Published in final edited form as:
Methods Mol Biol. 2010 ; 639: 39–55. doi:10.1007/978-1-60761-702-0_3.

Gene Regulation During Cold Stress Acclimation in Plants

Viswanathan Chinnusamy, **Jian-Kang Zhu**, and **Ramanjulu Sunkar**

Abstract

Cold stress adversely affects plant growth and development and thus limits crop productivity. Diverse plant species tolerate cold stress to a varying degree, which depends on reprogramming gene expression to modify their physiology, metabolism, and growth. Cold signal in plants is transmitted to activate CBF-dependent (C-repeat/drought-responsive element binding factor-dependent) and CBF-independent transcriptional pathway, of which CBF-dependent pathway activates CBF regulon. CBF transcription factor genes are induced by the constitutively expressed ICE1 (inducer of CBF expression 1) by binding to the *CBF* promoter. ICE1–CBF cold response pathway is conserved in diverse plant species. Transgenic analysis in different plant species revealed that cold tolerance can be significantly enhanced by genetic engineering CBF pathway. Posttranscriptional regulation at pre-mRNA processing and export from nucleus plays a role in cold acclimation. Small noncoding RNAs, namely micro-RNAs (miRNAs) and small interfering RNAs (siRNAs), are emerging as key players of posttranscriptional gene silencing. Cold stress-regulated miRNAs have been identified in *Arabidopsis* and rice. In this chapter, recent advances on cold stress signaling and tolerance are highlighted.

Keywords

Cold stress; second messengers; CBF regulon; CBF-independent regulation; ICE1; posttranscriptional gene regulation

1. Introduction

Temperature profoundly influences the metabolism of organisms and thus is a key factor determining the growing season and geographical distribution of plants. Cold stress can be classified as chilling (<20°C) and freezing (<0°C) stress. Temperate plants have evolved a repertoire of adaptive mechanisms such as seed and bud dormancy, photoperiod sensitivity, vernalization, supercooling (prevention of ice formation in xylem parenchyma cells up to homogenous ice nucleation temperature, −40°C), and cold acclimation. In cold acclimation, plants acquire freezing tolerance on prior exposure to suboptimal, low, nonfreezing temperatures. The molecular basis of cold acclimation and acquired freezing tolerance in *Arabidopsis* and winter cereals has been studied extensively. Plants modify their metabolism and growth to adapt to cold stress by reprogramming gene expression during cold acclimation (1,2). This chapter briefly covers cold stress signaling, transcriptional and posttranscriptional regulation of gene expression in cold acclimation process, and the genetic engineering of crops with enhanced cold tolerance.

2. Cold Stress Sensing

Thus far, the identity of stress sensor in plants is unknown. The fluid mosaic physical state of the plasma membrane is vital for the structure and function of cells, as well as to sense temperature stress. The plasma membrane undergoes phase transitions, from a liquid crystalline to a rigid gel phase at low temperature and to a fluid state at high temperature. Thus, a decrease in temperature can rapidly induce membrane rigidity at microdomains.

Further, protein folding is influenced by temperature changes. Temperature-induced changes in the physical state of membranes and proteins are expected to change the metabolic reactions and thus the metabolite concentrations. Therefore, plant cells can sense cold stress through membrane rigidification, protein/nucleic acid conformation, and/or metabolite concentration (a specific metabolite or redox status).

In alfalfa and *Brassica napus*, cold stress-induced plasma membrane rigidification leads to actin cytoskeletal rearrangement, induction of Ca^{2+} channels, and increased cytosolic Ca^{2+} level. These events induce the expression of *cold-responsive* (*COR*) genes and cold acclimation. Further, a membrane rigidifier (DMSO) can induce *COR* genes even at 25°C, whereas a membrane fluidizer (benzyl alcohol) prevents *COR* gene expression even at 0°C (3,4). Genetic evidence for plants sensing cold stress through membrane rigidification is from the study of the *fad2* mutant impaired in the oleic acid desaturase gene of *Arabidopsis*. In wild-type *Arabidopsis* plants, diacylglycerol (DAG) kinase is induced at 14°C. The *fad2* mutant (more saturated membrane) and transgenic *Arabidopsis* overexpressing linoleate desaturase gene showed the expression of DAG kinase at 18 and 12°C, respectively (5).

3. Second Messengers and Signaling

Cytosolic Ca^{2+}levels act as second messenger of the cold stress signal (6). Calcium may be imported into the cell or released from intracellular calcium stores. Patch-clamp studies of cold-induced potential changes of the plasma membrane in *Arabidopsis* mesophyll protoplasts showed the cold-activatedcalcium-permeable channel involved in the regulation of cytosolic Ca^{2+}signatures (7). Membrane rigidification induced cytosolic Ca^{2+}signatures; and *COR* gene expression was impaired by gadolinium, a mechanosensitive Ca^{2+} channel blocker, which suggests the involvement of mechanosensitive Ca^{2+} channels in cold acclimation (4). Pharmocological studies implicated cyclic ADP-riboseand inositol-1,4,5-triphosphate (IP_3)-activated intracellular calcium channels in *COR* gene expression (4). Calcium influx into the cell appears to activate phospholipase C (PLC) and D (PLD), which produce IP_3 and phosphatidic acid, respectively. IP_3 can further amplify Ca^{2+}signatures by activation of IP_3-gated calcium channels (8). Genetic analysis revealed that loss-of-function mutants of *FIERY1* (*FRY1*) inositol polyphosphate 1-phosphatase show significantly higher and sustained levels of IP_3 instead of the transient increase observed in wild-type plants. This situation leads to higher induction of *COR* genes and CBFs, the upstream transcription factors (9). In addition, the *ca*lcium e*x*changer 1 (*cax1*) mutant of *Arabidopsis*, which is defective in a vacuolar Ca^{2+}/H^{+} antiporter, exhibited enhanced expression of C-repeat binding factor/dehydration responsive element binding (*CBF/DREB*) proteins and their target *COR* genes (10). Therefore, cytosolic Ca^{2+} signatures are upstream of the expression of *CBFs* and *COR* genes in cold stress signaling.

Cold acclimation induces accumulation of ROS such as H_2O_2, both in chilling-tolerant *Arabidopsis* and chilling-sensitive maize plants. ROS can act as a signaling molecule to reprogram transcriptome probably through induction of Ca^{2+} signatures and activation of mitogen-activated protein kinases (MAPKs) (11) and redox-responsive transcription factors. *Arabidopsis frostbite1* (*fro1*) mutant, which is defective in the mitochondrial Fe-S subunit of complex I (NADH dehydrogenase) of the electron transfer chain, shows a constitutively high accumulation of ROS. This high accumulation of ROS in *fro1* results in reduced *COR* gene expression and hypersensitivity to freezing stress, probably because of desensitization of cells by the constitutively high ROS expression (12).

Cold stress-induced second messenger signatures can be decoded by different pathways. Calcium signatures are sensed by calcium sensor family proteins, namely calcium-dependent protein kinases (CDPKs), calmodulins (CaMs), and salt overly sensitive 3-like (SOS3-like)

or calcineurin B-like (CBL) proteins. In a transient expression system in maize leaf protoplast, a constitutively active form of an *Arabidopsis* CDPK (AtCDPK1) activated the expression of barley *HVA1* ABA-responsive promoter::*LUC* reporter gene suggesting that AtCDPK is a positive regulator in stress-induced gene transcription (13). Genetic and transgenic analyses implicated CDPKs as positive regulators, but a calmodulin, a SOS3-like or a CBL calcium binding protein, and a protein phosphatase 2C (AtPP2CA) are negative regulators of gene expression and cold tolerance in plants. Components of MAPK cascades are induced or activated by cold and other abiotic stresses. Genetic and transgenic analyses showed that MAPKs act as a converging point in abiotic stress signaling. ROS accumulation under these stresses might be sensed through a MAPK cascade (14). ROS activates the AtMEKK1/ANP1 (MAPKKK)–AtMKK2 (MAPKK)–AtMPK4/6 (MAPK) MAPK cascade, which positively regulates cold acclimation in plants (11). Many of these phosphorylated proteins show activation or induction of gene expression under multiple stress conditions, and genetic modification results in alteration of multiple stress responses. These results suggest that the proteins act as connecting nodes of stress signal networks. Identification of the target proteins or transcription factors of protein kinase or phosphatase cascades will shed further light on stress signaling.

4. Transcriptional Regulation

Chilling-tolerant plants reprogram their transcriptome in response to acclimation temperature. Cold-regulated genes constitute about 4–20% of the genome in *Arabidopsis* (15). The promoter region of many *COR* genes of *Arabidopsis* contains C-repeat (CRT)/DREs, initially identified in the promoter of *r*esponsive to *d*ehydration *29A* (*RD29A/COR78/LTI78*). As well, ABA-responsive elements are present in many cold-induced genes. Genetic screens using dehydration and cold stress-responsive promoter-driven *LUCIFERASE* (*RD29A::LUC* and *CBF3::LUC*) led to the isolation of mutants, which unraveled cold-responsive transcriptional networks.

4.1. CBF Regulons and Cold Tolerance

Yeast one-hybrid screens to identify CRT/DRE binding proteins led to the identification of CRT/DREBs (CBFs/DREBs) in *Arabidopsis*. CBFs belong to the ethylene-responsive element binding factor/APETALA2 (ERF/AP2)-type transcription factor family. *Arabidopsis* encodes three *CBF* genes (*CBF1*/*DREB1B*, *CBF2*/*DREB1C*, and *CBF3*/*DREB1A*), which are induced within a short period of exposure to cold stress. CBFs bind to *CRT/DRE cis*-elements in the promoters of *COR* genes and induce their expression (16,17). Ectopic expression of *CBFs* in transgenic *Arabidopsis* induced the expression of *COR* genes at warm temperatures and induced constitutive freezing tolerance. These transgenic *Arabidopsis* plants were also tolerant to salt and drought stresses (17–19). Microarray analysis of *CBF*-overexpressing transgenic plants identified several CBF target genes involved in signaling, transcription, osmolyte biosynthesis, ROS detoxification, membrane transport, hormone metabolism, and stress response (20,21). Transgenic overexpression of *Arabidopsis* CBFs is sufficient to induce cold tolerance in diverse plant species (Table 3.1). Further, CBF homologs have been identified from several chilling-tolerant and chilling-sensitive plant species and transgenic analysis confirmed their pivotal role in cold acclimation (Table3.1).

These evidences suggest that a CBF transcription network plays a pivotal role in cold acclimation of evolutionarily diverse plant species. Transcriptome analysis of transgenic tomato and *Arabidopsis* plants overexpressing *LeCBF1* and *AtCBF3* revealed that CBF regulons from freezing-tolerant and freezing-sensitive plant species differ significantly (35).

Constitutive overexpression of CBFs under the transcriptional control of the 35S cauliflower mosaic virus promoter in transgenic plants resulted in severe growth retardation under normal growth conditions in diverse plant species such as *Arabidopsis* (18,19,34,36), *B. napus* (22), tomato (23,24), potato (29), and rice (31). Inhibition of metabolism and change in growth-regulating hormones appears to be important causes of the growth inhibition of CBF-overexpressing plants. Reduction in the expression of photosynthetic genes appears to reduce photosynthesis and growth under cold stress. Transgenic plants constitutively overexpressing *CBFs* showed higher induction of the *STZ/ZAT10* zinc finger transcription factor gene, which appears to repress genes involved in photosynthesis and carbohydrate metabolism and thus reduce the growth of these transgenic plants (21). Microarray analysis revealed that cold stress regulates several genes involved in biosynthesis or signaling of hormones such as ABA, gibberellic acid (GA), and auxin, which suggests the importance of these hormones in coordinated regulation of cold tolerance and plant development (15). GA promotes important processes in plant growth and development, such as seed germination, growth through elongation, and floral transition. Growth retardation of transgenictomato plants constitutively overexpressing *AtCBF1* was reversed by GA_3 treatment (24). This finding suggested a link between CBFs and GA in cold stress-induced growth retardation. During cold stress, growth retardation appears to be regulated by CBFs through nuclear-localized DELLA proteins, which repress growth in *Arabidopsis*. GA stimulates the degradation of DELLA proteins and promotes growth. CBFs enhance the expression of GA-inactivating GA2-oxidases, and thus allow the accumulation of the DELLA protein repressor of GA1-like 3 (RGL3), which leads to dwarfism and late flowering. Further, mutant plants of *DELLA* genes encoding GA-insensitive [GAI] repressor of GA1–3 [RGA] were significantly less freezing tolerant than were wild-type plants after cold acclimation. This finding suggests that DELLAs might contribute significantly to cold acclimation and freezing tolerance (37).

4.2. Regulators of CBF Expression

Transcription of CBF genes is induced by cold stress. Hence, constitutive transcription factors present in the cell at normal growth temperatures may induce the expression of CBFs on activation by cold stress. A systematic genetic analysis by *CBF3::LUC* bioluminescent genetic screening led to the identification of a constitutively expressed and nuclear-localized transcription factor, *i*nducer of *C*BF *e*xpression 1 (ICE1) in *Arabidopsis*. *ICE1* encodes a MYC-type basic helix-loop-helix (bHLH) transcription factor, can bind to MYC recognition elements in the *CBF3* promoter, and induces the expression of *CBF3* during cold acclimation. The *ice1* mutant is defective in both chilling and freezing tolerance, whereas transgenic *Arabidopsis* overexpressing *ICE1* showed enhanced freezing tolerance (38). Transcriptome analysis revealed the dominant *ice1* mutant with impaired expression of about 40% of cold-regulated genes, in particular 46% of cold-regulated transcription factor genes (15). Therefore, ICE1 is a master regulator that controls CBF and many other cold-responsive regulons. Overexpression analysis showed that ICE2 (At1g12860, a homolog of ICE1) induces the expression of CBF1 and confers enhanced freezing tolerance in *Arabidopsis* after cold acclimation (39). In wheat, the ICE1 homologs *TaICE141* and *TaICE187* are constitutively expressed and activate the wheat CBF group IV, which are associated with freezing tolerance. Overexpression of *TaICE141* and *TaICE187* in *Arabidopsis* enhanced *CBF* and *COR*gene expression and enhanced freezing tolerance only after cold acclimation. This finding suggests that similar to *Arabidopsis* ICE1, wheat ICE1 also needs to be activated by cold acclimation (40).

ICE1 appears to negatively regulate the expression of MYB15 (an R2R3-MYB family protein) in *Arabidopsis*. MYB15 is an upstream transcription factor that negatively regulates *CBF* expression. Transgenic *Arabidopsis* overexpressing *MYB15* showed reduced

expression of *CBFs* and freezingtolerance, whereas *myb15* T-DNA knockout mutants showed enhanced cold induction of *CBFs* and enhanced freezing tolerance. In a yeast two-hybrid system, ICE1 interacted with MYB15 (41). Further, the expression of MYB15 is increased in *ice1* mutants (R236H and K393R) (41,42). Thus, the ICE1-MYB15 interaction appears to play a role in regulating CBF expression levels during cold acclimation (41).

Although ICE1 is expressed constitutively, only on exposure to low temperature does it induce transcription of the CBF and other cold stress-responsive genes (38,40). Posttranslational modifications play a key role in regulating the activity of ICE1 under cold stress. Cold stress activates ICE1 sumoylation (42) and negatively regulates ICE1 levels by targeted proteolysis (43). The *Arabidopsis High expression of Osmotically responsive* gene 1 (*HOS1*) encodes a RING finger ubiquitin E3 ligase. The nuclear localization of *HOS1* is enhanced by cold stress. HOS1 physically interacts with ICE1 and targets ICE1 for polyubiquitination and proteolysis of ICE1 after 12 h of cold stress. Overexpression of HOS1 in transgenic *Arabidopsis* results in a substantial reduction in level of ICE1 protein and that of its target genes, as well as hypersensitivity to freezing stress. Thus, HOS1 mediates ubiquitination of ICE1 and plays a critical role in maintaining the level of ICE1 target genes in the cell during cold acclimation (43). Sumoylation of proteins prevents the proteasomal degradation of target proteins. The null mutant of *Arabidopsis* SUMO E3 ligase, *SAZ1* (SAP and Miz1), exhibits reduced cold induction of *CBFs* and the target *COR* genes, as well as hypersensitivity to chilling and freezing stresses. SIZ1 catalyzes SUMO conjugation to K393 of ICE1 during cold acclimation and thus reduces polyubiquiti-nation of ICE1. Mutation in a K393 residue of ICE1 impairs its activity (42). Hence, SIZ1-mediated sumoylation facilitates ICE1 stability and activity, whereas HOS1 mediation reduces ICE1 protein levels during cold acclimation.

Stomata play a crucial role in regulating photosynthesis and transpiration. Recently, the *scream-D* dominant mutant and *ice1* mutant were found to be the same as R236H, which results in constitutive stomatal differentiation in the epidermis, and the entire epidermis differentiates into stomata. Thus, ICE1 is required for controlled stomatal development. ICE1protein interacts and forms a dimer with other bHLH transcription factors, SPEECHLESS (SPCH), MUTE, and FAMA, which regulate stomatal development. ICE1 may act as a link between the formation of stomata and the plant response to environmental cues (44).

Recently, members of the calmodulin binding transcription activator (CAMTA) family proteins have been identified as transcriptional regulators of *CBF2* expression. Cold-induced expression of CBF2 was considerably lower in *camta3* mutant as compared to WT plants. The CAMTA3 protein binds to conserved DNA motifs present in *CBF2* promoter and regulates *CBF2* expression. The *camta1/camta3* double mutant exhibited hyper-sensitivity to freezing stress as compared to WT plants. Since CAMTA proteins can interact with calmodulins, cold-induced calcium signals may regulate *CBFs* expression through CAMTA proteins (45).

4.3. CBF1, CBF2, and CBF3 Play Different Roles in Cold Acclimation

Microarray analysis revealed that CBFs regulate about 12% of the cold-responsive transcriptome. Overexpression of CBFs enhances osmolyte accumulation, reduces growth, and enhances abiotic stress tolerance (Table 3.1). Constitutive overexpression studies of transgenic *Arabidopsis* suggested that CBF1, CBF2, and CBF3 have redundant functional activities (36). However, the *ice1* mutant, impaired mainly in *CBF3* but not *CBF1* and *CBF2*, showed chilling and freezing hypersensitivity (38). Studies of the *cbf2* T-DNA insertion mutant of *Arabidopsis* revealed that CBFs have different functions in cold acclimation. *cbf2* null mutants showed increased expression of *CBF1* and *CBF3* and

enhanced tolerance to freezing (with or without cold acclimation), dehydration, and salt stresses. Further, *CBFs* show a temporal difference in expression, with the cold-induced expression of *CBF1* and *CBF3* preceding that of *CBF2*. These results suggest that CBF2 negatively regulates *CBF1* and *CBF3* to optimize the expression of downstream target genes (45). In potato (*Solanum tuberosum*), overexpression of *AtCBF2* failed to confer freezing tolerance (29). Transgenic analysis of *CBF1* and *CBF3* RNAi lines revealed that both CBF1 and CBF3 are required for the full set of CBF regulon expression and freezing tolerance (46).

Besides CBF2, the C2H2 zinc finger transcription factor *ZAT12* negatively regulates the expression of *CBF1*, *CBF2*, *and CBF3* during cold stress. *Arabidopsis* transgenic plants overex-pressing *ZAT12* showed decreased expression of *CBFs* under cold stress (47). *los2* mutant plants showed an enhanced and more sustained induction of *ZAT10/STZ* during cold stress and enhanced cold sensitivity. *LOS2* encodes a bifunctional enolase that negatively regulates the expression of *ZAT10* (48).

Transgenic *Arabidopsis* plants overexpressing *AtMKK2* showed constitutive expression of *CBF2*, which suggests that the *CBF2* expression is probably positively regulated by a MAPK signaling cascade (11). *Arabidopsis FIERY2* (*FRY2*), which encodes an RNApolymerase II C-terminal domain (CTD) phosphatase, appears to act as a negative regulator of *CBFs* and their target *COR* genes because the *fry2* mutant showed enhanced expression of *CBFs* and *COR* genesunder cold stress and ABA. Since the *fry2* mutant is hypersensitive to freezing despite enhanced expression of *CBFs*, FRY2 may positively regulate the expression of certain genes critical for freezing tolerance (49). The maintenance of an optimal level of CBFs at an appropriate time is necessary, because constitutive overexpression affects growth and development significantly. Further, *CBF* expression is under the control of a circadian clock. The maximal cold-induced increase in transcription of *CBFs* occurs when cold stress is imposed 4 h after dawn. Transgenic plants overexpressing arrhythmic CCA1 showed no temporal difference in the cold induction of *CBF* expression (50).

4.4. CBF-Independent Regulons

Genetic and transgenic analyses revealed that several classes of transcription factors besides CBFs play an important role in cold acclimation. The *eskimo1* (*esk1*) mutant of *Arabidopsis* was identified through freezing tolerance genetic screening. The *esk1* mutant accumulated constitutively high levels of proline and exhibited constitutively freezing tolerance. *ESK1* is constitutively expressed and encodes the protein domain of unknown function (23). Transcriptome comparison of CBF2-overexpressing plants and *esk1* mutants showed that different sets of genes are regulated by CBF2 and ESK1. However, the mechanism of action of ESK1 in freezing tolerance has yet to be revealed (51).

P_{RD29A}::*LUC* reporter gene-based genetic screening led to the identification of two constitutively expressed transcription factors, HOS9 (a homeodomain protein) and HOS10 (an R2R3-type MYB), which are necessary for cold tolerance in *Arabidopsis*. *hos9* and *hos10* mutants are less freezing tolerant than wild-type *Arabidopsis* (52,53). Transcriptome analysis revealed distinct CBF and HOS9 regulons (52). HOS10 probably regulates ABA-dependent cold acclimation pathways, because HOS10 positively regulates *NCED3* (9-*cis*-epoxycarotenoid dioxygenase) and thus ABA accumulation during cold stress (53).

Gene expression analysis revealed several transcription factors induced during cold acclimation. Transgenic analysis of cold-inducible transcription factors helped in validation of functions of some transcription factors in cold tolerance. Constitutive overexpression of the soybean C2H2-type zinc finger protein SCOF1 in *Arabidopsis* transgenic plants

enhanced the expression of *COR* genes and conferred constitutive freezing tolerance. SCOF1 interacts with soybean G-box binding factor 1 (SGBF1) and may enhance the DNA binding activity of the SGBF1. *SGBF1* is induced by both cold and ABA (54). Overexpression of the cold-regulated rice transcription factors *MYB4* (an R2R3-type MYB) and *OsMYB3R-2* (an R1R2R3 MYB) enhanced freezing tolerance of *Arabidopsis* (55,56).

Some members of the abiotic, plant hormone, and pathogen-inducible ERF family play a crucial role in abiotic and biotic stress tolerance. The pepper ERF/AP2-type transcription factor *Capsicum annuum* pathogen and freezing tolerance-related protein 1 (*CaPF1*) is induced by cold, osmotic stress, ethylene, and jasmonic acid. Transgenic *Arabidopsis* overexpressing *CaPF1* showed induction of pathogen-responsive as well as *COR* genes and exhibited enhanced tolerance to stress by freezing and to pathogens (*Pseudomonas syringae* pv *tomato* DC3000) (57). Similarly, *Triticum aestivum ERF1* (*TaERF1*] was induced by cold, drought salinity, ABA, ethylene, salicylic acid, and infection by *Blumeria graminis f.* sp. *Tritici* pathogen in wheat. Transgenic *Arabidopsis* overexpressing *TaERF1* exhibited enhanced tolerance to cold, salt, and drought stresses, as well as pathogens (58). Genes encoding the A-5 subgroup AP2 domain protein from *Physcomitrella patens* (PpDBF1) (59) and soybean (GmDREB3) (60) are cold induced, and overexpression of these genes conferred enhanced cold tolerance.

In wheat, *w*heat *l*ow-*t*emperature-*i*nduced *protein* 19 (WLIP19), encoding a basic-region leucine zipper protein, is induced by cold, drought, and ABA. WLIP19 activates the expression of *COR* genes in wheat. Transgenic tobacco overexpressing *Wlip19* showed significant freezing tolerance. WLIP19 was found to interact and form a heterodimer with *T.aestivum ocs*-element *b*inding*f*actor 1 (*TaOBF1*), a bZIP transcription factor (61). The plant-specific transcription factor NAC (NAM, ATAF, and CUC) family plays a key role in stress response. Overexpression of cold stress-inducible rice *SNAC2* in transgenic rice resulted in high cell membrane stability under cold stress. Microarray analysis showed upregulation of several stress-regulated genes in *SNAC2*-overexpressing plants (62). These results suggest that several transcriptional networks operate during cold acclimation and cold stress tolerance of plants.

5. Posttranscriptional Gene Regulation

Posttranscriptional regulation at pre-mRNA processing, mRNA stability, and export from nucleus plays critical roles in cold acclimation and cold tolerance (2).

5.1. Messenger RNA Processing

Pre-mRNA processing and exports constitute important mechanisms of regulation of gene expression in eukaryotes. Pre-mRNA undergoes various nuclear processes such as the addition of a 5′ methyl cap and poly(A) tail and intron splicing. Splicing is necessary to remove introns and to synthesize translationally competent mRNAs. Primary transcripts with more than one intron can undergo alternative splicing to produce functionally different proteins from a single gene. In plants, about 20% of genes undergo alternative splicing. Although most alternative splicing events are uncharacterized in plants, but it appears to play an important role in the regulation of photosynthesis, flowering, grain quality in cereals, and plant defense response. Recent studies have implicated intron splicing in abiotic stress response. In wheat, cold stress induction of two early cold-regulated (e-cor) genes coding for a ribokinase (7H8) and a C3H2C3 RING finger protein (6G2) undergo stress-dependent splicing. Both of these genes are regulated by intron retention under cold stress, whereas 6G2 intron retention is also regulated by drought stress. However, homologs of these genes did not show stress-regulated intron retention in *Arabidopsis*. Interestingly, barley homologs of 7H8 and 6G2 showed stress-dependent intron retention under cold

stress, whereas barley albino mutants defective in chloroplast development failed to retain introns in these genes under cold stress (63). The *Arabidopsis COR15A* gene encoding a chloroplast stromal protein with cryoprotective activity plays an important role in conferring freezing tolerance to chloroplasts (64). The *Arabidopsis stabilized1* (*sta1*) mutant is defective in the splicing of the cold-induced *COR15A* pre-mRNA and is hyper-sensitive to chilling, ABA, and salt stresses. *STA1* encodes a nuclear pre-mRNA splicing factor and is upregulated by cold stress. STA1 catalyzes splicing of *COR15A*, which is necessary for cold tolerance (65). Further, pre-mRNA of serine/arginine-rich (SR) proteins, which are involved in the regulation or execution of mRNA splicing, undergo alternate splicing under cold and heat stresses in *Arabidopsis* (66). Further, in addition to a change in splicing pattern, expression levels of *AtSR45a* and *AtSR30*, SF2/ASF-like SR proteins, are also increased by high light and salinity stresses in *Arabidopsis* (67). Thus, the stress-regulated alternate splicing machinery may in turn change the splicing pattern of some of the stress-responsive genes.

5.2. Small RNAs

Small noncoding RNAs, namely micro-RNAs (miRNAs) and small interfering RNAs (siRNAs), act as ubiquitous repressors of gene expression in animals and plants. Small RNAs are incorporated into the argonaute (AGO) family of proteins containing the RNA-induced silencing complex (RISC) or RNA-induced transcriptional silencing (RITS) complex. The RISC-containing miRNA/siRNA induces posttranscriptional gene silencing by cleavage of mRNA and translational repression. Transcriptional gene silencing is mainly mediated by siRNAs. Cold stress-upregulated and -downregulated miRNAs have been identified in *Arabidopsis*. Abiotic stress-induced or -upregulated small RNAs can downregulate their target genes, which are likely negative regulators and/or determinants of the stress response. In contrast, stress-downregulated small RNAs can upregulate their target mRNAs, which are likely positive regulators and/or determinants of stress tolerance (68).

Accumulation of ROS is induced by abiotic stresses. Super-oxide dismutases catalyze conversion of the superoxide radical into H_2O_2, which is then detoxified by ascorbate peroxidase. The miR398 expression is reduced and that of its target genes *CSD1* and *CSD2* enhanced under oxidative stress in *Arabidopsis*. Under normal conditions, miR398 targets the *CSD* mRNAs for cleavage, and thus stress-induced reduction in miR398 expression results in accumulation of CSD transcripts. Because miR398 and its target sequence on the *CSD* mRNAs are conserved across plant species, miR398 appears to play a ubiquitous role in ROS detoxification under abiotic stresses (69). siRNAs derived from double-stranded RNAs (dsRNAs) formed from the mRNAs encoded by a natural *cis*-antisense gene pair are called natural antisense transcript-derived siRNAs (nat-siRNAs). One of the nat-siRNAs derived from a *cis*-nat pair of *SRO5* and *P5CDH* (Δ1-pyrroline-5-carboxylate dehydrogenase) regulates oxidative stress and osmolyte accumulation under salt stress in *Arabidopsis*. Salt stress-induced expression of *SRO5* leads to SRO5-P5CDH dsRNA, which is then processed by DCL2, RDR6, SGS3, and DNA-dependent RNA polymerase IV (NRPD1A) to generate a 24-nt nat-siRNA. The 24-nt nat-siRNA targets the cleavage of P5CDH and thus accumulation of proline. Oxidative stress also induces the expression of *SRO5* and the 24-nt SRO5-P5CDH nat-siRNA and decreases P5CDH transcript levels (70,71). These results suggest that small RNAs play a key role in gene regulation in the cold and other abiotic stress response of *Arabidopsis*.

6. Conclusions and Perspectives

Significant progress has been made to unravel the molecular basis of cold acclimation in model plant *Arabidopsis* and winter cereals. During cold acclimation, plants reprogram their gene expression through transcriptional, posttranscriptional, and posttranslational

mechanisms. The ICE1-CBF transcriptional cascade plays crucial role in cold acclimation in diverse plant species. Transgenic analysis revealed that genetic engineering of CBF pathway can improve cold tolerance across plant species. Recently, several components of CBF-independent transcriptional pathway of cold acclimation have been identified. Besides transcriptional regulation, plants employ diverse posttranscriptional regulatory mechanisms to regulate their gene expression during cold acclimation. Several cold stress-regulated miRNAs have been identified in *Arabidopsis* and rice. Characterization of cold-regulated miRNAs will help understand the role of posttranscriptional regulation of mRNA stability in cold stress response of plants. Cold-induced transcriptome differs significantly among leaf, root, and reproductive (pollen) tissues. Most of the mechanisms of cold acclimation were studied in vegetative stages of *Arabidopsis*. Further studies on transcriptional networks in reproductive tissues will identify key regulators of cold tolerance. Epigenetic processes play a key role in the regulation of plant development and stress responses. Further studies on the function of epigenetic processes, such as DNA methylation and chromatin modifications, and epigenetic stress memory will be necessary.

References

1. Yamaguchi-Shinozaki K, Shinozaki K. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annu Rev Plant Biol. 2006; 57:781–803. [PubMed: 16669782]
2. Chinnusamy V, Zhu J, Zhu JK. Cold stress regulation of gene expression plants. Trends Plant Sci. 2007; 12:444–451. [PubMed: 17855156]
3. Orvar BL, Sangwan V, Omann F, Dhindsa R. Early steps in cold sensing by plant cells: the role of actin cytoskeleton and membrane fluidity. Plant J. 2000; 23:785–794. [PubMed: 10998189]
4. Sangwan V, Foulds I, Singh J, Dhindsa RJ. Cold activation of *Brassica napus* BN115 promoter is mediated by structural changes in membranes and cytoskeleton, and requires Ca^{2+} influx. Plant J. 2001; 27:1–12. [PubMed: 11489178]
5. Vaultier MN, Cantrel C, Vergnolle C, Justin A-M, Demandre C, Benhassaine-Kesri G, Cicek D, Zachowski A, Ruelland E. Desaturase mutants reveal that membrane rigidification acts as a cold perception mechanism upstream of the diacylglycerol kinase pathway in *Arabidopsis* cells. FEBS Lett. 2006; 580:4218–4223. [PubMed: 16839551]
6. Knight MR. Signal transduction leading to low-temperature tolerance in *Arabidopsis thaliana*. Philos Trans R Soc Lond B Biol Sci. 2002; 357:871–875. [PubMed: 12171650]
7. Carpaneto A, Ivashikina N, Levchenko V, Krol E, Jeworutzki E, Zhu JK, Hedrich R. Cold transiently activates calcium-permeable channels in *Arabidopsis* mesophyll cells. Plant Physiol. 2007; 143:487–494. [PubMed: 17114272]
8. Vergnolle C, Vaultier MN, Taconnat L, Renou JP, Kader JC, Zachowski A, Ruell E. The cold-induced early activation of phospholipase C and D pathways determines the response of two distinct clusters of genes in *Arabidopsis* cell suspensions. Plant Physiol. 2005; 139:1217–1233. [PubMed: 16258011]
9. Xiong L, Lee BH, Ishitani M, Lee H, Zhang C, Zhu JK. FIERY1 encoding an inositol polyphosphate 1-phosphatase is a negative regulator of abscisic acid and stress signaling in *Arabidopsis*. Genes Dev. 2001; 15:1971–1984. [PubMed: 11485991]
10. Catala R, Santos E, Alonso JM, Ecker JR, Martinez-Zapater JM, Salinas J. Mutations in the Ca^{2+}/H^{+} transporter CAX1 increase *CBF/DREB1* expression and the cold-acclimation response in *Arabidopsis*. Plant Cell. 2003; 15:2940–2951. [PubMed: 14630965]
11. Teige M, Scheikl E, Eulgem T, Doczi R, Ichimura K, Shinozaki K, Dangl JL, Hirt H. The MKK2 pathway mediates cold and salt stress signaling in *Arabidopsis*. Mol Cell. 2004; 15:141–152. [PubMed: 15225555]
12. Lee BH, Lee H, Xiong L, Zhu JK. A mitochondrial complex I defect impairs cold-regulated nuclear gene expression. Plant Cell. 2002; 14:1235–1251. [PubMed: 12084824]
13. Sheen J. Specific Ca^{2+}-dependent protein kinase in stress signal transduction. Science. 1996; 274:1900–1902. [PubMed: 8943201]

14. Pitzschke A, Hirt H. Mitogen-activated protein kinases and reactive oxygen species signaling in plants. Plant Physiol. 2006; 141:351–356. [PubMed: 16760487]
15. Lee BH, Henderson DA, Zhu JK. The *Arabidopsis* cold-responsive transcriptome and its regulation by ICE1. Plant Cell. 2005; 17:3155–3175. [PubMed: 16214899]
16. Stockinger EJ, Gilmour SJ, Thomashow MF. Arabidopsis thaliana CBF1 encodes an AP2 domain-containing transcription 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 USA. 1997; 94:1035–1040. [PubMed: 9023378]
17. Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA-binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression in *Arabidopsis*. Plant Cell. 1998; 10:1391–1406. [PubMed: 9707537]
18. Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF. *Arabidopsis CBF1* overexpression induces COR genes and enhances freezing tolerance. Science. 1998; 280:104–106. [PubMed: 9525853]
19. Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress inducible transcription factor. Nat Biotech. 1999; 17:287–291.
20. Fowler S, Thomashow MF. *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell. 2002; 14:1675–1690. [PubMed: 12172015]
21. Maruyama K, Sakuma Y, Kasuga M, Ito Y, Seki M, Goda H, Shimada Y, Yoshida S, Shinozaki K, Yamaguchi-Shinozaki K. Identification of cold-inducible downstream genes of the *Arabidopsis* DREB1A/CBF3 transcriptional factor using two microarray systems. Plant J. 2004; 38:982–993. [PubMed: 15165189]
22. Jaglo KR, Kleff S, Amundsen KL, Zhang X, Haake V, Zhang JZ, Deits T, Thomashow MF. Components of the *Arabidopsis* C-repeat/dehydration responsive element binding factor cold-response pathway are conserved in *Brassica napus*and other plant species. Plant Physiol. 2001; 127:910–917. [PubMed: 11706173]
23. Hsieh TH, Lee JT, Charng YY, Chan MT. Tomato plants ectopically expressing *Arabidopsis* CBF1 show enhanced resistance to water deficit stress. Plant Physiol. 2002; 130:618–26. [PubMed: 12376629]
24. Hsieh TH, Lee JT, Yang PT, Chiu LH, Charng YY, Wang YC, Chan MT. Heterology expression of the *Arabidopsis* C-repeat/dehydration response element binding factor 1 gene confers elevated tolerance to chilling and oxidative stresses in transgenic tomato. Plant Physiol. 2002; 129:1086–1094. [PubMed: 12114563]
25. Kasuga M, Miura S, Shinozaki K, Yamaguchi-Shinozaki K. A combination of the A*rabidopsis DREB1A* gene and stress-inducible *RD29A* promoter improved drought- and low-temperature stress tolerance in tobacco by gene transfer. Plant Cell Physiol. 2004; 45:346–350. [PubMed: 15047884]
26. Pellegrineschi A, Reynolds M, Pacheco M, Brito RM, Almeraya R, Yamaguchi-Shinozaki K, Hoisington D. Stress-induced expression in wheat of the *Arabidopsis thaliana DREB1A* gene delays water stress symptoms under greenhouse conditions. Genome. 2004; 47:493–500. [PubMed: 15190366]
27. Oh SJ, Song SI, Kim YS, Jang HJ, Kim SY, Kim M, Kim YK, Nahm BH, Kim JK. *Arabidopsis CBF3/DREB1A* and *ABF3* in transgenic rice increased tolerance to abiotic stress without stunting growth. Plant Physiol. 2005; 138:341–351. [PubMed: 15834008]
28. Al-Abed D, Madasamy P, Talla R, Goldman S, Rudrabhatla S. Genetic engineering of maize with the *Arabidopsis DREB1A/CBF3* gene using split-seed explants. Crop Sci. 2007; 47:2390–2402.
29. Pino MT, Skinner JS, Park EJ, Jeknic Z, Hayes PM, Thomashow MF, Chen THH. Use of a stress inducible promoter to drive ectopic *AtCBF* expression improves potato freezing tolerance while minimizing negative effects on tuber yield. Plant Biotechnology J. 2007; 5:591–604.
30. Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, Dubouzet EG, Miura S, Seki M, Shinozaki K, Yamaguchi-Shinozaki K. *OsDREB* genes in rice, *Oryza sativa* L., encode transcription activators

that function in drought-, high-salt- and cold-responsive gene expression. Plant J. 2003; 33:751–763. [PubMed: 12609047]

31. Ito Y, Katsura K, Maruyama K, Taji T, Kobayashi M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K. Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. Plant Cell Physiol. 2006; 47:141–153. [PubMed: 16284406]
32. Qin F, Sakuma Y, Li J, Liu Q, Li Y-Q, Shinozaki K, Yamaguchi-Shinozaki K. Cloning and functional analysis of a novel *DREB1/CBF* transcription factor involved in cold-responsive gene expression in *Zea mays*. Plant Cell Physiol. 2004; 45:1042–1052. [PubMed: 15356330]
33. Savitch LV, Allard G, Seki M, Robert LS, Tinker NA, Huner NP, Shinozaki K, Singh J. The effect of over-expression of two Brassica CBF/DREB1-like transcription factors on photosynthetic capacity and freezing tolerance in *Brassica napus*. Plant Cell Physiol. 2005; 46:1525–1539. [PubMed: 16024910]
34. Welling A, Palva ET. Involvement of CBF transcription factors in winter hardiness in birch. Plant Physiol. 2008; 147:1199–1211. [PubMed: 18467468]
35. Zhang X, Fowler SG, Cheng H, Lou Y, Rhee SY, Stockinger EJ, Thomashow MF. Freezing-sensitive tomato has a functional CBF cold response pathway, but a CBF regulon that differs from that of freezing-tolerant *Arabidopsis*. Plant J. 2004; 39:905–919. [PubMed: 15341633]
36. Gilmour SJ, Fowler SG, Thomashow MF. *Arabidopsis* transcriptional activators CBF1, CBF2, and CBF3 have matching functional activities. Plant Mol Biol. 2004; 54:767–781. [PubMed: 15356394]
37. Achard P, Gong F, Cheminant S, Alioua M, Hedden P, Genschik P. The cold-inducible CBF1 factor-dependent signaling pathway modulates the accumulation of the growth-repressing DELLA proteins via its effect on gibberellin metabolism. Plant Cell. 2008; 20:2117–2129. [PubMed: 18757556]
38. Chinnusamy V, Ohta M, Kanrar S, Lee B.-h, Hong X, Agarwal M, Zhu JK. ICE1, a regulator of cold induced transcriptome and freezing tolerance in *Arabidopsis*. Genes Dev. 2003; 17:1043–1054. [PubMed: 12672693]
39. Fursova OV, Pogorelko GV, Tarasov VA. Identification of ICE2, a gene involved in cold acclimation which determines freezing tolerance in *Arabidopsis thaliana*. Gene. 2009; 429:98–103. [PubMed: 19026725]
40. Badawi M, Reddy YV, Agharbaoui Z, Tominaga Y, Danyluk J, Sarhan F, Houde M. Structure and functional analysis of wheat ICE (Inducer of CBF Expression) genes. Plant Cell Physiol. 2008; 49:1237–1249. [PubMed: 18635580]
41. Agarwal M, Hao Y, Kapoor A, Dong CH, Fujii H, Zheng X, Zhu JK. A R2R3 type MYB transcription factor is involved in the cold regulation of *CBF* genes and in acquired freezing tolerance. J Biol Chem. 2006; 281:37636–37645. [PubMed: 17015446]
42. Miura K, Jin JB, Lee J, Yoo CY, Stirm V, Miura T, Ashworth EN, Bressan RA, Yun DJ, Hasegawa PM. SIZ1-mediated sumoylation of ICE1 controls *CBF3/DREB1A* expression and freezing tolerance in *Arabidopsis*. Plant Cell. 2007; 19:1403–1414. [PubMed: 17416732]
43. Dong CH, Agarwal M, Zhang Y, Xie Q, Zhu JK. The negative regulator of plant cold responses, HOS1, is a RING E3 ligase that mediates the ubiquiti-nation and degradation of ICE1. Proc Natl Acad Sci USA. 2006; 103:8281–8286. [PubMed: 16702557]
44. Kanaoka MM, Pillitteri LJ, Fujii H, Yoshida Y, Bogenschutz NL, Takabayashi J, Zhu JK, Torii KU. *SCREAM/ICE1* and *SCREAM2* specify three cell-state transitional steps leading to *Arabidopsis* stomatal differentiation. Plant Cell. 2008; 20:1775–1785. [PubMed: 18641265]
45. Doherty CJ, Van Buskirk HA, Myers SJ, Thomashow MF. Roles for Arabidopsis CAMTA transcription factors in cold-regulated gene expression and freezing tolerance. Plant Cell. 2009; 21:972–984. [PubMed: 19270186]
46. Novillo F, Alonso JM, Ecker JR, Salinas J. CBF2/DREB1C is a negative regulator of CBF1/DREB1B and CBF3/DREB1A expression and plays a central role in stress tolerance in Arabidopsis. Proc Natl Acad Sci USA. 2004; 101:3985–3990. [PubMed: 15004278]
47. Novillo F, Medina J, Salinas J. *Arabidopsis* CBF1 andCBF3havea different function than CBF2 in cold acclimation and define different gene classes in the CBF regulon. Proc Natl Acad Sci USA. 2007; 104:21002–21007. [PubMed: 18093929]

48. Vogel JT, et al. Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of *Arabidopsis*. Plant J. 2005; 41:195–211. [PubMed: 15634197]
49. Lee H, Guo Y, Ohta M, Xiong L, Stevenson B, Zhu JK. *LOS2*, a genetic locus required for cold responsive transcription encodes a bi-functional enolase. EMBO J. 2002b; 21:2692–2702. [PubMed: 12032082]
50. Xiong L, Lee H, Ishitani M, Tanaka Y, Stevenson B, Koiwa H, Bressan RA, Hasegawa PM, Zhu JK. Repression of stress-responsive genes by FIERY2, a novel transcriptional regulator in *Arabidopsis*. Proc Natl Acad Sci USA. 2002; 99:10899–10904. [PubMed: 12149453]
51. Fowler SG, Cook D, Thomashow MF. Low temperature induction of Arabidopsis CBF1, 2 and 3 is gated by the circadian clock. Plant Physiol. 2005; 137:961–968. [PubMed: 15728337]
52. Xin Z, Mandaokar A, Chen J, Last RL, Browse J. *Arabidopsis ESK1* encodes a novel regulator of freezing tolerance. Plant J. 2007; 21:786–799. [PubMed: 17316173]
53. Zhu J, Shi H, Lee BH, Damsz B, Cheng S, Stirm V, Zhu JK, Hasegawa PM, Bressan RA. An *Arabidopsis* homeodomain transcription factor gene, *HOS9*, mediates cold tolerance through a CBF-independent pathway. Proc Natl Acad Sci USA. 2004; 101:9873–9878. [PubMed: 15205481]
54. Zhu J, Verslues PE, Zheng X, Lee BH, Zhan X, Manabe Y, Sokolchik I, Zhu Y, Dong CH, Zhu JK, Hasegawa PM, Bressan RA. *HOS10* encodes an R2R3-type MYB transcription factor essential for cold acclimation in plants. Proc Natl Acad Sci USA. 2005; 102:9966–9971. [PubMed: 15994234]
55. Kim JC, Lee SH, Cheong YH, Yoo CM, Lee SI, Chun HJ, Yun DJ, Hong JC, Lee SY, Lim CO, Cho MJ. A novel cold-inducible zinc finger protein from soybean, SCOF-1, enhances cold tolerance in transgenic plants. Plant J. 2001; 25:247–259. [PubMed: 11208017]
56. Vannini C, Locatelli F, Bracale M, Magnani E, Marsoni M, Osnato M, Mattana M, Baldoni E, Coraggio I. Overexpression of the rice *Osmyb4* gene increases chilling and freezing tolerance of *Arabidopsis thaliana* plants. Plant J. 2004; 37:115–127. [PubMed: 14675437]
57. Dai X, Xu Y, Ma Q, Xu W, Wang T, Xue Y, Chong K. Overexpression of a *R1R2R3 MYB* gene, *OsMYB3R-2*, increases tolerance to freezing, drought, and salt stress in transgenic *Arabidopsis*. Plant Physiol. 2007; 143:1739–1751. [PubMed: 17293435]
58. Yi SY, Kim JH, Joung YH, Lee S, Kim WT, Yu SH, Choi D. The pepper transcription factor *CaPF1* confers pathogen and freezing tolerance in *Arabidopsis*. Plant Physiol. 2004; 136:2862–2874. [PubMed: 15347795]
59. Xu ZS, Xia LQ, Chen M, Cheng XG, Zhang RY, Li LC, Zhao YX, Lu Y, Ni ZY, Liu L, Qiu ZG, Ma YZ. Isolation and molecular characterization of the *Triticum aestivum* L. ethylene-responsive factor 1 (*TaERF1*) that increases multiple stress tolerance. Plant Mol Biol. 2007; 65:719–732. [PubMed: 17874224]
60. Liu N, Zhong NQ, Wang GL, Li LJ, Liu XL, He YK, Xia GX. Cloning and functional characterization of PpDBF1 gene encoding a DRE-binding transcription factor from *Physcomitrella patens*. Planta. 2007; 226:827–838. [PubMed: 17541631]
61. Chen M, Xu Z, Xia L, Li L, Cheng X, Dong J, Wang Q, Ma Y. Cold-induced modulation and functional analyses of the DRE-binding transcription factor gene, GmDREB3, in soybean (*Glycine max* L.). J Exp Bot. 2009; 60:121–135. [PubMed: 18988621]
62. Kobayashi F, Maeta E, Terashima A, Kawaura K, Ogihara Y, Takumi S. Development of abiotic stress tolerance via bZIP-type transcription factor LIP19 in common wheat. J Exp Bot. 2008; 59:891–905. [PubMed: 18326864]
63. Hu H, You J, Fang Y, Zhu X, Qi Z, Xiong L. Characterization of transcription factor gene *SNAC2* conferring cold and salt tolerance in rice. Plant Mol Biol. 2008; 67:169–181. [PubMed: 18273684]
64. Mastrangelo AM, Belloni S, Barilli S, Ruperti B, Fonzo ND, Stanca AM, Cattivelli L. Low temperature promotes intron retention in two *e-cor* genes of durum wheat. Planta. 2005; 221:705–715. [PubMed: 15666155]
65. Nakayama K, Okawa K, Kakizaki T, Honma T, Itoh H, Inaba T. *Arabidopsis* Cor15am is a chloroplast stromal protein that has cryoprotective activity and forms oligomers. Plant Physiol. 2007; 144:513–523. [PubMed: 17384167]

66. Lee BH, Kapoor A, Zhu J, Zhu JK. STABILIZED1, a stress-upregulated nuclear protein, is required for pre-mRNA splicing, mRNA turnover, and stress tolerance in *Arabidopsis*. Plant Cell. 2006; 18:1736–1749. [PubMed: 16751345]

67. Palusa SG, Ali GS, Reddy ASN. Alternative splicing of pre-mRNAs of *Arabidopsis* serine/arginine-rich proteins: regulation by hormones and stresses. Plant J. 2007; 49:1091–1107. [PubMed: 17319848]

68. Tanabe N, Yoshimura K, Kimura A, Yabuta Y, Shigeoka S. Differential expression of alternatively spliced mRNAs of Arabidopsis SR protein homologs, atSR30 and atSR45a, in response to environmental stress. Plant Cell Physiol. 2007; 48:1036–1049. [PubMed: 17556373]

69. Sunkar R, Chinnusamy V, Zhu J, Zhu JK. Small RNAs as big players in plant abiotic stress responses and nutrient deprivation. Trends Plant Sci. 2007; 12:301–309. [PubMed: 17573231]

70. Sunkar R, Kapoor A, Zhu JK. Posttranscriptional induction of two Cu/Zn superoxide dismutase genes in *Arabidopsis* is mediated by downregulation of miR398 and important for oxidative stress tolerance. Plant Cell. 2006; 18:2051–2065. [PubMed: 16861386]

71. Borsani O, Zhu J, Verslues PE, Sunkar R, Zhu JK. Endogenous siRNAs derived from a pair of natural *cis*antisense transcripts regulate salt tolerance in *Arabidopsis*. Cell. 2005; 123:1279–1291. [PubMed: 16377568]

Table 3.1

Abiotic stress tolerance of transgenic plants overexpressing CBFs

Gene	Transgenic plant	Stress tolerance of transgenic plants	References
AtCBF1/2/3	*Brassica napus*	Constitutive overexpression enhanced both basal and acquired freezing tolerance	(22)
AtCBF1	Tomato	Constitutive overexpression enhanced oxidative stress tolerance under chilling stress; enhanced tolerance to water-deficit stress	(23,24)
AtDREB1A/CBF3	Tobacco	Transgenic plants expressing *RD29A*::*DREB1A* exhibited enhanced chilling and drought tolerance	(25)
AtDREB1A/CBF3	Wheat	Transgenic plants expressing *RD29A* promoter::*AtDREB1A* gene showed delayed water stress symptoms	(26)
AtCBF3	Rice	Constitutive overexpression resulted in enhanced tolerance to drought and high salinity and a marginal increase in chilling tolerance	(27)
AtDREB1A/CBF3	Maize	*RD29A*::*CBF3* transgenic plants are more tolerant to cold, drought, and salinity	(28)
AtCBF1	Potato	Constitutive or stress-inducible expression of *CBF1* or *CBF3* but not *CBF2* conferred improved freezing tolerance to frost-sensitive *Solanum tuberosum*	(29)
OsDREB1	*Arabidopsis*	Overexpression in *Arabidopsis* induced target *COR* genes and conferred enhanced tolerance to freezing and drought stresses	(30)
OsDREB1A/B	Rice	Constitutive expression conferred improved tolerance to cold, drought, and salinity	(31)
ZmDREB1	*Arabidopsis*	Overexpression in *Arabidopsis* induced *COR* genes and conferred tolerance to freezing and drought	(32)
BnCBF5 and BnCBF 17	*B. napus*	Overexpression led to increased constitutive freezing tolerance, increased photochemical efficiency and photosynthetic capacity	(33)