al. 2015; Ke et al. 2021). In rice, the neutral ceramidase OsCDase preferentially hydrolyzes d18:1Δ4-Cer over t18:0-Cer (Pata et al. 2008).

Notably, OsCDase exhibits reverse ceramidase activity, catalyzing the formation of C26- and C28-phytoceramides in yeast when induced by growth in galactose (Pata et al. 2008). NCER2 likely functions as a Cer synthase, according to the sphingolipid profiles in mutant plants and the response of mutants to fumonisin B1 (FB1), a mycotoxin that blocks Cer synthase activity (Zienkiewicz et al. 2019). It will be interesting to know whether NCER2 and other ceramidases exhibit both ceramidase and reverse ceramidase activity like OsCDase. These ceramidases maintain the balance of Cer and LCB in the ER and Golgi. For example, AtNCER1 localizes to the ER (Li et al. 2015) and AtTOD1 localizes to the Golgi (Chen et al. 2015), while AtACER and OsCDase localize both to the ER and Golgi (Pata et al. 2008; Wu et al. 2015b).

Glycosylation of Cers at the C-1 hydroxyl group produces GlcCers or GIPCs (Luttgeharm et al. 2016). GLUCOSYLCERAMIDE SYNTHASE (GCS) transfers a glucose residue from UDP-glucose to a hCer to produce GlcCer via beta-glycosidic linkage (Leipelt et al. 2001; Melser et al. 2010). GLYCOLIPID TRANSFER PROTEIN 1 (GLTP1) can transfer GlcCer *in vitro* (West et al. 2008). GlcCer can also be degraded. We recently reported that *Arabidopsis* GLUCOSYLCERAMIDASE 3 (GCD3) preferentially hydrolyzes GlcCers that contain long acyl

chains (Dai et al. 2019). By contrast, GIPCs are synthesized from Cers or hCers in the Golgi. The Cers and hCers are first modified by INOSITOLPHOSPHORYLCERAMIDE SYNTHASEs (IPCSs) to add a head group derived from phosphatidylinositol (PI) to form inositolphosphoceramides (IPCs) (Mina et al. 2010). Then, a glucuronic acid (GlcA) moiety is added via a (1,4)-linkage by INOSITOL PHOSPHORYLCERAMIDE GLUCURONOSYLTRANSFERASE 1 (IPUT1) (Rennie et al. 2014). Further glycosylation of the head group is mediated by GIPC MANNOSYL TRANSFERASE 1 (GMT1) or GLUCOSAMINE INOSITOL PHOSPHORYLCERAMIDE TRANSFERASE 1 (GINT1) and requires the GOLGI-LOCALIZED NUCLEOTIDE SUGAR TRANSPORTERs (GONST1/2) or UDP-N-ACETYL-D-GLUCOSAMINE TRANSPORTER 1 (UGNT1) (Mortimer et al. 2013; Fang et al. 2016; Ebert et al. 2018; Ishikawa et al. 2018; Jing et al. 2021; Moore et al. 2021). In cabbage (*Brassica oleracea*), GIPCs can be cleaved by GIPC-SPECIFIC PHOSPHOLIPASE D (GIPC-PLD) at the D position of the ester linkage between inositol and phosphate to produce C1P (Hasi et al. 2020). In *Arabidopsis*, GIPCs can be hydrolyzed by NONSPECIFIC PHOSPHOLIPASE C4 (NPC4) (Yang et al. 2021).

Sphingolipid metabolism upon pathogen infection

Accumulating evidence shows that infection by various pathogens affects host sphingolipid metabolism (Table 1). For example, an avirulent strain (*avr*RPM1 DC3000) of the bacterial pathogen *Pseudomonas syringae* (*P. syringae*), which triggers ETI, induced significant LCB production at 2 hours post infection (hpi), and long-lasting elevated t18:0 levels until 24 hpi (Peer et al. 2010). By contrast, virulent *P. syringae* DC3000, which does not trigger ETI, induced a transient increase in t18:0, in which t18:0 levels were elevated at 2 hpi, but returned to basal levels at 5 hpi (Peer et al. 2010). It seems that the pathogen-triggered increase in t18:0 results from SBH1-mediated *de novo* biosynthesis of t18:0 from d18:0 (Peer et al. 2010). Neither Cers nor hCers showed significant changes upon infiltration with *P. syringae avr*RPM1 DC3000. During later stages of infection (two days post infection with virulent or avirulent *P. syringae*), besides t18:0, some LCBs, LCBs-P, Cers, and hCers were induced in treated plants, but no changes in GlcCer and GIPC contents occurred upon infection (Magnin-Robert et al. 2015). Notably, C16-Cer accumulation was more pronounced in the interaction with *P. syringae avr*RPM1 DC3000 compared with *P. syringae* DC3000, suggesting that ETI substantially reprograms sphingolipid metabolism (Magnin-Robert et al. 2015).

Indeed, activation of ETI by the R protein RESISTANT TO P. SYRINGAE 2 (RPS2) in *Arabidopsis* induced transcriptional and translational regulation of genes encoding sphingolipid metabolism-related enzymes such as IPCS2 and GONST1 (Yoo et al. 2019). In addition, *Xanthomonas campestris* pv. *campestris* infection of *Brassica oleracea* caused dynamic changes in sphingolipid

Table 1 Sphingolipid profiles in plants upon treatment with pathogens, defense hormones, or sphinganine-analog mycotoxins

Treatment	Species	Outcome	References
P. syringae, or *P. syringae avrRpm1*	*Arabidopsis*	High levels of LCBs, LCBs-P, and Cers	(Peer et al. 2010; Magnin-Robert et al. 2015)
B. cinerea	*Arabidopsis*	High levels of LCBs, LCBs-P, Cers and hCers	(Bi et al. 2014; Magnin-Robert et al. 2015; Zeng et al. 2021)
X. campestris	*Brassica oleracea*	A significant reduction in N-palmitoylsphinganine	(Tortosa et al. 2018)
V. dahliae	Cotton	Disruption of SL homeostasis, increased GIPC contents	(Xu et al. 2022)
SA or BTH	*Arabidopsis*	Subtle changes in the SL profile	(Simanshu et al. 2014; Shi et al. 2015; Huang et al. 2019; Zeng et al. 2021)
ET	*Arabidopsis*	Subtle changes in the SL profile	(Wu et al. 2015a)
MeJA	*Arabidopsis*	Subtle changes in the SL profile	(Huang et al. 2021)
BRs	Olive	Low levels of LCBs	(Corbacho et al. 2018)
ABA	*Arabidopsis*	High levels of LCB-Ps	(Guo et al. 2012)
FB1	*Arabidopsis*	Disruption of sphingolipid homeostasis, sharp increase in LCB contents	(Shi et al. 2007; Markham et al. 2011; Saucedo-Garcia et al. 2011; Kimberlin et al. 2013, 2016; Wu et al. 2015b; Shao et al. 2019; Zeng et al. 2022)
AAL-toxin, or FB1	Tomato, duckweed, tobacco callus, maize, banana, or cotton	Disruption of SL homeostasis, sharp increase in LCB contents	(Abbas et al. 1994; Shao et al. 2019; Gutierrez-Najera et al. 2020; Chen et al. 2021; Xie et al. 2021; Xu et al. 2022)

P. syringae, *Pseudomonas syringae*; *B. cinerea*, *Botrytis cinerea*; *X. campestris*, *Xanthomonas campestris*; *V. dahliae*, *Verticillium dahliae*; SA, salicylic acid; ET, ethylene; MeJA, methyl jasmonate; BRs, brassinosteroids; ABA, abscisic acid; FB1, fumonisin B1; SL, sphingolipid; LCBs, long-chain bases; Cers, ceramides; hCers, hydroxyceramide

metabolism (Tortosa et al. 2018). Infection with the necrotrophic fungus *Botrytis cinerea* induced remarkable accumulation of Cers in *Arabidopsis* (Bi et al. 2014; Magnin-Robert et al. 2015).

Hormone signaling also mediates the effects of pathogen infection on sphingolipid metabolism. For example, we recently showed that loss of function of ENHANCED DISEASE SUSCEPTIBILITY (EDS1) or PHYTOALEXIN DEFICIENT 4 (PAD4) prevents Cers from accumulating in wild-type plants and *acd5* mutants upon *B. cinerea* infection, partly through the SA pathway (Zeng et al. 2021). Interestingly, SA and EDS1 signaling also affect LCB accumulation and LCB-associated cell death (König et al. 2022; Zeng et al. 2022). Indeed, both SA and its analog benzothiadiazole affected sphingolipid metabolism (Shi et al. 2015). Moreover, treatment with methyl jasmonate (MeJA) elevated Cer levels in wild type and *acd5*, and ethylene modulated sphingolipid synthesis in *Arabidopsis* upon FB1 treatment, indicating that JAs and ethylene are involved in *B. cinerea*-induced Cer accumulation (Wu et al. 2015a; Huang et al. 2021).

Cell death and immunity responses

Treatment with exogenous LCBs stimulates plant cell death and immune responses such as calcium influx, ROS production, expression of defense genes, and callose deposition (Table 2 and Fig. 2). Furthermore, treatment with exogenous t18:0 increased endogenous sphingolipids and inhibited aphid infestation in *Arabidopsis* plants (Begum et al. 2016).

Table 2 Exogenous treatments of sphingolipids or sphingolipid metabolism inhibitors affecting plant cell death and immunity

Treatment and concentration	Samples	Response	References
d18:0, d18:1, t18:0, or d17:1 (0.3–100 μM)	*Arabidopsis* leaves, seedlings	ROS, cell death, expression of defense genes	(Shi et al. 2007; Peer et al. 2011; Saucedo-Garcia et al. 2011; Glenz et al. 2019; Zeng et al. 2022)
d18:0, d18:1, or t18:0 (10–50 μM)	Tobacco leaves, seedlings, BY-2 cells	Calcium increase, ROS, cell death, expression of defense genes	(Takahashi et al. 2009; Lachaud et al. 2010; Lachaud et al. 2011; Rivas-San Vicente et al. 2013)
t18:0 (2–25 μM)	*Arabidopsis* plants, cells	Calcium-dependent cell death, callose deposition, resistance of plants to *P. syringae* and aphids	(Lachaud et al. 2013; Begum et al. 2016; Liu et al. 2020a)
d18:0, or t18:0-P (100 μM)	*Arabidopsis* leaves	Effects on the response to *P. syringae* or *B. cinerea*	(Magnin-Robert et al. 2015)
t18:0 (1 μM)	Tobacco leaves	ROS, cell death, resistance of plants to *Phytophthora parasitica* var. *nicotianae*	(Seo et al. 2021)
t18:0-P (10 μM)	*Arabidopsis* leaves	PTI- and SA-induced stomatal closure	(Gupta et al. 2020a, b)
d18:0, d18:1, t18:0, d18:0-P, or d18:1-P (20 μM); t18:0-P (40 μM); DMS or SKi (5–25 μM)	Tobacco BY-2 cells	Effects on cryptogein-induced ROS in cells	(Coursol et al. 2015)
C2 Cer (30–50 μM), C6 Cer (100 μM), or C2 Cer-P (10 μM)	*Arabidopsis*, rice protoplasts	Cer induces ROS-, calcium-, caspase-, and mitochondrial membrane potential-associated cell death; Cer-P rescues Cer-induced cell death	(Liang et al. 2003; Yao et al. 2004; Bi et al. 2014; Zhang et al. 2020)
GIPCs (100 μg/mL)	*Arabidopsis* leaves	Enhanced EV secretion and flg22-induced ROS	(Liu et al. 2020a)
FB1 (0.3–50 μM)	Various plants	Multiple defense responses, e.g., ROS, cell death, SA, JA, ET, calcium, extracellular ATP deletion, vacuolar membrane disruption, MAPK	(Zeng et al. 2020; Iqbal et al. 2021)
C2 Cer (20 μM), or FB1 (7 μM)	Tobacco protoplasts	Potassium loss-associated cell death	(Peters and Chin 2007)
Myriocin (0.1 μM), FB1 (5 μM), PDMP (50 μM), D609 (100 μM), or DMS (30 μM)	*Arabidopsis* seedlings	Effects on callose levels	(Iswanto et al. 2020)

LCB, long-chain base; LCB-P, long-chain base-phosphate; Cer, ceramide; Cer-P, ceramide-phosphate; SA, salicylic acid; ROS, reactive oxygen species; myriocin, an inhibitor of serine palmitoyltransferase; FB1, Fumonisin B1, an inhibitor of ceramide synthase; PDMP, DL-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol hydrochloride, an inhibitor of glucosylceramide synthase; D609, tricyclodecan-9-yl-xanthogenate, an inhibitor of phosphatidylcholine specific phospholipase C and sphingomyelin synthase; DMS, D-erythroN,N-dimethylsphingosine, an inhibitor of LCB kinase; SKi, 2-(*p*-hydroxyanilino)-4-(*p*-chlorophenyl)thiazole, an inhibitor of sphingosine kinase

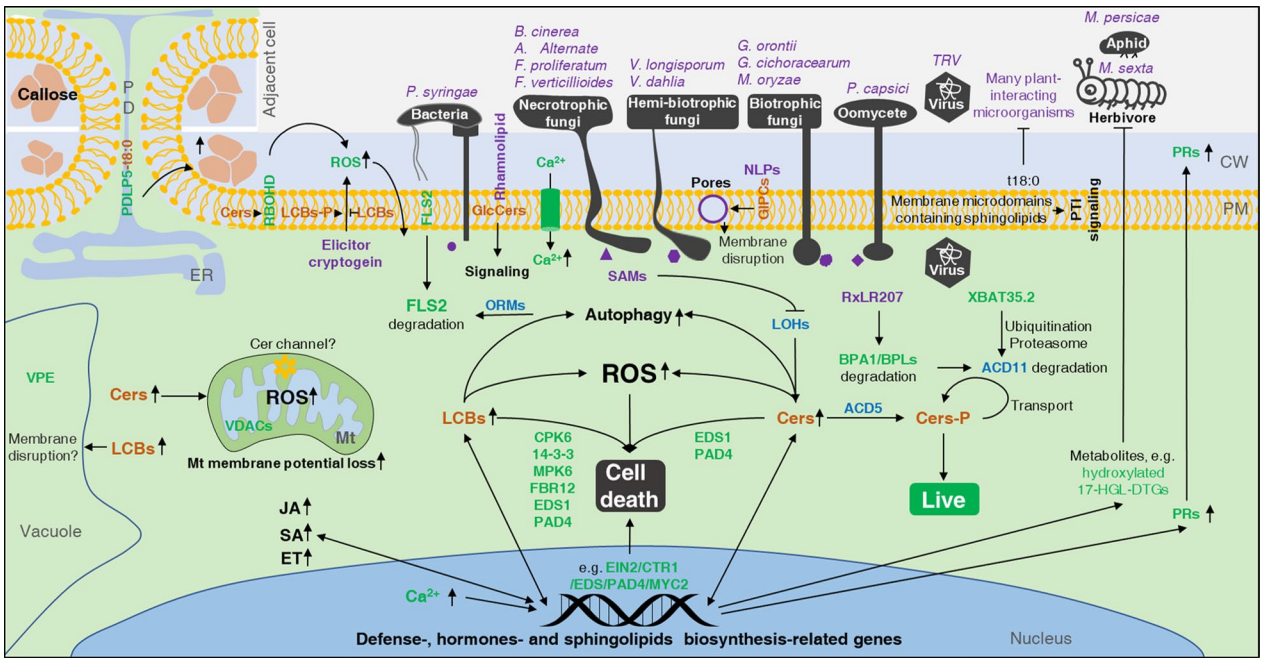

Fig. 2 An overview of sphingolipid functions in plant immunity. ABA: abscisic acid; ACD5, ceramide kinase; ACD11, ceramide-P transporter; Cers, ceramides; Cer-P: ceramide-phosphate; CPK3: CALCIUM-DEPENDENT KINASE; CTR1, CONSTITUTIVE TRIPLE RESPONSE 1; CW: cell wall; EDS1: ENHANCED DISEASE SUSCEPTIBILITY; EIN2: ETHYLENE INSENSITIVE 2; ER: endoplasmic reticulum; ET: ethylene; FB1: Fumonisin B1, an inhibitor of ceramide synthase; FBR12, FB1-RESISTANT 12; flg22: flagellin-derived peptide flagellin 22; FLS2: FLAGELLIN SENSING 2; GIPCs: glycosyl inositol phosphoceramides; GlcCers: glucosylceramides; JA: jasmonic acid; LCBs, long-chain bases; LCBs-P, long-chain base-phosphate; LOH: LAG ONE HOMOLOGUES, ceramide synthases; MPK6: mitogen-activated protein kinase 6; Mt, mitochondrion; NLPs: necrosis and ethylene-inducing peptide 1-like proteins; ORMs: orosomucoid-like proteins; PAD4: PHYTOALEXIN DEFICIENT 4; PCD: programmed cell death; PD: plasmodesmata; PDLP5: PD-LOCATED PROTEIN 5; PM: plasma membrane; PRs, PATHOGENESIS-RELATED proteins; RBOHD: RESPIRATORY BURST OXIDASEHOMOLOGUE D; ROS, reactive oxygen species; SA: salicylic acid; SAMs: sphinganine-analog mycotoxins; TRV, tobacco rattle virus; VDACs: voltage-dependent anion channels; VPE: vacuolar processing enzyme; SL: sphingolipid; t18:0: phytosphingosine or 4-hydroxysphinganine

Among the LCBs, d18:0 and t18:0 are strongly elevated in plants treated with the Cer synthase inhibitor FB1 (Saucedo-Garcia et al. 2011). A study in tobacco (*Nicotiana tabacum*) BY-2 cells showed that d18:0 triggers a rapid increase in cytosolic calcium within the first minute and increased nuclear calcium within 10 min; the nuclear calcium signature is responsible for the cell death induced by d18:0 (Lachaud et al. 2010). The d18:0-triggered calcium increase subsequently resulted in PM NADPH oxidase-dependent production of the ROS H_2O_2 (Lachaud et al. 2011). It appears that the ROS induced by d18:0 treatment activates expression of defense-related genes, rather than being implicated in programmed cell death (PCD) (Lachaud et al. 2011). Although both d18:0 and t18:0 induce defense-related gene expression in an EDS1-dependent manner, only d18:0 triggers PCD in an EDS1-dependent manner (Zeng et al. 2022).

MAP KINASE 6 (MPK6) activation by LCBs such as d18:0 and t18:0 mediates cell death and immunity in *Arabidopsis* (Saucedo-Garcia et al. 2011). Treatment with d18:0 slightly reduced electrolyte leakage triggered by infection with virulent *P. syringae* DC3000, and strongly reduced disease symptoms and electrolyte leakage triggered by avirulent *P. syringae* DC3000 *avr*RPM1 (Magnin-Robert et al. 2015). In addition to MPK6, CALCIUM-DEPENDENT KINASE (CPK3) positively regulates LCB-mediated cell death in *Arabidopsis* (Lachaud et al. 2013). Treatment with t18:0 actives CPK3 to phosphorylate its binding partners, the 14-3-3 proteins, leading to disruption of the CPK3–14-3-3 complex and CPK3 degradation (Lachaud et al. 2013).

Treatment with t18:0 increases the protein level of PD-LOCATED PROTEIN 5 (PDLP5), induces PDLP5-dependent callose accumulation and decreased PD permeability, and enhances pathogen resistance to the hemibiotrophic pathogens *Verticillium dahlia* and *P. syringae* (Liu et al. 2020b). PDLP5 specifically binds t18:0, but not d18:0, d18:1, or t18:1, indicating that t18:0- or t18:0-based sphingolipid species selectively recruit PDLP5 to regulate PD (Liu et al. 2020b). In line with this, wild-type plants treated with d18:1 or t18:1 showed no

effect on plant responses to *V. dahlia* or *P. syringae* (Liu et al. 2020b).

In contrast to free LCBs, phosphorylated LCBs do not induce PCD and ROS in plant cells (Shi et al. 2007; Saucedo-Garcia et al. 2011). Indeed, phosphorylated and free LCBs can have opposite effects on cell death and ROS generation (Shi et al. 2007). For example, t18:0 induces defenses, but t18:0-P delayed ROS production upon challenge with *B. cinerea* and dramatically reduced the *P. syringae*-induced oxidative burst (Magnin-Robert et al. 2015). Paradoxically, LCB-P also promotes ROS production in plants treated with the proteinaceous elicitor cryptogein, which triggers early production of ROS, possibly through RESPIRATORY BURST OXIDASE HOMOLOGUE D (RBOHD) at the PM in tobacco BY-2 cells (Coursol et al. 2015). LCB-P application or overexpression of LCBK1 increased cryptogein-induced ROS production, whereas treatment with LCBs or LCB kinase inhibitors suppressed cryptogein-induced ROS production, indicating that LCBs and their phosphorylated derivatives differentially regulate cryptogein-induced ROS (Coursol et al. 2015).

Another controversial observation is that phosphorylated LCBs can offset the cell death stimulated by LCBs in plant cells. However, a recent study reported that elevated levels of phosphorylated LCBs do not antagonize LCB- or FB1-induced plant cell death; rather, the antagonistic effects of LCBs-P after simultaneous application of non-phosphorylated LCBs can be ascribed to reduced uptake of non-phosphorylated LCBs into the tissue (Glenz et al. 2019). The researchers also found that phosphorylated LCBs did not antagonize PCD in the hypersensitive response (Glenz et al. 2019).

Some synthetic short-chain Cers are used in Cer treatments since they cross the cell membrane and simulate the accumulation of C16 Cer in the cell during apoptosis in plant and animal cells (Liang et al. 2003; Hernandez-Corbacho et al. 2015). C2 Cer stimulates cell death in *Arabidopsis* (Liang et al. 2003). C2 Cer induces mitochondrial membrane potential loss within 2 h, followed by the release of cytochrome c from mitochondria at 8 h of C2 Cer treatment, resulting in cell death (Yao et al. 2004). Treatment of wild-type protoplasts with C2 Cer rapidly produced mitochondria-localized ROS, which can be blocked by an inhibitor of mitochondrial membrane potential loss (Bi et al. 2014). In vitro, C2 or C6 Cer can increase the NADPH oxidase activity of RbohD (Li et al. 2022). C2 Cer-induced cell death required protein kinase activity and new protein synthesis (Bi et al. 2014). By contrast, phosphorylated C2 Cer partially blocks these early events induced by C2 Cer, thus rescuing the plant cell (Liang et al. 2003; Yao et al. 2004; Bi et al. 2014).

The C2 Cer-induced ROS seem to not be responsible for cell death, as a study using *Arabidopsis* suspension cultures showed that inhibition of ROS had no effect on cell survival (Townley et al. 2005). Instead, C2 Cer-stimulated cell death depended on the generation of a calcium transient (Townley et al. 2005). Similarly, C6 Cer-induced cell death requires an increase in calcium levels and calcium signal transduction in rice protoplasts (Zhang et al. 2020). Moreover, caspase-3-like protease activity and VOLTAGE-DEPENDENT ANION CHANNELs (VDACs) are involved in C6 Cer-induced cell death (Zhang et al. 2020). A previous study reported that potassium loss is a critical step in cell death induced by C2 Cer or FB1 treatment in tobacco protoplasts (Peters and Chin 2007).

In addition, application of GIPCs influences PTI responses, such as the production of extracellular vesicles containing antimicrobial compounds. Mutants affecting the membrane microdomain protein TETRASPANIN 8 have lower amounts of cellular GIPCs and secrete fewer extracellular vesicles upon flagellin-derived peptide flagellin 22 (flg22) treatment. GIPCs partially restore extracellular vesicle secretion and the ROS burst in the *TETRASPANIN 8* knockout mutant upon flg22 treatment (Liu et al. 2020a).

Besides sphingolipids, sphingolipid metabolism inhibitors such as myriocin, fumonisins, *Alternaria alternata* toxins (AALs), DL-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol hydrochloride, tricyclodecan-9-yl-xanthogenate, and D-erythroN,N-dimethylsphingosine, are useful tools to study the role of endogenous sphingolipids in plant cell. For example, resistance of *Nicotiana benthamiana* against *Pseudomonas cichorii* was compromised by application of the SPT inhibitor myriocin and in *NbLCB2*- and *NbLCB1*- silenced plants, suggesting that sphingolipid biosynthesis is necessary for the nonhost resistance of *N. benthamiana* against *P. cichorii* (Takahashi et al. 2009).

Indeed, some pathogens produce and employ sphingolipid inhibitors, like fumonisins and AALs, to cause plant disease. For example, FB1 strongly disturbs sphingolipid metabolism in plants, causing a decrease in VLCF sphingolipid levels, a strong increase in LCBs, and induction of C16 Cer-containing sphingolipids (Shi et al. 2007; Markham et al. 2011) (Table 1). Two recent reviews have described the multiple defense responses of plants to FB1 in detail (Zeng et al. 2020; Iqbal et al. 2021) (Table 2). Recent studies highlighted the functions of FB1 in the interaction between plant hosts and FB1-producing pathogens. One group identified FB1 as a potential virulence factor of *Fusarium proliferatum* in modulating banana fruit defense responses (Xie et al. 2021). They found that FB1 contamination inhibited the defense ability of

banana fruit via decreasing phenylalanine ammonia lyase, β-1,3-glucanase and chitinase activities, and triggered the cell death of banana peel, increasing the aggressiveness of *F. proliferatum* on banana fruit (Xie et al. 2021). Another group determined FB1 is one of the toxins in *V. dahlia*, which causes cotton Verticillium wilt (Xu et al. 2022). They revealed that FB1 phenocopies the symptoms induced by *V. dahlia* and FB1 biosynthesis contributes to *V. dahliae* pathogenicity (Xu et al. 2022). Uncovering the multiple functions of FB1 in various plant host–pathogen systems remains an important topic for future study.

A recent study reported that application of sphingolipid pathway inhibitors (Table 2) altered glucosylhydroxyceramides and related sphingolipids by disturbing the secretory machinery for the GPI-anchored PD β-1,3-glucanase protein, resulting in an overaccumulation of callose and affecting symplasmic channel connectivity (Iswanto et al. 2020). Together, the use of commercial sphingolipids and sphingolipid metabolism inhibitors has helped reveal the multiple functions of sphingolipids in plant immunity.

Defense hormones

Many *Arabidopsis* mutants with abnormal sphingolipid contents show autoimmunity, cell death, and senescence phenotypes, along with constitutively elevated SA levels and activation of SA-dependent responses (Table 3). Until recently, it has been hard to judge which sphingolipid induced SA signaling; however, recent studies have suggested that LCBs, Cer, hCer, or GIPC are responsible for activating SA signaling.

Loss of ORM function leads to strong accumulation of LCBs, Cers, and hCers, and triggers constitutive induction of SA-dependent gene expression and tolerance to *P. syringae* strain DG3 compared with wild-type plants (Li et al. 2016). The induction of SA pathways is associated with the LCB composition of the sphingolipids. The *Arabidopsis sbh1 sbh2* double mutant, which accumulates large amounts of dihydroxylated LCBs but lacks trihydroxy LCBs, shows high expression of SA-signaling genes and manifests necrotic spots and premature death (Chen et al. 2008). In line with this, tobacco plants in which *SPT* was silenced had higher levels of d18:0 and d18:1, and lower levels of t18:0; these plants induced SA and SA-dependent genes and showed increased susceptibility to the necrotrophic fungus *Alternaria alternata* f. sp. *lycopersici* (Rivas-San Vicente et al. 2013). Furthermore, disruption of NCER2 induces t18:0 and SA accumulation, indicating that high levels of t18:0 trigger SA signaling (Zienkiewicz et al. 2019). However, in *sld1 sld2* mutants, which contained undetectable LCB Δ8 unsaturation and more d18:0 and t18:0, the SA level was similar to that in wild-type (Chen et al. 2012; Liu et al. 2020b), implying that reduced Δ8 LCB unsaturation may offset induction of SA by d18:0 and t18:0. Interestingly, we previously found that *acer* mutant plants, which contain less t18:0 and more Cers compared with wild type, accumulate less SA and are more susceptible to *P. syringae* (Wu et al. 2015b).

The activation of the SA pathway in *acd5*, *acd11*, *erh1*, and *fah1 fah2* mutants likely results from Cer accumulation, although other groups of sphingolipids cannot be excluded (Wang et al. 2008; Konig et al. 2012; Bi et al. 2014; Simanshu et al. 2014; Zeng et al. 2021). Among them, *acd5*, *erh1*, and *fah1/2* mutants exhibited enhanced resistance to the biotrophic fungal pathogen *Golovinomyces cichoracearum* (Wang et al. 2008; Konig et al. 2012). Similarly, virus-induced gene silencing of *ACD11* in *Arabidopsis* leaves enhanced resistance to the oomycete pathogen *Phytophthora capsica*. However, when infected with the hemi-biotrophic pathogens *P. syringae* or *V. longisporum*, the *erh1* and *fah1/2* mutants showed no difference in resistance compared with wild type (Wang et al. 2008; Konig et al. 2012). For necrotrophic fungi, *acd5* and *acd11* mutants are substantially more susceptible to *Botrytis* species than the wild type due to the cell death phenotype (Van Baarlen et al. 2007; Bi et al. 2014; Zeng et al. 2021).

Overexpression of *LOH1*, *LOH2*, or *LOH3* induced SA, highlighting the role of LOH2-produced C16 Cer in SA metabolism (Luttgeharm et al. 2015; Zeng et al. 2021). The contribution of VLCFA Cers to cell death phenotypes cannot be ignored, since *acd5 loh2* had a stronger phenotype than *acd5* (Bi et al. 2014). Induction of the SA pathway and cell death by accumulation of Cers, especially C16 Cers, required EDS1 and PAD4 signaling (Brodersen et al. 2002; Wang et al. 2008; Zeng et al. 2021). Overaccumulation of C16 Cer elevated the PAD4 and EDS1 protein levels in both the nucleus and cytoplasm; therefore, nuclear-localized PAD4 and EDS1 likely promote the expression of *SID2* and SA signaling genes (Zeng et al. 2021). We note that Cer-associated cell death cannot be completely ascribed to SA accumulation or SA signaling, as depleting SA (in the *salicylic acid induction deficient* 2–1 mutant) or blocking SA signaling pathway (in the *nonexpresser of pathogenesis-related genes* 1–1 mutant) did not fully abolish the *acd5* cell death phenotype (Greenberg et al. 2000; Zeng et al. 2021).

Modification of GIPC is also associated with activation of the SA pathway. For example, the *gonst1*, *gonst1 gonst2*, *iput1*, and *gmt1* mutants, which have defects in GIPC glycosylation or mannosylation, show high expression of SA-signaling genes (Mortimer et al. 2013; Fang et al. 2016). The vast compensatory changes in the sphingolipidome in the *iput1* mutants may also be responsible for cell death and SA induction (Tartaglio et al. 2017). However, abnormal glycosphingolipid mannosylation seems

Table 3 Sphingolipid metabolism-related proteins involved in plant immunity

Protein	Experimental condition	Immune response	Mechanisms	References
AtLCB2b	Expression of *AtFBR41* in tomato	*A. alternata* (R)	AAL-toxin resistance	(Shao et al. 2019)
NbLCB1	VIGS of *NbLCB1*	*P. cichoriiis* (S)	Cell death	(Takahashi et al. 2009)
NbLCB2	VIGS of *NbLCB2*	*P. cichoriiis* (S), *A. alternata* (S)	Cell death, SA	(Takahashi et al. 2009; Rivas-San Vicente et al. 2013)
	NbLCB2 OE	*P. cichoriiis* (R)	Cell death	(Takahashi et al. 2009)
BcLCB2	Expression of *BcLCB2* in *Nicotiana tabacum*	*R. solanacearum* (R)	Cell death	(Gan et al. 2009)
OsLCB2a1	*OsLCB2a1* OE	*M. persicae* (R)	Callose, wax, SA	(Begum et al. 2016)
AtORM1/2	*orm1 amiR-ORM2*, *ORM1 RNAi*, *ORM2 RNAi*, and *orm1 or orm2*	*P. syringae* (R), enhanced ROS response to flg22	SA, degradation of FLS2 by selective autophagy	(Li et al. 2016; Yang et al. 2019a)
	ORM1 OE and *ORM2* OE	*P. syringae* (S), reduced ROS response to flg22		
AtLCBK1	*lcbk1-2*, *lcbk1-3*	*P. syringae* (S), reduced stomata response to flg22, or SA	Induction of stomatal closure by t18:0-P, and crosstalk with SA	(Gupta and Nandi 2020; Gupta et al. 2020)
AtSPHK1	*sphk1-2*, *SPHK1*-KD	No treatment	Callose	(Iswanto et al. 2020)
AtSPHK1	Expression of *AtSPHK1* in tobacco BY-2 cells	Proteinaceous elicitor cryptogein (R)	ROS	(Coursol et al. 2015)
AtDPL1	*dpl1*	*P. syringae* and *P. syringae avrRPM1* (S), and *B. cinerea* (R)	Cell death, SA, JA	(Magnin-Robert et al. 2015)
OsSPL1	*OsSPL1*-OE	*P. syringae* (S)	*PR* genes	(Zhang et al. 2014)
AtSLD1/2	*sld1 sld2*	*P. syringae* (R), and *V. dahlia* (R)	Callose	(Ning-Jing et al. 2019)
AtLOH1	*loh1-2*	Cytotoxins NLP proteins (R)	GIPCs as receptors of NLPs	(Lenarčič et al. 2017)
AtLOH1/2/3	LOH1/2/3 OE	No treatment	SA	(Luttgeharm et al.2015)
AtFAH1/2	*fah1/fah2*, *fah1c* (CRISPR/Cas9-based mutant) *fah2*	*P. syringae* (R), *G. cichoracearum* (R), cytotoxins NLP proteins (R), reduced long-term ROS response to rhamnolipids, reduced ROS response to flg22 and chitin	GIPCs as receptors of NLPs, PTI	(Konig et al. 2012; Lenarčič et al. 2017; Schellenberger et al. 2021; Ukawa et al. 2022)
OsFAH1/2	*OsFAH1/2*-KD	*M. oryzae* (S)	PTI	(Nagano et al. 2016)
PpS4H	*s4h*	No treatment	Callose	(Gomann et al. 2021)
AtACD5	*acd5*	*P. syringae* (S), *B. cinerea* (S), *G. cichoracearum UCSC1* (R)	SA, ROS, cell death	(Greenberg et al. 2000; Wang et al. 2008; Bi et al. 2014)
AtACD11	*acd11*, VIGS of *AtACD11*	*P. capsici* (R)	SA, ROS, cell death	(Brodersen et al. 2002; Li et al. 2019)
AtACER	*acer-1* and amiR-*ACER*-1	*P. syringae* (S), reduced ROS response to flg22	PTI signaling	(Wu et al. 2015b)
AtNCER1	*ncer1*	No treatment	JA	(Zienkiewicz et al. 2019)
AtNCER2	*ncer2*	No treatment	SA	(Zienkiewicz et al. 2019)
AtIPCS2	*erh1*	*G. cichoracearum* (R), *P. syringae* (S)	Cell death, SA	(Wang et al. 2008)
AtGONST1	*gonst1-2*	No treatment	SA, ROS, cell death, cellulose	(Mortimer et al. 2013)
AtGONST2	*gonst2-1*	*G. orontii* (R), *B. cinerea* (S)	SA, ROS, cell death, cellulose	(Jing et al. 2021)
IPUT1	*pLAT52:IPUT1 iput1/iput1*	No treatment	SA, ROS, cell death	Tartaglio et al. 2017)
AtGMT1	*gmt1-1*, *gmt1-3*	No treatment	SA, ROS, cell death	(Fang et al. 2016)
AtGCS	*gcs-2*	No treatment	Callose	(Iswanto et al. 2020)

R, resistant; S, sensitive; KD, knockdown; OE, overexpressing line; *At*, *Arabidopsis thaliana*; *Os*, *Oryza sativa*; *Bc*, *Brassica campestris*; *Pp*: *Physcomitrium patens*; *P. syringae*, *Pseudomonas syringae*; *B. cinerea*, *Botrytis cinerea*; *A. alternata*, *Alternaria alternata*; *F. verticillioides*, *Fusarium verticillioides*; *V. dahlia*, *Verticillium dahlia*; *G. orontii*, *Golovinomyces orontii*; *G. cichoracearum*, *Golovinomyces cichoracearum*; *M. oryzae*, *Magnaporthe oryzae*; *M. persicae*, *Myzus persicae*; *P. capsica*, *Phytophthora capsici*; *R. solanacearum*, *Ralstonia solanacearum*; *M. sexta*, *Manduca sexta*; SPT, serine palmitoyltransferase; LCB, long-chain base; LCB-P, long-chain base-phosphate; Cer, ceramide; hCer, hydroxyceramide; SA, salicylic acid; ROS, reactive oxygen species; LCB2b, subunit of SPT; LCB2, subunit of SPT; LCB2a1, subunit of SPT; ORM1/2, orosomucoid-like proteins; LCBK1, LCB kinase; SPHK1, sphingosine kinase; DPL1,dihydrosphingosine-1-phosphate lyase1; SPL1, sphingosine-1 phosphate lyase; SLD1/2, sphingoid LCB desaturase; LOH1, ceramide synthase; FAH1/2, fatty acid alphahydroxylase; S4H, LCB C-4 hydroxylase; ACD5, ceramide kinase; ACD11, ceramide-P transporter; ACER, alkaline ceramidase; IPCS2, inositol phosphorylceramide synthase 2; GONST1/2, Golgi nucleotide sugar transporter; IPUT1, inositol phosphorylceramide glucuronosyltransferase; GMT1, GIPC mannosyl-transferase; GINT1, GIPC transferase; GCS, glucosylceramide synthase

to have little effect on the overall sphingolipid composition in *gonst1* callus, corroborating suggested roles for GIPC modification in plant cell death and activation of the SA pathway (Mortimer et al. 2013). In addition, depletion of SA largely suppressed necrotic lesion phenotypes in *gonst1* mutants (Mortimer et al. 2013). GONST2 has a similar function to GONST1, and the *gonst2-1* mutant has increased resistance to the biotrophic pathogen *Golovinomyces orontii* but not the necrotrophic pathogen *B. cinerea* (Jing et al. 2021).

In addition to SA, JAs are involved in defense responses in mutants with altered sphingolipid contents (Table 3). JA regulates plant defenses against fungi and insects (Howe et al. 2018). In *acd5* mutants, JAs accumulate and JA signaling is active (Huang et al. 2021; Zeng et al. 2021). Surprisingly, *acd5* showed a similar phenotype to wild-type in *Spodoptera exigua* resistance (Huang et al. 2021). Loss of function of the JA signaling genes *JASMONATE RESISTANT1* or *CORONATINE INSENSITIVE1* delayed cell death of *acd5* but failed to inhibit cell death later in development (Huang et al. 2021). Indeed, Me-JA increases Cer levels and thus accelerates cell death in *acd5* mutants through an SA-dependent pathway (Huang et al. 2021).

Additional studies have explored the effects of JA and SA levels on sphingolipids and defenses. For example, in *erh1* mutants, which accumulate SA and Cer, no significant difference in jasmonate-isoleucine (JA-Ile) content was detected relative to wild-type plants (Wang et al. 2008). By contrast, JA-Ile accumulated in *ncer1* mutants, which displayed JA-Ile-dependent early leaf senescence and an increase in hCers (Zienkiewicz et al. 2019). Higher levels of SA and JA in *ncer1 ncer2* double mutants (compared with that in the single mutants) resulted in a more serious cell death phenotype, suggesting that these two hormones synergistically meditate cell death when sphingolipid homeostasis is disrupted (Zienkiewicz et al. 2019).

OsLCB2a-overexpressing transgenic plants accumulated LCB and Cers compared with wild type, and induced SA-dependent genes but repressed JA-related genes; these plants also showed inhibited aphid infestation (Begum et al. 2016). Conversely, overexpression of SPHINGOSINE-1-PHOSPHATE LYASE in tobacco dramatically reduced SA-dependent gene expression and increased susceptibility to *P. syringae* pv. *tabaci*, while JA-responsive gene expression was slightly enhanced (Zhang et al. 2014). JA-Ile synthesis and JA-dependent signaling pathways were only enhanced in the *dpl1-1* mutant under pathogen infection (Magnin-Robert et al. 2015). The plants showed sensitivity to *P. syringae* but tolerance to *B. cinerea*, suggesting the role of sphingolipids in JA-dependent immunity (Magnin-Robert et al. 2015).

Our understanding of the molecular mechanisms underlying the crosstalk between sphingolipids and defense hormones is still rudimentary. A previous study reported that the synthesis of chloroplast glycerolipids, particularly molecular species containing C16 fatty acids, was reduced in *sbh1 sbh2* mutants (Chen et al. 2008). The authors hypothesized that this may be due to the increased demand for palmitic acid to support sphingolipid biosynthesis in LCB C-4 hydroxylase mutants (Chen et al. 2008). Since the main steps in SA and JA biosynthesis mainly occur in chloroplasts, disruption of sphingolipid metabolism may affect SA or JA levels by affecting chloroplast lipids (Kachroo et al. 2001; Nandi et al. 2003; Chandra-Shekara et al. 2007).

The important defense-related hormone ethylene also interacts with sphingolipids. For example, ethylene-dependent signaling pathways are required for FB1-induced cell death in *Arabidopsis* (Asai et al. 2000; Plett et al. 2009; Mase et al. 2013; Wu et al. 2015a). Ethylene signaling negatively regulates FB1-induced cell death by inhibiting sphingolipid biosynthesis (Wu et al. 2015a). Conversely, sphingolipids affect ethylene signaling. Unsaturation of VLCFA-containing Cers protects plant from hypoxia-induced damage by modulating ethylene signaling through association with CONSTITUTIVE TRIPLE RESPONSE1, a negative regulator of the ethylene signal transduction pathway (Xie et al. 2015). Further research will be needed to understand the crosstalk between ethylene and sphingolipid signaling in plant–pathogen interactions.

Brassinosteroids (BRs) are also important defense-related hormones (Burger and Chory 2019) and emerging research suggests that BRs affect sphingolipid metabolism. For example, BR signaling modulates sphingolipid metabolism during early fleshy-fruit growth of olive (*Olea europaea* L. cv *Picual*) (Corbacho et al. 2018). In olives, the application of exogenous BRs (24-epibrassinolide) significantly reduced the LCB content and application of a BR biosynthesis inhibitor (brassinazole) increased the LCB content (Corbacho et al. 2018). The BR receptor BR INSENSITIVE 1 (BRI1) interacts with BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) to regulate PTI (Albrecht et al. 2012; Belkhadir et al. 2012). This led to speculation that BRs may coordinate PTI and LCB signaling during immunity. Indeed, a recent study reported that FB1 and *V. dahliae* treatment suppressed an IQ67-domain (IQD) protein-encoding gene *GhIQD10*, which confers Verticillium wilt resistance in cotton in a BR- and pathogenesis-related protein-dependent manner (Xu et al. 2022). Overexpression of *GhIQD10* significantly enhanced resistance of cotton to FB1 (Xu et al. 2022). These findings imply that LCB accumulation, induced by FB1 and *V. dahlia*, may affect BR signaling. In addition,

hydroxyl groups of sphingolipid acyl chains affect the abundance of BAK1 in the *Arabidopsis* PM, suggesting the association between 2-hydroxy sphingolipids and BR signaling (Ukawa et al. 2022). In-depth studies will be required to investigate how BRs and sphingolipids interact with each other upon pathogen attack.

Abscisic acid (ABA), which is traditionally associated with abiotic stress responses, has an antagonistic role to SA during plant immunity and controls stomatal immunity (Burger and Chory 2019). ABA application inhibits sphingolipid accumulation and cell death in *acd5* mutants by an antagonistic interaction with SA (Yang et al. 2019a, b). However, ABA treatment rapidly induces the accumulation of several LCB-Ps in wild-type plants (Guo et al. 2012). Interestingly, LCB-P interacts with ABA and SA in the positive regulation of stomatal closure (Guo et al. 2012; Nakagawa et al. 2012; Gupta and Nandi 2020), strongly underlining the critical roles of LCB-P in stomatal immunity.

PTI, ETI, and ETS

Perception of PAMPs and DAMPs requires plant cell-surface immune receptors, such as FLAGELLIN SENSING 2 (FLS2), which functions as the receptor for the flg22. In addition to negatively regulating sphingolipid biosynthesis, *Arabidopsis* ORM proteins act as selective autophagy receptors to mediate the FLS2 degradation through interaction with FLS2 and the autophagy-related protein AUTOPHAGY 8 (ATG8) (Yang et al. 2019a). We previously reported that LCBs trigger autophagy (Zheng et al. 2018). Since t18:0 is rapidly induced by bacteria, negative feedback regulation of FLS2-dependent PTI signaling may be mediated by autophagy induced by t18:0.

LCB-P is involved in PTI-induced stomatal closure. Application of t18:0-P triggers stomatal closure and rescues the loss-of-PTI phenotype of *lcbk1* mutant plants, suggesting that LCBK1 positively regulates stomatal immunity via phosphorylating t18:0 in *Arabidopsis* (Gupta et al. 2020). Moreover, LCBK1 interacts with the Polycomb-group repressor complex2 protein MEDEA, which suppresses both PTI and ETI (Gupta et al. 2020), highlighting the role of LCB metabolism in immunity.

Sphingolipids seem to participate in the perception of invasion-related pattern molecules. Rhamnolipids are extracellular amphiphilic metabolites produced by several bacteria, especially *Pseudomonas* and *Burkholderia* species. Rhamnolipids modulate bacterial surface motility, biofilm development, and thus successful colonization of hosts (Perneel et al. 2008; Abdel-Mawgoud et al. 2010; Irorere et al. 2017). In addition, rhamnolipids trigger an atypical immune response and resistance in *Arabidopsis* to the opportunistic pathogen *Pseudomonas aeruginosa* (Schellenberger et al. 2021). Pre-treatment with rhamnolipids can enhance plant disease resistance. The *fah1/2* mutant, but not *loh1*, showed a reduced long-term ROS response upon rhamnolipid treatment and the enhanced resistance of plants to *P. syringae* triggered by rhamnolipid pretreatment is compromised in *fah1/2* mutant plants (Schellenberger et al. 2021). This study suggests an important role of PM sphingolipid composition, possibly GlcCers, in the immune response triggered by rhamnolipids (Schellenberger et al. 2021).

ACD11 is a sphingolipid metabolic enzyme that plays essential roles in plant immunity and susceptibility. Disruption of ACD11 in *Arabidopsis* triggers an autoimmunity that requires the RPS4-like R protein LAZARUS5 (LAZ5) (Palma et al. 2010). The necrotrophic fungus *Sclerotinia sclerotiorum* may manipulate plant sphingolipid pathways guarded by LAZ5 to trigger cell death and cause disease (Barbacci et al. 2020). How altered sphingolipid levels induce autoimmunity through LAZ5 and how the protein acts in ETI signaling remain unknown.

By contrast, ACD11-mediated resistance and susceptibility are well described. In *Arabidopsis*, ACD11 interacts with Golgi-localized XBAT35.2, a defense protein belonging to a subfamily of RING-type E3s. XBAT35.2 mediates proteasome-dependent degradation of ACD11 to induce ROS and defense responses (Liu et al. 2017). In addition, XBAT35.2 plays an opposing role in abiotic stress through the regulation of ACD11 degradation (Li et al. 2020a). ACD11 degradation can be manipulated by pathogens. The *Phytophthora capsici* effector RxLR207, which is essential for virulence of the pathogen, targeted and promoted the degradation of BINDING PARTNER OF ACD11 1 (BPA1) and its homologs, to disrupt ACD11 stabilization in a 26S proteasome-dependent manner, eventually leading to enhanced ROS, cell death, and defense responses in *Arabidopsis* (Li et al. 2019). ACD11, BPA1, and its homologs seem to be susceptibility factors that are hijacked by *P. capsici* to aid in the transition from biotrophic to necrotrophic infection, by producing the RxLR207 effector (Li et al. 2019).

Functions of sphingolipids in membranes

In some cases, a small change in sphingolipids is enough to cause cell death or plant immunity. For example, the sphingolipid contents were not significantly changed in the At*LCB1* RNAi plants, but the plants exhibited obvious necrotic lesions (Chen et al. 2006). In addition, relative to wild-type plants, *LOH1*- and *LOH3*-overexpressing plants had enhanced biomass and higher levels of SA, despite little change in total sphingolipid contents and composition in these plants (Luttgeharm et al. 2015). One possibility is that plant sphingolipids subtly shape membrane microdomains or alter membranes in some way. In fact, a set of evidence has demonstrated

that membrane sphingolipids control immunity-related proteins, or directly sense pathogen-producing factors (Fig. 2).

During plant–microbe interactions, nanometer-scale membrane platforms may act as signaling hubs and enable the formation of specific spatio-temporal assemblies of PRRs and other complex constituents; these domains allow the physical separation of genetically overlapping pathways (Ott 2017). The larger-scale membrane sub-compartments have been called 'lipid rafts', 'nanodomains', and 'microdomains' (Ott 2017). The concept of lipid rafts was initially based on the self-organizing capacity of sterols in model membranes and a comparably crude biochemical separation of PMs named detergent-resistant membranes (DRMs) (Ott 2017). Further study showed that DRMs were a biochemical counterpart of membrane rafts (Raffaele et al. 2009). DRMs are mainly formed by sphingolipids, sterols, and some glycerolipids, and they have much higher sphingolipid contents than vacuolar membranes and the PM. Moreover, some sphingolipid species from the PM were concentrated in DRMs in plants (Mongrand et al. 2004; Laloi et al. 2007; Lefebvre et al. 2007; Cacas et al. 2016; Carmona-Salazar et al. 2021).

The terms 'microdomains' and 'nanodomains' have been synonymously used, and some confusion about terminology has emerged (Ott 2017). For instance, larger membrane structures such as PAMP-induced focal protein accumulation have been referred to as microdomains (Bhat et al. 2005). The composition of the microdomain directly (lipid–protein interactions) and indirectly (changes to the physical membrane) regulates the functions of receptors and ion channels in microdomains (Quinville et al. 2021).

Emerging research has revealed the role of plant sphingolipids in regulation of microdomains. For example, microdomain-associated endocytic pathways mediate RbohD dynamics upon salt stress (Hao et al. 2014), implying that disturbance of sphingolipid metabolism may alter RbohD turnover. In rice, 2-hydroxy-sphingolipids produced by OsFAH1 and OsFAH2 regulate the formation of microdomains, which are critical for the organization of defense proteins localized in these microdomains, such as RbohB (Nagano et al. 2016). The rice Os*fah1/2* plants are susceptible to the hemibiotrophic fungus *Magnaporthe oryzae* (*M. oryzae*) due to impaired ROS production (Nagano et al. 2016). It is interesting that Os*fah1/2* plants displayed similar SA levels to wild-type plants, indicating that this process is independent of SA (Nagano et al. 2016). Similarly, deletion of FAH1 and FAH2 downregulates RBOHD and PRRs in the PM of *Arabidopsis* and suppresses the ROS burst after flg22 and chitin treatment, indicating that 2-hydroxy sphingolipids are crucial for the organization of PM nanodomains and the ROS burst during PTI (Ukawa et al. 2022).

Plants overexpressing BAX-INHIBITOR-1 (AtBI-1), which interacts with FAH1 and FAH2, exhibited enrichment of 2-hydroxy fatty acid-containing GlcCer in microdomains. Among the DRM proteins in *BI-1*-overexpressing cells, the abundance of FLOTILLIN HOMOLOG and HYPERSENSITIVE-INDUCED REACTION PROTEIN3 markedly decreased (Ishikawa et al. 2015). This reduction in protein content enhanced tolerance to SA or oxidative stress in *AtBI-1*-overexpressing plants since these two proteins function in plant defense and cell death (Ishikawa et al. 2015). In addition to microdomain regulation, BI-1 also participates in autophagy by interacting with the core autophagy-related protein AUTOPHAGY 6 to regulate autophagic activity induced by *N* gene-mediated resistance to tobacco mosaic virus (Xu et al. 2017). How sphingolipids in microdomains interact with autophagy upon pathogen infection remains ambiguous.

Arabidopsis mutants in sphingolipid synthesis are useful tools to understand the structure and function of membrane microdomains in PD (Gonzalez-Solis et al. 2014). PDLP5 can be recruited by t18:0-based sphingolipids, stimulating callose accumulation at PDs in *Arabidopsis* upon infection with the fungal pathogen *V. dahlia* or the bacterium *P. syringae* (Liu et al. 2020b). Alteration in glucosylhydroxyceramides or related sphingolipid composition through application of sphingolipid pathway inhibitors disturbed the secretory machinery for the GPI-anchored PdBG2 protein. This resulted in over-accumulation of callose and affected symplasmic channel connectivity (Iswanto et al. 2020). The *acd5* plants showed less callose deposits than wild-type plants in response to *B. cinerea* infection or the PAMP chitin (Bi et al. 2014), but not flg22 (Yang et al. 2019b). The callose deposit defect in *acd5* at early time points when Cer content is almost unchanged indicates that Cer-P may engage in callose deposition and PD regulation.

SA also controls PDs (Han and Kim 2016). For instance, SA induces the expression of *PDLP5* (Lee et al. 2011; Wang et al. 2013). Moreover, SA triggers the compartmentalization of lipid raft nanodomains by modulating the lipid raft-regulatory protein remorin, without significantly changing sterol and sphingolipid abundance (Huang et al. 2019). By inducing SA signaling, plants restrict the spreading of virus and PD cargoes by increasing lipid order and closing PD (Huang et al. 2019). Since disturbing sphingolipid metabolism activates SA signaling, the contribution of SA in regulating PD should be considered in future studies.

Moreover, sphingolipid metabolism affects PD ultrastructure (Yan et al. 2019). PHLOEM UNLOADING

MODULATOR encodes an enzyme potentially required for the biosynthesis of sphingolipids with VLFA (Yan et al. 2019). Lack of this protein disturbs the formation of ER–PM tethers, resulting in the majority of PD lacking a visible cytoplasmic sleeve (Yan et al. 2019).

GIPCs, the largest group of sphingolipids in plants, act as receptors in the outer layer of the PM to recognize necrosis and ethylene-inducing peptide 1-like proteins (NLPs) microbial toxins, proteins produced by plant pathogenic bacteria, fungi, and oomycetes (Lenarčič et al. 2017). NLPs are cytotoxic in eudicot plants because they have selective binding affinity for the eudicot plant-specific GIPCs (Lenarčič et al. 2017). The oomycete NLP cytolysin disrupts plant membranes, a multistep process that includes electrostatic-driven GIPC recognition, shallow membrane binding, protein aggregation, and transient pore formation (Pirc et al. 2022). NLP-induced damage is caused by small membrane ruptures rather than membrane reorganization or large-scale defects (Pirc et al. 2022). Interestingly, *V. dahliae* seems to increase GIPC contents to make cotton more sensitive to its NLP toxins (Xu et al. 2022). A very recent study revealed the cytotoxic activity of NLPs on several monocot plant species, including onion, lily, and maize (Steentjes et al. 2022). GIPC composition may correlate with NLP sensitivity (Steentjes et al. 2022).

The salt-sensing mechanism mediated by GIPCs has also been revealed. GIPCs bind Na^+ to gate Ca^{2+} influx channels (Jiang et al. 2019). We speculate that some molecules produced by pathogens may bind to GIPCs, or indirectly affect GIPCs, thus determining the action of Ca^{2+} influx channels.

GIPCs also function in plant endosymbiosis, which relies on the development of specialized sphingolipid-containing membranes that encapsulate the endosymbiont and facilitate nutrient exchange (Moore et al. 2021). A recent study reported that the successful colonization of *Medicago truncatula* roots by arbuscular mycorrhizal fungi requires the sphingolipid glycosyltransferase MtGINT1 expressed in the host to synthesize GIPCs (Moore et al. 2021). Lack of MtGINT1 strongly impaired nodulation and arbuscular mycorrhizal symbiosis, leading to the senescence of symbiosomes and arbuscules, indicating that local reprogramming of GIPC glycosylation is required for endosymbionts to persist within the host cell (Moore et al. 2021).

Based on the large amount of evidence, it is important to consider GIPC metabolism as an essential factor in plant immunity. Importantly, considering that DRMs contain 44% GIPCs (Carmona-Salazar et al. 2021), and plant sphingolipids in extracellular vesicles are nearly pure GIPCs (Liu et al. 2020a), the function of DRMs-localized and extracellular GIPCs in plant immunity needs to be investigated thoroughly.

Antimicrobial activity of sphingolipids

Sphingolipids may be exported into the apoplastic space by plant cells to influence microbial growth. Recently, t18:0 was detected in wheat (*Triticum aestivum*) root exudates and was identified as a potential allelochemical that defends against *Fusarium oxysporum* f. sp. *niveum* (*Fon*) infection in a wheat/watermelon (*Citrullus lanatus*) companion cropping system (Li et al. 2020b). Indeed, t18:0 inhibits *Fon* and affects the rhizosphere soil microbial community of watermelon (Li et al. 2020b). Another group found that t18:0 reduced *in vitro* growth of a range of plant-interacting microorganisms, including the plant pathogenic fungus *Verticillium longisporum* (causing wilt, e.g. in canola, *Brassica napus* ssp. *napus*), *Fusarium graminearum* (causing wheat head blight) and *Sclerotinia sclerotiorum* (causing stem rot), the plant symbiotic fungal endophyte *Serendipita indica*, and the bacterial pathogens *P. syringae* and *Agrobacterium tumefaciens*, along with the related beneficial strain *Rhizobium radiobacter* (Glenz et al. 2022). Notably, t18:0 kills *P. syringae*, but its phosphorylated counterpart, t18:0-P, fails to inhibit growth of *P. syringae* (Glenz et al. 2022). When co-infiltrated into *Arabidopsis* leaves, t18:0 reduces disease symptoms and inhibits growth of *P. syringae*, with no visible negative effect on host tissue (Glenz et al. 2022). These findings indicate the potential of elevated LCB levels to enhance plant pathogen resistance.

Conclusions and future perspectives

Recently, remarkable advances have revealed the critical role of sphingolipids in regulating plant immunity. However, several pressing questions remain to be addressed in order to gain a comprehensive understanding of sphingolipids in regulating plant immunity. Plant sphingolipid metabolism may be employed by the host or pathogen to favor their own interests. Therefore, it is important to model the regulatory networks by which plant sphingolipids respond to different pathogens, for us to understand their roles in the interactions. Moreover, although many diverse functions of sphingolipids have been uncovered, and the functions of a large body of sphingolipid metabolic enzymes in plant immunity have been revealed, most of the molecular mechanisms remain unclear, particularly their connections to known signaling mechanisms like PTI, ETI, and hormone pathways. It would be useful to know how sphingolipids in the PM, particularly microdomains, act to perceive pathogens and transduce signals during plant–pathogen interactions.

Sphingolipid-mediated cell death in eukaryotes remains a hot topic for research. In animals, C16 Cers

form specialized Cer channels, without the need for proteins, in mitochondrial outer membranes but not in the PM (Siskind and Colombini 2000; Siskind et al. 2006; Samanta et al. 2011; Colombini 2016; Perera et al. 2016), or directly and specifically bind VDAC2 on mitochondria (Dadsena et al. 2019) to trigger cell death. It will be interesting to investigate whether sphingolipids mediate cell death in plants through these pathways (Fig. 2). In addition, more effort needs to be exerted to investigate whether sphingolipids participate in ETI-triggered cell death, for example, controlling the Ca^{2+}-permeable channels formed by R proteins (Bi et al. 2021; Jacob et al. 2021; Ngou et al. 2021).

Sphingolipids are a large group of lipids with high structural diversity. In *Arabidopsis*, for example, more than 300 sphingolipid species have been identified (Liu et al. 2021). Corresponding advanced analytical methods for sphingolipids in various plant species, in particular crops, remain to be established. Theoretically, sphingolipid metabolism is conserved in eukaryotes and the genes that encode enzymes in plant sphingolipid metabolism should be easy to characterize. However, revealing the entire sphingolipid metabolism and transport pathway will require more effort and resources, not only in model plants such as *Arabidopsis* but also in other plants. Progress in characterizing and measuring sphingolipids and investigating their turnover will set the stage for researchers to understand the roles sphingolipids play in plant immunity. It is hard but meaningful to appreciate the relationship between structural diversity and functional significance of sphingolipids in plant immunity. For example, understanding which sphingolipid modifications influence their functions in plant immunity may pave the way for us to genetically remodel sphingolipid profiles to improve disease resistance in crops in the future.

Understanding sphingolipids in plant pathogens and insects will also help us to reveal the interaction between hosts and their foes. For example, inhibition of Cer synthase by noncontrolled hydroxylated diterpene derivatives causes a severe toxic reaction in *Nicotiana attenuata*, as well as in herbivores, whereas the controlled hydroxylated diterpene strategy allows plants to gain resistance to their insect herbivores without Cer-triggered autotoxicity (Li et al. 2021).

Some fungi use sphinganine-analog mycotoxins (SAMs) to destroy Cer synthase function in plants; therefore, Cer synthases seem to be important for plant immunity. A recent study revealed the mechanisms of self-protection employed by the FB1 producer *Fusarium verticillioides* (Janevska et al. 2020). Similar strategies could be used to modify Cer synthases in crops to avoid damage by SAMs. In addition, artificial pesticides could be designed to target Cer synthases in pathogenic fungi or insects, without affecting normal function of the enzymes in plants and animals. In another example, *M. oryzae* VLCFs are needed to organize septin GTPases, which are essential for appressorium-mediated infection (He et al. 2020). VLCF biosynthesis inhibitors show effective, broad-spectrum fungicidal activity against rice blast (mediated by *M. oryzae*), and a wide range of fungal pathogens of maize, wheat, and locusts, without affecting their respective hosts (He et al. 2020). It is possible that sphingolipids, especially those containing VLCFs, are recruited to build fungal infection structures. Impairing the sphingolipid metabolism of pathogenic fungi or insects accurately may be a useful way to control damage they caused.

In concluding this review, we would like to point out that, although we have focused our discussion on how sphingolipids affect plant responses to biotic stresses, the functions of sphingolipids in development and abiotic stresses cannot be overlooked, since they may also determine the outcomes of defense in natural environments. Furthermore, there are numerous examples in which sphingolipids affect animal–microbe interactions (Seo et al. 2011; Maceyka and Spiegel 2014; Tafesse et al. 2015; Rolando et al. 2016). The identification of cross-kingdom principles will substantially inform our understanding of sphingolipids in plants and animals. Investigating sphingolipids, and their mechanisms underlying defense responses will provide a holistic understanding of plant and animal immunity that can be harnessed to control diseases.

Abbreviations

3-KSR: 3-Ketodihydrosphingosine reductase; ABA: Abscisic acid; BAK1: BRI1-ASSOCIATED RECEPTOR KINASE 1; BRI1: BR INSENSITIVE 1; BRs: Brassinosteroids; CDases: Ceramidases; Cer: Ceramide; Cer-P: Ceramide-phosphate; CPK3: CALCIUM-DEPENDENT KINASE; d18:0: Sphinganine or dihydrosphingosine; d18:1: Sphingosine; d18:2: Sphingadiene; D609: Tricyclodecan-9-yl-xanthogenate; DAG: Diacylglycerol; DAMPs: Damage-associated molecular patterns; DMS: D-erythro-N,N-dimethylsphingosine, an inhibitor of LCB kinase; DPL1: Dihydrosphingosine-1-phosphate lyase1; EDS1: ENHANCED DISEASE SUSCEPTIBILITY; ER: Endoplasmic reticulum; ET: Ethylene; ETI: Effector-triggered immunity; ETS: Effector-triggered susceptibility; EVs: Extracellular vesicles; FA: Fatty acid; FAHs: Fatty acid alphahydroxylase; FB1: Fumonisin B1, an inhibitor of ceramide synthase; flg22: Flagellin-derived peptide flagellin 22; FLS2: FLAGELLIN SENSING 2; GCD: Glucosylceramidases; GCS: Glucosylceramide synthase; GINT1: Glucosamine inositol phosphorylceramide transferase; GIPC: Glycosyl inositol phosphoceramide; GIPCPLD: GIPC-specific phospholipase D; GlcCer: Glucosylceramide; GMT1: GIPC mannosyl-transferase; GONST: Golgi-localized nucleotide sugar transporters; hCer: Hydroxyceramide; IPCS: Inositolphosphorylceramide synthases; IPUT1: Inositol phosphorylceramide glucuronosyltransferase; IQD10: IQ67-domain protein 10; JA: Jasmonic acid; JA-Ile: Jasmonate-isoleucine; KD: Knockdown; LCB: Long-chain base; LCB-P: Long-chain base-phosphate; LCF: Long-chain fatty acid; LOH: LAG ONE HOMOLOGUES, ceramide synthases; MAPKs: Mitogen-activated protein kinases; MeJA: Methyl jasmonate; NLPs: Necrosis and ethylene-inducing peptide 1-like proteins; NLR: Nucleotide binding-leucine rich repeat; NPC4: A nonspecific phospholipase C4; OE: Overexpressing line; ORM: Orosomucoid-like proteins; PAD4: PHYTOALEXIN DEFICIENT 4; PAMPs: Pathogen-associated molecular patterns; PCD:

Programmed cell death; PD: Plasmodesmata; PDLP5: PD-LOCATED PROTEIN 5; PDMP: DL-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol hydrochloride, an inhibitor of glucosylceramide synthase; PI: Phosphatidylinositol; PLM: Phloem unloading modulator; PM: Plasma membrane; *Pp*: *Physcomitrium patens*; PRRs: Membrane pattern recognition receptors; PTI: PAMP-triggered immunity; RBOHD: RESPIRATORY BURST OXIDASE HOMOLOGUE D; ROS: Reactive oxygen species; SA: Salicylic acid; SAMs: Sphinganine-analog mycotoxins; SBH1/2: Sphingoid base hydroxylases; Ski: 2-(p-hydroxyanilino)-4-(p-chlorophenyl)thiazole, an inhibitor of sphingosine kinase; VDACs: Voltage-dependent anion channels; SL: Sphingolipid; SLD1/2: Sphingoid LCB desaturase; SPHK1: Sphingosine kinase; SPL1: Sphingosine-1 phosphate lyase; SPP1: Phyto-S1P phosphatase; SPT: Serine palmitoyltransferase; ssSPTa/b: Small subunits of SPT; t18:0: Phytosphingosine or 4-hydroxysphinganine; t18:1: 4-Hydroxysphingenine; UGNT1: UDP-*N*-acetyl-D-glucosamine transporter; VLCF: Very-long-chain fatty acid.

Acknowledgements
We apologize to all colleagues whose work could not be cited due to space limitations.

Author contributions
HYZ and NY wrote the manuscript. HYZ drew the figures. Both authors read and approved the final manuscript.

Funding
This work was supported by the National Natural Science Foundation of China (32070196), the Natural Science Foundation of Guangdong Province (2019B1515120088).

Availability of data and materials
Not applicable.

Declarations

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 19 February 2022 Accepted: 14 May 2022
Published online: 01 June 2022

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