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**Update**

# From sensing to acclimation: The role of membrane lipid remodeling in plant responses to low temperatures

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## Abstract

Low temperatures pose a dramatic challenge to plant viability. Chilling and freezing disrupt cellular processes, forcing metabolic adaptations reflected in alterations to membrane compositions. Understanding the mechanisms of plant cold tolerance is increasingly important due to anticipated increases in the frequency, severity, and duration of cold events. This review synthesizes current knowledge on the adaptive changes of membrane glycerolipids, sphingolipids, and phytosterols in response to cold stress. We delve into key mechanisms of low-temperature membrane remodeling, including acyl editing and headgroup exchange, lipase activity, and phytosterol abundance changes, focusing on their impact at the subcellular level. Furthermore, we tabulate and analyze current gycerolipidomic data from cold treatments of Arabidopsis, maize, and sorghum. This analysis highlights congruencies of lipid abundance changes in response to varying degrees of cold stress. Ultimately, this review aids in rationalizing observed lipid fluctuations and pinpoints key gaps in our current capacity to fully understand how plants orchestrate these membrane responses to cold stress.

## Introduction

Cold shapes plants' native ranges and life strategies (Das et al. 2021). In crop plants, cold limits distribution, growing season, quality, and yield (Powell et al. 2012; Jha et al. 2017), because it disrupts multiple cellular processes, including membrane composition, and forces metabolic adjustments. Extensive research has identified thousands of transcripts and metabolites responding to temperature drops, highlighting the complexity of cold tolerance (Kaplan et al. 2007; Vu et al. 2022). Plants have evolved remarkable adaptive mechanisms to cope with natural seasonal fluctuations, but climate change enhances this challenge. Climate change has already altered the timing and location of cold waves due to reduced sea ice affecting the polar vortex and jet stream (Tachibana et al. 2019; Zhang et al. 2020). Coupled with damage from cold spells following crop de-hardening in response to warmer springs (Snyder and Paulo de Melo-Abreu 2005), there is a pressing need to better understand these mechanisms.

The word "cold" is a general one, and because plants efficiently distinguish different levels of stress, additional words have been adopted. Here, we differentiate chilling from freezing based on the temperature threshold (chilling: above 0°C, freezing: below 0°C). Additionally, "cold acclimation" refers to adaptive changes plants undergo after nonlethal cold exposure that allow them to tolerate subsequent colder temperatures, independent of the specific temperatures used, which vary by species/variety.

Membranes are one of the first responders to cold stress, sensing not only its presence but also its severity and triggering a cascade of responses (Mori et al. 2018). This update review delves into the physical properties of membranes and their constituent lipids (Section I). We then explore how these lipids are modified and transported (Section II), how cold affects them at the subcellular level (Section III), and identify trends in glycerolipid responses to cold stress (Section IV). Finally, we conclude by highlighting outstanding questions in the field.

## Section 1: The properties of lipids and their effects on membranes

Here we describe the physical properties of plant membrane lipids that form the rationale for lipid exchanges as an adaptive response to temperature. Understanding these principles is essential for rationalizing the many observed lipid exchanges and engineering lipid-based interventions for agriculture and biotechnology. We include recent updates from experimental and molecular dynamics simulations on membrane permeability and the roles of hexagonal II ($H_{II}$) phase.

Plant membranes, like all membranes, serve the cell as diffusion barriers, capacitors, and platforms for signaling (Hope 1956; Sussman and Harper 1989). These functions rely on the membrane's ability to provide a permeability barrier to passive diffusion of water and ions. Membrane permeability is in turn directly affected by temperature (Mills and Needham 2005). Increasing temperatures increase molecular motion within a membrane, causing disorder and increasing leakage (Fig. 1A, "liquid disordered") (Frallicciardi et al. 2022). Similarly, decreasing temperatures also increase leakage, though through different mechanisms. Initially, decreasing temperatures decrease molecular motion, pushing the membrane toward a more ordered phase, with less leakage (Fig. 1A, "liquid ordered") (Ghysels et al. 2019). As temperatures continue to lower, membranes can experience increased lateral phase separation of their lipid components

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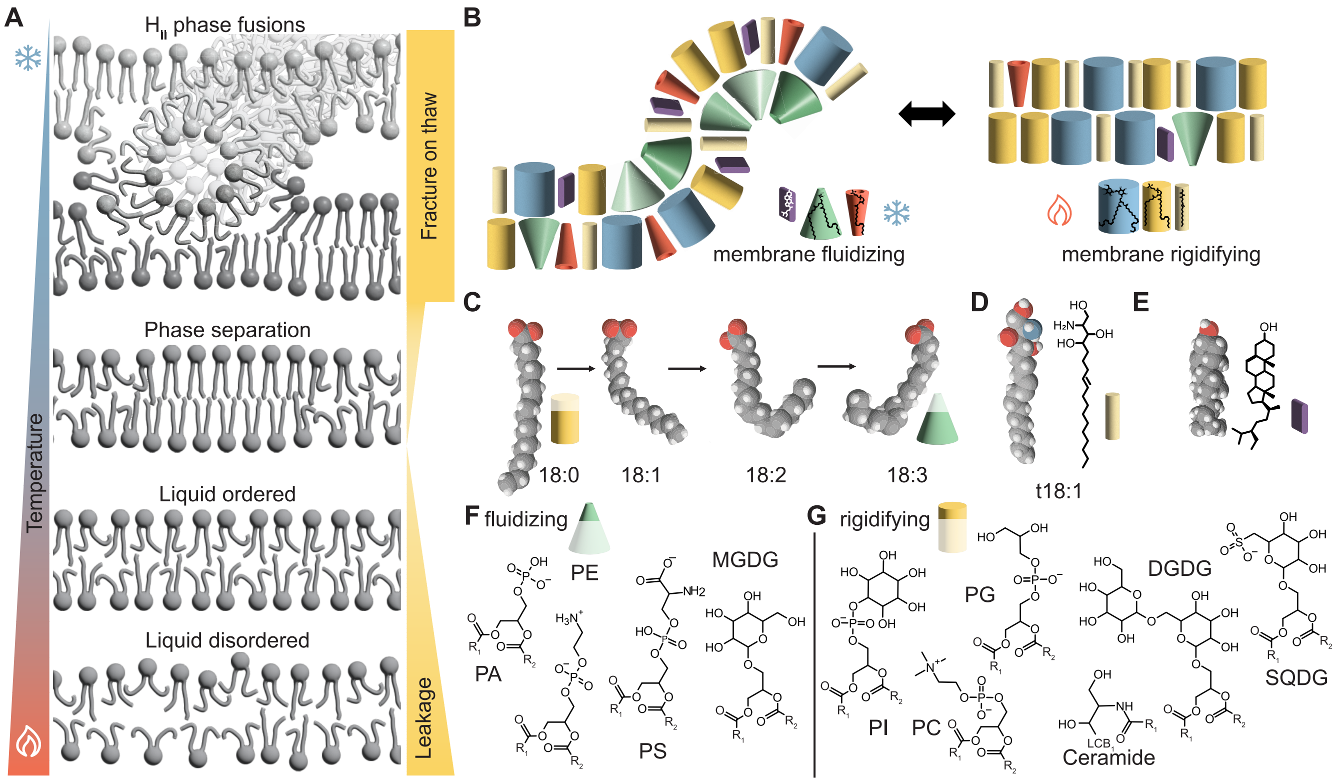

**Figure 1.** The interaction of temperature with lipid membranes. **A)** Membrane phases observed at different temperatures. The lamellar phases can be disordered (highest passive leakage), ordered (low passive leakage), or include phase-separated gel regions (very low passive leakage). During severely low temperatures accompanied by dehydration, $H_{II}$ phase lipid fusions are observed. **B)** Shapes of lipids contribute to the phases they form individually and thus their influence on bilayer properties including fluidity, lateral pressure, and mechanical strength. The shape is influenced by the ratio of the hydrophilic headgroup to the hydrophobic tails. Conical lipids (green cone) include many species of PA, PE, PS, and MGDG; inverted conical lipids (orange cone) include lysolipids; shorter lipids (purple rectangle) include phytosterols and in some species shorter chain glycerolipids. Cylindrical lipids include PI, PC, PG, DGDG, SQDG, LCBs, and ceramides. **C)** Progressive desaturation of an 18-carbon fatty acyl group showing the change in its shape to be increasingly wide and short, promoting more rigidifying or fluidizing properties as indicated by a cylinder or cone, respectively. Coloring is standard, with red indicating oxygen, grey carbon, and white hydrogen. The same scale and coloring are also represented in **(D)** the structure of the most common long-chain base in Arabidopsis, and **(E)** the structure of sitosterol, a representative phytosterol. Structures of headgroups and glycerol for the most common membrane fluidizing lipids **(F)** and membrane rigidifying lipids **(G)**. Abbreviations: $H_{II}$, hexagonal II; LCB, long chain base; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; R, remainder group; SQDG, sulfoquinovosyldiacylglcyerol.

(Fig. 1A, "phase separation"). The precise temperatures at which phase separations are experienced change with composition, as reviewed by Shaw et al. (2021). This is more likely in membranes that already have lateral segregation of lipids, such as the plasma membrane (Grosjean et al. 2015; Mamode Cassim et al. 2019), or the thylakoid and mitochondrial inner membranes, which rely on the presence of nonbilayer phases (Garab et al. 2022). These membranes likely have increased leakage as temperatures cool due to their uneven distribution of surface pressure (Mills and Needham 2005; Shinoda 2016; Cordeiro 2018). The final mechanism of low-temperature-induced membrane damage is the formation of $H_{II}$ phase membrane fusions (Fig. 1A, "$H_{II}$ phase fusions"). Freeze-induced dehydration of closely apposed membranes prompts a critical phase transition from a lamellar to a $H_{II}$ phase, observed in the plasma membrane, chloroplast, and vacuolar membranes from multiple non-acclimated, freezing-tolerant species (Webb et al. 1994; Uemura et al. 1995; Uemura and Steponkus 1997; Nagao et al. 2008). Although $H_{II}$ is now recognized as a common membrane property (Jouhet 2013; Garab et al. 2022), the return of freezing-induced $H_{II}$ to lamellar phase is coincident with irreversible cellular damage (Gordon-Kamm and Steponkus 1984); thus, it is likely these $H_{II}$ phases are unresolvable.

Recent advances in modeling have improved our understanding of the passive permeability of a membrane. Based on physical experiments, we understood passive permeability depended on the area per lipid (Mathai et al. 2008; Nagle et al. 2008), and their phase, with gel phases having significantly less permeability than other phases (Guler et al. 2009). Lipid packing influences a membrane's ability to exclude water and to maintain a specific phase at a given temperature (Mathai et al. 2008; Shinoda 2016; Ghysels et al. 2019). Thus, the spatial agreement between the sizes of the lipid headgroups and fatty acids (FAs) within the membrane bilayer significantly influences its physical properties, and plants make multiple lipid exchanges during temperature stress that result in less membrane damage (Fig. 1B; Uemura et al. 1995; Uemura and Steponkus 1997). Recent molecular dynamics simulations coupled with stopped-flow kinetic experiments by Frallicciardi and colleagues suggest that at least yeast plasma membranes are significantly more ordered than previously believed and challenge the importance of lipid surface area–based models (Frallicciardi et al. 2022). Plant glycerolipid properties are excellently reviewed elsewhere (Garab et al. 2022; Stephanie et al. 2022) and are summarized in Box 1 and 2. In the following paragraphs, we describe more recently studied properties of sphingolipids and phytosterols, the 2 other major plant membrane lipid structural classes.

**Box 1.** Glycerolipid properties

- Conical lipids modify lateral pressure and increase flexibility of biological membranes.
- Cylindrical lipids decrease lateral pressure, permeability, and increase rigidity.
- Longer tails increase lateral phase separation, decreasing permeability.
- Shorter tails increase membrane permeability and flexibility.
- More double bonds increase fatty acyl width (Fig. 1C).

**Box 2.** Glycerolipid properties cont.

- Headgroup size is relative to most common tail sizes, for example, small (Fig. 1F) and wide (Fig. 1G).
- Lysolipids with a single acyl group have wide heads and narrow tails.
- Charged headgroups decrease passive ion transport.
- Sugar headgroups can provide phosphate-free membrane hydration shells.
- Lipid headgroups often drive specific protein interactions.

Sphingolipids are a diverse structural class of lipids connected by the presence of a long chain base (LCB; Fig. 1D) and concentrated in the outer leaflet of the plasma membrane. The simplest sphingolipid is simply an LCB, which may be bonded to a fatty acyl group and a headgroup to form more complex sphingolipids. A sphingolipid's fatty acyl group is modified similarly to those of glycerolipids, though they may also be hydroxylated, typically at the C2 position (reviewed in Huby et al. 2020). A sphingolipid LCB may be desaturated and hydroxylated like its FA, while free LCBs are typically hydroxylated at C1 and may be hydroxylated at additional positions, they may alternatively be phosphorylated at C1 (Huby et al. 2020). Sphingolipids tend to have longer, more saturated acyl groups than most glycerolipids, increasing the packing density, phase separation, and thickness of the plasma membrane (Mamode Cassim et al. 2019). Larger sphingolipid headgroups are common, including the most common class in plants: glycosylinositolphosphorylceramides (GIPCs; Cacas et al. 2012). GIPCs preferentially associate with phytosterols to make large, ordered regions in artificial membranes (Grosjean et al. 2015; Mamode Cassim et al. 2021), and this role has been suggested for them in vivo as well.

In concert with glycerolipids and sphingolipids, free phytosterols form a critical part of cell membranes, where their relatively short and flat structure (Fig. 1E) increases order and facilitates bidirectional regulation of membrane temperature response (Hartmann 1998). They preferentially associate with some membrane lipids, and in doing so enhance phase separation of lipid mixtures, including, but not limited to, sphingolipids (Grosjean et al. 2015). This feature is crucial for maintaining the high integrity, low permeability, and specialized domain structure of plasma membranes (Dufourc 2008; Grosjean et al. 2015). Similarly, phytosterols can have sugar head groups, including sterylglucosides and acylated sterylglucosides (Rogowska and Szakiel 2020). Phytosterols also have multiple potential signaling roles (Clouse 2002; Du et al. 2022). Currently, no coherent model exists to link biological lipid mixtures— including hundreds to thousands of different glycerolipid, phytosterol, and, in the plasma membrane, sphingolipid components—to membrane fluidity.

## Section 2: An overview of membrane remodeling

Membrane remodeling is necessary for temperature tolerance. Here, we discuss the major mechanisms through which subcellular membrane lipids are modified, acyl editing and headgroup exchange, in a subcellular context moving from the endoplasmic reticulum (ER) to energy-making organelles.

Glycerolipid modification occurs predominantly in the ER. Phosphatidic acid (PA), diacylglycerol (DAG), and phosphatidylcholine (PC) are central glycerolipid metabolites serving as intermediates for acyl chain modification and exchange of complex lipid headgroup classes (Li-Beisson et al. 2013). FAs synthesized in the plastid are incorporated into PA and DAG via the parallel operation of 2 glycerolipid synthesis pathways: the eukaryotic pathway (incorporating 18C FAs at the 2 positions of the glycerol backbone) in the ER and the prokaryotic pathway (incorporating 16C FAs at the middle position of glycerol) in the chloroplast (Fig. 2), though the prokaryotic pathway was lost in some plants over evolutionary time (Mongrand et al. 1998). For a comprehensive review of prokaryotic and eukaryotic lipid synthesis, readers are directed to (Holzl and Dormann 2019) and (Jacquemyn et al. 2017), respectively.

Regardless of which glycerolipid synthesis pathway is available to the plant, the ER is the predominant site for acyl editing. Acyl editing is the exchange of fatty acyl groups between membrane lipid classes via acyl-CoA intermediates (Bates et al. 2012). Acyl-CoA itself can be elongated in the ER by a multi-enzyme complex (Batsale et al. 2023), while acyl groups attached to PC can be desaturated by membrane-bound FA desaturases in the ER and then transferred to other lipid headgroups (Miquel and Browse 1992; Nguyen et al. 2019). Similar modifications occur to both the LCB and acyl group of sphingolipids (Luttgeharm et al. 2016). Alternatively, to acyl editing, FAs may be catabolized via peroxisomal β-oxidation (Graham 2008) or shuttled into triacylglycerol (TAG). TAG, a nonpolar glycerolipid, is synthesized from DAG and an acyl donor in the ER membrane and accumulates in lipid droplets (Bates et al. 2009; Guzha et al. 2023). In unstressed vegetative tissues, only trace amounts of TAG are accumulated (Lu et al. 2020).

Lipid modification in the ER also occurs through headgroup exchange. PA and DAG produced from de novo glycerolipid synthesis are converted to the phospholipids PC, phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylethanolamine (PE), and phosphatidylserine (PS) in reactions that take place in the ER membrane (Somerville and Browse 1991). In addition to phospholipids, ceramides are also synthesized in the ER (Chen et al. 2006), which are further glycosylated to produce a diverse family of sphingolipids (Liu et al. 2021a).

Lipid transfer between the ER and the plasma membrane, mitochondria, plastid, and other associated membranes is critical for cellular function (Fig. 2, dashed lines) and is reviewed by (Michaud and Jouhet 2019). Vesicular trafficking via the secretory pathway is a well-characterized route for phospholipid transport between the ER and the plasma membrane (Jürgens 2004), as well as the dominant route for sphingolipid transport (Kajiwara et al. 2008). Recent data show lipids can also be transported via membrane contact sites, especially between the ER, plastid envelope, mitochondria, and plasma membranes (Leterme and Michaud 2022).

Energetic organelle membranes are the sites of distinct lipid synthesis, modification, and transfer. The chloroplast is both a

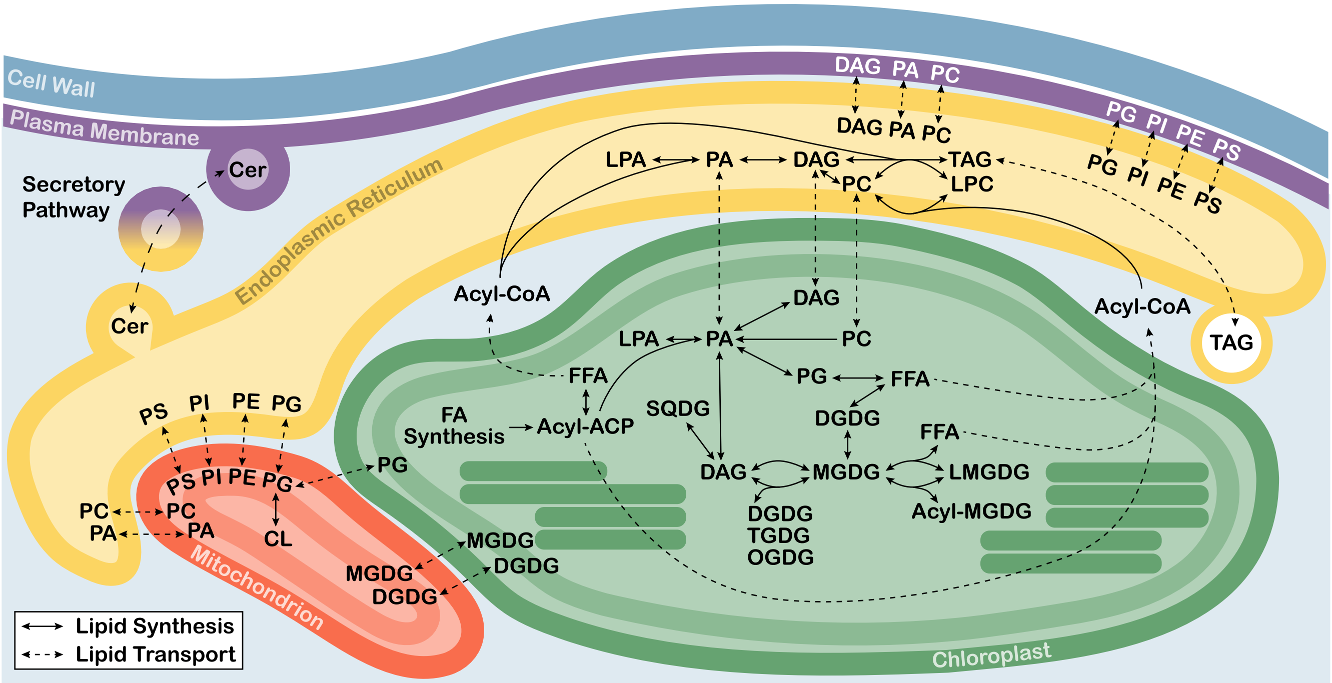

**Figure 2.** Lipid modification and transfer occurs within and between distinct organellar membranes. Lipid synthesis (solid lines) begins with FAs synthesized in the chloroplast, which are activated to acyl-CoAs and then incorporated into membrane lipids through de novo lipid synthesis in the chloroplast or ER or through acyl editing in the ER. FFAs and PUFAs released via lipase activity in the chloroplast, ER, and plasma membranes are reincorporated as activated acyl-CoAs into new lipids. Lipids are transported across distinct membrane compartments via vesicular or nonvesicular trafficking (dashed lines). Note that indicated transport may be indirect. For space reasons, the location of lipids in the figure is indicative of the organellar compartment but not the specific suborganellar membranes where the lipids are synthesized. Abbreviations: Cer, ceramide; CL, cardiolipin; DAG, diacylglycerol; DGDG, digalactosyldiacylglycerol; ER, endoplasmic reticulum; FFA, free fatty acid; LMGDG, lysomonogalactosyldiacylglycerol; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; MGDG, monogalactosyldiacylglycerol; OGDG, oligogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; SQDG, sulfoquinovosyldiacylglycerol; TAG, triacylglycerol; TGDG, trigalactosyldiacylglycerol.

location of lipid synthesis and a reservoir of unique lipids required for efficient photosynthesis (Li and Yu 2018), namely monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), PC, PG, sulfoquinovosyldiacylglycerol (SQDG), and trace amounts of PI (Garab et al. 2022; Stephanie et al. 2022). De novo lipid synthesis can occur in the chloroplast membranes via the prokaryotic pathway (Holzl and Dormann 2019). Additionally, PA, PC, and DAG originating from the ER are efficiently transported to the chloroplast (Wang et al. 2013; Marechal and Bastien 2014; Yin et al. 2015). Transport of PA and DAG from the ER to the chloroplast is a requirement of photosynthetic lipid synthesis, even in plants that retain a chloroplast-localized de novo lipid synthesis pathway (Kunst et al. 1988; LaBrant et al. 2018).

The addition of UDP-galactose to DAG produces the most abundant lipid in the plastid, MGDG (Awai et al. 2001). MGDG subsequently can be converted to DGDG by the addition of a second galactose (Dörmann et al. 1995). FAs can be released from either MGDG or DGDG and then reincorporated into other chloroplast lipids or exported for recycling into glycerolipids in the ER (Ellinger et al. 2010; Higashi et al. 2018). The chloroplast membranes also contain low levels of anionic lipids, namely the phospholipid PG and the sulfolipid SQDG, which are essential for photoautotrophic growth (Yu and Benning 2003). A third anionic lipid, glucuronosyl diacylglycerol, is present during phosphate deprivation (Okazaki et al. 2013). The acyl groups of PG can be desaturated by chloroplast envelope desaturases (Gao et al. 2009), and the resultant FAs can be incorporated into the ER acyl editing pool (Wang et al. 2017). Transfer of PG through membrane contact sites is highly likely (Babiychuk et al. 2003; Liu et al. 2023). Bulk transfer of galactolipids, particularly DGDG, likely occurs through the same sites, observed primarily during phosphate starvation (Michaud et al. 2016).

Most of the membrane lipids in mitochondria are glycerolipids, predominately cardiolipin (CL), PC, and PE, with smaller amounts of PI, PS, PG, PA, MGDG, and DGDG (Liu et al. 2023). CL is unique to the mitochondria and is synthesized through condensation of CDP-DAG and PG, generating a 4-acyl chain lipid that fluidizes the membrane (Bligny and Douce 1980; Katayama et al. 2004; Unsay et al. 2013).

## Section 3: Membrane dynamics in temperature stress

In this section, we describe membrane changes resulting from chilling and freezing, including head group and FA composition and phase transitions, and briefly touch on lipids affecting signaling within the context of the plasma, mitochondrial, ER, and chloroplast membranes. The topic of signaling lipids is broad, encompassing both lipids and lipid-derived products. For further information, we refer readers to additional reviews on this subject (Wang and Chapman 2013, Hou et al. 2016; Ali et al. 2018; Cook et al. 2021; Wu et al. 2022; Liang et al. 2023).

The plasma membrane is a first responder to temperature stress. Chilling temperatures rigidify the plasma membrane, triggering calcium flow into the cytoplasm through mechanosensitive ion channels MID1-COMPLEMENTING ACTIVITY1 and 2 (Knight

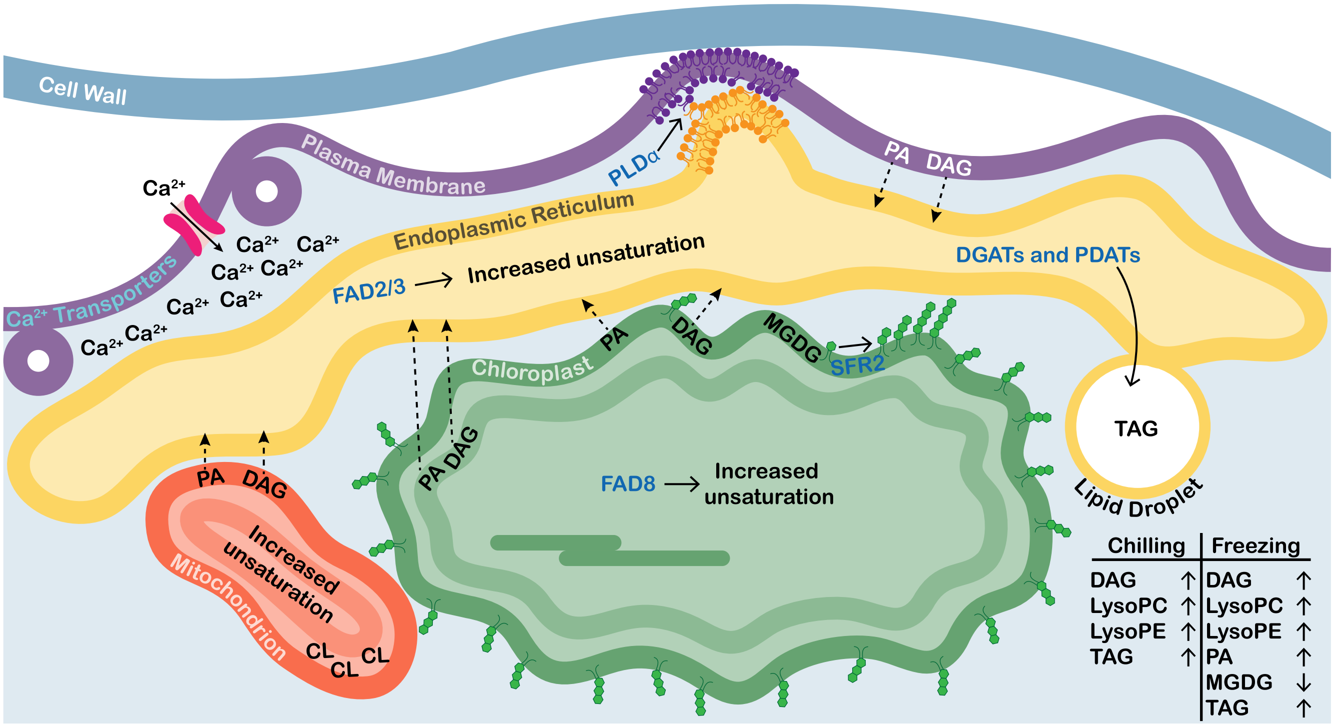

**Figure 3.** The impact of freezing on organelle morphology, functionality, and lipid remodeling. Low temperatures rigidify the plasma membrane and cause it to pull away from the cell wall. This mechanical pressure causes freeze-induced vesicles on the plasma membrane. Cytoplasmic dehydration, coupled with PLDα-driven lipase activity, promotes the formation of the $H_{II}$ phase between organelles. Together, these structural changes cause an influx of calcium, and this along with membrane damage induces changes to the lipid composition of the cell. The ER serves as the central hub for lipid modification, where lipase-derived products (PA and DAG) are utilized for lipid synthesis, acyl editing, or conversion into TAG for storage. Additionally, the ER aids in increasing membrane lipid desaturation via FAD2/3 to maintain integrity. Simultaneously, mitochondria and chloroplasts enhance membrane unsaturation. CL levels increase in the inner membrane of the mitochondria, altering mitochondrial architecture. The chloroplast outer envelope enzyme SFR2 catalyzes the production of oligogalactolipids via the transfer of galactose head groups from MGDG. This process is crucial for freezing tolerance. The inset table at the bottom right summarizes *Arabidopsis thaliana* lipids consistently observed in chilling and freezing as shown in Section 4, Tables 1 and 2. Arrow direction indicates increased (↑) or decreased (↓) lipid levels during cold compared with control. Locations of lipids in the inset table are shown, while lysolipids are not because their location is unknown. Abbreviations: CL, cardiolipin; DAG, diacylglycerol; DGATs, DIACYLGLYCEROL ACYLTRANSFERASES; ER, endoplasmic reticulum; FAD2/3, FATTY ACID DESATURASE 2 or 3; HII, hexagonal II; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PDATs, PHOSPHOLIPID: DIACYLGLYCEROL ACYLTRANSFERASES; PE, phosphatidylethanolamine; PLDα, PHOSPHOLIPASE Dα; SFR2, SENSITIVE TO FREEZING 2; TAG, triacylglycerol.

et al. 1996; Mori et al. 2018), and membrane rupture (Yamazaki et al. 2008a). A recent report suggests that ANNEXIN1 also allows calcium influx during chilling (Liu et al. 2021b), though how has yet to be identified, possibly due to the protein family's multifunctional roles (Laohavisit and Davies 2009). The calcium flux triggers multiple transcription cascades, resulting in additional protein and lipid changes to maintain membrane fluidity and integrity (Fig. 3; Takahashi et al. 2013). Importantly, the sudden burst of intracellular calcium initiates plasma membrane resealing specifically during freezing through both lipid remodeling and protein recruitment (Yamazaki et al. 2008a). Additionally, the plasma membrane can quickly accumulate freeze-induced vesicles, which have been shown to mitigate the dehydration and mechanical stress imposed during freezing (Yamazaki et al. 2008b).

The plasma membrane consists of glycerolipids, sphingolipids, and phytosterols, with sphingolipids concentrated on the outer leaflet. The glycerolipids experience notable modifications upon chilling through the actions of phospholipases (Ruelland et al. 2002; Li et al. 2009a) and DAG kinases (Arisz et al. 2013), which catalyze the production of PA. PA serves as a signaling molecule and a central metabolite, acting as an intermediate of headgroup remodeling (Testerink and Munnik 2011), and PA levels were recently correlated with freezing tolerance (Vu et al. 2022). PA rapidly accumulates within minutes of chilling stress and directly affects transcription of cold-responsive genes and binds to regulatory proteins (reviewed in Wu et al. 2022). When present in low levels in freezing temperatures, PA can act as a signaling molecule; however, if PA accumulates to high levels during freezing, membrane damage and cell death result (Welti et al. 2002; Zheng et al. 2016). Failure to make necessary lipid adjustments during cold acclimation leads to a transition of the plasma membrane from a stable lamellar phase to $H_{II}$ phase fusions with other membranes during freezing (Section I, Fig. 1A), which ruptures the cell upon thawing (Fig. 3, Uemura et al. 1995; Li et al. 2004). Importantly, this transition of the plasma membrane to $H_{II}$ is dependent on PHOSPHOLIPASE Dα (PLDα), which favors PC hydrolysis. In the absence of PLDα, the PC to PE ratio becomes unbalanced (Welti et al. 2002). This change favors $H_{II}$ and could contribute to plasma membrane damage (Jouhet 2013).

Sphingolipids and phytosterols serve as both structural components of the plasma membrane and signalers of its health (Rogowska and Szakiel 2020; Haslam and Feussner 2022). During chilling, total sphingolipid levels increase, including the most abundant sphingolipids, GIPCs (Nagano et al. 2014). Simultaneously, total LCB levels decline (Guillas et al. 2013). LCB kinases that promote degradation of LCBs are known to be important for cold tolerance (Dutilleul et al. 2012; Dutilleul et al. 2015; Huang et al. 2017), possibly to avoid LCB induction of cell death responses known to occur in

**Table 1.** Chilling Arabidopsis glycerolipid changes

| °C | Trt | Ctrl | V. | PG | PC | L-PC | PE | L-PE | PI | PS | PA | MGDG | DGDG | SQDG | DAG | TAG | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 21 d 10°C lt 8°C dk | 21 d 22°C lt 17°C dk | Col | — Total; ↑ 16:0, 18:3; ↓ 16:1, 18:0, 18:1, 18:2 | ↓ Total; ↑ 18:3; ↓ 16:0, 18:0, 18:2 | nd | ↓ Total; ↑ 18:1, 18:3; ↓ 16:0, 18:0, 18:2 | nd | — Total;↓ 18:2 | nd | nd | ↑ Total; ↑ 16:3, 18:3; ↓ 16:0, 16:1, 16:2, 18:1, 18:2 | — Total; ↑ 16:2, 16:3, 18:3; ↓ 16:0, 18:0, 18:1, 18:2 | — Total; ↑ 18:3; ↓ 18:0, 18:1, 18:2 | nd | nd | 7 d; soil; ESI-MS/MS; t.test; (Li et al. 2015) |
| 6 | 37 d 6°C | 11 d 25°C | Col | — Total; ↑ 32:0, 34:3; ↓ 32:1, 34:1, 34:2, 34:4 | ↑ Total; ↑ 34:3, 36:5, 36:6; ↓ 34:1, 34:2, 36:2, 36:3 | ↑ Total; ↑ 16:0, 18:3 | ↑ Total; ↑ 34:3, 36:3, 36:4, 36:5, 36:6, 42:3; ↓ 34:2, 36:2 | ↑ Total; ↑ 16:0, 18:1, 18:2, 18:3 | ↑ Total; ↑ 34:3, 36:6; ↓ 34:1, 34:2, 36:2, 36:3, 36:4 | — Total; ↑ 42:3, 42:4, 44:3; ↓ 34:2, 34:3, 36:2, 36:3, 36:5, 38:2, 38:3, 40:2, 42:2 | — Total↑ 36:6; ↓ 34:1, 34:2, 34:4, 36:2 | ↓ Total; ↑ 34:6; ↓ 34:3, 34:4, 34:5, 36:5, 36:6 | ↑ Total; ↑ 34:6, 36:4, 36:6; ↓ 34:1, 34:2, 34:3, 34:5, 36:3, 36:5 | nd | nd | nd | 14 d; soil; ESI-MS/MS; t.test; some growth conditions differed between experiments (Chen and Thelen 2013) |
| 4 | 1 d 4°C | 1 d 22°C | Col | — Total; ↑ 32:1, 34:0, 34:3, 36:5; ↓ 34:1 | — Total; ↑ 34:3, 38:6; ↓ 38:3 | ↑ Total; ↑ 16:0 | ↑ Total; ↑ 34:4, 36:4, 36:5, 36:6, 38:5, 38:6, 40:3, 42:3, 42:4 | ↑ Total; ↑ 16:0, 18:2 | — Total; ↑ 32:2, 32:3, 34:3; ↓ 36:2 | — Total; ↑ 42:3, 42:4; ↓ 34:2, 36:4 | — Total↑ 34:1 | — Total; ↓ 34:1, 34:2, 34:3, 34:4, 34:5, 36:2, 36:3, 36:4, 36:5, 38:5 | — Total; ↓ 34:1, 36:4 | nd | nd | nd | 42 d; soil; ESI-MS/MS; t.test; (Barajas-Lopez et al. 2021) |
| 4 | 3 d 4°C | 3 d 23°C lt 19°C dk | Col | — Total; ↑ 34:3, 34:4; ↓ 34:1 | — Total; ↑ 36:5, 36:6; ↓ 36:2, 36:3 | ↑ Total; ↑ 16:0, 18:2, 18:3; ↓ 18:1 | — Total; ↑ 36:4, 36:5, 36:6; ↓ 34:1, 36:2 | ↑ Total; ↑ 18:3 | — Total; ↓ 34:1, 36:2 | nd | ↑ Total ↑ 34:2, 36:2, 36:4, 36:5, 36:6 | ↑ Total; ↑ 34:6, 36:6; ↓ 34:1, 34:2 | ↑ Total; ↑ 34:3, 34:6, 36:6; ↓ 34:1 | nd | nd | nd | 35 d; soil; ESI-MS/MS; t.test; (Welti et al. 2002) |

*(continued)*

**Table 1.** Continued

| °C | Trt | Ctrl | V. | PG | PC | L-PC | PE | L-PE | PI | PS | PA | MGDG | DGDG | SQDG | DAG | TAG | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 14 d 4°C | 14 d 20° C lt 18°C dk | Col | nd | — Total; ↑ 36:4, 36:5, 36:6, 38:4, 38:5, 38:6; ↓ 34:1, 34:2, 36:1, 36:2, 36:3 | ↑ Total; ↑ 16:0, 18:2, 18:3 | ↑ Total; ↑ 36:4, 36:5, 36:6, 38:5; ↓ 34:1, 36:2, 38:1 | nd | — Total | nd | nd | ↓ Total; ↑ 34:2, 34:3, 38:4, 38:6; ↓ 34:1, 34:4, 34:5, 34:6, 36:3, 36:5 | — Total; ↑ 34:3, 36:4, 36:7; ↓ 32:0, 32:3, 34:2, 34:3, 36:5 | — Total | ↑ Total; ↑ 36:3, 36:4, 36:5, 36:6, 38:6 | ↑ Total; ↑ 50:X, 52:X, 54:X, 56:X, 58:X, 60:X | 42 d; soil; UPLC FT_MS; t.test; (Degenkolbe et al. 2012) |
| 4 | 14 d 4°C | 14 d 20° C lt 18°C dk | Alc, C24, Can, Cvi, Ita, Kn, Ms, N6, N13, N14, Oy, Ran, Rsch, Sah | nd | ↑ Total*; ↑ 36:4, 36:5, 38:4, 38:5, 38:6; ↓ 34:1, 36:1, 36:2, 36:3 | ↑ Total*; ↑ 16:0, 18:2 | ↑ Total*; ↑ 36:3, 36:4, 36:5, 36:6, 38:5; ↓ 34:1, 36:2, 38:1 | nd | — Total* | nd | nd | ↓ Total*; ↓ 34:4, 34:5, 36:3, 36:5 | — Total* | — Total* | ↑ Total*, ↑ 36:5 | ↑ Total*; ↑ 50:X, 52:X, 54:X, 56:X, 58:X, 60:X | 42 d; soil; UPLC FT_MS; t.test; (Degenkolbe et al. 2012) |

Arrows indicate direction of significant changes compared to control, —indicates insignificant changes, and nd indicates no data. Asterisks indicate the most common change in studies with multiple varieties of species. Only high abundance lipid species changes were reported. Temperatures of cold treatment are indicated, with light and dark temperatures differentiated if relevant. The control growth and treatment conditions are indicated, as is the genomic variety. Significance of changes indicated are either from published statistical analyses, our analysis (t-test) of available raw datasets, or for 4 publications (Zheng et al. 2016; Gu et al. 2017; Marla et al. 2017; Tan et al. 2018) by visual use of graphs where non-overlapping error bars on bar graphs or wide differences from heat maps compared to control values. The "notes" category includes the age of the plants at the beginning of treatment, growth support as soil or medium, the method of MS analysis, the statistical test used, and the reference. Abbreviations: °C, degrees Celsius; Ctrl, control; DAG, diacylglycerol; DGDG, digalactosyldiacylglycerol; dk, dark; L-PC, lysophosphatidylcholine; L-PE, lysophosphatidylethanol; lt, light; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; SQDG, sulfoquinovosyldiacylglycerol; TAG, triacylglycerol; Trt, treatment; V., variety.

response to other stresses (Lambour et al. 2022). Together, sphingolipids and phytosterols can form lateral phase separations known as detergent-resistant regions or nanodomains (Grosjean et al. 2015), and phytosterol changes in response to chilling have also been documented in a few species (Rogowska and Szakiel 2020; Du et al. 2022) and are correlated with increased survival (Vu et al. 2022). Currently, the role of nanodomains in cold tolerance remains unknown, and alternative explanations for phytosterol changes exist (Dufourc 2008; Aboobucker and Suza 2019).

In addition to head group modifications, the plasma membrane requires increased acyl chain desaturation to tolerate freezing (Uemura et al. 1995), though these reactions primarily occur in the ER. Glycerolipid desaturases FATTY ACID DESATURASE2 (FAD2) (Miquel et al. 1993) and FAD3 (Matos et al. 2007) as well as sphingolipid desaturases D8 SPHINGOLIPID DESATURASE (Chen et al. 2012) and ACYL DESATURASE 2 (Chen and Thelen 2013; Smith et al. 2013) have been implicated in maintaining the plasma membrane desaturation level during cold acclimation.

The ER orchestrates desaturation and lipid synthesis to maintain endomembrane integrity during low temperatures. The ER is the primary site for eukaryotic lipid synthesis and lipid modification (Section 2). While the ER can undergo low temperature–induced biophysical changes to form multiplex lamellae (Fujikawa and Takabe 1996; Kobayashi et al. 2014), much of the work done on the ER focuses on how its enzymes affect membrane integrity in multiple subcellular compartments (Fig. 3).

As cellular membranes adapt to low temperatures, lipids are transported to the ER for remodeling (Section 2). Desaturation is critical for tolerance and mediated by ER FA desaturases, including FAD2 and FAD3 (Browse and Somerville 1991). Evidence of the importance of ER FADs includes decreased chilling tolerance in FAD2 loss-of-function mutants (Miquel et al. 1993). Protoplasts supplemented with polyunsaturated PC (indicating increased FAD3 activity) show enhanced survival at freezing temperatures due to reduced plasma membrane lysis (Steponkus et al. 1988). Similarly, overexpression of ER FADs also enhances chilling tolerance in multiple species (e.g. Wang et al. 2019; Wang et al. 2021). Cold stress also activates acyl editing in the ER to increase acyl chain length and decrease fluidity (Box 1). This is observed in both rice and Arabidopsis, where longer acyl chains on PA accumulate, indicating a preference for remodeling longer chains under cold conditions (Zheng et al. 2016). Additionally, overexpression of 3-KETOACYL-COA SYNTHASE 1, which lengthens acyl chains, enhances plasma membrane integrity (Chen et al. 2020), underscoring the role of acyl editing in adapting membrane lipid compositions to fluctuating temperatures.

A final type of ER lipid restructuring in response to low temperatures is converting membrane lipids into TAG. There are multiple hypotheses for the role of TAG accumulation in abiotic stress, as it is essential. At least 3 enzymes were shown to have a role in TAG production during low temperatures, 2 of which have phenotypic consequences depending on the severity of the temperature ramp applied (Arisz et al. 2018; Tan et al. 2018; Demski et al. 2020; Shomo et al. 2024). TAG accumulation may buffer plant reductant systems (Schmid-Siegert et al. 2016; Yu et al. 2021), remove acyl groups from the membrane rapidly (Lu et al. 2020), be an energy or reductant storage critical for recovery (Yu et al. 2021), or some combination of these possibilities.

Mitochondrial lipid saturation level is important for respiratory function. Like other membranes, mitochondrial membranes balance their acyl saturation level for functional responses, including respiration and efficient electron transfer (Fig. 3, Caiveau et al. 2001). In *fad2* loss-of-function cell cultures treated with low temperatures, the mitochondrial membranes were more rigid (Matos et al. 2007), implying that saturation level is critical for cold responses. Additionally, CL is vital for mitochondrial structure and function in Arabidopsis, protecting against damage from environmental stresses (Pineau et al. 2013; Pan et al. 2014). While not directly linked to low-temperature tolerance, the shared physiological responses to cold stress and other stressors suggest CL may be a target for mitochondrial temperature adaptation. Mutants affecting the desaturation of CL precursors also impact low-temperature tolerance (Chen and Thelen 2013), highlighting the importance of CL in mitochondrial resilience.

The chloroplast responds to low temperatures through unique lipid modifications. Cold tolerance is frequently studied in green shoot tissues because soil or substrate is frozen. Chloroplast membranes are directly damaged by freezing (Uemura and Steponkus 1997) and are extensively remodeled to maintain photosynthetic capacity and their structure (Fig. 3, reviewed in Cook et al. 2021). PA and DAG production is necessary for chloroplast lipid biosynthesis (Dubots et al. 2012), and they are generated in response to chilling and freezing (Welti et al. 2002; Vu et al. 2022). The stromal DAG lipase ADIPOSE TRIGLYCERIDE LIPASE-LIKE is upregulated in low temperatures (Yu et al. 2023), and loss-of-function mutations in PLDα and PLDδ show changes to plastid-derived PA and altered freezing tolerance (Welti et al. 2002; Li et al. 2009b). Chloroplast-specific FAs do not accumulate in large amounts in PA, suggesting that PA is a temporary store for movement into TAG (Tan et al. 2018). SENSITIVE TO FREEZING2 also produces DAG during low temperatures, which is processed into TAG (Moellering et al. 2010).

Unique lipids are generated in chloroplasts in response to chilling and freezing stress. Under non-stress conditions, the ratio of MGDG to DGDG in Arabidopsis is approximately 2:1 (Block et al. 1983). During freezing, the ratio of MGDG:DGDG is shifted to approximately 1.25:1 by SENSITIVE TO FREEZING2, which uses MGDG as a substrate to processively synthesize oligogalactolipids on the chloroplast outer envelope, also yielding DAG, which is further catalyzed to TAG (Moellering et al. 2010; Roston et al. 2014). While the biophysical properties of oligogalactolipids remain unknown, their ability to form lamellar phases may prevent $H_{II}$ formation and membrane fusion with opposing extraplastidic membranes (Chng et al. 2022). A second unique lipid generated in response to cold is acyl-MGDG, which contains a third acyl chain added to the headgroup via ACYLATED GALACTOLIPID ASSOCIATED PHOSPHOLIPASE1 (Nilsson et al. 2015). Like oligogalactolipids, acyl lipids increase after temperatures drop below 0, suggesting their importance to membrane freezing (Vu et al. 2022), though their physical properties are yet to be studied. Furthermore, the origin of the substrate MGDG remains unclear. The photosynthetic thylakoid membranes are the largest source of MGDG, but the enzymes generating oligogalactolipids or acyl-MGDG are located at the outer envelope membrane (Roston et al. 2014) or cytoplasm (Nilsson et al. 2015), respectively. Thus, it is currently unclear to what extent thylakoidal MGDG is modified by these enzymes in the cold. This lack of knowledge is partly due to our poor understanding of lipid transport to the thylakoid membrane (García-Cerdan et al. 2020). Lastly, the acyl chains of PG exclusive to the chloroplast have also been implicated in chilling tolerance. Cold-tolerant species tend to have PG composed of more *cis*-unsaturated FAs, while the PG in sensitive plants has more saturated and *trans*-unsaturated FAs (Murata 1983, Murata et al. 1992). When the ability of PG to incorporate *cis*-unsaturations is blocked (Wolter et al. 1992; Gao et al. 2020), normally chilling-tolerant Arabidopsis becomes sensitive, suggesting that *cis*-unsaturated FAs in PG are essential for chilling tolerance.

Table 2. Freezing Arabidopsis glycerolipid changes

| °C | Trt | Ctrl | V. | PG | L-PG | PC | L-PC | PE | L-PE | PI | PS | PA | MGDG | DGDG | DAG | TAG | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| −6 | 3 d_4°C<br>↳ 5h_rp<br>↳ 2 h_-6°C | 3 d 4°C | Col | ↑ Total; ↑ 32:0, 32:1, 34:0, 34:1, 34:2, 34:3, 36:3, 36:4, 36:5, 36:6; ↓ 34:4 | — Total; ↑ 16:0, 18:1, 18:2, 18:3 | — Total; ↑ 36:6; ↓ 34:1, 34:2 | ↑ Total; ↑ 16:0, 18:2, 18:3 | — Total; ↓ 34:2, 34:3 | ↑ Total; ↑ 16:0, 18:2, 18:3 | ↑ Total; ↑ 34:2, 34:3, 36:5 | ↓ Total; ↓ 34:2, 34:3, 36:3, 38:2, 38:3, 40:2, 40:3 | ↑ Total; ↑ 34:1, 34:2, 34:3, 34:4, 34:6, 36:2, 36:3, 36:4, 36:5, 36:6 | ↓ Total; ↓ 34:6, 36:6 | ↑ Total; ↑ 34:6, 36:6; ↓ 34:3 | nd | nd | 42 d; soil; ESI-MS/MS; Q test; (Zheng et al. 2016) |
| −6 | 10 d_7°C ↳ 3 h_rp<br>↳ 1d_-2°C<br>↳ 5 h_rp<br>↳ 1 d_-6°C | 10 d 7°C | Col | nd | nd | nd | nd | nd | nd | nd | nd | nd | ↓ Total | ↑ Total | — Total | ↑ Total; ↑ 16:3; ↓ 18:2 | 14 d; medium; GC, ESI-MS/MS; (Moellering et al. 2010) |
| −8 | 3 d<br>23°C lt<br>21°C dk<br>↳ 3 h_rp<br>↳ 2 h_-2°C<br>↳ 3 h_rp ↳ 40 m_-8°C | 3 d 23°C lt 21°C dk | Col | — Total | ↑ Total | ↓ Total; ↓ 34:1, 34:2, 34:3, 36:4, 36:5 | ↑ Total | ↑ Total; ↑ 34:2, 34:3, 36:5; ↓ 36:3 | ↑ Total | — Total; ↑ 34:2, 34:3 | — Total; ↑ 34:3, 36:4, 36:5; ↓ 36:3, | ↑ Total; ↑ 34:1, 34:2, 34:3, 34:4, 34:5, 34:6, 36:1, 36:2, 36:3, 36:4, 36:5, 36:6 | ↓ Total; ↓ 34:5, 34:6, 36:5, 36:6 | ↑ Total; ↑ 34:3, 34:6, 36:6 | ↑ Total; ↑ 18:1/16:3, 18:2/16:0, 18:3/16:0, 18:3/16:1, 18:3/16:3 | ↑ Total; ↑ 16:0, 18:1, 18:2, 18:3 | 28 d; soil; ESI-MS/MS; t.test; (Tan et al. 2018) |
| −8 | 3 d_4°C ↳ 2hr_-8°C | 3 d 23°C lt 21°C dk | Col | ↑ Total | ↑ Total | ↓ 34:1, 36:2, 36:3, 36:4, 36:5, 38:3, 38:5; ↑ 40:3, 40:4, 40:5 | ↑ Total | nd | ↑ Total | nd | nd | ↑ Total; ↑ 32:0, 34:1, 34:2, 34:3, 34:4, 34:5, 34:6, 36:2, 36:3, 36:4, 36:5, 36:6 | ↓ Total | nd | nd | nd | 35 d; soil; ESI-MS/MS; t.test; (Du et al. 2010) |
| −8 | 3 d_4°C<br>↳ 2 h_rp<br>↳ 2 h_-2°C<br>↳ 6 h_rp<br>↳ 2 h_-8°C | 3 d 4°C | Col | ↓ Total; ↓ 34:0, 34:1, 34:2, 34:3, 34:4 | nd | ↓ Total; ↓ 34:2, 34:3, 34:4, 36:2, 36:3, 36:4, 36:5, 36:6, 38:2, 38:3, 38:4 | ↑ Total; ↑ 16:0, 18:0, 18:1, 18:2, 18:3 | ↓ Total; ↓ 34:2, 34:3, 34:4, 36:3, 36:4, 36:5, 36:6, 38:4 | ↑ Total; ↑ 16:0, 18:1, 18:2, 18:3 | — Total; ↑ 34:4, 36:5, 36:6 | nd | ↑ Total; ↑ 34:1, 34:2, 34:3, 34:4, 34:6, 34:5, 36:2, 36:3, 36:4, 36:5, 36:6 | ↓ Total | — Total; ↑ 38:6 | nd | nd | 35 d; soil; ESI-MS/MS; t.test; (Welti et al. 2002) |

(*continued*)

**Table 2.** Continued

| °C | Trt | Ctrl | V. | PG | L-PG | PC | L-PC | PE | L-PE | PI | PS | PA | MGDG | DGDG | DAG | TAG | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| −8 | 3 d_4°C ↳ 2 h_rp ↳ 2 h_-2°C ↳ 6 h_rp ↳ 2 h_-8°C | 3 d 4°C | Col & WS | ↓ Total; ↓ 32:0, 32:1, 34:1, 34:2, 34:3, 34:4 | ↑ Total; ↑ 16:0, 16:1 | ↓ Total; ↓ 34:2, 34:3, 36:2, 36:3, 36:4, 36:5, 36:6 | ↑ Total; ↑ 16:0, 18:0, 18:1, 18:2, 18:3 | ↓ Total; ↓ 34:2, 34:3, 36:4, 36:5, 36:6 | ↑ Total; ↑ 16:0, 18:1, 18:2, 18:3 | — Total | nd | ↑ Total; ↑ 34:1, 34:2, 34:3, 34:6, 36:2, 36:3, 36:4, 36:5, 36:6 | ↓ Total; ↓ 36:4 | — Total | nd | nd | 28 d; soil; ESI-MS/MS; t.test; (Li et al. 2008) |
| −10 | 3 d_4°C ↳ 3h_rp ↳ 2 h_-2°C ↳ 4 h_rp ↳ 40m_-10° C | 3 d 23°C lt 21°C dk | Col | — Total | — Total | ↓ Total; ↓ 34:1, 34:2, 34:3, 36:4, 36:5 | ↑ Total | ↑ Total; ↑ 34:2, 34:3, 36:4, 36:5; ↓ 34:1, 36:3 | ↑ Total | — Total; ↑ 34:2, 34:3 | — Total; ↑ 34:3, 36:3, 36:4, 36:5 | ↑ Total; ↑ 34:1, 34:2, 34:3, 34:4, 34:5, 34:6, 36:1, 36:2, 36:3, 36:4, 36:5, 36:6 | ↓ Total; ↓ 34:5, 34:6, 36:5, 36:6 | — Total; ↑ 34:3, 34:6, 36:6 | ↑ Total; ↑ 18:1/16:3, 18:2/16:0, 18:3/16:0, 18:3/16:1, 18:3/16:3 | ↑ Total ↑ 16:0, 18:1, 18:2, 18:3 | 28 d; soil; ESI-MS/MS; t.test; (Tan et al. 2018) |
| −12 | 3 d_4°C ↳ 10hr_-12°C | 3 d 4°C | Col | ↓ Total; ↓ 32:0, 32:1, 34:1, 34:2, 34:3, 34:4 | — Total; ↑ 16:0, 18:2; ↓ 18:3 | ↓ Total; ↓ 34:1, 34:2; 34:3, 36:2, 36:3, 36:4, 36:5, 36:6 | ↑ Total; ↑ 16:0, 18:0, 18:1, 18:2, 18:3 | ↓ Total; ↓ 34:2; 34:3, 36:2, 36:3, 36:4, 36:5, 36:6, 40:2, 42:2, 42:3 | ↑ Total; ↑ 16:0, 16:1, 18:1, 18:2, 18:3 | ↓ Total; ↑ 34:2 | ↓ Total; ↓ 34:2, 34:3, 36:3, 38:2, 38:3, 40:2, 40:3 | ↑ Total; ↑ 32:0, 34:1, 34:2, 34:3, 34:4, 34:6, 36:2, 36:3, 36:4, 36:5, 36:6 | ↓ Total; ↓ 34:6, 36:6 | ↓ Total; ↓ 34:3, 36:6 | nd | nd | 42 d; soil; ESI-MS/MS; Qtest; (Zheng et al. 2016) |
| −12 | 3 d_4°C ↳ 10hr_-12°C | 3 d_4° C ↳ 5 h_rp ↳ 2 h_-6° C | Col | ↓ Total; ↓ 32:0, 32:1, 34:1, 34:2, 34:3, 34:4 | — Total; ↑ 18:2; ↓ 18:3 | ↓ Total; ↓ 34:1, 34:2, 34:3, 34:4, 36:2, 36:3, 36:4, 36:5, 36:6 | ↑ Total; ↑ 16:0, 18:0, 18:1, 18:2, 18:3 | ↓ Total; ↓ 34:2, 34:3, 34:4, 36:3, 36:4, 36:5, 36:6, 42:2, 42:3 | ↑ Total; ↑ 16:0, 16:1, 18:1, 18:2; ↓ 18:3 | ↓ Total; ↓ 34:2, 34:3, 36:5, 36:6 | ↓ Total; ↓ 34:2, 34:3, 36:5, 40:2, 40:3 | ↑ Total; ↑ 32:0, 34:1, 34:2, 34:3, 34:4, 34:6, 36:2, 36:3, 36:4, 36:5, 36:6 | ↓ Total; ↓ 34:6, 36:5, 36:6 | ↓ Total; ↓ 34:3, 34:6, 36:5, 36:6 | nd | nd | 42 d; soil; ESI-MS/MS; Qtest; (Zheng et al. 2016) |

All terms and abbreviations are identical to Table 1. Arrows indicate direction of significant changes compared to control, —indicates insignificant changes, and nd indicates no data. Asterisks indicate the most common change in studies with multiple varieties of species. Only high abundance lipid species changes were reported. Temperatures of cold treatment are indicated, with light and dark temperatures differentiated if relevant. The control growth and treatment conditions are indicated, as is the genomic variety. ↳ indicates the transition between steps in a multistep freezing treatment, ramps in temperature are explicit. An underscore links temperatures and time in the same step. Significance of changes indicated are either from published statistical analyses, our analysis (t-test) of available raw datasets, or for 4 publications (Zheng et al. 2016; Gu et al. 2017; Marla et al. 2017; Tan et al. 2018) by visual use of graphs where non-overlapping error bars on bar graphs or wide differences from heat maps compared to control values. The "notes" category includes the age of the plants at the beginning of treatment, growth support as soil or medium, the method of MS analysis, the statistical test used, and the reference. Abbreviations: °C, degrees Celsius; Ctrl, control; DAG, diacylglycerol; DGDG, digalactosyldiacylglycerol; dk, dark; L-PC, lysophosphatidylcholine; L-PE, lysophosphatidylethanol; lt, light; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; Rp, ramp; SQDG, sulfoquinovosyldiacylglycerol; TAG, triacylglycerol; Trt, treatment; V., variety.

**Table 3.** Chilling sorghum and maize glycerolipid changes

| °C | Trt | Ctrl | V. | PG | L-PG | PC | L-PC | PE | L-PE | PI | PS | PA | MGDG | DGDG | SQDG | DAG | TAG | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 1d 10°C | 0 h 10°C | *S.b.* M-81E | ↑ Total;<br>↑ 16:1, 18:1, 18:2;<br>↓ 16:0, 18:0, 18:3 | nd | ↑ Total;<br>↑ 16:0, 18:1, 18:2, 18:3;<br>↓ 18:0 | nd | ↓ Total;<br>↑ 18:1, 18:2, 18:3;<br>↓ 16:0, 18:0 | nd | nd | nd | nd | ↑ Total;<br>↑ 18:0, 18:2;<br>↓ 16:0, 18:1, 18:3 | ↓ Total;<br>↑ 16:0, 18:1, 18:2, 18:3;<br>↓ 18:0 | ↓ Total;<br>↑ 16:0, 18:1, 18:2, 18:3;<br>↓ 18:0 | nd | nd | 4-leaf; soil; GC; Duncan's test; (Guo et al. 2016) |
| 10 | 1d 10°C | 0 h 10°C | *S.b.* Roma | ↓ Total;<br>↑ 6:0, 18:0;<br>↓ 16:1, 18:1, 18:2, 18:3 | nd | ↑ Total;<br>↑ 18:0;<br>↓ 16:0, 18:1, 18:2, 18:3 | nd | ↓ Total;<br>↑ 18:0, 18:2, 18:3;<br>↓ 16:0, 18:1 | nd | nd | nd | nd | ↑ Total;<br>↑ 18:0;<br>↓ 16:0, 18:1, 18:2, 18:3 | ↓ Total;<br>↑ 18:0, 18:3;<br>↓ 16:0, 18:1, 18:2 | ↑ Total;<br>↑ 18:0, 18:2;<br>↓ 16:0, 18:1, 18:3 | nd | nd | 4-leaf; soil; GC; Duncan's test; (Guo et al. 2016) |
| 10 | 12 h 10°C lt 8°C dk | 12 h 28° C lt 25° C dk | *S.b.* BTx623 | ↑ Total;<br>↑ 32:1, 34:4, 36:4, 36:5 | nd | ↓ Total;<br>↑ 36:5, 36:6 | ↑ Total | nd | nd | nd | nd | ↓ Total | ↑ Total;<br>↑ 34:3, 34:6, 36:6, 38:5, 38:6;<br>↓ 34:2, 36:3, 36:4, 36:5 | — Total;<br>↑ 34:4, 34:6;<br>↓ 34:1, 34:2, 36:1, 36:4, 36:5 | ↑ Total;<br>↑ 32:0, 34:3 | nd | — Total | 10 d; soil; ESI-MS/MS; heatmap; (Marla et al. 2017) |
| 10 | 12 h 10°C lt 8°C dk | 12 h 28° C lt 25° C dk | *S.b.* RTx430 | ↓ Total;<br>↑ 36:2, 36:4, 36:5;<br>↓ 34:4 | nd | ↑ Total;<br>↑ 36:5, 36:6 | ↑ Total | nd | nd | nd | nd | ↓ Total | ↓ Total;<br>↑ 38:4;<br>↓ 34:X, 36:X, 38:5, 38:6 | ↓ Total;<br>↑ 34:1;<br>↓ 36:3, 36:4, 36:5, 36:6, 38:6 | — Total;<br>↓ 34:3 | nd | — Total | 10 d; soil; ESI-MS/MS; heatmap; (Marla et al. 2017) |
| 10 | 12 h 10°C lt 8°C dk | 12 h 28° C lt 25° C dk | *S.b.* SQR | ↓ Total;<br>↑ 36:4, 36:5;<br>↓ 32:0, 34:0, 34:1, 34:2, 36:3 | nd | — Total;<br>↑ 36:5, 36:6 | ↑ Total | nd | nd | nd | nd | ↓ Total | ↓ Total;<br>↓ 34:2, 34:3, 36:3, 36:4 | ↓ Total;<br>↑ 34:1, 34:2, 36:5;<br>↓ 34:3, 36:3, 36:6, 38:6 | ↓ Total;<br>↓ 34:3 | nd | — Total | 10 d; soil; ESI-MS/MS; heatmap; (Marla et al. 2017) |

(*continued*)

**Table 3.** Continued

| °C | Trt | Ctrl | V. | PG | L-PG | PC | L-PC | PE | L-PE | PI | PS | PA | MGDG | DGDG | SQDG | DAG | TAG | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 12 h 10°C lt 8°C dk | 12 h 28° C lt 25° C dk | *S.b.* HKZ | ↓ Total; ↑ 36:5, 36:6; ↓ 32:1, 34:0, 34:1, 34:2, 34:3, 34:4 | nd | ↓ Total; ↑ 36:5, 36:6 | ↑ Total | nd | nd | nd | nd | ↓ Total | ↓ Total; ↓ 34:X, 36:X, 38:X | ↓ Total; ↓ 34:3, 34:4, 36:3, 36:6, 38:4, 38:6 | ↑ Total; ↑ 32:0, 34:3, 36:6 | nd | — Total | 10 d; soil; ESI-MS/MS; heatmap; (Marla et al. 2017) |
| 10 | 12 h 10°C lt 8°C dk | 12 h 28° C lt 25° C dk | *S.b.* Kao | ↓ Total; ↑ 32:1, 36:3, 36:4, 36:5; ↓ 34:1, 34:3, 34:4 | nd | ↓ Total; ↑ 36:5, 36:6 | ↑ Total | nd | nd | nd | nd | ↓ Total | ↓ Total; ↑ 38:5; ↓ 34:X, 36:X | ↓ Total; ↑ 34:1, 34:2, 34:6, 36:5, 38:5; ↓ 36:3, 36:4, 36:6, 38:6 | ↑ Total; ↑ 34:3 | nd | ↑ Total | 10 d; soil; ESI-MS/MS; heatmap; (Marla et al. 2017) |
| 10 | 12 h 10°C lt 8°C dk | 12 h 28° C lt 25° C dk | *S.b.* NSZ | ↓ Total; ↓ 32:1, 34:0, 34:1, 34:2, 34:3, 34:4, 36:3, 36:4 | nd | ↓ Total; ↑ 36:5, 36:6 | ↑ Total | nd | nd | nd | nd | ↓ Total | ↓ Total; ↓ 34:X, 36:X | ↓ Total; ↑ 36:3, 36:5; ↓ 34:4, 34:6, 36:1, 36:4, 36:6, 38:4, 38:5 | — Total; ↓ 36:6 | nd | — Total | 10 d; soil; ESI-MS/MS; heatmap; (Marla et al. 2017) |
| 10 | 36 h 10°C lt 8°C dk | 12 h 10° C lt 8°C dk | *S.b.* RTx430 | ↑ Total; ↑ 32:1, 34:0, 34:1, 34:2, 34:4; ↓ 36:4 | nd | nd | nd | nd | nd | nd | nd | nd | ↑ Total; ↑ 34:5, 34:6, 46:5, 46:6; ↓ 34:3, 38:6 | ↑ Total; ↑ 34:2, 34:3, 34:4, 36:3, 36:5, 36:6, 38:5, 38:6; ↓ 34:1, 38:4 | — Total | nd | nd | 10 d; soil; ESI-MS/MS; heatmap; (Marla et al. 2017) |
| 10 | 36 h 10°C lt 8°C dk | 12 h 10° C lt 8°C dk | *S.b.* NSZ | ↓ Total; ↓ 32:0, 32:1, 34:1, 34:3, 34:4 | nd | nd | nd | nd | nd | nd | nd | nd | ↓ Total; ↓ 34:3, 34:4, 34:6, 36:X, 38:X | ↓ Total; ↑ 36:1; ↓ 34:3, 34:4, 36:3, 36:5, 36:6, 38:6 | ↓ Total; ↓ 32:0, 34:3, 36:6 | nd | nd | 10 d; soil; ESI-MS/MS; heatmap; (Marla et al. 2017) |
| 10 | 36 h 10°C lt 8°C dk | 12 h 10° C lt 8°C dk | *S.b.* RTx430 | ↓ Total; ↑ 32:1, 34:0, 34:1, 34:2, 36:5; ↓ 34:3 | nd | nd | nd | nd | nd | nd | nd | nd | ↓ Total; ↓ 34:3, 34:4, 34:6, 36:X, 38:X | ↓ Total; ↑ 36:3; ↓ 34:1, 34:2, 36:4 | — Total; ↓ 34:3 | nd | nd | 10 d; soil; ESI-MS/MS; heatmap; (Marla et al. 2017) |

(*continued*)

Table 3. Continued

| °C | Trt | Ctrl | V. | PG | L-PG | PC | L-PC | PE | L-PE | PI | PS | PA | MGDG | DGDG | SQDG | DAG | TAG | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 36 h 10°C lt 8°C dk | 12 h 10°C lt 8°C dk | *S.b.* NSZ | ↓ Total; ↑ 36:5; ↓ 32:0, 32:1, 34:0, 34:1, 34:2, 34:3, 34:4 | nd | nd | nd | nd | nd | nd | nd | nd | ↓ Total; ↓ 34:X, 36:X, 38:X | ↓ Total; ↑ 34:2, 34:3, 34:4, 34:6; ↓ 36:1, 36:2, 36:3, 36:4, 36:5, 36:6, 38:4, 38:5, 38:6 | ↓ Total; ↓ 32:0, 34:3, 36:6 | nd | nd | 10 d; soil; ESI-MS/MS; heatmap; (Marla et al. 2017) |
| 10 | 3 d 10°C | 3 d 22°C | *Z.m.* He334 | nd | nd | ↑ Total; ↑ 34:1, 36:3, 36:4; ↓ 34:3, 36:5, 36:6 | nd | ↑ Total; ↑ 34:2, 34:3, 36:4 | nd | ↓ Total; ↓ 34:3, 36:5 | — Total | ↑ Total; ↑ 34:2, 36:6 | nd | nd | nd | nd | nd | 21d; soil; GC-MS & LC-MS; (Gu et al. 2018) |
| 5 | 3 d 5°C | 3 d 22°C | *Z.m.* He334 | ↑ Total; ↑ 34:2, 34:4; ↓ 36:2, 36:6 | ↓ Total | ↓ Total; ↓ 34:2, 34:3, 36:1, 36:2, 36:3, 36:5, 36:6 | ↑ Total | ↓ Total; ↑ 34:1; ↓ 34:2, 34:3, 36:2, 36:3, 36:4, 36:5, 36:6 | ↑ Total | ↓ Total; ↑ 36:4, 36:5, 36:6; ↓ 34:1,34:2, 34:3, 36:2, 36:3 | ↓ Total | ↑ Total; ↑ 34:X, 36:X | ↓ Total; ↑ 34:4, 34:6, 36:1; ↓ 34:3 | ↑ Total; ↑ 34:3, 34:4, 36:3; ↓ 34:2, 34:5, 36:4, 36:5 | ↓ Total; ↓ 34:3, 34:4, 36:2, 36:4, 36:6 | nd | nd | 14 d; soil; ESI-MS/MS; t.test; (Gu et al. 2017) |
| 5 | 3 d 5°C | 3 d 22°C | *Z.m.* He334 | — Total; ↑ 36:4; ↓ 34:1, 34:2, 34:3, 36:2 | ↑ Total | ↓ Total; ↓ 36:4, 36:5 | — Total | — Total; ↑ 36:4, 36:5; ↓ 34:1, 36:2, 36:3 | — Total | — Total; ↑ 36:4; ↓ 34:2, 34:3, 36:4 | — Total; ↑ 34:1, 36:4; ↓ 36:3, 36:5 | ↑ Total; ↑ 34:2, 36:3, 36:4; ↓ 34:1, 34:3 | ↓ Total; ↓ 34:2, 34:3, 36:6 | — Total; ↑ 34:2, 36:4; ↓ 36:3, 36:5, 36:6 | — Total; ↑ 36:6; ↓ 34:2, 34:4, 36:4 | nd | nd | 14 d; soil; roots; ESI-MS/MS; SPSS statistics; (Zhao et al. 2021) |
| 4 | 3 d 4°C | 3 d 10°C | *Z.m.* He334 | nd | nd | ↓ Total; ↓ 34:1, 34:3, 36:3 | nd | ↓ Total; ↓ 34:2, 34:3, 36:4 | nd | — Total; ↑ 36:4, 36:5, 36:6; ↓ 34:1, 34:2 | ↓ Total | ↑ Total; ↑ 34:1, 34:2, 34:3, 36:4, 36:5 | nd | nd | nd | nd | nd | 21 d; soil; GC-MS & LC-MS; (Gu et al. 2018) |
| 4 | 3 d 4°C | 3 d 22°C | *Z.m.* He334 | nd | nd | ↓ Total; ↓ 34:3, 36:3 | nd | ↓ Total; ↓ 34:2 | nd | ↓ Total; ↑ 36:6; ↓ 34:2, 34:3 | ↓ Total | ↑ Total; ↑ 34:1, 34:2, 34:3, 36:4, 36:5 | nd | nd | nd | nd | nd | 21 d; soil; GC-MS & LC-MS; (Gu et al. 2018) |

(continued)

**Table 3.** Continued

| °C | Trt | Ctrl | V. | PG | L-PG | PC | L-PC | PE | L-PE | PI | PS | PA | MGDG | DGDG | SQDG | DAG | TAG | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 2 d 4°C | 2 d 25°C | *Z.m.* LH244 | — Total; ↑ 32:2, 32:3, 36:4, 36:5 | nd | ↑ Total; ↑ 34:4, 36:6; ↓ 32:0, 34:1, 34:2, 34:3, 36:1, 36:2, 36:3, 38:3, 42:2 | ↑ Total; ↑ 18:2, 18:3 | — Total; ↑ 38:2, 40:2, 42:1, 42:3, 42:3; ↓ 38:5 | ↑ Total; ↑ 18:2 | ↓ Total; ↑ 36:5, 36:6 ↓ 32:0, 34:1, 34:2, 34:3, 36:2 | — Total; ↑ 34:2; ↓ 40:2 | ↑ Total; ↑ 36:5, 36:6 | ↓ Total; ↓ 34:2, 34:4, 36:3, 36:4, 36:5, 36:6 | — Total; ↓ 34:2, 36:3, 36:4 | — Total; ↓ 32:0, 34:0, 36:2 | ↑ Total; ↑ 36:5, 36:6 | — Total; ↑ 50:2, 52:6; ↓ 52:3, 52:4, 54:4, 54:5, 54:6, 54:7 | 14 d; soil; HPLC-MS/MS; t-test; (Gao et al. 2024) |

All terms and abbreviations are identical to Table 1. Arrows indicate direction of significant changes compared to control, —indicates insignificant changes, and nd indicates no data. Asterisks indicate the most common change in studies with multiple varieties of species. Only high abundance lipid species changes were reported. Temperatures of cold treatment are indicated, with light and dark temperatures differentiated if relevant. The control growth and treatment conditions are indicated, as is the genomic variety. Significance of changes indicated are either from published statistical analyses, our analysis (t-test) of available raw datasets, or for 4 publications (Zheng et al. 2016; Gu et al. 2017; Marla et al. 2017; Tan et al. 2018) by visual use of graphs where non-overlapping error bars on bar graphs or wide differences from heat maps compared to control values. The "notes" category includes the age of the plants at the beginning of treatment, growth support as soil or medium, the method of MS analysis, the statistical test used, and the reference. Abbreviations: °C, degrees Celsius; lt, light; dk, dark; Trt, treatment; Ctrl, control; V., variety; PG, phosphatidylglycerol; PC, phosphatidylcholine; L-PC, lysophosphatidylcholine; PE, phosphatidylethanolamine; L-PE, lysophosphatidylethanol; PI, phosphatidylinositol; PS, phosphatidylserine; PA, phosphatidic acid; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol; *S.b.*, *sorghum*; *Z.m.*, maize. Note (Zhao et al. 2021) tested root tissue, while others tested leaf tissue.

In addition to membrane lipid remodeling to maintain chloroplast ultrastructure and function, proteinaceous components also help to physically stabilize internal chloroplast membranes during low temperatures. The COLD REGULATED COR15A and COR15B proteins are induced during low temperatures and translocate to the stroma, where they bind to MGDG on the inner envelope to maintain the structural integrity of the envelope and chloroplast morphology (Artus et al. 1996; Thalhammer and Hincha 2014).

## Section 4: Glycerolipid changes upon cold stress severity

In this section, we examine patterns of glycerolipid fluctuations in response to low temperatures, as revealed through lipidomic studies of Arabidopsis, maize, and sorghum. This analysis highlights the influence of stress severity, genetics, and species-specific responses. The reviewed work reported wild-type plants under cold stress analyzed by mass spectrometry to improve the similarity in reported lipids. Our discussion centers on the overall abundance of headgroups (classes), though specific FA combination changes within each class are documented in the tables. Sphingolipids and phytosterols are excluded due to limited data on their cold-induced alterations (refer to Section 3 for details).

Lipid changes vary with cold severity. In nature, Arabidopsis ecotypes can be found in a variety of climates (1001 Genomes Consortium 2016); however, in cold studies, methodological convenience outweighs biological relevance. Arabidopsis cold acclimation and chilling treatments are often conducted near 4°C (refrigerator, Table 1), while freezing treatments vary in severity and duration (Table 2) based in part on available equipment. Sorghum and maize, less cold-tolerant than Arabidopsis, can also acclimate to low temperatures depending on growth stage and breeding location (Pál et al. 2020; Barnes et al. 2023; Hernández et al. 2023). Available mass spectrometry equipment and protocols also affect sensitivity to specific lipids.

Lipidomic analyses of Columbia ecotype Arabidopsis reveal a nuanced response to cold stress. During chilling, variations occurred within a single lipid class depending on the specific treatment conditions (Table 1). For instance, PA levels remained relatively unchanged under milder chilling conditions, for example, 6 °C for 37 days (Chen and Thelen 2013) or 4 °C for 1 day (Barajas-Lopez et al. 2021), but significantly increased when the stress intensified, for example, 4 °C for 3 days (Welti et al. 2002), while MGDG levels varied during chilling without a discernable pattern (Table 1). Other nonbilayer-forming lipids, lysoPC, lysoPE, and DAG, tended to consistently increase in chilling stress (Table 1; Fig. 3), congruent with their roles in membrane flexibility, lipid metabolism, and signaling (Sections 1, 2, 3). Cold acclimation followed by freezing often induced more consistent changes across entire lipid classes, including PA (increased) and MGDG (decreased), and increases in the same set of nonbilayer-forming lipids: lysoPC, lysoPE, and DAG. Because MGDG favors the $H_{II}$ phase (Fig. 1; Section 1) and is degraded and modified by multiple enzymes during freezing (Section 3), the decrease in MGDG levels (Table 2; Fig. 3) may improve tolerance. In seeming contrast to MGDG changes, PA also favors the $H_{II}$ phase (Fig. 1; Section 1), but its levels increased during freezing (Table 2; Fig. 3). This apparent discrepancy is likely a matter of abundance, as excessive PA increase during freezing is detrimental (Section 3). Consistent with its essential role in cold tolerance (Section 3), TAG levels consistently increased with additional cold, independent of the condition (Tables 1 and 2; Fig. 3). Together, these changes suggest that Arabidopsis can interpret cold severity and respond appropriately.

Another explanation for the variations in lipid level changes between studies is the age of the plant. During early developmental stages, plants are more sensitive to cold (Lyons 1973) and may be less protected by acclimation (Limin and Fowler 2006). The study applying chilling to the youngest plants (Chen and Thelen 2013) showed more significant lipid changes than chilling in older plants, comparing PC, PI, and DGDG levels (Table 1). In freezing, studies performed at 28 days (Li et al. 2008; Tan et al. 2018) were less likely to have statistically relevant changes in PG, PI, PS, and DGDG compared with studies performed at 35 or 42 days (Zheng et al. 2016). Though it is impossible to exclude the role of other experimental differences, these observations suggest the age of Arabidopsis is important for lipid responses.

Compared with Arabidopsis, there are relatively fewer cold lipidomics studies in sorghum and maize (Table 3). One pattern arising from them is consistent with the conclusion of Arabidopsis cold severity sensing. Sorghum variety RTx430 reversed the direction of PG, MGDG, and DGDG lipid level changes between 12 and 36 hours of 10 °C exposure (Marla et al. 2017). Maize variety HE334 showed increases in PC and PE levels at 10 °C and decreases at 4 or 5 °C (Gu et al. 2017; Gu et al. 2018). These lipid level reversals suggest that all plants respond to cold severity with differing lipid changes.

Genetic variance contributes to altered lipid patterns. Genetic variance across genotypes increases the variation of lipid changes in response to cold conditions. An Arabidopsis study involving 15 ecotypes illustrates this phenomenon, where both common and unique lipid changes were reported between ecotypes (Degenkolbe et al. 2012). In sorghum, cold responses of PG, PC, DGDG, and SQDG levels were affected by genetic variance (Table 3) (Guo et al. 2016; Marla et al. 2017). Within groups of similarly tolerant sorghum varieties (HKZ, Kao, NSZ), changes in PC, MGDG, DGDG, and SQDG levels in response to cold were more similar (Marla et al. 2017). In maize, comparisons between He334 and LH244 showed differences in changes to PC, PE, and PS levels (Gu et al. 2017; Gu et al. 2018; Gao et al. 2024), though these studies also differ in the age of the plants at onset and duration of cold application, preventing a strong conclusion. Between species, the only consistent lipid change is that of PA, which tends to increase (Tables 1, 2, and 3), consistent with the recent identification of PA as 1 of 4 lipids whose changes correlate with improved temperature tolerance (Vu et al. 2022). Together, the Arabidopsis and sorghum studies strongly suggest the importance of genetic and species variation. A remaining question is whether this is due to the differing impact of the same cold temperature applied to plants with different tolerance levels or if it truly underpins a different metabolic strategy for tolerance. Part of the answer may come from continuing genome-wide association studies, which can identify genes among varieties of a species associated with temperature tolerance and lipid metabolism (Li et al. 2019; Pranneshraj et al. 2022). Another will have to come from continued careful measurements of lipid behaviors during temperature stress.

In summary, lipid responses to cold were affected by genetic variance, plant age, stage, and severity and length of applied cold. In addition to these biological effects, noise was added from differing methodological choices made, including extraction and mass spectrometry equipment and methods used and choice of lipids analyzed. We also note differences in application of abiotic stresses likely play a factor: for example, light intensity, water sufficiency, time of day, etc. We refer readers to appropriate lipid extraction (Bengtsson et al. 2021; Mahboub et al. 2021) and lipidomics methods (Gutbrod et al. 2021; Tamura et al. 2021) and encourage thorough descriptions of growth parameters and analysis of as many common headgroup classes (Tables 1, 2, and 3) as is practical.

## Concluding remarks

In summary, this review delves into the intricate adaptations of plant membrane lipids to cold stress, highlighting the vital roles of glycerolipids, sphingolipids, and phytosterols. We explore how biochemical pathways, including acyl editing, headgroup exchange, and abundance changes, equip plants to survive chilling conditions. Additionally, our analysis of lipidomic data from Arabidopsis, maize, and sorghum underscores the complexity and variability of lipid responses across different species and stress intensities. Despite these advances, significant knowledge gaps remain. See *Outstanding Questions*, particularly, regarding the functional impacts of specific lipids, the subcellular dynamics and transport of lipids, and the specificity of membrane metabolic strategies of cold tolerance. Addressing these gaps is crucial for enhancing our predictive capabilities and improving plant resilience against increasingly frequent and severe cold events.

Outstanding questions box:

- What are the roles of $H_{II}$ phase and plasma membrane nanodomains during freezing?
- What are the roles of phytosterols outside the plasma membrane during cold?
- How do unusual lipids that change levels during cold (TGDG, acyl-MGDG, CL, and phosphorylated LCBs) impact membrane temperature tolerance?
- What happens to organelle membranes other than chloroplast membranes in the cold?
- Are thylakoid lipids remodelled by enzymes in other membranes? If so, how does transport occur?
- What is the role of TAG accumulation in cold tolerance?
- How relevant is comparing plant varieties with different tolerance levels at the same temperature when they acclimate at different temperatures in their native environments?
- Do differences in how lipids respond to cold observed between species reflect a different metabolic strategy for tolerance?

Advances box:

- Computational modeling of lipid environments continually increases in effectiveness. Recent data suggest the increased importance of lateral phase separations. This combines with recent studies showing phytosterols and sphingolipids form temperature-dependent lateral phase separations in artificial membranes.
- Membrane contact sites are better established, improving the understanding of lipid transport between multiple subcellular compartments.
- Multiple studies of TAG accumulation during low temperatures imply hypotheses for its utility in cold stress.
- Multiple recent cold lipidomic studies in Arabidopsis, sorghum, and maize allow comparisons between studies.

## Author contributions

All authors drafted a section of the manuscript and edited the whole manuscript.

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## Data availability

No new data were generated or analysed in support of this research.

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