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Review article

Low-temperature tolerance in land plants: Are transcript and membrane responses conserved?

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ABSTRACT

Plants' tolerance of low temperatures is an economically and ecologically important limitation on geographic distributions and growing seasons. Tolerance for low temperatures varies significantly across different plant species, and different mechanisms likely act in different species. In order to survive low-temperature stress, plant membranes must maintain their fluidity in increasingly cold and oxidative cellular environments. The responses of different species to low-temperature stress include changes to the types and desaturation levels of membrane lipids, though the precise lipids affected tend to vary by species. Regulation of membrane dynamics and other low-temperature tolerance factors are controlled by both transcriptional and post-transcriptional mechanisms. Here, we review low-temperature induced changes in both membrane lipid composition and gene transcription across multiple related plant species with differing degrees of low-temperature tolerance. We attempt to define a core set of changes for transcripts and lipids across species and treatment variations. Some responses appear to be consistent across all species for which data are available, while many others appear likely to be species or family-specific. Potential rationales are presented, including variance in testing, reporting and the importance of considering the level of stress perceived by the plant.

1. Introduction

Low-temperatures negatively affectplant growth, development, and productivity. Plant species display a broad range of tolerance to low-temperatures, which is a major factor influencing the spatial distribution of plant species and limits the expansion of the growing regions of crop species into areas otherwise suitable for crop production. Low-temperature stress has two distinct components: chilling, usually defined as lower than normal growing temperatures for a given species but higher than 0 °C, and freezing, defined as less than 0 °C A number of economically important crops such as maize, soybean, rice, cotton, and tomato are sensitive to chilling [1]. Chilling tolerant plants of temperate origin usually require exposure to moderate cool temperatures to increase their low-temperature tolerance through a process called cold-acclimation [2]. This explains why winter-hardy crops like wheat, barley, oats, and rye are sensitive to low temperature during many life stages, including flowering. These species have a vernalization requirement [3,4], which prevents them from flowering prior to spring.

A reduction in temperature causes two direct effects at the molecular level. It variably reduces enzyme activity, and it reduces membrane flexibility [5]. An important example of the variable reduction in enzyme activity is photosynthesis. As plants are chilled, the light reactions of photosynthesis are relatively stable while the dark reactions enzymes are reduced in activity. This leads to photoinhibition of photosystem I and sometimes II [6,7], and allows production of reactive oxygen species (ROS) [8]. One of the effects of ROS is to cause lipid

Abbreviations: ROS, reactive oxygen species; DEGs, differentially expressed genes; Col-0, Columbia ecotype of Arabidopsis; PLD, phospholipase D; Fv, variable photosynthetic fluorescence; Fm, maximum photosynthetic fluorescence; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; PG, phosphatidylglycerol; PI, phosphatidylinositol

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peroxidation, which reduces membrane fluidity [9]. Low temperatures also directly reduce membrane flexibility. Rigid membranes are easily damaged, resulting in increased electrolyte leakage [5]. Genetic and metabolic studies have been critical to identifying plant responses, which in Arabidopsis include changes in membrane lipid abundance and composition [10–14], especially desaturation [15–18], accumulation of solutes [19–21] and membrane-stabilizing proteins [22,23], and increases in ROS scavengers [9,22]. In wheat, non-specific lipid transport proteins are also implicated [24]. At the subcellular level, it has long been known that changes to the plasma membrane composition are necessary, as it is the barrier between the cell and its environment [14,25]. These changes include both glycerolipids and sphingolipids, a special class of membrane lipids that accumulate only on the outer surface of the plasma membrane [26]. Damage of internal membranes in response to low temperatures was classically observed [27], and we now know in Arabidopsis, remodeling of galactolipids in the chloroplast envelopes is necessary to prevent catastrophic membrane failure during freezing stress [28,29].

Many of the cellular changes in response to chilling are regulated by a series of cold-responsive transcriptional cascades. The major regulators were discovered by a combination of yeast one hybrid analysis and genetic studies in Arabidopsis and include C-repeat binding factors (CBFs), which are also referred to as dehydration responsive element-binding factors (DREBs) [30–32]. CBFs are activated by Inducer of CBF expression 1 (ICE1) [33] and inhibited by MYB15, an R2R3-MYB family protein [34]. Low-temperature responses are tied into calcium signaling through the calmodulin binding transcription activator (CAMTA) family proteins that directly regulate the CBFs [35], and responses also appear to be regulated by the circadian clock [36,37]. DREB/CBF-like genes appear to play a similar role in regulating cold responses in grasses and have been identified in every grass genome sequenced to date [38]. Constitutive over-expression of these genes results in increased cold tolerance combined with marked growth retardation in maize, rice, wheat, barley, and cassava [39–42]. In Arabidopsis, expression of CBF3 allows increased low-temperature tolerance, although it can also cause growth retardation during normal conditions [43,44]. Arabidopsis expression of CBF homologs from freezing tolerant (wheat, barley, and *B. napus*) and freezing sensitive species (rice, maize, and tomato) has been shown to provide enhanced freezing tolerance [45,46]. In contrast, expression of *AtCBF3* or *LeCBF1* in tomato does not increase freezing tolerance in that species [46], implying that the CBF protein DNA binding specificity is more conserved than the composition of the downstream CBF target gene regulation. This likely causes phenotypic differences in CBF over-expression responses in freezing-tolerant Arabidopsis and susceptible tomato species.

Low temperatures damage plant membranes similarly, regardless of the species, though varied responses have been recorded. Some of these can potentially include changes that do not necessarily increase fitness to low temperature, and instead are results of energy scavenging from cell damage or death. It would be useful if the variable responses to low temperature could be removed, leaving a core set of responses required for fitness to low temperature. A potential way to do that is to compare data acquired across multiple species. In this review, we explore the hypothesis that there is a core set of changes to transcription and lipid levels in response to low-temperature stress across published reports. We focus on changes at the transcriptional and lipid metabolite level, because of the established importance of each and the presence of multiple publications describing each in detail. From the studies surveyed, we show that a subset of transcriptional changes seem to be conserved across plant species, implying that a core response is conserved. However, conservation of changes in lipids is less clear. We highlight both the conserved and non-conserved nature of transcript and lipid changes, point out the wide variety of experimental designs and reporting standards, and conclude that either the variety of approaches is too wide or a core lipid response does not exist. Finally, we suggest some additional reporting standards which will facilitate future comparisons for similar multi-study comparisons.

2. Transcriptional changes due to low temperature

During the past two decades, improvements in sequencing technologies have enabled extensive use of transcript profiling to understand gene expression changes and their regulation. This has enabled researchers to study genome-wide patterns of gene expression changes to various internal and external stimuli [47]. Low-temperature stress produces wide-ranging changes in the overall transcriptome of every species examined to date. Microarray and RNA-seq based studies have consistently identified between 10–15 percent of genes as differentially expressed in response to low-temperature in Arabidopsis, rice, and maize [48–50]. Early studies using cDNA microarrays which measured only a subset of expressed genes in Arabidopsis, rice, wheat and several other plants identified smaller numbers cold-responsive (COR) genes [51–53]. In Arabidopsis, Seki and colleagues reported 19 COR genes, while Fowler and Thomashow identified 306 COR genes, 45 of which were regulated by CBF1 [52,54]. Expression profiling of cassava apical shoots, lead to the identification of early responsive genes including photosynthesis-related, signal transduction components, and transcription factors [55]. Genome-wide analysis of low-temperature tolerance in grapefruit showed down-regulation of transcripts related to photosynthesis, defense, cell wall, and secondary metabolism, while membrane proteins, lipid metabolism, phytohormone and cold-responsive transcription factors were up-regulated [56].

The set of three CBF genes found in the Arabidopsis genome are clustered in the same region of Chromosome 5 [57] and play an essential role in mediating responses to cold. CBF genes are highly induced in Arabidopsis during the early response to low-temperature stress, reaching peak expression after 1–3 h [58]. However, mutations of different upstream regulators have different effects on CBF expression, with CBF3 induction disrupted in *ice1* mutants, CBF2 induction disrupted in both *ice1* and *ice2* mutants, and CBF1 induction increased in an *ice2* over-expressing line, but not disrupted in an *ice2* mutant background [33,59–62]. The CAMTA3 transcription factor has also been identified as a positive regulator of CBF2 [35]. However, only a small subset of low-temperature-responsive genes is regulated by CBFs. One study concluded that of 2000 identified low-temperature-responsive transcripts, about 170 were regulated by CBFs [63]. Early low-temperature induced transcription factors like ZAT12, ZAT10, HSFC1, ZF, ICE1 and CZF1 are also known to regulate expression of cold responsive genes and each other, and may be responsible for the large proportion of transcriptional responses to cold which are not CBF dependent [33,63,64].

To test our hypothesis that there is a core set of low-temperature responsive transcripts, we reviewed the low-temperature transcriptome across plant species, considering only published studies with transcriptome data in repositories NCBI-SRA or ArrayExpress (Table 1; Fig. 1). Given the breadth of different ideal growth conditions across the plant species sampled, we see a broad range of temperatures used as stress treatment in these studies (0 °C to 23 °C). The duration of the low-temperature treatment also varied from as little as 30 min to as long as 7 days. There were also differences across studies in the selection of the developmental stage at the initiation of stress, with the earliest stress applied at germination (sorghum) and the latest stress applied at two years after planting (tea). The vast majority of studies worked with only a single genetic background, while several compared multiple accessions or cultivars [65–71] or closely related species [72,73].

The parameters used in each considered study, as well as the primary findings of each, are listed in Table 1. Genes related to photosynthesis and chloroplast development were consistently repressed in response to low-temperature, though in some species this occurred after 24 h and may not be a primary response [74–76]. The most conserved set of genes upregulated in response to low-temperature stress belonged to the CBFs, WRKYs, and AP2/EREBP transcription factors. Calcium

Table 1
Summary of gene expression profiling across plant species under low-temperature stress.

Species	Published conclusions	Temp & duration	Age/Stage	Sequencing platform	Raw data	Ref
Medicago sativa	AP2-EREBP, CCAAT, WRKY, MYB, bZIP, bHLH, NAC, and AUX/IAA TF's up-regulated.	4 °C for 3 h	8 weeks	GA-II 100 PE	SRP064230	[77]
Vigna subterranea	Gene co-expression analysis showed enrichment of genes related to stress response, carbohydrate and lipid metabolic processes.	23 °C and 18 °C for 5 days	NI	Microarray	GSE72255	[78]
Glycine max	Photosynthesis down-regulated.	4 °C for 24 h	3 weeks	Microarray	MEXP-3164*	[79]
Lotus japonicus	Lipid, cell wall, phenylpropanoid, sugar, and proline regulation genes up-regulated, photosynthetic process and chloroplast development genes repressed.	9 °C/5 °C day/night for 24 h	3 weeks	Hiseq 1500 PE	PRJNA288510	[80]
Fragaria x ananassa	Photosynthesis down-regulated.	2 °C 0, 3, 24, 72, 240 h (Stress at ZT4)	9 weeks (1 week at 10 °C)	Microarray	GSE73488	[81]
Poncirus trifoliata	AP2, WRKY, and NAC up-regulated at all points, photosynthesis genes repressed after 72 hr.	0, 6, 24, 72 h	3 months	HiSeq 2000 PE	SRP056728	[75]
Populus simonii	CBFs and WRKYs significantly induced, Calcium/calmodulin-mediated signal transduction, ABA homeostasis and transport, and antioxidant defense systems were significantly up-regulated while photosynthesis genes were repressed.	4 °C for 10 h	1-year cutting	Microarray	GSE43872	[82]
Jatropha curcas	β-amylase, gibberellin oxidase, non-specific lipid-transfer protein, AP2/ERF, SAD, FAD2, FAD8, and ABC transcription factors strongly upregulated.	12 °C for 12, 24, 48 h	2 weeks	Hiseq 2000	SRR653198	[83]
Manihot esculenta	AP2-EREBP involved in an early response. Majority of the stress-responsive genes were primarily expressed in mature leaves, stem cambia, and fibrous roots rather than apical buds and young leaves.	7 °C for 4, 9 h	3 months	Microarray	GSE31073	[55]
Vitis amurensis	Many heat shock proteins and TFs were cold induced. Alternative splicing increased with prolonged cold stress.	0 °C 3, 12, 48 h	2 years	GA-II PE	SRP026302	[84]
Camellia sinensis	Photosynthesis up-regulated in CT4 and FT4 groups; while repressed or unchanged in CT8 and FT8.	4 °C for 4, 8 h	2 years	Hiseq 2000	SRP051838	[76]
Physcomitrella patens	AP2/EREBP consistently up-regulated. AP2, Carbohydrate metabolism, PUFA desaturases show early response while photosynthesis is repressed as a late response, ABA increases after 24 hr along with DEGs for ABA.	3.5 °C for 1, 3, 8, 24 h (Stress at 4 pm)	4-week-old gametophores	Microarray	MTAB-2165*	[74]
Elaeis guineensis	CBF, ICE1, AP2, bZIP, NAC, WRKY induced.	8 °C for 0.5, 1, 4, 8 hr, 1, 7 days	1 year	Hiseq 2000 PE	SRR1612397	[85]
Zea mays	Dysregulation of circadian rhythm and down-regulation of genes related to photosynthesis.	8 °C/6 °C for 24 h (Stress at ZT14)	V3 stage	Microarray	MTAB-1252	[86]
Arabidopsis thaliana	ABA, GA, and auxin biosynthesis genes are regulated by cold stress. TFs up-regulated as early response and metabolism genes as a late response.	0 °C for 3, 6, 24 h (Stress at 12 pm)	2 weeks	Microarray	GSE3326	[48]
Arabidopsis thaliana†	Cold-tolerant Col-0 ecotype showed fewer gene expression changes compared to Cvi, known to grow in warmer regions.	10 °C for 3 hr	3 weeks	Microarray	GSE41935	[65]
Solanum lycopersicum†	CBF up-regulated during all time points while ICE1 was induced at 1 hr and repressed at 12 hr.	4 °C for 0, 1, 12 h (Stress at 12 pm)	8 weeks	GA-II SE	SRP057825	[66]
Musa spp†	A smaller number of differentially expresses genes in cold-tolerant plantain compared to sensitive banana. Expression pattern of ICE1 and MYBS3 was different in an extended time-course experiment.	10 °C for 0, 3 and 6 h.	Six-leaf stage	Hiseq 2000	SRP047347	[72]
Oryza sativa†	Differential constitutive gene expression prior to stress. During recovery, tolerant genotypes showed quick and efficient reversion of gene expression, whereas the sensitive genotype displayed slower transcriptional recovery.	4 °C for 2, 8, 24, 48 h	S3-stage seedlings	Microarray	GSE38023	[71]
Triticum aestivum†	Differential response in spring and winter wheat using crown and leaf tissues.	4 °C for 2days	3 weeks	Microarray	GSE11774	[70]
Sorghum bicolor†	Orthologs of AtCBF3 upregulated in tolerant genotype under chilling, while it remained lowly expressed in cold-sensitive BTx623.	10 °C/8 °C day/night (Stress at 8 pm)	10-day-old seedling	HiSeq 2500 PE	SRP110161	[68]
Sorghum bicolor†	CBF, AP2/ERF, and ERF up-regulated.	14 °C for 7 days	Germinating seeds	GA-II SE		[67]
Cicer arietinum†	Energy metabolism/photosynthesis repressed in leaves and flowers of both genotypes.	5 °C for 7 days	2 weeks DAF	Microarray	GSE7504	[69]

The column labeled "Temp & duration" lists the lowest temperature and longest duration of the low-temperature stress. If provided, time of day at which stress is applied is shown in parenthesis. The column labeled "Age/stage" lists the age or developmental stage of the plants when stress is applied (including any development during cold acclimation); time of cold acclimation is also shown in parenthesis. Days (d), night (n); hour (hr); week (wk). Locations of raw data are provided using either Sequence Read Archive (SRA) or Gene Expression Omnibus (GEO) identifiers. * indicates microarray datasets deposited in ArrayExpress, † indicates studies where more than one genotype was profiled. No information (NI), days after flowering (DAF).

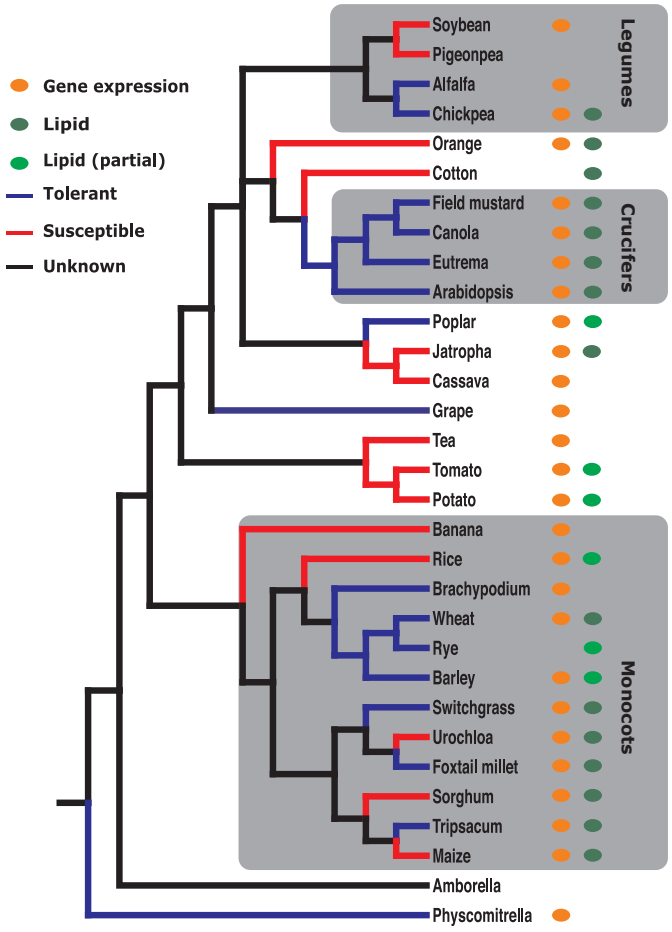

Fig. 1. Phylogenetic tree displaying plant species with low-temperature gene expression data surveyed. Orange ovals show the availability of low-temperature gene expression data, dark-green ovals show low-temperature stress lipid data, and light-green ovals show partial lipid data. Branches are colored to highlight freezing tolerance, blue (ability to withstand less than 0 °C, data collected from USDA Plants website (https://plants.usda.gov/java/), susceptibility, red, or unknown tolerance, black. Grey boxes group the following species: legumes represent species belonging to Fabaceae, crucifers represent members of Brassicaceae, and monocots represent the monocotyledons group including poaceae and musaceae. Scientific names of species identified by common names above: soybean (*Glycine max*), pigeonpea (*Cajanus cajan*), alfalfa (*Medicago sativa*), chickpea (*Cicer arietinum*), orange (*Citrus* × *sinensis*), cotton (*Gossypium hirsutum*), field mustard (*Brassica napus*), canola (*Brassica rapa*), eutrema (*Eutrema salsugineum*), Arabidopsis (*Arabidopsis thaliana*), poplar (*Populus simonii*), jatropha (*Jatropha curcas*), cassava (*Manihot esculenta*), grape (*Vitis amurensis*), tea (*Camellia sinensis*), tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), banana (*Musa sps*), rice (*Oryza sativa*), brachypodium (*Brachypodium distachyon*), wheat (*Triticum aestivum*), rye (*Secale cereale*), barley (*Hordeum vulgare*), switchgrass (*Panicum virgatum*), urochloa (*Urochloa fusca*), foxtail millet (*Setaria italica*), sorghum (*Sorghum bicolor*), tripsacum (*Tripsacum dactyloides*), maize (*Zea mays*), amborella (*Amborella trichopoda*), and physcomitrella (*Physcomitrella patens*).

binding proteins play a role in the early chilling-stress signal transduction, with the majority induced at early time points. In multiple species including Arabidopsis, sorghum, maize, and *Physcomitrella patens* the magnitude of gene expression changes under chilling stress increases from onset to 24 h of stress [48,52,64,73,74]. In Arabidopsis, after 24 h the number of identified differentially expressed genes (DEGs) decreased slowly [48,52,64,74]. One possible explanation for this observation is that if the expression of a few transcripts greatly increases, the expression of all other transcripts may appear to decrease, as both microarray and sequencing based methods for analyzing transcript expression normalize against measures of total abundance.

The timing of transcriptional responses to low temperature can also vary across species. For a specific example, consider MYBS3, a transcription factor believed to suppress CBF1 signaling after prolonged low temperature exposure. In rice, CBF1 responds to low temperature within 6 h, while MYBS3 (*Os10g0561400*) accumulates over the next 72 h [87]. Similarly, this gene was specifically up-regulated in a low-temperature tolerant rice accession [71]. Two homologs of OsMYBS3 in maize (*GRMZM5G813892* and *GRMZM2G034110*), and their shared sorghum ortholog (*Sobic.001G297500*) showed down-regulation after 24 h of low-temperature stress [73], while the same sorghum gene was up-regulated in both susceptible and tolerant sorghum genotypes after 36 h of stress [68]. Banana showed down-regulation of MYBS3 expression between 3 and 24 h, and then showed recovery of expression 48 h post stress. In contrast, MYBS3 expression in plantain did not show significant down-regulation until 6 h post-stress and the expression had already started to recover at 24 h post-stress.

Differences in timing of transcriptional responses can also be seen through comparing homologs with early responses in different species and by comparing changes in timing between multiple ecotypes of a single species. In *P. patens*, genes which showed early transcriptional responses to low temperature tended to lack homologs in other land plants, while genes with later transcriptional responses to low temperature were more likely to be homologous to genes in other land plant genomes [74]. Even when the same gene was conserved across species or between different accessions of the same species, in many cases the pattern of transcriptional response to low temperature is not also conserved. Initial transcriptional changes under low temperature appear to be more conserved between conserved orthologous gene pairs in maize and sorghum while later transcriptional responses were often specific to only one member of a conserved orthologous gene pair [73]. Waters, et al. reported significant amounts of variation in transcriptional responses to low temperature between different maize accessions and between different alleles within hybrid plants [50]. In two studies of sorghum, fewer low-temperature responsive DEGs were identified in chilling-tolerant than in chilling-sensitive accessions [67,68]. Barah et al., reported larger numbers of DEGs in response to low temperature stress in the Cvi ecotype (Cape Verde Islands) adapted to a warmer temperature regime than in the cold-tolerant Col-0 ecotype (Columbia). Relatively few genes were consistently modulated in all ten Arabidopsis ecotypes tested and the majority of DEGs (~75%) showed ecotype-specific expression patterns. The increase in DEGs in sensitive accessions and ecotypes underscores that the transcriptional response of a gene to low temperature does not necessarily indicate that the gene plays a beneficial role in low temperature survival. A study on cis-acting promoter elements in dehydration and chilling stress suggests the existence of diverse transcriptional regulation of chilling-response genes in Arabidopsis, rice, and soybean, in contrast to conserved dehydration-induced promoter elements [79]. To summarize our attempt to find similarities in transcriptional responses to low temperature across species, we identified only transcription factors with well-established roles in the low temperature stress response, and even for these both the timing and the magnitude of the transcriptional responses varied.

We next examined the degree of conservation between similarly designed low-temperature stress studies within the same species, focusing on Arabidopsis and sorghum, where multiple published high quality transcriptome and lipid datasets exist. In Arabidopsis, two studies were compared which both used the Col-0 ecotype and where transcriptional responses were analyzed using microarrays. Lee et al., moved two-week old plants to 0 °C at 12 PM while Barah et al. used three-week old plants and 10 °C as stress treatment. Three hours post stress, Lee et al. identified 132 DEGs (p-value < 0.01), while Barah et al. identified 185 DEGs (p-value < 0.01). Only 10 common DEGs were identified in both studies (Fig. 2a; Supplemental Table 1). Using a more relaxed p-value cut-off (< 0.05), Barah et al. identified 977 DEGs of which 31 overlapped with the set identified by Lee et al. DEGs

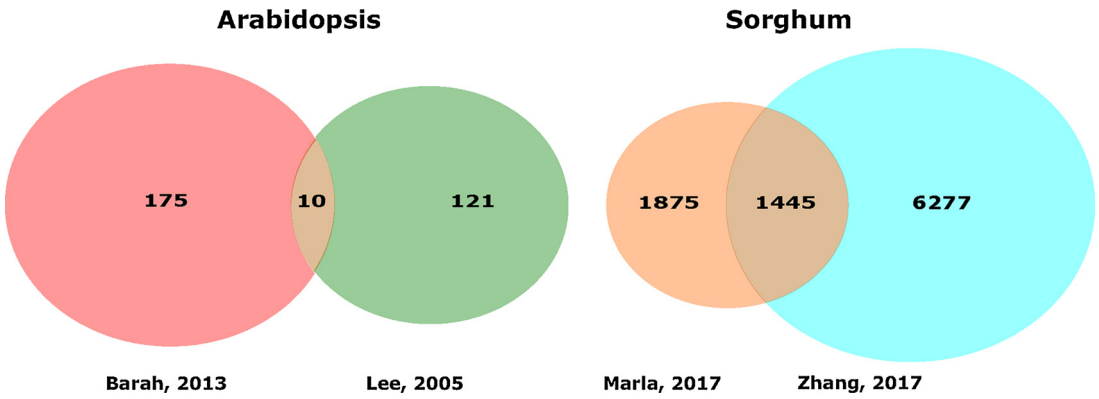

Fig. 2. Few differentially expressed genes are identical in two sets of similarly-designed low-temperature stress studies.
Low-temperature treatments of the studies were as follows: Barah et al. treated 2-week-old Col-0 Arabidopsis at 10 °C for 3 h [65]. Lee et al. treated 3-week-old Col-0 Arabidopsis at 0 °C for 3 h [48]. Marla et al. treated ten-day-old sorghum seedlings at 10 °C/8 °C for 36 h [68]. Zhang et al. treated 12-day old sorghum seedlings at 6 °C for 24 h [73]. In both sorghum experiments, low-temperature stress was initiated at the beginning of the night cycle.

identified in both studies include CBF1, CBF3, CML38, ERF4, ZAT10, CYP707A3, and a lipase implicated in oxylipin metabolism (AT1G02660). That there was not more overlap in the highest confidence DEGs is surprising. We suggest that differences in experimental set up may be a major factor responsible for the low degree of commonality observed between these two studies. In addition, differences in statistical methods and p-values employed may play a role, particularly as the use of stringent, multiple-testing-corrected p-values increases the frequency of false negatives in gene expression analyses.

To gain more insight into the pathways associated with differentially expressed genes conserved in multiple ecotypes, we performed GO analysis using the DavidGO package [88] on two sets of genes selected from Barah et al. [65]. While Barah et al. previously examined differences in GO terms enriched among genes that showed differential expression in individual ecotypes, here we used the same dataset to test for differences in functional annotations between genes which showed consistent transcriptional responses to low temperature stress. We defined this as occurring in at least seven of the ten investigated ecotypes. We also tested for ecotype-specific transcriptional responses to low temperature, which we defined as differentially expressed in only one tested ecotype. Genes related to circadian rhythm, cold acclimation, transcription, and the response to red light were overrepresented among the conserved DEGs while DEGs unique to individual Arabidopsis ecotypes were enriched for defense response to bacterium, response to salicylic acid, response to wounding, and genes whose products were predicted to be located in the chloroplast stroma (Table 2). CBF3 was up-regulated at 3 h post-stress in all ten accessions tested, while CBF1 and CBF2 were induced in all except ecotype Cvi, known to be grown in warmer climates [65]. Similarly, all three CBFs were up-regulated at the same time point in another study in Arabidopsis [48].

From sorghum, we compared three studies which used the chilling-sensitive genotype BTx623 [67,68,73]. Both Marla et al. and Zhang et al. initiated the stress treatment with start of the dark cycle on samples of similar developmental stage (10–12 day seedling), while in Chopra et al, cold stress began as soon as the seeds were imbibed. 24 h after the initiation of stress, Zhang et al., identified 7722 DEGs in BTx623, while Marla et al, identified 3320 DEGs at 36 h after the initiation of stress, with slightly less than 50% of these DEGs also being identified by Zhang et al. (Fig. 2b, Supplemental Table 1). Differences in the duration of low-temperature stress (24 h in case of Zhang et al, and 36 h in Marla et al) and the stress temperature (6 °C in case of Zhang et al, and 10 °C/8 °C in Marla et al) may explain the number of non-overlapping DEGs between the studies. However, the proportion of DEGs which were consistently identified in both sorghum studies is greater than the proportion of DEGs consistently identified in both Arabidopsis studies. Potential explanations include the relatively similar experimental set-ups, similar data analysis pipelines, and the shared use of next-generation sequencing technology rather than distinct microarray platforms. Overall, the comparison of these studies

Table 2
Enriched GO terms from conserved and unique DEGs from low-temperature stress transcriptome in ten Arabidopsis ecotypes.

Type	GO Term	Number	Count	%	P-value	FE	FDR
Conserved DEG[1]	Cold acclimation	GO:0009631	5	6.7	4.30E-05	25.2	1.20E-03
(at least 7 ecotypes)	Circadian rhythm	GO:0007623	7	9.3	1.90E-06	18.5	1.40E-04
	Response to red light	GO:0010114	4	5.3	1.40E-03	17.7	3.40E-02
	Negative regulation of transcription	GO:0045892	5	6.7	1.80E-03	9.6	3.50E-02
	Response to cold	GO:0009409	9	12	1.90E-05	7.7	6.70E-04
	Regulation of transcription	GO:0006355	25	33.3	5.60E-07	3	8.00E-05
	Transcription, DNA-templated	GO:0006351	21	28	1.80E-05	2.9	8.60E-04
	Transcription factor activity, sequence-specific DNA binding	GO:0003700	21	28	2.10E-07	3.7	1.70E-05
	DNA binding	GO:0003677	17	22.7	6.10E-04	2.5	2.50E-02
	Transcription factor activity, transcription factor binding	GO:0000989	3	4	7.90E-04	69.9	2.20E-02
Unique DEGs[1]	Response to bacterium	GO:0009617	38	0.8	2.10E-07	2.4	4.00E-04
	Response to salicylic acid	GO:0009751	45	1	3.00E-05	1.9	1.90E-02
	Defense response to bacterium	GO:0042742	71	1.6	1.30E-05	1.7	1.20E-02
	Response to wounding	GO:0009611	53	1.2	7.10E-05	1.7	3.30E-02
	Chloroplast stroma	GO:0009570	141	3.1	4.10E-05	1.4	1.70E-02

[1] DEGs used for DAVID GO annotation [88] were from [65]. DEGs consistently modulated in at least seven ecotypes under low-temperature were considered conserved, while DEGs unique in each ecotype were considered unique. FE (fold enrichment). False-discovery rate (FDR) is given after multiple testing corrections using the Benjamini-Hochberg method [89].

emphasizes the fact that even though a core set of genes respond to low-temperature in a conserved fashion, over half of the genes identified as differentially expressed will vary, possibly due to small variations in experimental set-up or data analysis.

3. Glycerolipid changes due to low temperature

To survive low temperature stress, plants must maintain membrane integrity and fluidity under changing temperature regimes. Integrity is dependent on fluidity, as membranes must maintain an optimal fluidity to avoid leakage or fracture. Glycerolipids make up the majority of the membranes and are composed of a polar "head" attached through a glycerol to two fatty acid "tails". Fatty acids can be fully saturated, having no double bonds along the tail, or be desaturated at specific locations. The level of saturation influences how a fatty acid fits into the rest of the membrane and impacts the fluidity of membranes at different temperatures. In addition, the relative size of a lipid's "head" group in relationship to its "tails" also affects fit and fluidity [90]. Thus, changes to headgroups or fatty acids can affect overall membrane fluidity. Glycerolipids are the most frequently quantified type of lipid in studies of membrane responses to low temperature and therefore are the only lipid class considered here. There is emerging evidence for a role of sphingolipids in low-temperature tolerance [26,101], however they have been much less frequently quantified in studies of low temperature stress [102], limiting the feasibility of drawing conclusions about a core set of responses from cross species comparisons at this time. Some reported glycerolipid adaptations to low temperature include increasing levels of desaturation of the fatty acid tails [15,103,104] and conversions between different whole lipid head groups like increasing triacylglycerol, also known as oil, or removing lipids, such as monogalactosyldiacylglycerol (MGDG), that destabilize membranes [28,105]. We predicted that, as in the transcript analysis above, a core set of lipid changes in response to low temperature stress may be conserved across plant species. To test this hypothesis, we chose to focus on reports from sorghum, maize, wheat, Arabidopsis, pea, and tomato, as these species are well represented in the literature and include multiple monocots and eudicot taxa. Few papers quantified both transcriptional and glycerolipid-based changes in response to stress, generally reporting one or the other. However for each selected species at least one transcript dataset and multiple publications quantifying lipid abundance for multiple lipid headgroups were available (Table 3, Fig. 1). Some species had abundant reports with complete datasets, such as Arabidopsis, for these we chose to consider publications with the most similarity in both experimental design and lipid quantification.

Lipid desaturation is a commonly observed response to low-temperature stress and assists in maintaining membrane fluidity at low temperatures. Of the publications considered (Table 3), several reported overall desaturation increases in total lipids under low-temperature treatment [95,96,98]. Other publications showed desaturation changes of specific lipids. Marla et al. showed that desaturation of total PC increased, specifically 36:5 and 36:6 [68]. Tarazona et al., showed more desaturated lipids were retained after low-temperature treatment, along with an increase in sphingolipid desaturation [96]. Additionally, Spicher et al., concluded that increases in desaturated MGDG and DGDG were important for low-temperature tolerance [99]. The exception was one paper listed in Table 3 which reported no change in overall lipid desaturation [97]. Fatty acid desaturation has been studied as a potential route of understanding and improving low-temperature tolerance. Studies across multiple plant species show the improvement of low-temperature tolerance after the expression or overexpression of either endogenous or exogenous desaturase genes [106–108]. Improving tolerance during low temperature through increased fatty acid desaturation may be a near universal rule of life, as desaturase genes from sunflower also improve the salt and freezing tolerance of yeast [109].

In Table 3, we summarize changes reported in major membrane lipids, MGDG, digalactosyldiacylglycerol (DGDG), and phosphatidylcholine (PC), lipids important for signaling, phosphatidylinositol (PI) and phosphatidic acid (PA), those important for photosynthesis, sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG), and lastly phosphatidylethanolamine (PE) which is abundant in mitochondrial inner membranes and important for respiration. We also calculate lipid ratios of MGDG/DGDG, MGDG/PC, and PC/PE (Supplemental Table 2) and display the changes to these ratios in Table 3. We note the process in analyzing lipids across species was cumbersome, as lipid data reporting was not standardized. Data was calculated and displayed differently, and not all publications included equivalent measures. We encourage the inclusion of raw data with publications, or in a public repository [110,111].

Galactolipids are the most abundant glycerolipids in nature [112]. Of the galactolipids, MGDG is the most abundant membrane lipid found in chloroplast membranes, making up 52 mol% of the thylakoids [113]. Reports are mixed about how MGDG changes under low-temperature stress, even among related species. Some decreases in MGDG were reported in maize, sorghum, Arabidopsis and wheat [68,92,95]. In general, papers that saw a decrease in MGDG suggested that removing MGDG, a non-bilayer forming lipid, could help to stabilize membranes. In corroboration of that idea, at least two enzymes modify MGDG in response to low-temperature stress and recovery [105,114], also implying that it may be important to reduce the amount of MGDG in membranes exposed to low temperature. DGDG is the second most abundant lipid found in chloroplast membranes, making up 26 mol% of thylakoids [113]. Unlike MGDG, DGDG is a bilayer-forming lipid [115]. Reports of changes in DGDG were mixed. In papers where an increase in DGDG was observed in response to low temperature stress, it was suggested that an increase in bilayer-forming lipids helps to stabilize membranes [92,94,95]. Additionally, papers quantifying specific forms of DGDG found that during freezing, more saturated forms were lost or reduced more often than less saturated forms [96]. SQDG is an anionic lipid important for photosynthesis, and can partially substitute for PG in photosynthetic membranes, and SQDG and PG changes have been observed to be negatively correlated [116]. There were a wide range of reported responses of SQDG abundance to low-temperature stress with approximately even representation of reported decreases, increases, and no changes. SQDG is not required for plant growth under normal conditions because Arabidopsis mutants lacking SQDG synthesis genes can survive as long as they are not phosphate stressed [117]. Few studies have speculated as to the role of the observed low-temperature SQDG abundance response, perhaps because of the lack of consistent patterns.

Phospholipids make up the bulk of extra-plastidic membranes. Of all phospholipids, PC is the most abundant and is also found in the outer envelope of the chloroplast [113]. Thus, it is particularly interesting that reports of changes in PC are so divided. PC levels were shown to increase in wheat and the non-thylakoid fraction of isolated pea membranes [94,95,97]. In contrast, PC abundance was reported to decline under low-temperature treatment in several studies [10,11,92,100], and in one study comparing two wheat genotypes, it decreased only in the low-temperature tolerant line [94]. The two reports in Arabidopsis attribute the decrease in PC abundance to conversion into PA through the activity of two phospholipase D (PLD) isoforms: PLDα and δ [10,11]. They investigate their roles through loss-of-function Arabidopsis mutants, showing that PLDα is important for tolerance, and PLDδ is important for stress recovery [10,11]. In maize, Gu et al., suggested that the decrease of PC combined with an increase in DGDG, resulting from shuttling of PC into galactolipids under low-temperature stress [92]. When reported, lyso-phospholipids and PA nearly always showed an increase in abundance in response to low-temperature stresses [10,11,92]. This increase could be explained by the action of PLDs turning PC into PA [10,11] or through the activity of diacylglycerol kinase [118]. Diacylglycerol kinase was suggested to

Table 3
Lipid changes after low temperature in multiple species.

Species	Genotype	Relative abundance after low temperature											Temp & Duration	Light Intensity	Day (h)	Age/ Stage	Ref
		MG	DG	SQ	PC	PE	PA	PG	PI	MG/DG	MG/PC	PC/PE					
Sorghum bicolor	BTx623	↑	↓	ND	NS	ND	↓	NS	ND	↑	↑	ND	10 °C d/ 8 °C n, 12 h	700–800– µmol/ m^2/s	12	V3	[68]
	RTx430	↓	↓	ND	NS	ND	↓	NS	ND	NS	↓	ND					
	Niu Sheng Zui	↓	↓	ND	↓	ND	↓	NS	ND	NS	NS	ND					
	Hong Ke Zi	↓	↓	ND	NS	ND	↓	NS	ND	NS	↓	ND					
	Shan Qui Red	↓	↓	ND	NS	ND	↓	NS	ND	↓	↓	ND					
	Kaoling	↓	↓	ND	NS	ND	↓	NS	ND	NS	NS	ND					
	BTx623	NS	NS	NS	NS	↑	NS	NS	ND	NS	NS	↓	6 °C, 24 h	ND	13	3 leaf	[91]
Zea mays	He344	↓	↑	NS	NS	NS	↑	NS	NS	↓	↑	↑	5 °C, 3 d	ND	16	2.5 weeks	[92]
	CM 7	↓	NS	ND	ND	ND	ND	ND	ND	ND	ND	ND	5 °C, 4 d & 6 d	no light	16	V3.5	[93]
	Co 151	↓	NS	ND	ND	ND	ND	ND	ND	ND	ND	ND					
	S125	↓	NS	ND	ND	ND	ND	ND	ND	ND	ND	ND					
	EPI	↓	NS	ND	ND	ND	ND	ND	ND	ND	ND	ND					
	B73	NS	NS	NS	NS	NS	NS	NS	ND	NS	NS	NS	6 °C, 24 h	ND	13	3 leaf	[91]
Triticum aestivum	Miranovskaja	↓	↑	↑	↓	ND	ND	NS	ND	↓	NS	ND	1.5 °C, 4 wk	ND	16	8 weeks	[94]*
	Penjamo	↓	↓	↑	↑	ND	ND	↑	ND	↑	↓	ND					
	Manitou	↓	↑	↓	↑	↑	ND	ND	ND	↓	↓	↑	4 °C, 2 wk	120 µmol/m^2/s	16	4 weeks	[95]
Arabidopsis thaliana	Columbia	NS	NS	ND	↓	↓	↑	↓	NS	↓	↑	↓	−8 °C, 2 h	30 µmol/m^2/s	12	4.5 weeks	[11]
	Wassilewskija	↓	NS	ND	↓	↓	↑	↓	NS	↓	↑	↓					
	Columbia	↑	NS	NS	↓	↓	ND	NS	NS	↑	↑	NS	10 °C, 3 wk	120 µmol/m^2/s	16	4 weeks	[95]
	Columbia	NS	NS	ND	↓	↓	↑	↓	NS	↓	↑	↓	−8 °C, 2 h	30 µmol/m^2/s	12	5.5 weeks	[10]
	Columbia	↓	↓	↓	↑	↑	ND	↓	↑	ND	ND	ND	−2 °C, 12 h	no light	12	4.5 weeks	[96]†
Pisum sativum	Feltham First	↓	NC	↑	↑	ND	ND	NC	ND	NS	↓	ND	4 °C/7 °C, season	60 w/m^2	16	3 leaf stage	[97]†
	Feltham First	↑	↑	↑	↑	ND	ND	↑	ND	NS	↓	ND	5 °C–10 °C, season	natural		3 leaf stage	[98]†
Solanum lycopersicum	M82	↓	↓	ND	ND	↑	ND	ND	ND	NS	ND	ND	10 °C/8 °C, 6 d	250 µmol/m^2/s	16	5–6 weeks	[99]†
	Sibirskie Skorospelye	↓	↓	↓	↓	↓	ND	↓	↓	↑	↑	NS	6 °C, 5 d	10 klx and 2.5 klx	16	3–4 true leaves	[100]†

The Temp & duration column gives the lowest temperature and the longest duration of the low-temperature stress. Ages/stages gives the age or stage of the plant, including any time spent in cold acclimation, when stress is applied. Day (d), night (n), hour (hr), week (wk), not determined (ND). NC (no change) indicates that cold and control samples were reported to exhibit equivalent values without any reported statistical testing. NS (not significant) indicates that a statistical test was conducted, but any pattern observed was not statistically significant. Monogalactosyldiacylglcyerol (MG), digalactosyldiacylglycerol (DG), sulfoquinovosyldiacylglycerol (SQ), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylinositol (PI). * represents a study done on isolated thylakoid membranes and † represents studies lacking statistical analysis. Lipid ratios represent reported ratios or were calculated for studies reporting quantified lipid abundance. No change (NC) was indicated for lipid ratios if the difference was less than 5%.

activate in response to membrane rigidification in isolated Arabidopsis cells [18] and may respond to low temperature. PA and lyso-lipids are known to be signals during many developmental stages of plant growth and various environmental stresses [119], so this change may be a functional part of the low-temperature response. PG is required for photosynthesis [116], and is important for photochemical and electron transport inside the thylakoids and coordinating Photosystem II reaction centers [120]. PG is synthesized in the chloroplast, endoplasmic reticulum, and the mitochondria in plant cells [121–123]. Mutant plants lacking PGP1, one of two PG synthases, have chloroplast defects [124], while mutants lacking both PG synthases have embryonic defects, are albino, and have chloroplast and mitochondrial defects [125]. PG levels decreased under low-temperature treatment in most reported datasets, although some individual studies also reported increases or the absence of statistically significant changes. During low-temperature stress, the dark reactions of photosynthesis lag and this leads to a buildup of ROS through the saturation of photosystem I and in high light conditions photosystem II [8]. Thus, one potential benefit of decreasing levels of PG in the plant during low-temperature stress is that the electron transport chain has been shown to be less efficient with reduced PG [120]. It is unclear how the decrease in PG occurs, but it could be hydrolyzed and recycled in response to stress conditions. However, Welti et al. suggest that PLDα, a lipase known to be active in response to freezing stress is not responsible for the reduction in PG [10]. PE levels were frequently observed to decrease in response to low temperatures. In one instance, the specific fatty acid groups 36:4 and 36:5 were observed to decrease dramatically [10]. In contrast, one publication reported a PE level increase specifically in 34:2 and 36:4 PC [99]. PE is a non-bilayer forming lipid [115], so decreasing overall PE abundance may help improve membrane stability. Saturated PE is more likely to form bilayers than desaturated PE, which could mean that decreases in more desaturated PE levels are a functional adaptation to low-temperature [126]. PI derivatives are important secondary signaling molecules during times of stress. PI-kinases have been found to be active under low temperatures upstream of phospholipase C, which cleaves the entire lipid headgroup and creates diacylglycerol and inositol-1,4,5-triphosphate [127]. A phosphatidylinositol transfer-associated protein from maize was overexpressed in Arabidopsis and conferred tolerance to low-temperature stress [128]. However, when measured, the abundance of PI was generally found to be unchanged under low-temperature stress. Only one report noted an increase in PI abundance in response to stress treatment and one a decrease. This may be because PI changes are typically ephemeral and may not have been captured at the limited timepoints sampled.

Few general trends of glycerolipid responses were observed across multiple treatments, genotypes, and species, and in every case exceptions also existed. MGDG levels were the most consistent, showing decreases in response to cold across many treatments and species, with

the exception of reports in sorghum, Arabidopsis, or pea, four of which increased and two of which were non-significant (Table 3). No additional consistent trends were found when separately considering data from studies in monocots or eudicots, or from treatments above freezing. Ratios of changes in major membrane lipid classes also did not explain changes in response to low-temperature stress.

One challenge in a broad, multistudy comparison of lipid changes is a lack of consistency with which lipid head groups are assayed. In many studies, only lipid subsets of interest to specific research questions were assayed (Table 3). A second challenge is the difficulty of defining equivalent development stages across species. Studies varied from 2 week old corn [91] to reproductive stage Arabidopsis [10]. Within individual species, the largest age variance was in wheat, where there was a difference of four weeks. In addition, the studies shown in Table 3 vary in growth conditions and the parameters of the low temperature treatment, including temperature, length of stress, and amount of light during treatment. In spite of sharing all of the same challenges, comparison of transcript data across species did reveal a core set of changes, while lipid data did not. Most transcript-based studies assayed a comprehensive or nearly comprehensive set of annotated genes, while few publications reported even all of the major membrane lipids. Thus, we consider two possibilities, i) there is no core lipid response to low temperature, and ii) a core lipid response exists but cannot be defined using these studies with too much diversity in testing procedures, developmental stage, and lipid reporting.

We next compared different reports from the same species. We again utilized data from Arabidopsis and sorghum because of their relatively abundant lipid and transcript datasets, and representation of the monocots and eudicots [10,11,68,91,95,96] (Table 3). Changes in abundance of individual lipid headgroups were generally consistent across studies. With the exception of one study, Arabidopsis PC and PE levels decreased in response to low temperature stress across two genotypes. PG levels also decreased consistently in studies of Arabidopsis with the exception of Li et al 2015, in which no statistically significant changes were identified [95]. Sorghum PA levels were decreased in most reports, while Arabidopsis PA levels increased. In sorghum DGDG levels decreased across all genotypes, chilling sensitive and tolerant alike, though in one study the change was not statistically significant, whereas in Arabidopsis changes in DGDG levels were generally not statistically significant. MGDG levels did not exhibit a clear pattern of response to low-temperature stress in Arabidopsis, however, changes in MGDG levels in sorghum showed differences between lines which were largely consistent with the classification of these lines as chilling tolerant or chilling sensitive (Table 3). Generally, decreases in MGDG abundance were observed after low-temperature treatment especially in accessions that were chilling tolerant, Niu Sheng Zui, Hong Ke Zi, Shan Qui Red, and Kaoling [68]. The exception was the chilling-sensitive line BTx673, which either increased post low-temperature treatment [68] or decreased so slightly as to not be statistically significant [91].

Upregulation of multiple membrane lipid desaturases occurs in low temperatures [15,16], some of which seem to function primarily during chilling stress [17]. Engineering increases in lipid desaturation increases low-temperature tolerance [106,129,130], and mutants decreasing desaturation increase low-temperature susceptibility [15,17,103]. A long-standing hypothesis is that the membrane rigidifying effect of low-temperatures is directly relieved by increasing membrane desaturation [131]. Reporting of desaturation levels across lipid headgroups varies, in part because of inherent differences between direct mass-spectrometry measurement and derivatization-based methods. First, we discuss conclusions from two similar studies on sorghum using each study's own approach to quantifying desaturation, then we compare multiple Arabidopsis studies using a ratio between reported desaturation index levels in control and treated plants to compare overall desaturation changes between species. Marla et al. showed an increase in PC desaturation after chilling in all sorghum accessions tested, including both low-temperature tolerant and sensitive lines [68]. These changes primarily came from an increase in 36:5 and 36:6 PC levels relative to levels of less desaturated forms of PC. Statistically significant increases in desaturation levels were also observed for PI and PA in five out of six sorghum accessions tested. Yan et al. and also observed increased desaturation of PC in response to cold, including an increase in 18:3 containing PC, consistent with the observation of Marla et al. However, the difference was not sufficient to be statistically significant, and neither were differences in PI or PA [91]. In contrast, desaturation levels were observed to decrease in PE, PG, SQDG and DGDG. The two experimental setups were similar (Table 3), both using young seedlings of the same accession and within 4 °C of the same temperature, however the timing of the study was dramatically different with respect to the diurnal clock with a 12- vs a 24-hour sampling time.

We reasoned that if lipid desaturation directly relieves membrane stiffness caused by low temperatures, then there would be more lipid desaturation at lower temperatures. To avoid multi-species comparisons and differences between desaturation indices, we calculated desaturation indices from multiple Arabidopsis investigations with different temperature usage (Fig. 3). We also included one mass-spectrometry study with self-reported desaturation indices from a similar calculation [132]. Surprisingly, a correlation is not observed between temperature and desaturation index. Data from three reports have a desaturation index ratio below one, indicating decreasing desaturation during low temperatures [132–134]. Data from six reports showed an increase in total desaturation during low temperatures, but there was no trend of increasing desaturation with increasingly low temperatures. Because membrane fluidity must be preserved, we conclude that desaturation is not the only mechanism preventing membrane stiffening at low temperatures. Similarly, because desaturation is known to be required for low-temperature survival, the desaturation of specific lipid types must be needed for other reasons, allowing tolerance. Alternatively, a final possibility is that differences in experimental setup and analysis obscure interpretation. Controls varied between studies with some research groups using unstressed plants and some cold-acclimated plants. Also, low-temperature treatments differed in length from 2 h to 37 days.

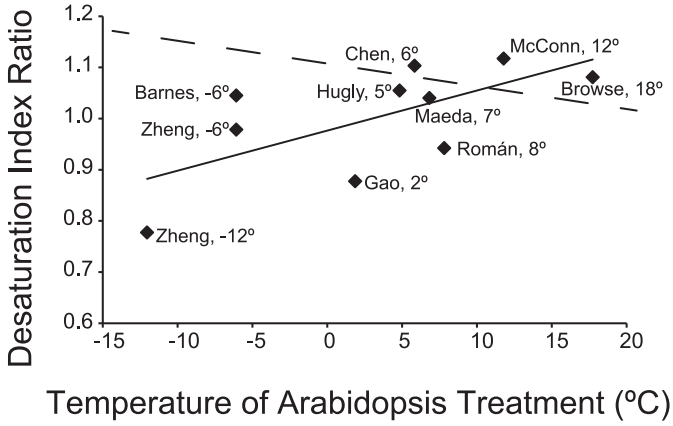

Fig. 3. Overall lipid desaturation does not correlate with low-temperature tolerance.
Total fatty acid desaturation index ratios in Arabidopsis across a range of temperatures are graphed from publications reporting data as quantified values [17,105,132,133,166,167]. Desaturation index ratios displayed are a ratio of low-temperature desaturation index to control temperature desaturation index. Where desaturation indices were not reported, they were calculated as follows: (X:0*0) + (X:1*1) + (X:2*2) + (X:3*3), where X:0, X:1, X:2 and X:3 indicate the amount of each fatty acid quantified as a molar percentage of total lipids for the lipid headgroup class under consideration. A linear trend line is shown with an R squared value of 0.4259 (solid line). The anticipated trend of fatty acid desaturation with temperature is also shown (dashed line).

Table 4
Response of lipid metabolism genes to low-temperature stress.

Citation		Marla	Gu	Li	Li	
Species		Sorghum	Maize	Arabidopsis	Wheat	CBF Regulated
Gene	MGD (1,2,3)	↑	↑	↑	↑	No
	DGD (1,2)	↑	↑	↑	↑	↑
	SQD (1,2)	↑	↑	↓	↑	No
	FATB	↑	↑	ND	↑	No
	LACS	↑	↑	ND	↑	No
	PLDα	↑	↑	ND	↓	No
	C/E Kinase	↑	↑	ND	↓	↓
	FAD (2,3)	↓	↑	↓	↑	No
	FAD (5,6,7,8)	↓	↑	↑	↑	No
	PAP	↑	↑	ND	↓	No

Transcript analyses from three papers containing four species of plants, also found in Table 3, are compared here [68,92,95]. CBF regulation was determined from [63]. Abbreviations are as follows: MGD – MGDG synthase; DGD – DGDG synthase; SQD – SQDG synthase; FATB – Acyl-ACP Thioesterase B; LACS – Long-Chain Acyl Co-A Synthetase; PLDα – Phospholipase D alpha; C/E Kinase – Choline ethanolamine kinase; FAD – Fatty acid desaturase; PAP – Phosphatidic acid phosphatase.

Relatively few papers performed both transcript and lipid analysis [68,92,95]. Given the lack of similarity of lipid responses to low temperatures between these papers, the level of similarity observed in transcripts of lipid genes is surprising (Table 4). MGD and DGD synthase transcript number increased for all species, while MGDG and DGDG lipid levels generally decreased under low-temperature stress. DGD synthase is directly upregulated as a response to cold as a component of the CBF regulon [63] (Table 4), presumably explaining its increased transcript levels. MGD synthase is not part of the CBF regulon, and instead it is regulated by multiple post-translational mechanisms [135,136]. High MGD synthase transcript levels could be a response to low MGDG levels as a feedback mechanism. Choline kinase is directly downregulated in response to cold (Table 4) [63]. In Arabidopsis studies shown in Table 3, PC generally decreased in response to cold. Unlike DGD and MGD synthases, choline kinase may be responding to cold temperatures mainly at the transcript level. Fatty acid desaturase transcripts had some of the least consistent changes (Table 4), though the direction of the desaturase transcript change generally matched the levels of lipid desaturation in that study. For example, Li et al., 2015 observed increases in all fatty acid desaturase transcripts, and also saw an increase in total desaturated fatty acids under low-temperature stress [95]. This implies that fatty acid desaturases are mostly transcriptionally controlled in response to low temperature. Surprisingly, transcriptional changes were more consistent across species than the lipid responses to low-temperature stress, even though most were absent from the CBF regulon [63]. We conclude that low-temperature tolerance has many non-transcriptional factors influencing it. It is likely that different species have different post-translational regulation or response levels, and this could be connected to their stress perception.

4. Reported differences in experimental setups

A major complicating factor in comparing both transcript and lipid changes in response to low temperature is the variety of growth conditions and stress treatment protocols used (Tables 1 and 3). Multiple aspects of low-temperature treatment affect the intensity of a plant's response. It has long been known that the severity of low-temperature treatments matter, and longer treatments of low-temperature stress result in increased plant damage [137]. For instance, 5 °C treatment of maize for two days was mostly survived, but after ten days nearly all plants died [138]. Sorghum exhibits even greater sensitivity to low temperatures, with significant reductions in growth observable when either air or soil temperatures are below 15 °C [139,140]. In contrast, Arabidopsis can complete its entire growth cycle and set seed at temperatures of 6 °C, but dies below −10 °C [141,142]. Many studies used different low temperatures to apply stress, even though they investigated the same species. Within included Arabidopsis studies, the duration of low temperature stress treatments varied from as little as two hours to as long as three weeks, while treatment temperatures varied from 10 °C to −12 °C [95,132]. A second factor known to affect the severity of a low-temperature stress is whether a plant was previously cold acclimated [2]. Cold acclimation is a well-recognized and commonly performed part of freezing stress tests, but is less frequently added to severe cold tests of plants that cannot withstand freezing. As demonstrated in Fig. 4, cold acclimation of maize seedlings at 16 °C increases their tolerance of 4 °C.

One of the most frequently reported growth conditions was plant age (Tables 1,3). However, the age of plants tested varied from young vegetative plants to plants at reproductive stages. This is an important factor in experimental design because in most species, different growth stages and tissue types have different levels of tolerance for low-temperatures [143–145]. Young seedlings are often more sensitive to low temperature than the same plants at more advanced stages of development [1], for example winter wheat is most tolerant at its full vegetative growth stage [146]. However in Arabidopsis, younger leaves have greater freezing tolerance than older leaves [21,147]. Other tissues also vary in tolerance. For example, Arabidopsis pollen grains are more sensitive to low temperature than leaf tissues [148], and the maize meristem is more resistant, especially while it remains below the soil surface and is buffered from fluctuations in air temperature prior to the six-leaf-stage [149].

The level of light provided during low-temperature treatment also affects the severity of a plant's perceived stress, as high light levels under low temperature conditions adds oxidative stress [150]. In Arabidopsis, oxidative stress response genes are upregulated in response to low-temperature stress [52]. Differences in light intensity during low temperature stress treatment can therefore have an impact on both molecular (transcriptional and lipid) and whole-plant responses to equivalent severities of treatment. In many cases light intensity levels during cold stress treatment and/or control conditions were not reported. In studies where light intensity was reported, values varied more than 10 fold from 30 μmoles/m2/s to 800 μmoles/m2/s. Thus, even studies employing equivalent temperatures as part of their low temperature treatment may subject plants to substantially different degrees of stress.

In addition to the tolerance changing effect of light levels during stress, the timing of the light/dark cycle before stress also changes tolerance. Studies have found that the expression levels of many genes known to be involved in mediating responses to low-temperature stress cycle diurnally [151]. Further, mutations disrupting the circadian clock in Arabidopsis also decrease survival rates in freezing stress treatments by 50% [152]. This strongly suggests that the time of day at which low temperatures are applied influences the level of tolerance observed in different experiments. We confirmed this with maize plants. When exposed to cold in the middle of the day, maize exhibited more severe stress phenotypes than equivalent plants cold treated at the end of the day (Fig. 4). This phenomenon is likely due to the interaction between low-temperature tolerance and circadian and diurnal rhythms. Many abiotic stress genes are connected to the clock and naturally cycle in addition to reacting to times of stress, with one transcriptome analysis finding extensive overlap between abscisic acid signaling and the circadian clock [153]. Abscisic acid is connected to many environmental stress responses; meaning time of day plants are stressed and sampled could greatly influence observed patterns of transcriptional responses. However, few studies report the time of day at which low-temperature stress treatments are initiated.

Finally, an additional consideration is the relative health of the plants prior to treatment, which is often a sum of other stresses

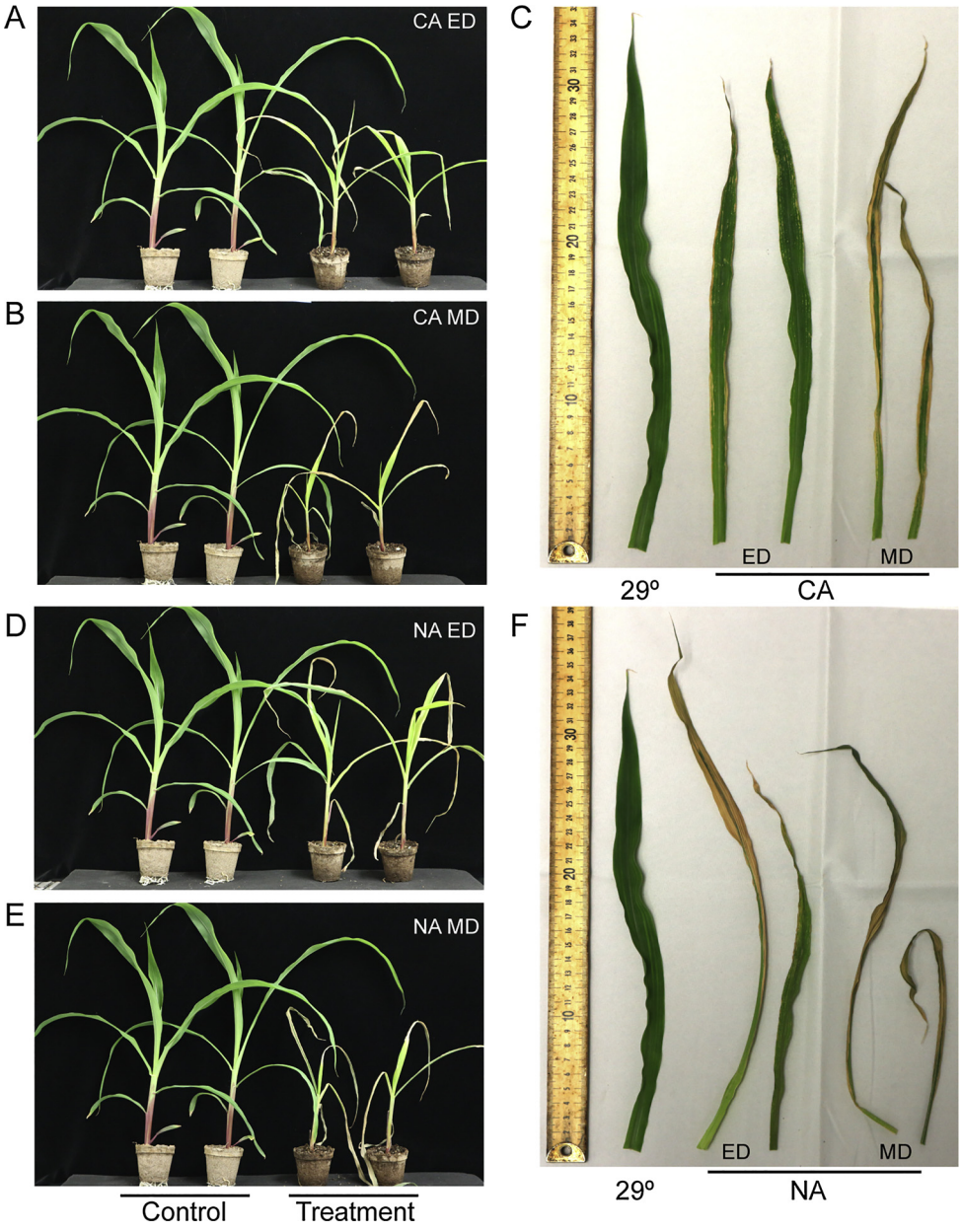

Fig. 4. Low-temperature tolerance is affected by cold acclimation and time of low-temperature stress.
For all portions of the figure: Normal growth conditions were 29 °C day/ 22 °C night with a 16 h:8 h day/night cycles. All maize is accession B73. CA represents plants cold acclimated at 16 °C with a 12 h:12 h day/night cycle for one week. NA represents plants that were not acclimated. ED represents plants moved to low-temperature stress at the end of the day cycle when growth chamber lights shut off. MD represents plants moved into low-temperature stress in the middle of the day cycle. Cold treatments were at 4 °C with a 12 h:12 h day:night cycle. All images were taken 3 days post recovery from stress. A) 20-day-old maize plants grown under normal conditions (left) or grown normally for 10 days, cold-acclimated at 16 °C for one week, and cold treated at the end of the day cycle (ED) for three days (right). B) Same as A, except plants were cold treated in the middle of the day cycle (MD). C) Second leaf excised from plants in A and B, 29° denotes control plants, CA denotes cold-acclimated and cold-treated plants. D) 20-day-old maize plants grown under normal conditions (left) or grown normally for 17 days, then cold treated without prior acclimation for three days (right). Cold treatment began at the end of the day cycle (ED). E) 20-day-old maize plants grown under normal conditions (left) or grown normally for 17 days, then cold treated without prior acclimation for three days (right). Cold treatment began in the middle of the day cycle (MD). F) Second leaf excised from plants in D and E, 29° denotes control plants, CA denotes cold-acclimated and cold-treated plants.

preexisting for the plant. In summary, the severity of a low-temperature treatment can differ greatly between experiments based on duration of treatment, prior cold acclimation, plant age, tissue type sampled, light levels during treatment, and position of the treatment within the diurnal cycle, and of course the temperature used to create the stress.

5. Physiological and phenotypic measurements to quantify tolerance/susceptibility to low-temperature stress

Low temperatures directly cause physiological, biochemical, cellular, and molecular changes that alter whole-plant processes. Plants exposed to low temperature show growth inhibition, dehydration, membrane damage, solute leakage, and metabolite imbalances [22]. As plants perceive changes in temperature, transcription factors and other primary responses are activated, causing gene expression and physiological, biochemical, cellular, and molecular trait changes.

Treatments with the same low temperatures may not always impose equivalent stress levels across plant species or genotypes because of native variation in the ability to withstand low temperatures. Thus, the level of stress experienced by plants in a given trial is critical to both quantify and report. Below we review phenotypic changes in response to low temperature, noting the level of consistency with which they are experienced by different species.

Impairment of CO_2 assimilation rates have been used as a proxy for photosynthetic activity in maize, sorghum, and several other panicoid grasses as a quantitative measure to infer differing levels of sensitivity to chilling stress [73]. Chlorophyll fluorescence measured as the ratio of F_v (variable fluorescence) over F_m (maximum fluorescence), is another photosynthetic property whose changes are relevant in understanding plants response to low temperature. Upon cold stress, F_v/F_m values are used to determine the maximum quantum efficiency of Photosystem II [154], which drops significantly in low-temperature-sensitive plants, whereas in low-temperature-tolerant plants, the values decrease only slightly [155,156].

Imaging plants provides a visual indication of the plants' perceived stress levels. Few studies sampled in Table 1 included images of plants after low-temperature treatment. Including close-up pictures of leaves or tissues of interest can improve interpretation of the plants' stress

levels, especially in larger bodied plants where whole-plant images are necessarily small and details are difficult to resolve (Figs. 4; 1A,B in [72] and Fig. 1A–C in [55]). With the advent of high-throughput plant phenotyping systems, automated phenotyping platforms have been used to study plants response to various biotic and abiotic stress [157]. In a proof-of-concept study, Humplik et al, developed automated screening for growth rate and photosynthetic efficiency by RGB and chlorophyll fluorescence imaging and verified the reliability of these image based data with ground truth measurements [158]. However, regular photography can also be effective. For example, Yang et al. showed that the leaves of low-temperature sensitive banana plants droop after 6 h of cold treatment at 10 °C, while the resistant plantain leaves remained normal [72]. These phenotypic changes and other physiological measurements corresponded well with the gene expression changes indicating differing sensitivity low-temperature [72].

Membrane injury levels are another marker of plant stress, and can be measured by membrane leakage and lipid oxidation [159,160]. Following low temperature stress treatments, electrolyte leakage was higher in low-temperature sensitive banana and *japonica* rice variety IR29, compared to resistant genotypes [71,72]. Malondialdehyde is an end-product of lipid peroxidation in cell membranes, and has been shown to accumulate in low-temperature sensitive genotypes in rice, cassava, and banana, particularly after prolonged stress [55,71,72].

Other physiological responses of low-temperature-stressed plants like chlorophyll content, amino acid accumulation, and relative water content can also be observed, however these responses are less consistent across accessions and/or species. Low temperature is known to inhibit chlorophyll synthesis and chloroplast formation in rice plants [161]. Chlorophyll content has a positive effect on low-temperature tolerance in rice. Low-temperature tolerant *japonica* rice varieties accumulate chlorophyll at a higher rate than sensitive *indica* lines under cold stress [162]. In cassava, An et al, reported that low-temperatures changed chloroplast ultrastructure by reducing thylakoid stacking and reducing or eliminating starch granules [55]. Most freezing tolerant plants accumulate one or more amino acids as cryoprotectants during low temperatures, though the precise amino acid profile varies. Relative increases in proline levels are common, in Arabidopsis proline and glutamine are hyperaccumulated [163]. Other species preferentially accumulate different sets of amino acids: bluegrass accumulates proline, tyrosine and arginine [164], and frost-resistant barley accumulates mainly γ-aminobutyric acid while its relative levels of proline and many other amino acids are reduced [165].

Comparisons of the severity of low-temperature stress experienced in different studies are currently challenging. However, as described above a number of phenotypic traits appear to show relatively universal changes in response to the degree of stress experienced by a plant – rather than the absolute temperature or other details of how the stress is applied. Specifically, plant imaging, ion leakage and accumulation of MDA are relatively species-independent measurements. Reporting values for some or all of these traits would be one way to improve cross-comparability across independent studies working with different genotypes, species, or stress treatment protocols.

6. Conclusions

Low-temperature is a key constraint on crop productivity and growing ranges. Unseasonably early or late cold can both reduce yield and decrease the quality of any surviving harvest. We used published studies to test the hypothesis that a core set of changes in transcript and lipid profiles induced by low temperature stress are conserved across plant species. A core set of transcriptional responses was indeed observed across many species. The CBF genes were consistently upregulated early in response to low-temperature stress, while photosynthesis and chloroplast related genes were consistently downregulated later. A subset of genes involved in lipid metabolism were also regulated consistently. In contrast, we were not able to identify any consistent changes in membrane lipid abundance across the species profiled in the literature.

Perception, signaling, and response to low-temperature stress is a complex process with multiple mechanisms and pathways converging to affect adaptation. The widespread variation in transcriptional and biochemical responses to low-temperature stress presumably results from two primary mechanisms: inherent genetic and physiological differences between how different species perceived and respond to low temperature or differences in how low temperature is applied and outcomes assayed across different studies. Genetic and physiological differences among species are interesting and could entirely explain the variation in lipid abundance, however, more consistent reporting of experimental design parameters and phenotypic responses to stress are needed to test this. Alternatively, accurate comparisons that take into account the perceived severity of the low temperature stress may identify the most relevant lipid changes to improve plant low temperature tolerance in a variety of species. Ultimately, multistudy analyses sample a wider range of treatment conditions and species than an individual report, enabling them to highlight the subset of biochemical and transcriptional changes that are functionally constrained.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.plantsci.2018.08.002.

References

[1] J.M. Lyons, Chilling injury in plants, Annu. Rev. Plant Physiol. 24 (1) (1973) 445–466.
[2] M.F. Thomashow, Molecular Genetics of Cold Acclimation in Higher Plants, in Advances in Genetics, Elsevier, 1990, pp. 99–131.
[3] Y.S. Teng, et al., Tic21 is an essential translocon component for protein translocation across the chloroplast inner envelope membrane, Plant Cell 18 (9) (2006) 2247–2257.
[4] K. Napp-Zinn, Vernalization: environmental and genetic regulation, in: J.G. Atherton (Ed.), Manipulation of Flowering, Butterworths, London, UK, 2018, pp. 123–132.
[5] D. Zoldan, et al., Understanding chilling tolerance traits using Arabidopsis chilling-sensitive mutants, Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change, Springer, 2012, pp. 159–173.
[6] S.P. Zhang, H.V. Scheller, Photoinhibition of photosystem I at chilling temperature and subsequent recovery in Arabidopsis thaliana, Plant Cell Physiol. 45 (11) (2004) 1595–1602.
[7] Y.J. Yang, et al., The effects of chilling-light stress on photosystems I and II in three Paphiopedilum species, Bot. Stud. 58 (1) (2017) 53.
[8] R. Awasthi, K. Bhandari, H. Nayyar, Temperature stress and redox homeostasis in agricultural crops, Front. Environ. Sci. 3 (2015) 11.
[9] A. Alonso, C.S. Queiroz, A.C. Magalhaes, Chilling stress leads to increased cell membrane rigidity in roots of coffee (Coffea arabica L.) seedlings, Biochim. Biophys. Acta 1323 (1) (1997) 75–84.
[10] R. Welti, et al., Profiling membrane lipids in plant stress responses role of phospholipase Dα in freezing-induced lipid changes in Arabidopsis, J. Biol. Chem. 277 (35) (2002) 31994–32002.
[11] W. Li, et al., Differential degradation of extraplastidic and plastidic lipids during freezing and post-freezing recovery in Arabidopsis thaliana, J. Biol. Chem. 283 (1) (2008) 461–468.
[12] T. Degenkolbe, et al., Differential remodeling of the lipidome during cold acclimation in natural accessions of Arabidopsis thaliana, Plant J. 72 (6) (2012) 972–982.
[13] G. Zheng, L. Li, W. Li, Glycerolipidome responses to freezing- and chilling-induced injuries: examples in Arabidopsis and rice, BMC Plant Biol. 16 (2016) 70.
[14] M. Uemura, R.A. Joseph, P.L. Steponkus, Cold-acclimation of Arabidopsis-Thaliana - effect on plasma-membrane lipid-composition and freeze-induced

lesions, Plant Physiol. 109 (1) (1995) 15–30.
[15] S. Hugly, C. Somerville, A role for membrane lipid polyunsaturation in chloroplast biogenesis at low temperature, Plant Physiol. 99 (1) (1992) 197–202.
[16] J.-M. Routaboul, S.F. Fischer, Trienoic fatty acids are required to maintain chloroplast function at low temperatures, Plant Physiol. 124 (4) (2000) 1697–1705.
[17] M. Chen, J.J. Thelen, ACYL-LIPID DESATURASE2 is required for chilling and freezing tolerance in Arabidopsis, Plant Cell 25 (4) (2013) 1430–1444.
[18] M.-N. Vaultier, et al., Desaturase mutants reveal that membrane rigidification acts as a cold perception mechanism upstream of the diacylglycerol kinase pathway in Arabidopsis cells, FEBS Lett. 580 (17) (2006) 4218–4223.
[19] T. Taji, et al., Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in Arabidopsis thaliana, Plant J. 29 (4) (2002) 417–426.
[20] S.J. Gilmour, et al., Overexpression of the Arabidopsis CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation, Plant Physiol. 124 (4) (2000) 1854–1865.
[21] L.A. Wanner, O. Junttila, Cold-induced freezing tolerance in Arabidopsis, Plant Physiol. 120 (2) (1999) 391–400.
[22] M.F. Thomashow, Plant cold acclimation: freezing tolerance genes and regulatory mechanisms, Annu. Rev. Plant Biol. 50 (1) (1999) 571–599.
[23] A. Thalhammer, et al., Interaction of two intrinsically disordered plant stress proteins (COR15A and COR15B) with lipid membranes in the dry state, Biochimica et Biophysica Acta (BBA)-Biomembranes 1798 (9) (2010) 1812–1820.
[24] Y. Yu, et al., Transcriptome analysis during seed germination of elite Chinese bread wheat cultivar Jimai 20, BMC Plant Biol. 14 (1) (2014) 20.
[25] U. Matsuo, et al., Responses of the plasma membrane to low temperatures, Physiol. Plant. 126 (1) (2006) 81–89.
[26] M. Chen, J.E. Markham, E.B. Cahoon, Sphingolipid Δ8 unsaturation is important for glucosylceramide biosynthesis and low-temperature performance in Arabidopsis, Plant J. 69 (5) (2012) 769–781.
[27] S. Fujikawa, Artificial biological membrane ultrastructural changes caused by freezing, Electron Microsc. Rev. 1 (1) (1988) 113–140.
[28] E.R. Moellering, B. Muthan, C. Benning, Freezing tolerance in plants requires lipid remodeling at the outer chloroplast membrane, Science 330 (6001) (2010) 226–228.
[29] R.L. Roston, et al., Structural determinants allowing transferase activity in SENSITIVE TO FREEZING 2, classified as a family I glycosyl hydrolase, J. Biol. Chem. 289 (38) (2014) 26089–26106.
[30] E.J. Stockinger, S.J. Gilmour, M.F. Thomashow, Arabidopsis thaliana CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit, Proc. Natl. Acad. Sci. 94 (3) (1997) 1035–1040.
[31] J.G. Dubouzet, et al., OsDREB genes in rice, Oryza sativa L., encode transcription activators that function in drought-, high-salt-and cold-responsive gene expression, Plant J. 33 (4) (2003) 751–763.
[32] Y.-G. Shen, et al., An EREBP/AP2-type protein in Triticum aestivum was a DRE-binding transcription factor induced by cold, dehydration and ABA stress, Theor. Appl. Genet. 106 (5) (2003) 923–930.
[33] V. Chinnusamy, et al., ICE1: a regulator of cold-induced transcriptome and freezing tolerance in Arabidopsis, Genes Dev. 17 (8) (2003) 1043–1054.
[34] M. Agarwal, et al., A R2R3 type MYB transcription factor is involved in the cold regulation of CBF genes and in acquired freezing tolerance, J. Biol. Chem. 281 (49) (2006) 37636–37645.
[35] C.J. Doherty, et al., Roles for Arabidopsis CAMTA transcription factors in cold-regulated gene expression and freezing tolerance, Plant Cell 21 (3) (2009) 972–984.
[36] S.G. Fowler, D. Cook, M.F. Thomashow, Low temperature induction of Arabidopsis CBF1, 2, and 3 is gated by the circadian clock, Plant Physiol. 137 (3) (2005) 961–968.
[37] C.-M. Lee, M.F. Thomashow, Photoperiodic regulation of the C-repeat binding factor (CBF) cold acclimation pathway and freezing tolerance in Arabidopsis thaliana, Proc. Natl. Acad. Sci. 109 (37) (2012) 15054–15059.
[38] D. Mao, C. Chen, Colinearity and similar expression pattern of rice DREB1s reveal their functional conservation in the cold-responsive pathway, PLoS One 7 (10) (2012) e47275.
[39] Y. Ito, et al., Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice, Plant Cell Physiol. 47 (1) (2006) 141–153.
[40] S. Morran, et al., Improvement of stress tolerance of wheat and barley by modulation of expression of DREB/CBF factors, Plant Biotechnol. J. 9 (2) (2011) 230–249.
[41] H.C. Nguyen, et al., Special trends in CBF and DREB2 groups in Eucalyptus gunnii vs Eucalyptus grandis suggest that CBF are master players in the trade-off between growth and stress resistance, Physiol. Plant. 159 (4) (2017) 445–467.
[42] F. Qin, et al., Cloning and functional analysis of a novel DREB1/CBF transcription factor involved in cold-responsive gene expression in *Zea mays* L, Plant Cell Physiol. 45 (8) (2004) 1042–1052.
[43] M. Jackson, et al., Costs and benefits of cold tolerance in transgenic Arabidopsis thaliana, Mol. Ecol. 13 (11) (2004) 3609–3615.
[44] Y. Zhen, P. Dhakal, M.C. Ungerer, Fitness benefits and costs of cold acclimation in Arabidopsis thaliana, Am. Nat. 178 (1) (2011) 44–52.
[45] K. Yamaguchi-Shinozaki, K. Shinozaki, Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses, Annu. Rev. Plant Biol. 57 (2006) 781–803.
[46] X. Zhang, et al., Freezing-sensitive tomato has a functional CBF cold response pathway, but a CBF regulon that differs from that of freezing-tolerant Arabidopsis, Plant J. 39 (6) (2004) 905–919.
[47] R. Lister, B.D. Gregory, J.R. Ecker, Next is now: new technologies for sequencing of genomes, transcriptomes, and beyond, Curr. Opin. Plant Biol. 12 (2) (2009) 107–118.
[48] B.-h. Lee, D.A. Henderson, J.-K. Zhu, The Arabidopsis cold-responsive transcriptome and its regulation by ICE1, Plant Cell 17 (11) (2005) 3155–3175.
[49] I. Makarevitch, et al., Transposable elements contribute to activation of maize genes in response to abiotic stress, PLoS Genet. 11 (1) (2015) e1004915.
[50] A.J. Waters, I. Makarevitch, J. Noshay, L.T. Burghardt, C.N. Hirsch, C.D. Hirsch, N.M. Springer, Natural variation for gene expression responses to abiotic stress in maize, Plant J. 89 (4) (2017) 706–717, https://doi.org/10.1111/tpj.13414.
[51] D. Mittal, D.A. Madhyastha, A. Grover, Gene expression analysis in response to low and high temperature and oxidative stresses in rice: combination of stresses evokes different transcriptional changes as against stresses applied individually, Plant Sci. 197 (2012) 102–113.
[52] S. Fowler, M.F. Thomashow, Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway, Plant Cell 14 (8) (2002) 1675–1690.
[53] D. Laudencia-Chingcuanco, et al., Genome-wide gene expression analysis supports a developmental model of low temperature tolerance gene regulation in wheat (Triticum aestivum L.), BMC Genomics 12 (1) (2011) 299.
[54] M. Seki, et al., Monitoring the expression pattern of 1300 Arabidopsis genes under drought and cold stresses by using a full-length cDNA microarray, Plant Cell 13 (1) (2001) 61–72.
[55] D. An, J. Yang, P. Zhang, Transcriptome profiling of low temperature-treated cassava apical shoots showed dynamic responses of tropical plant to cold stress, BMC Genomics 13 (1) (2012) 64.
[56] P. Maul, et al., Transcriptome profiling of grapefruit flavedo following exposure to low temperature and conditioning treatments uncovers principal molecular components involved in chilling tolerance and susceptibility, Plant Cell Environ. 31 (6) (2008) 752–768.
[57] J. Medina, et al., The Arabidopsis CBF gene family is composed of three genes encoding AP2 domain-containing proteins whose expression is regulated by low temperature but not by abscisic acid or dehydration, Plant Physiol. 119 (2) (1999) 463–470.
[58] J. Medina, R. Catalá, J. Salinas, The CBFs: three Arabidopsis transcription factors to cold acclimate, Plant Sci. 180 (1) (2011) 3–11.
[59] O.V. Fursova, G.V. Pogorelko, V.A. Tarasov, Identification of ICE2, a gene involved in cold acclimation which determines freezing tolerance in Arabidopsis thaliana, Gene 429 (1) (2009) 98–103.
[60] A. Kurbidaeva, T. Ezhova, M. Novokreshchenova, Arabidopsis thaliana ICE2 gene: phylogeny, structural evolution and functional diversification from ICE1, Plant Sci. 229 (2014) 10–22.
[61] Z. Ding, et al., Pattern of CsICE1 expression under cold or drought treatment and functional verification through analysis of transgenic Arabidopsis, Genet. Mol. Res. 14 (2015) 11259–11270.
[62] Y.S. Kim, et al., The unified ICE–CBF pathway provides a transcriptional feedback control of freezing tolerance during cold acclimation in Arabidopsis, Plant Mol. Biol. 89 (1–2) (2015) 187–201.
[63] S. Park, et al., Regulation of the Arabidopsis CBF regulon by a complex low--temperature regulatory network, Plant J. 82 (2) (2015) 193–207.
[64] J.T. Vogel, et al., Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of Arabidopsis, Plant J. 41 (2) (2005) 195–211.
[65] P. Barah, et al., Genome-scale cold stress response regulatory networks in ten Arabidopsis thaliana ecotypes, BMC Genomics 14 (1) (2013) 722.
[66] H. Chen, et al., A comparison of the low temperature transcriptomes of two tomato genotypes that differ in freezing tolerance: Solanum lycopersicum and Solanum habrochaites, BMC Plant Biol. 15 (1) (2015) 132.
[67] R. Chopra, et al., Transcriptome profiling and validation of gene based single nucleotide polymorphisms (SNPs) in sorghum genotypes with contrasting responses to cold stress, BMC Genomics 16 (1) (2015) 1040.
[68] S.R. Marla, et al., Comparative Transcriptome and Lipidome Analyses Reveal Molecular Chilling Responses in Chilling-Tolerant Sorghums, Plant Genome 10 (3) (2017).
[69] N.L. Mantri, et al., Transcriptional profiling of chickpea genes differentially regulated in response to high-salinity, cold and drought, BMC Genomics 8 (1) (2007) 303.
[70] M.O. Winfield, et al., Cold-and light-induced changes in the transcriptome of wheat leading to phase transition from vegetative to reproductive growth, BMC Plant Biol. 9 (1) (2009) 55.
[71] T. Zhang, et al., Comparative transcriptome profiling of chilling stress responsiveness in two contrasting rice genotypes, PLoS One 7 (8) (2012) e43274.
[72] Q.-S. Yang, et al., Comparative transcriptomics analysis reveals difference of key gene expression between banana and plantain in response to cold stress, BMC Genomics 16 (1) (2015) 446.
[73] Y. Zhang, D.W. Ngu, D. Carvalho, Z. Liang, Y. Qiu, R.L. Roston, J.C. Schnable, Differentially Regulated Orthologs in Sorghum and the Subgenomes of Maize, The Plant Cell 29 (8) (2017) 1938–1951, https://doi.org/10.1105/tpc.17.00354.
[74] A.K. Beike, et al., Insights from the cold transcriptome of Physcomitrella patens: global specialization pattern of conserved transcriptional regulators and identification of orphan genes involved in cold acclimation, New Phytol. 205 (2) (2015) 869–881.
[75] M. Wang, X. Zhang, J.-H. Liu, Deep sequencing-based characterization of transcriptome of trifoliate orange (Poncirus trifoliata (L.) Raf.) in response to cold

stress, BMC Genomics 16 (1) (2015) 555.
[76] C. Zheng, et al., Integrated RNA-Seq and sRNA-Seq analysis identifies chilling and freezing responsive key molecular players and pathways in tea plant (Camellia sinensis), PLoS One 10 (4) (2015) e0125031.
[77] Y. Shu, W. Li, J. Zhao, S. Zhang, H. Xu, Y. Liu, C. Guo, Transcriptome sequencing analysis of alfalfa reveals CBF genes potentially playing important roles in response to freezing stress, Genet. Mol. Biol. 40 (4) (2017) 824–833, https://doi.org/10.1590/1678-4685-GMB-2017-0053.
[78] V.S. Bonthala, et al., Identification of gene modules associated with low temperatures response in bambara groundnut by network-based analysis, PLoS One 11 (2) (2016) e0148771.
[79] K. Maruyama, et al., Identification of cis-acting promoter elements in cold-and dehydration-induced transcriptional pathways in Arabidopsis, rice, and soybean, Dna Res. 19 (1) (2011) 37–49.
[80] P.I. Calzadilla, et al., Transcriptome response mediated by cold stress in Lotus japonicus, Front. Plant Sci. 7 (2016) 374.
[81] G. Koehler, et al., Integrative "omic" analysis reveals distinctive cold responses in leaves and roots of strawberry, Fragaria× ananassa 'Korona', Front. Plant Sci. 6 (2015) 826.
[82] Y. Song, et al., Transcriptome profiling reveals differential transcript abundance in response to chilling stress in Populus simonii, Plant Cell Rep. 32 (9) (2013) 1407–1425.
[83] H. Wang, et al., Global analysis of transcriptome responses and gene expression profiles to cold stress of Jatropha curcas L, PLoS One 8 (12) (2013) e82817.
[84] W. Xu, et al., Transcriptome profiling of Vitis amurensis, an extremely cold-tolerant Chinese wild Vitis species, reveals candidate genes and events that potentially connected to cold stress, Plant Mol. Biol. 86 (4-5) (2014) 527–541.
[85] X. Lei, et al., RNA-seq analysis of oil palm under cold stress reveals a different C-repeat binding factor (CBF) mediated gene expression pattern in Elaeis guineensis compared to other species, PLoS One 9 (12) (2014) e114482.
[86] M. Jończyk, et al., Global analysis of gene expression in maize leaves treated with low temperature. II. Combined effect of severe cold (8 °C) and circadian rhythm, Plant Mol. Biol. 95 (3) (2017) 279–302.
[87] C.-F. Su, et al., A novel MYBS3-dependent pathway confers cold tolerance in rice, Plant Physiol. 153 (1) (2010) 145–158.
[88] G. Dennis, et al., DAVID: database for annotation, visualization, and integrated discovery, Genome Biol. (2003) 4.
[89] Y. Benjamini, Y. Hochberg, Controlling the false discovery rate – a practical and powerful approach to multiple testing, J. R. Stat. Soc. Ser. B-Methodol. 57 (1) (1995) 289–300.
[90] S. Melser, et al., Links between lipid homeostasis, organelle morphodynamics and protein trafficking in eukaryotic and plant secretory pathways, Plant Cell Rep. 30 (2) (2011) 177–193.
[91] L. Yan, Kenchanmane Raju, S.K. Lai, X. Zhang, Y. Dai, X. Rodriguez, O. Mahboub, S. Roston, R.L. Schnable, Parallel natural selection in the cold-adapted crop-wild relative Tripsacum dactyloides and artificial selection in temperate adapted maize, bioRxiv (2018), https://doi.org/10.1101/187575.
[92] Y. Gu, et al., Biochemical and transcriptional regulation of membrane lipid metabolism in maize leaves under low temperature, Front. Plant Sci. 8 (2017) 2053.
[93] Z. Kaniuga, et al., Degradation of leaf polar lipids during chilling and post-chilling rewarming of Zea mays genotypes reflects differences in their response to chilling stress. The role of galactolipase, Acta Physiol. Plant. 21 (1) (1999) 45–56.
[94] L. Vigh, et al., Effect of frost hardening on lipid and fatty acid composition of chloroplast thylakoid membranes in two wheat varieties of contrasting hardiness, Plant Physiol. 79 (3) (1985) 756–759.
[95] Q. Li, et al., Understanding the biochemical basis of temperature-induced lipid pathway adjustments in plants, Plant Cell 27 (1) (2015) 86–103.
[96] P. Tarazona, K. Feussner, I. Feussner, An enhanced plant lipidomics method based on multiplexed liquid chromatography–mass spectrometry reveals additional insights into cold-and drought-induced membrane remodeling, Plant J. 84 (3) (2015) 621–633.
[97] D.J. Chapman, J. De-Felice, J. Barber, Growth temperature effects on thylakoid membrane lipid and protein content of pea chloroplasts, Plant Physiol. 72 (1) (1983) 225–228.
[98] D. Chapman, J. De-Felice, J. Barber, Influence of winter and summer growth conditions on leaf membrane lipids of Pisum sativum L, Planta 157 (3) (1983) 218–223.
[99] L. Spicher, G. Glauser, F. Kessler, Lipid antioxidant and galactolipid remodeling under temperature stress in tomato plants, Front. Plant Sci. 7 (2016) 167.
[100] G. Novitskaya, T. Suvorova, T. Trunova, Lipid composition of tomato leaves as related to plant cold tolerance, Russ. J. Plant Physiol. 47 (6) (2000) 728–733.
[101] C. Dutilleul, et al., Phytosphingosine-phosphate is a signal for AtMPK6 activation and Arabidopsis response to chilling, New Phytol. 194 (1) (2012) 181–191.
[102] L.V. Michaelson, et al., Plant sphingolipids: their importance in cellular organization and adaption, Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 1861 (9) (2016) 1329–1335.
[103] M. Miquel, et al., Arabidopsis requires polyunsaturated lipids for low-temperature survival, Proc. Natl. Acad. Sci. U. S. A. 90 (13) (1993) 6208–6212.
[104] P.L. Steponkus, Advances in Low-Temperature Biology, (1993).
[105] A.C. Barnes, C. Benning, R. Roston, Chloroplast membrane remodeling during freezing stress is accompanied by cytoplasmic acidification activating SENSITIVE TO FREEZING 2, Plant Physiol. (2016) p. pp. 00286.2016.
[106] O. Ishizaki-Nishizawa, et al., Low-temperature resistance of higher plants is significantly enhanced by a nonspecific cyanobacterial desaturase, Nat. Biotechnol. 14 (8) (1996) 1003.
[107] H. Wada, Z. Combos, N. Murata, Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation, Nature 347 (6289) (1990) 200.
[108] C. Yu, et al., Overexpression of endoplasmic reticulum omega-3 fatty acid desaturase gene improves chilling tolerance in tomato, Plant Physiol. Biochem. 47 (11) (2009) 1102–1112.
[109] S. Rodríguez-Vargas, et al., Fluidization of membrane lipids enhances the tolerance of Saccharomyces cerevisiae to freezing and salt stress, Appl. Environ. Microbiol. 73 (1) (2007) 110–116.
[110] M. Sud, et al., Metabolomics Workbench: an international repository for metabolomics data and metadata, metabolite standards, protocols, tutorials and training, and analysis tools, Nucleic Acids Res. 44 (D1) (2016) D463–70.
[111] P. Bais, et al., PlantMetabolomics.org: a web portal for plant metabolomics experiments, Plant Physiol. 152 (4) (2010) 1807–1816.
[112] A.G. Lee, Membrane lipids: it's only a phase, Curr. Biol. 10 (10) (2000) R377–R380.
[113] M.A. Block, et al., Preparation and characterization of membrane fractions enriched in outer and inner envelope membranes from spinach chloroplasts. II. Biochemical characterization, J. Biol. Chem. 258 (21) (1983) 13281–13286.
[114] A.K. Nilsson, et al., Acylated monogalactosyl diacylglycerol: prevalence in the plant kingdom and identification of an enzyme catalyzing galactolipid head group acylation in Arabidopsis thaliana, Plant J. 84 (6) (2015) 1152–1166.
[115] J. Jouhet, Importance of the hexagonal lipid phase in biological membrane organization, Front. Plant Sci. 4 (2013) 494.
[116] B. Yu, C. Benning, Anionic lipids are required for chloroplast structure and function in Arabidopsis, Plant J. 36 (6) (2003) 762–770.
[117] B. Yu, C. Xu, C. Benning, Arabidopsis disrupted in SQD2 encoding sulfolipid synthase is impaired in phosphate-limited growth, Proc. Natl. Acad. Sci. 99 (8) (2002) 5732–5737.
[118] S.A. Arisz, et al., Rapid phosphatidic acid accumulation in response to low temperature stress in Arabidopsis is generated through diacylglycerol kinase, Front. Plant Sci. 4 (2013) 1.
[119] X. Wang, Lipid signaling, Curr. Opin. Plant Biol. 7 (3) (2004) 329–336.
[120] K. Kobayashi, K. Endo, H. Wada, Multiple Impacts of Loss of Plastidic Phosphatidylglycerol Biosynthesis on Photosynthesis during Seedling Growth of Arabidopsis, Front. Plant Sci. 7 (336) (2016).
[121] J. Andrews, J.B. Mudd, Phosphatidylglycerol synthesis in pea chloroplasts: Pathway and localization, Plant Physiol. 79 (1) (1985) 259–265.
[122] T.S. Moore, Phosphatidylglycerol synthesis in castor bean endosperm: kinetics, requirements, and intracellular localization, Plant Physiol. 54 (2) (1974) 164–168.
[123] R. Griebau, M. Frentzen, Biosynthesis of phosphatidylglycerol in isolated mitochondria of etiolated mung bean (Vigna radiata L.) seedlings, Plant Physiol. 105 (4) (1994) 1269–1274.
[124] E. Babiychuk, et al., Arabidopsis phosphatidylglycerophosphate synthase 1 is essential for chloroplast differentiation, but is dispensable for mitochondrial function, Plant J. 33 (5) (2003) 899–909.
[125] R. Tanoue, et al., Phosphatidylglycerol biosynthesis is required for the development of embryos and normal membrane structures of chloroplasts and mitochondria in Arabidopsis, FEBS Lett. 588 (9) (2014) 1680–1685.
[126] J.M. Seddon, Structure of the inverted hexagonal (HII) phase, and non-lamellar phase transitions of lipids, Biochimica et Biophysica Acta (BBA)-Reviews on Biomembranes 1031 (1) (1990) 1–69.
[127] E. Delage, et al., Arabidopsis type-III phosphatidylinositol 4-kinases β1 and β2 are upstream of the phospholipase C pathway triggered by cold exposure, Plant Cell Physiol. 53 (3) (2012) 565–576.
[128] X. Wang, et al., Isolation and functional characterization of a cold responsive phosphatidylinositol transfer-associated protein, ZmSEC14p, from maize (Zea may L.), Plant Cell Rep. 35 (8) (2016) 1671–1686.
[129] H. Kodama, et al., Genetic enhancement of cold tolerance by expression of a gene for chloroplast [omega]-3 fatty acid desaturase in transgenic tobacco, Plant Physiol. 105 (2) (1994) 601–605.
[130] T. Dominguez, et al., Increasing omega-3 desaturase expression in tomato results in altered aroma profile and enhanced resistance to cold stress, Plant Physiol. 153 (2) (2010) 655–665.
[131] N. Murata, D.A. Los, Membrane fluidity and temperature perception, Plant Physiol. 115 (3) (1997) 875–879.
[132] G. Zheng, L. Li, W. Li, Glycerolipidome responses to freezing-and chilling-induced injuries: examples in Arabidopsis and rice, BMC Plant Biol. 16 (1) (2016) 70.
[133] J.P. Gao, J.G. Wallis, J. Browse, Mutations in the prokaryotic pathway rescue the fatty acid biosynthesis1 mutant in the cold, Plant Physiol. 169 (1) (2015) 442-+.
[134] A. Roman, et al., Non-redundant contribution of the plastidial FAD8 omega-3 desaturase to glycerolipid unsaturation at different temperatures in Arabidopsis, Mol. Plant 8 (11) (2015) 1599–1611.
[135] J. Sarkis, et al., The influence of lipids on MGD1 membrane binding highlights novel mechanisms for galactolipid biosynthesis regulation in chloroplasts, FASEB J. 28 (7) (2014) 3114–3123.
[136] J. Rocha, et al., Structural insights and membrane binding properties of MGD1, the major galactolipid synthase in plants, Plant J. 85 (5) (2016) 622–633.
[137] J.P.F. Sellschop, S.C. Salmon, The influence of chilling, above the freezing point, on certain crop plants, J. Agric. Res. 37 (1928) 0315–0338.
[138] J.L. Harper, Studies in seed and seedling mortality, New Phytol. 55 (1) (1956) 35–44.
[139] G. Burow, et al., Genetic dissection of early-season cold tolerance in sorghum (Sorghum bicolor (L.) Moench), Mol. Breed. 28 (3) (2011) 391–402.
[140] J. Yu, M.R. Tuinstra, Genetic analysis of seedling growth under cold temperature stress in grain sorghum, Crop Sci. 41 (5) (2001) 1438–1443.
[141] M. Hasdai, et al., Differential responses of Arabidopsis ecotypes to cold, chilling

and freezing temperatures, Ann. Appl. Biol. 148 (2) (2006) 113–120.
[142] N. Khanal, B.A. Moffatt, G.R. Gray, Acquisition of freezing tolerance in Arabidopsis and two contrasting ecotypes of the extremophile Eutrema salsugineum (Thellungiella salsuginea), J. Plant Physiol. 180 (2015) 35–44.
[143] D.M. Hodges, et al., Sensitivity of maize hybrids to chilling and their combining abilities at two developmental stages, Crop Sci. 37 (3) (1997) 850–856.
[144] P. Revilla, et al., Inheritance of cold tolerance at emergence and during early season growth in maize, Crop Sci. 40 (6) (2000) 1579–1585.
[145] C. Ye, et al., Cold tolerance in rice varieties at different growth stages, Crop Pasture Sci. 60 (4) (2009) 328–338.
[146] A. Zech, A. Pauli, Cold resistance in three varieties of winter wheat as related to nitrogen fractions and total sugar 1, Agron. J. 52 (6) (1960) 334–337.
[147] T. Takagi, et al., The leaf-order-dependent enhancement of freezing tolerance in cold-acclimated Arabidopsis rosettes is not correlated with the transcript levels of the cold-inducible transcription factors of CBF/DREB1, Plant Cell Physiol. 44 (9) (2003) 922–931.
[148] J.-Y. Lee, D.-H. Lee, Use of serial analysis of gene expression technology to reveal changes in gene expression in Arabidopsis pollen undergoing cold stress, Plant Physiol. 132 (2) (2003) 517–529.
[149] P. Miedema, The effects of low temperature on zea mays, Advances in Agronomy, Elsevier, 1982, pp. 93–128.
[150] G.R. Gray, et al., Cold acclimation and freezing tolerance (a complex interaction of light and temperature), Plant Physiol. 114 (2) (1997) 467–474.
[151] Z. Bieniawska, et al., Disruption of the Arabidopsis circadian clock is responsible for extensive variation in the cold-responsive transcriptome, Plant Physiol. 147 (1) (2008) 263–279.
[152] M.A. Dong, E.M. Farré, M.F. Thomashow, Circadian clock-associated 1 and late elongated hypocotyl regulate expression of the C-repeat binding factor (CBF) pathway in Arabidopsis, Proc. Natl. Acad. Sci. 108 (17) (2011) 7241–7246.
[153] A. Matsui, et al., Arabidopsis transcriptome analysis under drought, cold, high-salinity and ABA treatment conditions using a tiling array, Plant Cell Physiol. 49 (8) (2008) 1135–1149.
[154] J. McFarlane, et al., Plant stress detection by remote measurement of fluorescence, Appl. Opt. 19 (19) (1980) 3287–3289.
[155] V. Bonnecarrère, et al., Response to photoxidative stress induced by cold in japonica rice is genotype dependent, Plant Sci. 180 (5) (2011) 726–732.
[156] S. Fonteyne, H. Muylle, P. Lootens, P. Kerchev, W. Van den Ende, A. Staelens, D. Reheul, I. Roldán-Ruiz, Physiological basis of chilling tolerance and early-season growth in miscanthus, Ann. Bot. 121 (2) (2018) 281–295, https://doi.org/10.1093/aob/mcx159.
[157] J.F. Humplík, et al., Automated phenotyping of plant shoots using imaging methods for analysis of plant stress responses–a review, Plant Methods 11 (1) (2015) 29.
[158] J.F. Humplík, et al., Automated integrative high-throughput phenotyping of plant shoots: a case study of the cold-tolerance of pea (Pisum sativum L.), Plant Methods 11 (1) (2015) 20.
[159] N.P. Sukumaran, C.J. Weiser, An excised leaflet test for evaluating potato frost tolerance, HortScience 7 (1972) 467–468.
[160] D.M. Hodges, et al., Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds, Planta 207 (4) (1999) 604–611.
[161] P. Sharma, N. Sharma, R. Deswal, The molecular biology of the low-temperature response in plants, Bioessays 27 (10) (2005) 1048–1059.
[162] J.-C. Glaszmann, R. Kaw, G.S. Khush, Genetic divergence among cold tolerant rices (Oryza sativa L.), Euphytica 45 (2) (1990) 95–104.
[163] D. Cook, et al., A prominent role for the CBF cold response pathway in configuring the low-temperature metabolome of Arabidopsis, Proc. Natl. Acad. Sci. U. S. A. 101 (42) (2004) 15243–15248.
[164] J. Dionne, et al., Amino acid and protein changes during cold acclimation of green-type annual bluegrass (Poa annua L.) ecotypes, Crop Sci. 41 (6) (2001) 1862–1870.
[165] E. Mazzucotelli, et al., Metabolism of gamma-aminobutyric acid during cold acclimation and freezing and its relationship to frost tolerance in barley and wheat, J. Exp. Bot. 57 (14) (2006) 3755–3766.
[166] J. Browse, P. McCourt, C. Somerville, A mutant of Arabidopsis deficient in c(18:3) and c(16:3) leaf lipids, Plant Physiol. 81 (3) (1986) 859–864.
[167] H. Maeda, et al., Tocopherols modulate extraplastidic polyunsaturated fatty acid metabolism in Arabidopsis at low temperature, Plant Cell 20 (2) (2008) 452–470.