Chapter 1
Cold-Induced Injuries and Signaling Responses in Plants

Jigeesha Mukhopadhyay and Aryadeep Roychoudhury

1.1 Introduction

The phenotypic manifestations of cold injury in plants are highly variable. Both low temperature and rapid fluctuations between heat and cold can severely affect the physiology of plants (Miura and Furumoto 2013). Cold stress inflicts damages to fruit trees, horticultural and landscape plants, as well as crop plants, posing a major threat to sustainable agriculture. Commercially important crop plants have been targeted for stress alleviation in order to increase productivity and yield through interspecific and intergeneric breeding which resulted in limited success; however, transgenic approaches to engineer cold-tolerant plants by manipulation of the key genes of the transcriptional and metabolic cascades have contributed to tolerance mechanisms in affected plants to some extent (Rihan et al. 2017). The present chapter highlights the physiological effects of cold stress and gene regulations on perceiving cold stress signals. Finally, the chapter discusses on the cold tolerance mechanisms, genetic engineering for tolerance, and acclimation that allows adaptation and successful breeding strategies for sustainable growth of plants in the face of cold injuries (Sanghera et al. 2011).

1.2 Cold Injuries: Chilling, Frost, and Freeze

Winter injury as well as freeze and frost injury are often synonymous. Cold injuries, however, are more severely manifested due to extreme temperature fluctuation, rather than prolonged low temperature conditions. Sudden temperature fluctuations

J. Mukhopadhyay · A. Roychoudhury (✉)
Post Graduate Department of Biotechnology, St. Xavier's College (Autonomous), Kolkata, West Bengal, India

S. H. Wani, V. Herath (eds.), *Cold Tolerance in Plants*,
https://doi.org/10.1007/978-3-030-01415-5_1

like rapidly falling temperature and hard freeze can result in stress development and injury in plants that have acquired dormancy, but have not yet fully acclimated (Guy 1990). Acclimation to below-freezing conditions can successfully occur, only if the temperature fall is gradual, whereas deacclimation can occur if extended periods of mild winter occur, and this poses a massive threat to plants if they are suddenly exposed to extremely low temperature conditions. Such deacclimated plants are vulnerable to tissue injury and cold stress (Kalberer et al. 2006). However, prolonged low temperatures, viz., during winter, can also severely damage plants, mainly when the temperature drops below a certain tolerance limit. Plants that are already physiologically weak may be, due to previous stress exposures or due to lack of hardiness and adaptability to the harsh conditions of a specific geographical locale, are more prone to suffer from winter injury (Arora and Rowland 2011). The manifestation of winter injury is highly variable, though buds show maximum susceptibility (Fig. 1.1).

Chilling injury can be defined as damage incurred to plants due to temperature exceeding the freezing point (32°F or 0°C). Maximum susceptibility to chilling injury is shown by plants inhabiting tropical or subtropical climes. Flowers, fruits, and leaves are affected in the sensitive species, and manifestation in the form of purple or reddish wilting leaves is common. Frost and freeze injury are closely related since both lead to membrane damage due to osmotic shock, dehydration stress, and ice crystal formation. Frost damage occurs during radiation freeze,

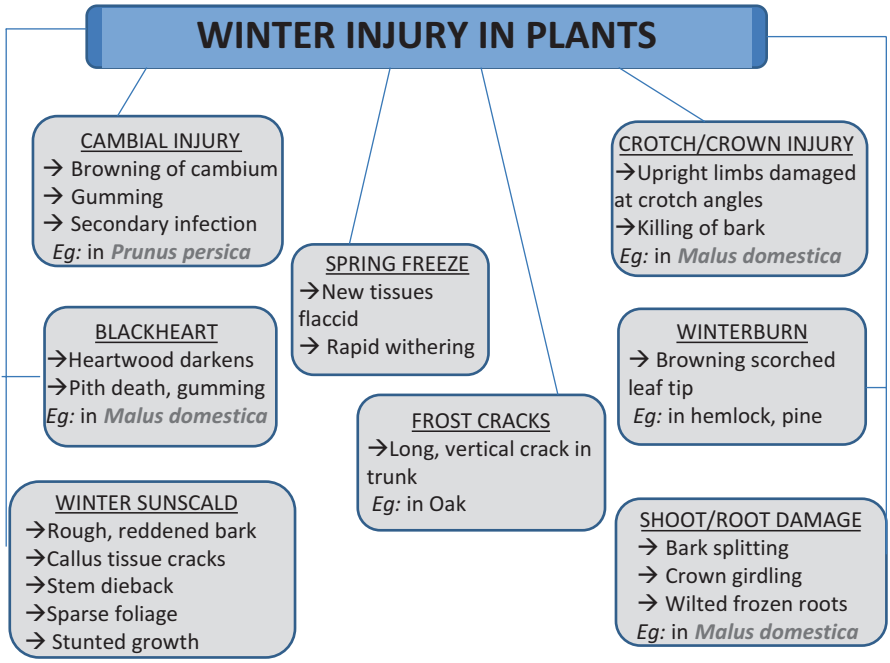

Fig. 1.1 Common manifestations of cold injury in the form of damages to root, shoot, and cambium in chilling-sensitive plants

mainly on calm and clear nights, when plants give off more heat than can be compensated for by the heat received by plants. Thus, it results in a temperature inversion, wherein cold air nearer to the ground is trapped by the warmer air layer above it (air temperature increases with altitude). When the air temperature at the plant level is near or below freezing, the temperature of the plant inevitably is lower than the ambient conditions. Freeze damage, on the other hand, occurs due to advective freezes, when an air mass with below-freezing temperature moves into and occupies an area, displacing warmer air. This causes the temperature of plants low enough to form ice crystals, hence damaging the tissues.

1.2.1 Freezing Injury in Plants

To generalize the term freezing injury, it is mainly concerned with the dysfunctions in physiology of the plant due to freezing of the water contained in plant tissues due to late spring and early fall frosts, low midwinter minima, and rapid temperature fluctuations. Freezing of tissue water is inevitably accompanied by ice formation, which may be intracellular or extracellular (Pearce 2001). Intracellular ice formation may be due to the following: (i) internal nucleation (large polysaccharides or proteins may act as nucleating agents for ice formation) and (ii) penetration (external ice crystals may penetrate into plant cells). Two types of freezing usually occur in plant cells and tissues: (i) vitrification (when rapid freezing of cells to very low temperatures causes the cellular content to get solidified into noncrystalline or amorphous state) and (ii) crystallization (ice crystallization due to gradual drop in temperature may be intracellular or extracellular). The more severe and damaging of the two is intracellular freezing, since it disrupts membrane integrity and can be lethal. Intracellular ice formation in susceptible tender plants is common; however, hardy plants before acclimation may also be affected. Intracellular ice may be formed spontaneously from centers of nucleation in the cytoplasm or may form in cell walls adjacent to intercellular spaces (apoplasm). Sometimes, ice may spread from cell to cell through plasmodesmatal connections. The plasmalemma can serve as a barrier to the entry of ice and hence can partially prevent dehydration, but cells and organelle tend to shrink and succumb to freezing injury to some extent. Thick cuticle can also serve as an effective shield that protects seedlings from external ice. Tissue damages due to freezing injury are characterized primarily by loss of membrane integrity, leakage of metabolites, and perturbations in plasmolysis as well as deplasmolysis.

1.2.1.1 Supercooling and Ice Nucleation

Some “deep supercooled” tissues in hardy plants may also show intracellular freezing. Deep supercooling is a mechanism by which plants avoid freezing injuries. The phenomenon by which water below freezing temperature still maintains its liquid

state is known as supercooling. Supercooling can occur in plants when the liquid held in the intercellular spaces does not make the transition from liquid to solid phase, and hence plants can avoid ice crystallization (Wisniewski et al. 2008). Some fruit trees and hardwoods are capable of supercooling down to –35°C; however, below –40°C, ice crystallization is spontaneous. Smaller crystals formed due to rapid freezing usually melt before causing cold injuries. Thus, water can indefinitely remain in supercooled state, unless the temperature falls below this homogeneous ice nucleation temperature or frost and soil ice invade plants through natural openings like stoma, lenticels, and wound sites. If external ice achieves nucleation, it rapidly spreads through vascular tissues, and the number and localization of nucleations depend on the initial extent of supercooling achieved by the plant. If supercooling is sufficient, multiple ice nucleation sites are available for the external ice to intrude.

Deep supercooling is achieved in some woody plants which involves supercooling of an aqueous fraction which is considerably isolated from seedling by an ice layer and is also divided into distinct compartments (Nuener et al. 2010). This compartmentalized pure water spontaneously freezes at –38°C. The presence of solutes depresses the spontaneous nucleation point as seen in lowering of supercooling by experimental addition of solutes to exotherm of shagbark hickory. In xylem parenchyma and flower bud tissues, ice penetration from adjacent frozen tissues is prevented by a barrier formed by undifferentiated cells between floral primordia in bud and nearby frozen stem tissues. Such barriers which prevent propagation of ice into healthy tissues may involve fine microcapillaries of cell wall, in addition to antinucleating chemicals in protoplasm.

1.2.1.2 Mechanism of Injury

(i) Intracellular freezing injury: intracellular freezing is a rapid process which results in flash freezing of cells which then allows ice crystals to propagate throughout protoplast and vacuole. Macromolecular assembly is disturbed due to mechanical tension and dehydration. Membrane integrity is hampered, and cellular compartmentalization is disrupted, resulting in leakage of hydrolyzing enzymes in the affected tissue. (ii) Extracellular freezing injury: extracellular ice imposes desiccation stress on the protoplasm, which is equivalent to drought stress, since water is removed from the cell to the extracellular ice. Dehydration of plant cells due to freezing injury can be lethal, primarily damaging the membrane of frost-injured cells. Following freezing, membrane proteins are rendered insoluble and protein dissociation into subunits occurs resulting in inactivation of enzymes, just as in case of drought stress. Membrane can thus be established as the primary site of desiccation stress. Membrane proteins are denatured due to a number of factors associated with freezing injury like pH imbalance, increased salt concentration, oxidation of sulfhydryl groups, and change in conformation due to water loss.

Tissue shearing in vascular tissues due to ice crystals has been observed in wheat crowns, azalea flower buds, and developing pear fruitlets. Freezing can also lead to shrinkage of protoplasts in injured plants. Lipoprotein membranes show fractures along hydrophobic regions, since intramolecular hydrogen bonds are weakened due to freezing injury.

1.2.2 Chilling Injury in Plants

Chilling injury is the damage incurred to chilling-sensitive plants at temperatures above the freezing point of tissues but lower than 15°C, i.e., injury at low but non-freezing temperature conditions. Plants which show visual manifestations of injury at temperatures exceeding 15°C are referred to as extremely chilling sensitive (Lukatkin et al. 2012). Accordingly, plants can be classified as (i) chilling-sensitive (severely damaged at temperatures above 0°C but below 15°C) and (ii) chilling-resistant (they are able to tolerate low temperature up to a tolerance threshold and show signs of injury only when ice formation occurs).

1.2.2.1 Mechanism of Chilling Injury

The physical phase transition of cellular membranes from flexible liquid crystalline to rigid gel structure at a temperature critical for chilling injury serves as a controlling response. Lowering of temperature in chilling-sensitive species leads to solidification of membrane lipids, which brings about contraction, causing cracks and channels and, consequently, increased permeability. This disturbed regulation of permeability leads to ionic imbalance and ion leakage from tissues. Enzyme activity is also hampered, since suitable temperature condition for optimum activity is not available. The temperature-induced phase change of membrane lipids is reversible till degenerative damage has been caused to the plant (Parkin et al. 1989).

1.3 Alterations in Cell Membrane: Marker for Chilling Stress Injury

The phase transition of cellular membranes from flexible, fluid state to rigidified solid state serves as a marker for detecting chilling-induced injury in plants. Such phase transition is characterized by the appearance of gel-like sites or microdomains in the plane of the lipid bilayer, which are partially or completely protein-free. Multiple membrane changes are detectable in stressed chilling-sensitive plants, viz., decrease in membrane elasticity, reduced compliance, preventing the inclusion of lipids in membrane composition, reduction of fluidity and hence

flexibility of membrane lipids, and inactivation of membrane-bound enzymes, including H^+-ATPase with increased lateral diffusion of phospholipids, sterols, and proteins in the plasma membrane (Kasamo et al. 1992; Kasamo and Noushi 1987).

Membrane functioning under chilling stress is dependent on the membrane lipids (Routaboul et al. 2000). In chilling-sensitive plants, membrane integrity is affected due to chilling-induced degradation of galactolipids and phospholipids, which result in an increased pool of free fatty acids. In stressed plants, a distinct change in molar ratio of sterols is observed, and increase in ratio of sterols/phospholipids resulted in decreased membrane fluidity on lowering of temperature (Whitaker 1993). A marked increase in unsaturated fatty acids, phospholipid accumulation in tissues, and depletion in sterols and sterol esters are physiological manifestations of chilling stress in sensitive species (Kojima et al. 1998; Kaniuga et al. 1999).

Membrane transport is severely affected due to reduced permeability associated with increased viscosity in response to low temperature. Hence, water uptake and sugar translocation were reduced in chilling-sensitive species. Distinct changes are also observed in the membrane proteins exposed to chilling stress (Fig. 1.2). Protein conformation is lost, and the nonprotein components of enzymes are released, resulting in changes in the allosteric control of activity and kinetic parameters. Low temperature-induced enzyme inactivation is also mediated by protein-lipid interactions in the membrane. Molecular ordering of membrane lipids changes due to low

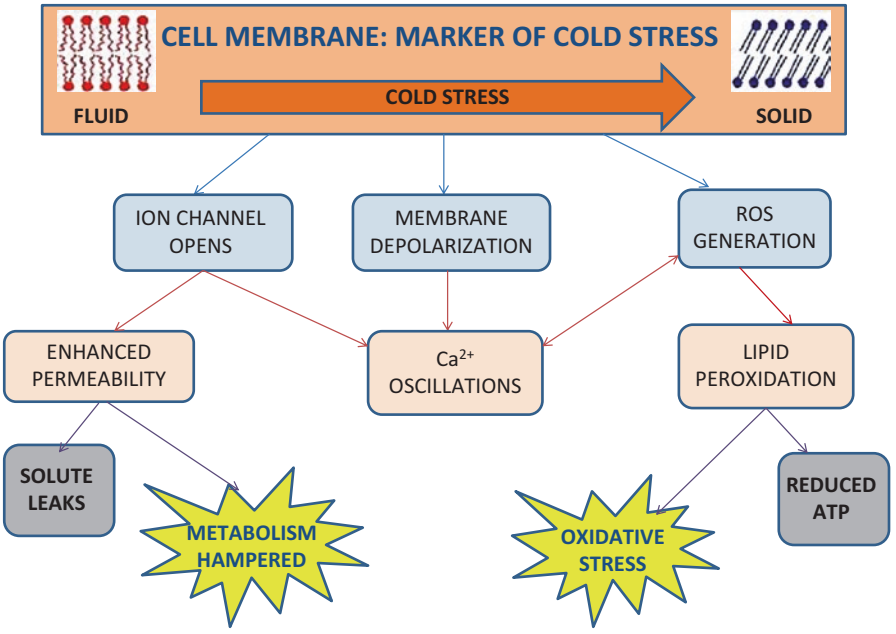

Fig. 1.2 Scheme for initiation of physiological changes on perception of cold stress cues with the plasma membrane serving as the main marker for detection of chilling stress; ROS (reactive oxygen species), ATP (adenosine triphosphate)

temperature exposures. These changes are accompanied by lowered ATP levels and increased membrane permeability. Hence, the membrane is the seat for the detection of chilling-induced injury in plants. Prolonged exposure to chilling stress disrupts membrane integrity and compartmentalization, solute leakage, and increase in the activation energy barrier for membrane-bound enzymes, thus jeopardizing the overall physiological status of the affected species.

1.4 Cold Perception and Downstream Signaling

Environmental cues perceived by the plant result in an intricate network of downstream signaling cascades. Different receptors at the cellular level are involved in receiving the external signals and, in turn, transfer them intracellularly. Plants are sensitive to both magnitude and rate of temperature fluctuations. Thermal responses in plants in the face of cold stress involve a complex intracellular machinery and genetic regulation. There are two principal transcriptional pathways that are activated in response to cold stress, C-repeat (CRT)/dehydration responsive element (DRE)-binding factor (CBF/DREB)-dependent and CBF/DREB-independent. The transcription factor, CBF, acts as a master regulatory player and is induced by the binding of trans-acting factors to the promoter regions of the *CBF* gene (Fowler and Thomashow 2002). The constitutively expressed ICE1 (Inducer of CBF Expression 1) binds to the corresponding cis element on the *CBF* promoter and elicits the ICE1-CBF cold-responsive pathway, which is conserved in diverse plant species (Chinnusamy et al. 2003).

1.4.1 Stress Perception Through Plasma Membrane Rigidification

The physiological responses of plants to stress are variable; however, membrane rigidification is a common response, since rapid fall in temperatures induces membrane to become rigid at microdomains. Signaling pathways involving calcium waves have also been worked out in alfalfa and *Brassica napus*, where cold stress induction led to actin cytoskeletal rearrangement and loss of fluidity of the plasma membrane, activation of Ca^{2+} channels, and, hence, rapid calcium oscillations (Orvar et al. 2000). Increased cytosolic Ca^{2+} levels induce the expression of *cold-responsive (COR)* genes, which can be activated artificially by a membrane rigidifier like dimethyl sulfoxide (DMSO) even at 25°C, while its expression is inhibited by a membrane fluidizer like benzyl alcohol even at 0°C (Sangwan et al. 2001). Ca^{2+} is the ubiquitous second messenger and is a major player in the cold-responsive signaling pathways (Knight et al. 1996), and mechanosensitive calcium channels have been found to be involved in cold acclimation. Intracellular calcium ion

channels implicated in *COR* expression are activated by cyclic ADP-ribose and inositol-1, 4, 5-triphosphate (IP_3). A typical Ca^{2+}-responsive signaling pathway consists of Ca^{2+}-activated phospholipase C and D (PLC, PLD) which produce IP_3 and phosphatidic acid, respectively, and, in turn, activate IP_3-gated calcium channels.

Rise in intracellular calcium levels can be perceived by calcium-dependent protein kinases (CDPKs) and calmodulins (CAMs) and salt overly-sensitive 3-like (SOS3-like) or calcineurin B-like (CBL) proteins. That CDPKs play a functional role in cold stress signaling was proved through a transient expression system in maize leaf protoplasts where a constitutively active form of an *Arabidopsis* CDPK (CDPK1) activated the expression of abscisic acid (ABA)-responsive promoter of *HVA1* gene (Sheen 1998). Hence, CDPKs were proved to have a positive role in mediating cold signaling; however, CAMs, CBLs, and SOS3-like proteins are negative regulators of such signaling cascades.

1.4.2 The CBF-COR Regulon: Transcriptional Machinery

Cold stress response is mediated by a gene regulatory network in which the CBFs are critical transcription factors, as they are involved in the control of the *COLD-REGULATED (COR)* genes through the CBF-COR regulon (Thomashow 1999). CBFs are also involved in the drought and salinity stress-responsive pathways, thereby proving that there exists an intricate cross-talk mechanism between the different forms of abiotic stress. The CBFs belong to the APETALA/ethylene response element-binding protein (AP2/EREBP) transcription factor family (Stockinger et al. 1997) and are modulated by upstream regulators like inducer of CBF expression 1 (ICE1), *high expression of osmotically responsive 1* (*HOS1*) gene, and MYB15 (Agarwal et al. 2006). ICE1 is a constitutively expressed myc-like bHLH (basic helix-loop-helix) transcription factor, which binds to *CBF3* gene promoter, inducing its expression, and is degraded via the ubiquitin-proteasomal pathway through the cold signaling attenuator HOS1, an E3 ubiquitin ligase (Dong et al. 2006).

The promoter regions of *COR* genes consist of one or multiple copies of the C-repeat/DRE with the highly conserved CCGAC core sequence. The CBFs or DREBs control ABA-independent expression of *COR* genes in response to cold stress, which indicates that ABA may be able to potentiate cold-induced CBF signaling, but ABA and cold stimuli may not be concurrent. CBF-DREB1 is involved in transcriptional response to cold as well as osmotic stress-regulated genes, whereas CBF/DREB2 is exclusively responsive to cold stress, and not to salinity or osmotic stress conditions, and is controlled by ICE1 transcription factor. This further provides an insight into the cross-talk of abiotic stress-responsive signaling pathways. Microarray analysis of CBF-overexpressing transgenic plants identified several CBF target genes involved in signaling, transcription, osmolyte biosynthesis, reactive oxygen species (ROS) detoxification, membrane transport, hormone metabolism, and stress response and can sufficiently induce cold tolerance in diverse plant species, e.g., *AtCBF1* of tomato enhanced oxidative stress tolerance under chilling

stress as well as enhanced tolerance to dehydration stress, whereas *AtCBF3* of rice resulted in enhanced tolerance to drought and high salt, together with a marginal increase in chilling tolerance.

The coordination of regulation of cold tolerance and plant development is regulated by signaling hormones like ABA, gibberellic acid (GA), and auxin (Lee et al. 2010). During cold stress, growth retardation appears to be regulated by CBFs through DELLA proteins, which are localized in the nucleus and represses growth in *Arabidopsis*, whereas GA-stimulated degradation of DELLA leads to promotion of growth enhanced by CBFs (Achard et al. 2008). ICE1, which regulates *CBF* expression, is constitutively expressed; however, only exposure to cold temperature leads to ICE1-induced expression of *CBF* and other cold stress-responsive genes. Overexpression of HOS1, a negative regulator of cold-responsive pathways, leads to substantial decline in ICE1 protein and its target genes, since HOS1 targets ICE1 to proteasomal degradation machinery via the ubiquitin pathway, and this leads to hypersensitivity to cold stress. The role of ICE1 in regulating photosynthesis and transpiration through stomatal development has also been investigated, and this establishes a bridge between stomatal development and responsiveness to environmental stress signals. ICE1 physically interacts and dimerizes with bHLH transcription factors like SPEECHLESS (SPCH), MUTE, and FAMA, thus regulating stomatal development (Kanaoka et al. 2008). ICE1 also negatively regulates expression of MYB15, which itself is a negative player in cold stress-responsive pathways in *Arabidopsis*. Overexpression of *MYB15* leads to reduced expression of *CBFs*, whereas *myb15* T-DNA knockout mutants showed enhanced cold induction of *CBFs* (Agarwal et al. 2006). Thus, regulation of CBF expression is dependent on the interaction between the positive and negative regulators of cold stress-responsive pathways.

Calmodulin-binding transcription activator (CAMTA) family proteins have also been studied as transcriptional regulators of CBF2 expression, which is proved through mutational studies involving *camta3* mutants, which, under cold stress, showed reduced levels of CBF2 in comparison to the wild-type plants. Further, double mutants of *camta/camta3* exhibit hypersensitivity to freezing stress in comparison to wild-type plants, establishing the role of CAMTA proteins in activating *CBF2* expression (Doherty et al. 2009).

1.4.3 CBF-Independent Regulons

Genetic screening led to the identification of transcription factors that mediate cold-responsive pathways and, subsequently, are responsible for conferring cold tolerance in *Arabidopsis*. These transcription factors include high expression of HOS9, a homeodomain protein, and HOS10, an R2R3-type MYB (myc-like basic helix-loop-helix), and transcriptome analysis showed distinct CBF and HOS9 regulons (Fig. 1.3). HOS10 acts through an ABA-dependent cold-responsive pathway, since it is responsible for the activation of ABA biosynthesis gene *NCED3* (9-cis-epoxycarotenoid dioxygenase) leading to ABA accumulation during cold stress (Zhu et al. 2004).

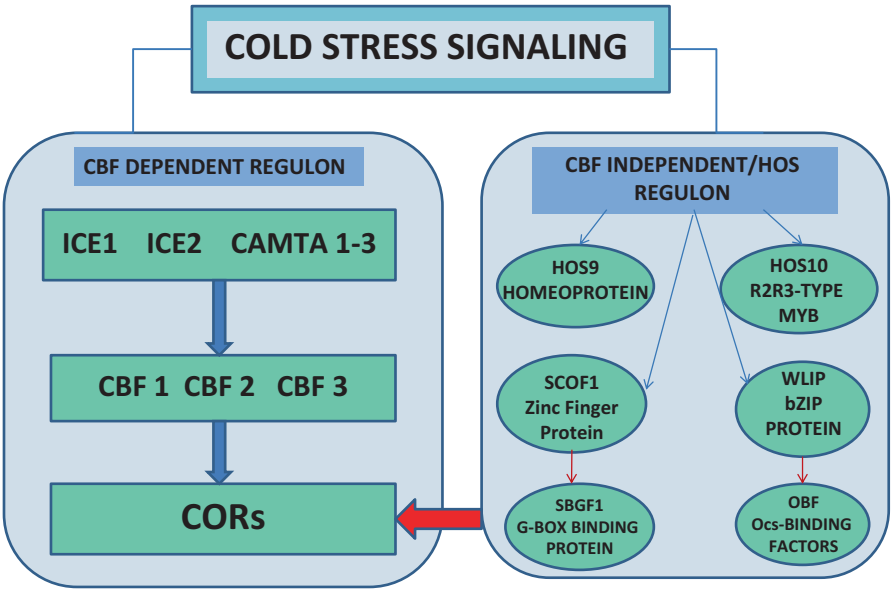

Fig. 1.3 The cold stress-responsive transcriptome showing control through CBF-dependent and CBF-independent regulons; CBF C-repeat/drought-responsive element-binding factor, ICE inducer of CBF expression, CAMTA calmodulin-binding transcription activator, COR cold responsive, HOS high expression of osmotically responsive genes, MYB myc-like beta helix-loop-helix, SCOF soybean cold-inducible factor, OBF ocs element-binding factor, WLIP wheat low temperature-induced protein

In response to freezing, the expression of *COR* genes in *Arabidopsis* transgenics was upregulated due to constitutive overexpression of soybean C2H2-type zinc finger protein, soybean cold activation factor (SCOF1), which, in turn, interacts with the conserved soybean G-box-binding factor 1 (SGBF1) (Kim et al. 2001). Another example of overlapping abiotic stress-responsive pathways is given by wheat low temperature-induced protein 19 (WLIP19), a basic leucine zipper protein (bZIP), which is induced by cold, drought, and ABA. WPLIP19 is involved in the activation of expression of *COR* genes in wheat by physically interacting and dimerizing with bZIP transcription factor TaOBF1in wheat (ocs element-binding factor 1 in *Triticum aestivum*). These observations altogether present examples of CBF-independent regulons involved in cold stress response.

1.5 Metabolic Changes in Cold Stress-Injured Plants

In cold-sensitive species, exposed to low temperature conditions, cytoplasmic gelling occurs due to increase in viscosity of the cytoplasm. This leads to impediment of biochemical reactions in the membrane, hence affecting the metabolic health of the plant. Prolonged exposure to cold stress leads to metabolic disorders, and

normal physiological processes of the plant are disturbed. One of the probable causes of metabolic changes is the uncoupling between energy obtained during respiration and its effective utilization. Chilling injury may also result in accumulation of toxic end products due to metabolic imbalance, which ensues after the temperature rises to normalcy, indicating that this is a secondary dysfunction associated with heating under post chilling conditions. Cold-induced metabolic changes can be simulated by ectopically expressing *CBF* genes under warm conditions, thereby proving that the CBF-dependent regulon has a key role in reconfiguration of the low temperature metabolome. This is consistent with the results from *Arabidopsis*, where the strain (Cape Verde Islands-1 ecotype) having a weak regulon and being deficient in CBF-regulated metabolites was incapable of cold acclimation (Cook et al. 2004).

The soluble sugars act as important signaling molecules during cold stress (Rolland et al. 2006). Compartmentalization of sugars is an important aspect of plant response to cold stress. In cereals exposed to freezing stress, fructans stored in vacuole are mobilized to fructose and then exported to the intracellular liquid, where it interferes with the adhesion between wet plant surface and extracellular ice. Hence, extracellular ice cannot invade into the living cell. In *Arabidopsis*, differential distribution of respiratory metabolites takes place during cold acclimation (Talts et al. 2004). A slight decrease in lipid hydrolysis has been reported in chilling-sensitive fruit tree like papaya, with concomitant increase in soluble solids. Cold-injured roots of sweet potato are unable to synthesize carotenoid pigments, and accelerated depletion of ascorbic acid has been reported in not only sweet potato but also cold-injured banana and pineapple. Dark coloration of fruit pulp, which is symptomatic of cold injury, has been observed in chilled banana due to accumulation of tannin and its oxidized products, as well as elevated levels of dihydroxyphenylalanine (DOPA) and tyrosine, whose polymerization and oxidation products result in dark pigmentation. Chilled sweet potato roots also showed a marked increase in chlorogenic acid. Similarly, pepper seeds also had higher levels of chlorogenic acid and total polyphenols.

Protein metabolome is also severely affected under cold stress. An active aspartate shuttle has been evidenced to play an important role in cold acclimation, in which pools of amino acids like asparagine, glutamine, and glutamate shift from the plastid to cytosol (Hoermiller et al. 2016). The experiments involved two distinct mutants in primary carbon metabolism (*pgm*, the starchless mutant of the plastidial phosphor glucomutase, and *spsa1*, a mutant defective in the dominant sucrose phosphate synthase), which demonstrate that inhibition of primary carbon metabolism limits the ability of the plants to respond to cold stress and also alter the patterns of intracellular allocation of metabolites in response to an imposed stress. The protein metabolome undergoes dramatic changes during cold stress response, since stress induces accumulation of soluble proteins to facilitate acclimation and tolerance. The level of high molecular weight glycoproteins also increase, as has been reported in mulberry bark cells and orchard grass tissue. Cold-induced cryoprotective proteins have been isolated from spinach chloroplast, and their nuclei also contained elevated levels of high molecular weight proteins at low temperature conditions,

whereas low molecular weight proteins are predominantly present under warmer conditions (Guy 1990). Cold stress response and acclimation leads to the activation of genes that can impart tolerance, and these genes are usually not expressed under normal conditions. Thus, synthesis of proteins that contribute to acclimation is increased in response to cold stress, and this was established through the isolation of translation competent polysomes from cold-acclimated tissues as well as through reported increase in rRNA and RNA polymerase activity during cold stress (Sarhan et al. 1997). Housekeeping proteins continue to be synthesized under low temperature conditions to maintain basal level of cellular metabolism. The ability to synthesize proteins faster in acclimated tissues is attributed to increased rRNA levels and protein synthesis capacity. However, it is to be noted that the set of proteins synthesized in response to low temperature stress is not as highly conserved as heat shock proteins, pointing toward the diversity of cold stress conditions and variability in stress response (Sakai and Larcher 1987). The changes in protein metabolism inevitably include altered activity of enzymes, especially those that are associated with the membrane. High molecular weight proteins tend to replace their smaller, low molecular weight counterparts during cold stress response, since they are more efficient considering kinetic parameters and K_m values and hence can impart tolerance. One such example is the invertase enzyme from wheat which shifts from lower to higher molecular weight forms during acclimation to low temperature. Increase in enzyme levels coincided with the increase in soluble protein content under low temperature conditions. Increase in activity of enzymes associated with respiratory pathways, such as glucose-6-phosphate dehydrogenase, lactate and isocitrate dehydrogenase are characteristic of cold-induced regulation of enzymes. Isozymic and conformational alterations in enzymes under cold stress exposure render them cryostable. In winter, new isozymic variants were reported for ATPases, esterases, acid phosphatases, leucine aminopeptidases, and peroxidases, and this winter-specific enzyme machinery contributed to freeze stability under adverse winter conditions.

Supramolecular interactions are also changed on exposure to low temperature, as evidenced by the oligomerization of light-harvesting chlorophyll *a/b* protein of Photosystem II and chlorophyll *a*-protein complex of Photosystem I in rye thylakoids. Studying the lipid profile revealed that there is a specific decrease in levels of *trans*-Δ^3-hexadecenoic acid of phosphatidyldiacylglycerol in thylakoid membranes due to the cold-induced oligomerization of light-harvesting complex (Krupa et al. 1987).

The cold-induced expression of distinct *GST* genes encoding glutathione S-transferase superfamily proteins, which contribute to cold tolerance, has been investigated in *Brassica oleracea* (Vijayakumar et al. 2016). Secondary metabolites also tend to accumulate in response to cold stress, along with elevated levels of sugar (Livingston et al. 2009), polyamines (Kovacs et al. 2010), anthocyanins (Christie et al. 1994), and glucosinolates (Baskar et al. 2012). In cold-injured tissues, activity of enzymes like glutathione S-transferase (GST), glutathione reductase (GR), superoxide dismutase (SOD), and glutathione peroxidase (GPX) increased manifold, along with elevated levels of nonenzymatic antioxidants like tripeptide thiols and vitamins (Janska et al. 2010), signifying that all these enzyme proteins are functionally important for cold tolerance.

1.6 Cytophysiological Changes Due to Cold Injury

Cell membrane damage and loss of compartmentalization are primary markers for cold-induced injury in plants. Besides cell membrane vesicularization, rupture of plasmalemma, destruction of endoplasmic reticulum, and alterations in Golgi body are also reported as a consequence of cold stress. Ultrastructure of mitochondria is also affected, leading to swelling and degeneration, matrix enlightenment, shortening of cristae, and decrease in their number, inevitably affecting oxidative phosphorylation (Yin et al. 2010). Prolamellar plastids were improperly formed, causing swelling and structural changes in chloroplast membranes. Thus, mitochondrial and chloroplast damage hampers both the photosynthetic and respiratory machineries. In the next sections, we probe into the physiological effects of cold injury and stress-induced alteration in photosynthetic efficiency and respiration, which eventually weakens the cold-susceptible plants physiologically.

1.6.1 Photosynthesis

The decline in the rate of photosynthesis, associated with cold stress, is related to fall in temperature and prolonged exposure to low temperature, and this effect persists long after the chilling-sensitive plants have been transferred to warmer conditions post chilling (Liu et al. 2004; Strauss et al. 2006). The loss of photosynthetic efficiency in cold-injured plants can be attributed to inhibition of solute (carbohydrate) transport from leaves via phloem; stomatal limitation; damaged photosynthetic machinery, especially the light-harvesting complex associated with Photosystem I (PSI); inhibition of electron transport; uncoupling of electron transfer and energy conservation; and finally loss of activity of key enzymes of the Calvin cycle and C_4-pathway. Light chilling (chilling of sensitive plants in presence of light) has more damaging impact than chilling in dark since the photosynthetic apparatus is affected mainly due to photoinhibition and photooxidation as a result of excess excitation energy sequestered from the photosynthetic machinery. Photoinhibition or decline in photosynthetic efficiency under excessive illumination and chilling is directly proportional with light intensity and fall in temperature.

Photooxidative damage incurred to photosystems housed in the chloroplast membranes is manifested by increased lipid peroxidation and degeneration of photosynthetic pigments like chlorophyll, carotene, and xanthophylls.

The effects on photosynthesis in cold-injured *Hibiscus* plants have been investigated (Paredes and Quiles 2015). The study involved chilling stressed illuminated plants of *Hibiscus rosa-sinensis*, and the involvement of chlororespiratory enzymes and ferredoxin-mediated cyclic electron flow was dissected in these cold-susceptible plants, where cold stress resulted in reduction of efficiency of photosynthesis and subsequently, electron transport. This study revealed the role of cyclic electron flow in protecting the photosystems during cold stress, as established by the increased

activity of electron donation by NADPH and ferredoxin to plastoquinone and elevated levels of PGR5 polypeptide which is an essential component of cyclic electron flow around PSI. Cold stress response also involved increase in levels of chlororespiratory enzymes NDH (NADPH dehydrogenase) complex and PTOX (plastid terminal oxidase). The study of photosynthetic potential in cold-stressed *Rhododendron chrysanthemum* (Zhou et al. 2017) revealed that cold stress could lead to a significant reduction in electron transport rate of Photosystem II (PSII), accompanied with an increase in excitation pressure (1-qP, where qP refers to the photochemical quenching). Photochemical efficiency of PSII was also affected in Mediterranean *Citrus albidus* L. and *Quercus ilex* L. (Oliveira and Penuelas 2005).

1.6.2 Mitochondrial Damage and Respiration

Respiratory rate decline during chilling stress is correlated with mitochondrial damage and lowering of kinetic energy and inhibition of respiratory enzymes. Decreased respiration coupled with increased utilization of energy-rich phosphates at chilling temperatures leads to decline in ATP levels. It has also been observed that cold stress shifts respiration to alternate pathways from the cytochrome path of electron transport in seedlings (Ribas-Carbo et al. 2000), since these accessory pathways functionally contribute to cold acclimation by reducing superoxide and reactive oxygen species (ROS) generation in mitochondria (Hu et al. 2010).

The mitochondrial respiratory chain in *Arabidopsis* has been studied under cold stress (Golzalez et al. 2007) which revealed a coordinated, tissue and developmentally dependent response of mitochondrial complex components encoded by the nuclear genome. Chilling leads to pronounced breakdown of mitochondrial proteins, mimicking protein degradation under drought stress, and often, modified or damaged versions of mitochondrial proteins (oxidized or S-nitrosylated forms) are produced abundantly under cold stress (Taylor et al. 2011). Proline is a key player in temperature stress recovery, where it serves as nitrogen and energy source, as well as a compatible osmolyte and effective ROS scavenger. Proline turnover takes place in the mitochondria where it is imported and converted into glutamate via the Pro/P5C cycle by proline dehydrogenase (PDH) and $^{1}\Delta$-pyrroline-5-carboxylate dehydrogenase (P5CDH); however, under temperature stress, the cycle may occur reciprocally as established by the findings that free proline accumulation in cold-treated cauliflower (*Brassica oleracea* var. *botrytis*) and PDH mutant plants impart frost resistance under cold stress (Hadi et al. 2011). Morphological changes in mitochondria under cold stress has been studied in chilled mung bean (*Vigna radiata*) suspension cells and cucumber root tips where extensive mitochondrial swelling during cold recovery was accompanied by appearance of internal translucence and vesicular structures (Lee et al. 2002). Aberrant ring-shaped mitochondria in chilled *Arabidopsis* mesophyll cells (Vella et al. 2012) and disorganized bursting mitochondria in recovering *Episcia reptans* (Murphy and Wilson 1981) were

reported in association with chilling injury. Respiratory declines due to oxidative damage in mitochondria coincide with upregulation of rescue pathways mediated by alternative oxidase (AOX) (Sugie et al. 2006), suggesting importance of AOX in cold acclimation (Fiorani et al. 2005; Armstrong et al. 2008). The redox homeostasis under cold stress is maintained by cold-responsive uncoupling protein 1 (UCP1) in *Arabidopsis* which facilitates photosynthesis under stress conditions by smooth running of the electron transport chain (Sweetlove et al. 2006), while other uncoupling proteins like plant uncoupling mitochondrial proteins 1, 4, and 5 (PUMP 1, 4, and 5) and cold shock proteins like CSP310 of wheat also play important roles (Kolesnichenko et al. 2002).

Accumulation of thermostable dehydrins in response to freezing and cold stress has also been reported in mitochondria of cereal crops like wheat and rye (Borovskii et al. 2002), whose function is to stabilize mitochondrial membrane and matrix proteins. Respiratory rate decline could also be associated with reduced capacity for respiratory NADH oxidation under cold stress, as has been investigated in leaves of *Solanum tuberosum* L., cv. Desiree (Svensson et al. 2002). This decrease is accompanied by reduced levels of NDA proteins (*nda* genes encode non-proton pumping respiratory chain NADH dehydrogenases) and internal rotenone-insensitive NADH oxidation, as has been observed in mitochondria isolated from cold-treated plants. These proteins are major players in flexible tuning of redox homeostasis in cytosol and matrix, in response to ATP demand; hence, reduced expression of *nda* genes during cold stress could possibly lead to respiratory energy dissipation. The ROS generated in response to cold stress lead to activation of antioxidant system in plants as has been discussed earlier. In order to scavenge ROS and protect plants from oxidative damage, plants have evolved efficient antioxidant systems which can be categorized as antioxidant enzymes; lipid-soluble, membrane-associated antioxidants like α-tocopherol, β-carotene, and ubiquinone; and water-soluble antioxidants like reduced glutathione (GSH) and ascorbate via ascorbate-glutathione cycle. Antioxidant response to cold stress has been thoroughly investigated in oil plants *Jatropha curcas* and *Jatropha macrocarpa* (Spano et al. 2017), in which antioxidant enzymes like ascorbate peroxidase, glutathione peroxidase, guaiacol peroxidase, and catalase contributed significantly to ROS scavenging and cold acclimation during cold-induced oxidative damages.

1.7 Interplay of Phytohormones during Cold Stress

Hormonal interplay during chilling stress involves intricate cross-talk between signaling pathways via the major phytohormones that play functionally distinct roles in mediating cold-responsive signaling. This signaling network integrates external stress cues into endogenous developmental programs, thus initiating an appropriate response for alleviation of the stress.

1.7.1 Gibberellin (GA)

GA is such a phytohormone whose signaling is regulated centrally by DELLA which is GRAS protein, whose five members have been characterized in *Arabidopsis*, viz., GIBBERELIC ACID INSENSITIVE (GAI), REPRESSOR OF GAI (RGA), and RGA-LIKE 1, 2, and 3 (RGL1, RGL2, and RGL3). Cold-induced expression of GA 2-oxidases leads to increased hydroxylation and inactivation of bioactive GA which could be correlated to suppressed growth and delayed flowering due to stress conditions. *CBF1* overexpression during cold stress response enhanced DELLA accumulation through transcriptional regulation of RGL3 (Achard et al. 2008). Furthermore, to establish that absence of GA and promotion of freezing tolerance coincide, mutational analysis was carried out, and it was shown that *Arabidopsis gai* mutant (a constitutive signaling mutant) was tolerant to freezing, whereas DELLA knockout lines *gai-t6* and *rga-24* were hypersensitive to freezing.

1.7.2 Brassinosteroid (BR)

BRs are plant polyhydroxysteroids which play an essential role in various developmental and physiological processes such as stem elongation, root growth, vascular differentiation, leaf epinasty, and reproduction (Fujioka and Yokota 2003; Kim and Wang 2010; Sasse 2003). The role of BRs in alleviating chilling stress has been extensively investigated in chilling-sensitive crops such as maize and cucumber (*Cucumis sativus*). That BRs functionally contribute to freezing tolerance is indicated by enhanced expression of *CBFs* and *CBF* target *COR47* in *Arabidopsis* upon exogenous application of BR following chilling, which successfully imparted cold tolerance. Regulation of antioxidant enzymes during cold storage by BR has also established the functional significance of BR in imparting cold stress tolerance in Washington Navel Orange (*Citrus sinensis* L.) (Ghorbani and Pakkish 2014).

1.7.3 Cytokinin (CK)

CKs are adenine derivatives with isoprenoid or aromatic side chains that control directional growth processes like gravitropism and are also involved in control of both CBF-dependent and CBF-independent regulons during cold stress response. *CYTOKININ RESPONSE FACTOR (CRF2)* and *CRF3* encoding APETALA2 transcription factors regulate *Arabidopsis* lateral root (LR) initiation when subjected to low temperature conditions (Jeon et al. 2016). Similarly, *CRF4* is cold inducible and imparts freezing tolerance as has been investigated in *Arabidopsis*. Analysis of the cold transcriptome reveals that cytokinin and a subset of two component signaling

system are involved in cold stress signaling and response which comprise histidine kinases, phosphotransfer proteins, and response regulators.

1.7.4 Abscisic Acid (ABA)

Abscisic acid is an isoprenoid hormone which has been christened the "universal stress hormone" since it is a key player in the response pathways to a plethora of abiotic stress conditions, besides its role in mediating seed dormancy and abscission (Nakashima et al. 2009). Its role in cold stress signaling has been investigated, particularly with respect to its regulation through the CBF-dependent pathway. A cold- and ABA-inducible transcription factor, MYB96, has been studied in *Arabidopsis* (Lee et al. 2015) which reveals that this transcription factor is involved in *CBF* induction and cold acclimation, which was compromised in *myb96* knockout lines, whereas cold-responsive pathways were upregulated by MYB96 overexpression. Moreover, MYB96 indirectly interacts with cold-responsive transcription factors which regulate *CBF* expression by binding to HEPTAHELICAL PROTEIN (HHP), HHP1, HHP2, and HHP3, which physically interact with ICE1, CAMTA, and ICE2, respectively (Chen et al. 2010).

Another regulator of *CBF* expression is the cold-inducible transcription factor Open Stomata 1 (OST1), a serine/threonine kinase, which is activated by ABA, and its role in cold stress response has been established by hypersensitivity to freezing in *ost1* knockout lines, whereas increased freezing resistance was achieved through overexpression. Hence, ABA ubiquitously participates in abiotic stress signaling and has a major role in stress tolerance in stress-injured plants.

1.7.5 Ethylene

The gaseous phytohormone, ethylene regulates many aspects of plant life cycle such as seed germination, leaf senescence, fruit ripening, and abscission, and its role in cold stress response has also been studied. However, the level of ethylene in response to cold stress was found to be variable from one species to the other, as evidenced by increased levels (through enhanced expression of ethylene biosynthetic genes) in cold-stressed *Arabidopsis* (Catala et al. 2014), in contrast to rapid decline in cold-treated *Medicago truncatula* (through reduction of ethylene precursor 1-aminocyclopropane-1-carboxylic acid or ACC) (Zhao et al. 2014). The role of ethylene as a negative regulator of cold-responsive signaling is indicated by studies in *M. truncatula* where administering ethylene biosynthesis inhibitor 2-aminoethoxyvinyl glycine (AVG) enhanced freezing tolerance post cold treatment, whereas hypersensitivity to freezing was reported on treatment with ethylene releaser ethephon, correlated with a compromised induction of expression of *MtCBF1*, *MtCBF3*, and *MtCAS15*, which belong to *COLD ACCLIMATION SPECIFIC* (*CAS*) gene family

equivalent to *COR* genes (Zhao et al. 2014). However, the positive role of ethylene in *Arabidopsis* freezing tolerance disputes this exclusivity of negative role of ethylene, as proved through mutational studies where ACS octuple mutant with extremely low levels of ethylene was hypersensitive to freezing (Catala et al. 2014). Another study on grapevine reported the cold-induced enhanced release of ethylene through modulation of expression of ETHYLENE RESPONSE FACTOR (ETR) 057, and the evidence was supported by enhanced cold tolerance on exogenous ACC application and reduced resistance to cold on treatment with aminoethoxyvinylglycine (AVG). Hence, though the role of ethylene in cold response remains controversial, it can be considered an important candidate regulator of cold stress signaling in susceptible plants.

1.7.6 Auxin

Biologically active auxins are indole acetic acid (IAA) and indole butyric acid (IBA) which essentially control all aspects of plant development, from embryogenesis to senescence, and are key players in intricate hormonal cross-talks influencing various developmental stages. The role of auxin in cold stress response is not clearly dissected; however, cold stress-mediated interruption of auxin transport has been established through analysis of auxin response and molecular mechanisms in *Arabidopsis*, where auxin signaling mutants *axr1* and *tir showed* reduced gravity response but normal response to cold treatment (equivalent to wild type). This indicated that stress affects auxin transport, without altering signaling (Shibasaki et al. 2009). Moreover, exogenous application of auxin analogues on canola (*Brassica napus*) stimulated accumulation of cryoprotective metabolites and soluble sugars, aiding in acclimation. Auxin influx and efflux carriers that determine spatial gradients of auxin concentration also influence auxin-regulated directional gravitropic growth. Such gravitropic growth is temperature-sensitive, as proved by its inhibition in *Arabidopsis* due to cold, correlated with altered distribution patterns of auxin in roots and repressed basipetal auxin transport caused by impaired intracellular cycling of auxin efflux carriers PIN2 and PIN3 (Shibasaki et al. 2009). Another interesting observation came from the study of SIZ1, a central regulatory component of cold signaling pathway which stabilizes ICE1 under cold conditions, by repressing its polyubiquitination (Miura et al. 2007), and negatively regulates phosphate starvation-induced root architecture remodeling through control of auxin patterning (Miura et al. 2011).

1.7.7 Melatonin

Melatonin (N-acetyl-5-methoxytryptamine), besides being an animal neurohormone, is also a regulator of plant developmental and stress-responsive pathways. The role of melatonin in alleviating cold stress has been investigated in Bermuda

grass (*Cynodon dactylon* L. Pers.), where exogenous application of melatonin prevented cold damage to Photosystem II through activation of multiple antioxidants and accumulation of freeze-protective secondary metabolites, amino acids, organic acids, soluble sugars, and sugar alcohols (Fan et al. 2015), besides maintaining membrane stability, improving process of Photosystem II, and inducing cold-responsive metabolic changes. Oxidative damage in wheat was also alleviated by application of melatonin to seedlings that enhanced the activities of superoxide dismutase, guaiacol peroxidase and ascorbate peroxidase, and other ROS scavengers. Cold stress tolerance by melatonin treatment in *Arabidopsis* is attributed to melatonin-mediated upregulation of *CBFs* and DREBs, as well as cold-responsive gene *COR15a*, transcriptional activator *CAMTA1*, and antioxidant genes *ZAT10* and *ZAT12*, following cold stress (Bajwa et al. 2013).

1.7.8 Salicylic Acid (SA)

SA is a phenolic compound, which is predominantly involved in triggering defense against biotic stress factors and pathogen hypersensitive response (HR). However, its role in abiotic stress signaling has also been studied, and it has been reported that SA accumulation and cold-induced growth suppression could be correlated, since SA-overaccumulating *constitutive expression of PR* (*cpr1*) mutant plants showed retarded growth under cold conditions, whereas SA deficiency led to cell cycle progression (G1/S phase transition) through enhanced transcription of cytokinin-regulated D-type cyclin CYCD3 in *Arabidopsis*. In chilling sensitive cucumber, SA was proposed to activate *CBF* expression, as established by the application of SA biosynthesis inhibitor, L-α-aminooxy-β-phenylpropionic acid (AOPP), which suppressed the expression of *CBFs* and *COR47* in cold-treated seedlings (Dong et al. 2014). SA-induced chilling and oxidative stress tolerance can also be correlated to gibberellin homeostasis, as studied in cold-stored tomato, through C-repeat/DREB factor pathway and antioxidant enzyme system (Ding et al. 2015). Foliar application SA also influenced the redox status of canola (*B. napus* L.) through activation of antioxidant enzymes during cold stress exposure.

1.7.9 Jasmonic Acid (JA)

JA is an oxylipin whose levels increase under cold stress, as has been reported in different plant species like rice, wheat, and *Arabidopsis* through increased expression of JA biosynthetic genes and repression of genes encoding JA catabolic enzymes. Exogenous application of JA imparts cold tolerance in *Arabidopsis* through enhanced *CBF* expression, whereas compromise in JA biosynthesis leads to hypersensitivity to freezing (Hu et al. 2013). Altered freezing tolerance is mediated by JASMONATE ZIM-DOMAIN (JAZ) proteins, JAZ1 and JAZ4, which

physically interact with ICE1 and ICE2, and JA-mediated cold-induced growth inhibition occurred through stabilization of DELLA proteins (Yang et al. 2012).

1.8 Freezing Stress Tolerance: Natural Mechanism and Engineering

1.8.1 Natural Mechanism: Dormancy and Vernalization

1.8.1.1 Dormancy and Cold Stress

Endodormancy is a distinct physiological process that is sensitive to, but does not need, cold or other external conditions to be induced (Faust et al. 1991), i.e., it is controlled by both internal factors and seasonal fluctuations in temperature and photoperiod. Hence, cold stress and its consequent acclimation can be closely associated with endodormancy. Endodormancy can result either in absence of cold or due to intermittent cold conditions, whereas continuous above freezing temperature results in endodormancy release. During chilling conditions of late autumn and early winter, potential accumulation of FT (Flowering Locus T) results (Lin et al. 2007) which influences the timing of endodormancy release and bud flush. FT is a mobile protein that travels from leaves to shoot apices, and hence, the formation of callose plugs in autumn inhibits their translocation. Such plugs are removed during freezing winter temperature through the activation of gibberellic acid-responsive genes that regulate GH17 proteins which break down callose, and hence FT translocation and accumulation can ensue (Rinne et al. 2001). Hence, endodormancy can be considered as one of the survival strategies of cold stressed plants in the temperate regions, and its key regulator *FT* has evolved from lineage-specific duplications. Furthermore, the functional role of bud set timing synchronized with endodormancy release is being investigated to determine how plants respond to selective environmental pressures, by combining genomics and functional and developmental approaches and correlating ecological mechanisms and phenotypic adaptations to adverse environmental conditions (Rohde and Bhalerao 2007).

1.8.1.2 Vernalization, Flowering, and Freezing Tolerance

When an extended period of cold makes plants competent for flowering, it is referred to as vernalization. Unlike endodormancy, shoot apices of vernalized plants maintain a basal level of mitotic division for supporting the vegetative condition. In *A. thaliana*, there is evidence of epigenetic control of vernalization responsiveness. The flowering repressor gene *FLC* and its five *MADS AFFECTING FLOWERING* (*MAF*) paralogs are epigenetically silenced by the Plant HomeoDomain-Polycomb Repressive Complex 2 (PHD-PRC2), which initiates trimethylation of histone 2 lysine 27 (H3K27me3) (Ratcliffe et al. 2003). This

complex becomes progressively localized to the first intron of *FLC* during cold exposure (Shindo et al. 2005; Angel et al. 2011; Strange et al. 2011) and initiates two noncoding transcripts from this intron [COOL-ASSISTED INTRONIC NONCODING RNA (COLDAIR)] and 3′-UTR (COOLAIR) which negatively regulate *FLC* transcription (Heo and Sung 2011). This consequently results in the *FT*-regulated change from vegetative to inflorescence identity, induction of MADS-box genes such as *FRUITFULL (FUL*) and *APETALA1 (AP1*), floral development, formation of siliques, and subsequently seeds.

Vernalization in crop species, wheat and barley (Pooideae subfamily), has also been investigated where the responsiveness is guided by the three loci, *VERNALIZATION1 (VRN1)*, *VRN2*, and *VRN3*. *VRN1* is homologous to the *Arabidopsis* floral development genes *AP1*, *CAULIFLOWER (CAL*), and *FUL*, and its expression is under epigenetic control through chromatin remodeling. Transcription factors interacting with the cis elements of the promoter regions of the *VRN1* gene mediate chromatin modifications that repress its expression prior to winter, whereas its long-day induction is prevented by the zinc finger *CO*-like gene *VRN2* (Alonso-Peral et al. 2011). Vernalization can be considered an accelerating factor for floral development and shortening of the juvenile or vegetative phase in plants. In winter cereals, the timing of vernalization saturation, deacclimation, downregulation of cold-induced genes, and reduced reacclimation potential are regulated by the *VRN1* locus, which indicate that the probability of freezing damage after warm season depends both on vernalization and photoperiod. This subsequently led to the investigation of the role of vernalization and the *VRN1* gene on freezing tolerance (Ergon et al. 2016). Thus, we can conclude that freezing tolerance, vernalization, and the onset of flowering are interlinked, and the genetic regulation shows overlapping activities of the key players in manipulating the plants to undergo flowering by overriding the stress cues.

1.8.1.3 Engineering Cold Stress Tolerance Through Transgenic Approach

In transgenic approach, several key regulatory players of cold stress-responsive pathways can be selected as candidates for manipulation and upregulation of cold-tolerant genes that can alleviate the damaging effects of low temperature injury in plants (Wani et al. 2008). The *CBF* genes are the key regulatory elements in cold-responsive signaling pathways and hence serve as potential targets of genetic manipulation to engineer stress tolerant plants. Transgenic *Arabidopsis* plants overexpressing *CBF1* showed freezing tolerance while avoiding the negative impact of cold stress on development and growth characteristics. Such overexpression also activates *COR* homologous genes in *Arabidopsis*. Constitutive overexpression of cold inducible transcription factors like CBF1 has been shown to impart cold stress tolerance, through introduction of *CBF1* cDNA into chilling-sensitive tomato (*Solanum lycopersicum*) under the control of strong CaMV35S promoter (Hsieh et al. 2002). Another candidate target is the *CBF4*, a close *CBF/DREB*1 homolog, whose overexpression alleviated both freezing and drought stress in *Arabidopsis.*

Arabidopsis DREB1A overexpression played the dual role of alleviation of damaging impact of both drought and low temperature stress in tobacco, and regulation of transgene expression through stress-inducible *RD29A* promoter minimized the cold-induced inhibition of plant growth (Kasuga et al. 2004). *DREB1A* gene and its two homologs were induced by low temperature stress, whereas expression of *DREB2A* and its homolog was observed in response to drought stress cues. Hence, DREBs present examples of integration of stress-responsive pathways involving both cold and dehydration cues.

CBFs are negatively regulated by an upstream transcription factor *MYB15* (an *R2R3-MYB* family protein) in *Arabidopsis* (Agarwal et al. 2006) which recognizes cis elements in the promoter of *CBF* called *MYB* recognition elements (*MYBRS*). Since it is a negative regulator of *CBF*, a transgenic approach involving *MYB* mutation resulted in enhanced cold acclimation by efficient expression of *CBFs* during cold stress, whereas *MYB* overexpression reduced freezing tolerance through negative regulation of *CBF*. The CBF regulon also provides the *COR* target for transgenic modification. *COR* gene expression and freezing tolerance in transgenics have been established through studies in which activation of *COR* by a zinc finger protein product of *SCOF-1* enhanced tolerance in soybean (Kim et al. 2001). On the other hand, the CBF-independent regulon presents the example of a protein eskimol (*ESK1* gene product) which, when mutated, resulted in constitutive cold tolerance (Xin and Browse 1998). Generating cold-tolerant transgenic plants through introduction of cold-shock proteins (CSPs) like CspA from *Escherichia coli* and CspB from *Bacillus subtilis* has successfully promoted stress adaptation in multiple species (Castiglioni et al. 2008).

Structural genes sometimes serve as targets for transgenic manipulation if they contribute to stress alleviation and tolerance. Genetically engineered tobacco plants overexpressing chloroplast glycerol-3-phosphate acyltransferase (*GPAT*) gene (which participates in desaturation of phosohatidyl glycerol fatty acid) from squash and *Arabidopsis* accumulate unsaturated fatty acids in the cell wall which enhanced tolerance to cold stress (Murata et al. 1992). Cold-induced expression of gene *TPP* (*trehalose-6-phosphate phosphatase*) is followed by accumulation of trehalose in rice tissues exposed to cold stress (Pramanik and Imai 2005). Hence, cold tolerance has been shown to be achieved through overexpression of *TPS* (*trehalose-6-phosphate synthase*) and *TPP* which enhanced trehalose accumulation in transgenic rice and tobacco. Transgenic *Arabidopsis* expressing plant phosphatase *AtPP2CA* enhanced cold acclimation response during freezing. Constitutive expression of kinase *NPK1* also improved tolerance to chilling as well as to other forms of abiotic stress (Kovtun et al. 2000). Overexpression of rice mitogen-activated protein kinase *OsMAPK5* conferred tolerance to a plethora of abiotic stress conditions including freezing stress, as observed in rice seedlings (Xiong and Yang 2003). Stress-responsive genes encoding calcineurin B-like protein-interacting protein kinases *OsCIPK03* and *OsCIPK12* are also involved in stress tolerance response in rice (Xiang et al. 2007). Calcium-dependent protein kinases are major players in different signal transduction pathways, and its cold-induced expression in rice leads to accumulation of the gene product OsCDPK7 and OsCDPK13, which serve as posi-

tive regulators of cold tolerance pathways (Wan et al. 2007). The *Late Embryogenesis Abundant* (*LEA*) genes also act as suitable targets for manipulation for their functional importance in abiotic stress response. Expression of the citrus gene-encoding *LEA* protein *CuCOR19* enhanced cold tolerance in transgenic tobacco (Hara et al. 2003), whereas introduction of wheat dehydrin gene *WCOR410* in strawberry imparted freezing tolerance (Houde et al. 2004). All these transcriptomic analyses and transgenic attempts established the significance of CBF-DREB-mediated cold stress responses.

1.8.1.4 Integration of Polyamines in Cold Tolerance Mechanisms

Intracellular accumulation of endogenous polyamines (PA) occurs in response to cold stress as they contribute to plant response to low temperature conditions. The increase in levels of diamine putrescine (Put) has been reported in cold-stressed *Arabidopsis* (Kaplan et al. 2004). The role of Put is to modulate ABA biosynthesis at the transcriptional level and hence regulate cold-induced, ABA-dependent responsive pathways. PA pre-treatment to seedlings of wheat showed alleviation of chilling injuries, where priming with spermidine (Spd) decreased the chilling stress-induced lipid peroxidation and membrane leakage, supported by endogenous increase in PA under low temperature conditions. The main function of PA is to maintain the catalytic activity of antioxidant enzymes and hence prevent oxidative damages associated with cold injury. Polycationic molecules also stabilize cellular membranes and minimize changes in membrane permeability, hence preventing loss of fluid. They also play a role in regulating hydrogen peroxide production, indirectly modulating plant defense mechanisms during chilling stress. Since high cellular levels of PA can be correlated to stress alleviation and tolerance, obtaining plants with elevated PA levels by genetically manipulating their biosynthesis can provide improved tolerance to abiotic stress conditions, including low temperature. For this, usually PA biosynthetic genes or those involved in the maintenance of PA homeostasis are targeted. *Arabidopsis* plants transformed with *SPDS* (*spermidine synthase*) cDNA from *Cucurbita ficifolia* under the control of strong, constitutive promoter, CaMV35S led to freezing tolerance (Kasukabe et al. 2004). *SPDS* overexpressor lines also exhibited enhanced activity of other PAs like spermine (Spm) and Put. In the PA biosynthetic pathway, overexpression studies have been conducted on cold-induced genes *ADC1* and *ADC2* (*arginine decarboxylase* 1 and 2) which promoted freezing tolerance in transgenic *Arabidopsis* and tobacco (Alcazar et al. 2005). Besides *ADC*, enhanced transcript levels of *SAMDC* (*S-adenosylmethionine decarboxylase*) have been reported in cold stress response. Transformation of tobacco plants with carnation *SAMDC* cDNA under strong promoter CaMV35S led to accumulation of Spd, Spm, and Put improving tolerance to multiple abiotic stress conditions like salinity and acidic and oxidative stress, other than freezing stress (Wi et al. 2006). The increased titers of Put on overexpression of *SAMDC* were actually the result of high Spd accumulation which was actively interconverted to Put by acetylation. PAs are also involved in integration of

signaling pathways modulating ROS- (hydrogen peroxide), NO- (nitric oxide), and ABA-mediated responses. PA levels are controlled by ABA, and this has been established through studies that showed impairment of stress-induced expression of *ADC2*, *SPDS1*, and *SPMS* (*spermine synthase*) genes in ABA-deficient and ABA-insensitive *Arabidopsis* mutants. This is, however, an example of an ABA-dependent cold-responsive pathway which is regulated by a bZIP (basic leucine zipper) family of transcription factors that interact with ABRE motifs, and this control is distinct from the CBF/DREB1 regulon which is ABA-independent.

1.9 Epigenetic Regulation of Chilling Responses

Low temperature-induced epigenetic modifications on perception of cold stress cues involve posttranslational histone modifications, DNA methylation, histone variant incorporation, and chromatin remodeling. Epigenetic modifications persist between generations through adaptive transgenerational plasticity and hence inherited by the progenies, through mitotic cell divisions, and transmitted to the next generation. Chromatin perception of ambient temperature has been investigated through study of *arp6* mutants of *Arabidopsis* (*ARP6* gene encodes a protein which is a subunit of the SWR1 chromatin-remodeling complex) (Kumar and Wigge 2010). SWI/SNF-type ATP-dependent chromatin remodeling complexes are evolutionarily conserved multiprotein machineries which regulate accessibility of DNA and chromatin structure to transcription factories. An ATPase component of this complex, BRM (BRAHMA), is a key target of ABA-dependent dephosphorylation through PP2C phosphatases, thereby indicating its role in ABA-dependent abiotic stress-responsive pathways. The presence of histone H2A.Z variant in the nucleosomes maintains the promoters in the repressed state till the perception of an activation signal (Li et al. 2015), i.e., the promoters of quiescent genes remain on standby mode till transcription initiation. Temperature-mediated changes in nucleosome composition and accessibility of nucleosomal DNA to promoter sequences have been analyzed by studying the H2A.Z occupancy at the *HSP70* promoter (HSPs or heat shock proteins maintain cells and protects proteins from denaturation during temperature stress) in response to varying ambient temperature which demonstrates that nucleosomes containing H2A.Z variant respond to varying ambient temperature conditions and in turn coordinate the transcriptome. Alterations in chromatin dynamics also mark cold stress responses in *Cannabis sativa* L., where the induction of *COR* genes is mediated by changes in chromatin structure (Mayer et al. 2015). In response to cold acclimation treatment, there was a marked initial increase in global DNA methylation that could be reverted during treatment only in cold-tolerant varieties. Significant increase in methylcytosine levels was also observed at the *COR* gene loci, on deacclimation which suggested an epigenetic mechanism of cold acclimation through locus-specific DNA methylation. Further, acetylation or deacetylation of H3K9 plays an important role in transcription regulation of *COR* genes (Pavangadkar et al. 2010), and its significance is proved through mutational

studies in *Arabidopsis* where mutants for histone deacetylases develop a hypersensitivity to freezing temperatures. Plants with defect in histone acetyltransferases are incapable of optimally inducing *COR* genes during cold acclimation (Vlaconasios et al. 2003). In cereal crops like rice (*Oryza sativa*), differential acetylation of Histone H3 at the regulatory region of *DREB1b* (*Drought Response Element Binding 1b*) promoter facilitates chromatin remodeling and transcription activation in response to cold stress (Roy et al. 2014). The rice ortholog of DREB1, *OsDREB1b*, is transcriptionally induced by cold stress; hence targeting this gene for overexpression confers increased tolerance to salinity and cold stress. On perception of cold stress cues, there is significant change in nucleosome arrangement at the upstream region of *OsDREB1b*, cis elements, and TATA box at the core promoter. Significant increase in acetylation levels of H3K14 and H3K27, hyperacetylation of H3K9, and enrichment of RNA Pol II occupancy at the promoter region lead to activation of transcription machinery through epigenetic mechanism. Hence, alterations of chromatin conformation lead to upregulation of the cold stress-responsive gene, increasing tolerance, and alleviation of stress.

1.10 Epigenetic Control of Vernalization Responses

Vernalization promoted flowering through epigenetic repression of flowering suppressor *FLC* in *Arabidopsis*, and such modifications are transmitted to the progeny (Jablonka and Raz 2009). There is also evidence of epigenetic memory of vernalization in winter cereals, which results from alterations in histone H3 lysine methylation levels throughout the extent of the *VRN1* gene. The increased H3K9 trimethylation and decreased H3K27 trimethylation are indicative of a vernalization-induced active chromatin state within *VRN1* gene (vernalization response is mediated by the stable induction of *VRN1* gene promoter). *VRN1* downregulates the floral repressor *VRN2* while allowing induction of the floral activator *FT* or *VRN3* which accelerates subsequent changes leading to floral development in cold-stressed plants. Vernalization and cold tolerance may also be mediated by DNA demethylation which results from the action of histone demethylases (Liu and Secombe 2015). Regulation of another key gene of the vernalization pathway is *FLC* (*FLOWERING LOCUS C*), which encodes a MADS-box transcription factor that represses genes involved in floral initiation, and hence the gene product is accumulated prior to cold adaptation by vernalization. This gene is also epigenetically controlled and remains transcriptionally silenced when temperature conditions are favorable, allowing expression of the downstream genes involved in floral initiation, such as *SOC1* (*SUPPRESSOR OF CONSTANS 1*) and *FT* (*FLOWERING LOCUS T*). Inhibition of *FLC* expression is correlated with elevated levels of repressive histone modifications through methylation of histone H3 lysine 9 and 27, as well as loss of histone modifications associated with active transcription such as H3 acetylation and H3 lysine 4 methylation (Bastow et al. 2004; Finnegan and Dennis 2007; Schmitz et al. 2008). The cellular memory of transcriptional repression of *FLC* is

maintained through successive cell divisions by mitotic inheritance of repressive histone modifications of the gene (Sung et al. 2006).

1.11 Role of microRNAs in Chilling Stress Response

MicroRNAs (miRNAs) are a class of small, noncoding RNAs that regulate target genes of abiotic stress-responsive pathways. The broad classification of small regulatory RNAs includes small interfering RNAs (siRNAs) along with miRNAs, which play a crucial role in plant development, and its functionality has been reported at the early phase of anther development when the susceptibility to cold stress is maximum. Small RNA (smRNA) and trans-acting small interfering RNA (tasiRNA) have been studied in thermosensitive genic male sterile (TGMS) lines of wheat (*Triticum aestivum*) which regulate auxin signaling pathway in relation to developmental response to cold stress (Tang et al. 2012). TGMS lines are hypersensitive to low temperature during meiosis stage. Under stress conditions, miRNAs guide anther development by mediating cold-induced abnormal activity of target *ARFs* (*Auxin Responsive Factors*), required for anther development under cold conditions.

Stress-induced miRNAs target repressors of stress-responsive pathways as well as positive regulators of signaling pathways which are inhibited by stress conditions. Accordingly, under stress conditions, there is an over- or under-expression of certain miRNAs which contributes to stress tolerance. Both miRNAs and siRNAs are loaded into AGO (ARGONAUTE) protein containing RISC (RNA-Induced Silencing Complex) which guides transcriptional or posttranscriptional target regulation through RNA-directed DNA methylation (RdDM), a pathway also involved in transgenerational inheritance of epigenetic stress markers and hence maintenance of stress memory. Cold stress results in the downregulation of *MET1*, leading to demethylation of mobile genetic elements (transposons) in *Zea mays* (Shan et al. 2013), whereas in *Arabidopsis* seedlings, subjection to cold stress leads to activation of retrotransposons. The siRNA pathway plays a key role in inhibition of retrotransposition in response to abiotic stress cues.

The miRNAs are involved in intricate overlapping stress regulatory networks (Khraiwesh et al. 2012). A specific example is presented by miR172 which plays the dual role of mRNA cleavage, as well as repression of translation (Jones-Rhoades et al. 2006). Elevated expression of this cold-inducible miRNA leads to repression of translation of *Arabidopsis* flowering development gene product AP2 (APETALA 2), resulting in early flowering and defects in floral organ identity (Chen et al. 2005; Axtell et al. 2006) during cold stress response. Another example is presented by cold-inducible miR169, which inhibits the expression of *XTH* (xyloglucan endotransglucosylase/hydrolase) genes, which function in cell elongation through increase in cell wall elasticity in response to stress cues.

Chloroplast is a major sensor of environmental stress cues, and tolerance during abiotic stress conditions is achieved through stabilization of lamellar mem-

brane systems of chloroplasts through alterations in lipid composition. The gh-miR397a-2, which targets chlorophyll a-binding protein P4, is upregulated in low temperature stress in cotton (*Gossypium hirsutum*) (Wang et al. 2016). Further, the role of miRNAs has been investigated in alleviation of oxidative stress, and miR398 has been implicated in targeting the mRNA of chloroplastic copper/zinc superoxide dismutase (CSD). The reduced expression level of miR398 correlated with the elevated CSD expression leads to scavenging of superoxide radicals and hence detoxification during oxidation stress.

The seedling tolerance to cold stress is also dependent on auxin signaling and modulation of auxin response through miR160g in cotton which targets ARFs (Auxin Responsive Factors) that bind Auxin Responsive Promoters (ARPs), thereby regulating the expression of auxin-responsive genes. Hence, downregulation of miR160g releases their repressing effect on ARFs during cold stress; otherwise, attenuation of plant growth and development may occur under stress conditions. Regulation of a specific miRNA miR319 during cold stress has been studied in sugarcane (Thiebaut et al. 2012). Small RNA transcriptomes have also been investigated to provide insights into miRNA-mediated wheat inflorescence development under cold stress by targeting floral development genes such as ARF, SPB (Squamosa Promoter Binding like Protein), MADS-box (MCM1, AG, DEFA, SRF), MYB, SPX (SYG1, Pho81, XPR1), TCP (TEOSINTE BRANCHED, Cycloidea and PCF), and PPR (Pentatricopeptide repeat) (Zhang et al. 2017). Hence, analysis of small RNA transcriptomes and their target genes provide a novel insight into abiotic stress tolerance mediated by miRNA which serve as an additional layer of gene regulation in cold stress response and hence increase productivity of commercially important crops.

1.12 Conclusion

The transcriptomic analysis of cold-tolerant plants has provided a thorough insight into the intricate genetic regulatory networks that lead to diverging cold-responsive signaling pathways during freezing/chilling stress. Such regulons have been investigated in order to identify suitable target genes that are chief players in mediating stress response and hence can be considered candidates for genetic manipulation for establishment of stable, tolerant transgenics of commercially important plants. Priming with polyamines is another approach through which stress alleviation has been possible; hence engineering pathways for enhancing polyamine levels endogenously other than external application and priming are novel tolerance conferring approaches. Physiologically, the photosynthetic and respiratory machinery are the most affected due to cold stress; thus such machineries have also been elaborately analyzed in order to engineer approaches to prevent chloroplast and mitochondrial damage and improve the scavenging of free radicals and reactive oxygen species through efficient antioxidant systems and maintenance of redox homeostasis in the face of cold stress. Membrane studies have also been conducted since they are the

primary markers of cold stress damage through osmotic imbalance and lipid peroxidation, along with alterations in metabolism which have been studied extensively. The overlapping genetic networks between drought and cold stress-responsive pathways provide insights into cross-talks between the different forms of abiotic stress; there are reported examples of many genes that participate in both pathways, hence serving as targets for manipulation. The interplay of phytohormones also provides a comprehensive view into the biochemical changes involved in cold stress response, and alteration of such hormone levels can regulate tolerance. Thus, integrating the current established understanding of the low temperature transcriptome, proteome, and metabolome, it is now possible to come up with approaches involving transgenics as well as epigenetic control so that the cytophysiological damage to cold stressed plants can be prevented. The novel techniques for imparting tolerance through microRNAs have opened up an alternative field for stress alleviation and tolerance engineering.

1.13 Future Perspectives

The complexity of the mechanisms of plant adaptation to stress arises from the multiple players involved in the elaborate stress-responsive transcriptome. An integrated understanding of the multiple aspects of cold-responsive pathways, the gene regulatory networks, the sensory mechanisms, and changes at the cytological, physiological, as well as metabolic levels will help in designing experimental approaches that can alleviate the damaging impact of cold stress on important fruit yielding, ornamental, as well as crop plants. The regulation of transcriptional response to stress, though has been deciphered to some extent, there still lies immense scope in probing further into the transcriptome and identifying novel genes of yet unidentified function in stress-responsive signaling pathways. The proteome and metabolome also need to be investigated extensively since accumulation of relatively newly characterized compatible solutes like glycinebetaine and trehalose indicates that genes encoding for enzymes synthesizing such metabolites can be novel targets of transgenic manipulation. Epigenetic regulations and their study in stress tolerance are in its nascent state and have to be investigated and researched on, in future, globally, in order to engineer novel techniques to confer cold stress tolerance in susceptible plants, exposed to freezing conditions due to their geographical locations as well as for adaptation to changing environment and climate. For this, microRNA approach has already been worked upon and has shown some promise in targeting various loci for strengthening plant tolerance to abiotic stress conditions. Thus, besides the utilization of regulon biotechnology, to dissect the CBF-COR dependent and independent regulons by research groups worldwide, the metabolome and epigenome also need to be extensively studied for a successful global and integrated research effort at alleviating cold stress.

Acknowledgments Financial assistance from the Council of Scientific and Industrial Research (CSIR), Government of India, through the research grant [38(1387)/14/EMR-II] to Dr. Aryadeep Roychoudhury is gratefully acknowledged.

References

Achard P, Renou JP, Berthomé R, Harberd NP, Genschik P (2008) Plant DELLAs restrain growth and promote survival of adversity by reducing the levels of reactive oxygen species. Curr Biol 18:656–660

Agarwal M, Hao Y, Kapoor A, Dong CH, Fujii H, Zheng X, Zhu JK (2006) A R2R3 type MYB transcription factor is involved in the cold regulation of CBF genes and in acquired freezing tolerance. J Biol Chem 281:37636–37645

Alcázar R, Garcia-Martinez JL, Cuevas JC, Tiburcio AF, Altabella T (2005) Overexpression of ADC2 in *Arabidopsis* induces dwarfism and late-flowering through GA deficiency. Plant J 43:425–436

Alonso-Peral MM, Oliver SN, Casao MC, Greenup AA, Trevaskis B (2011) The promoter of the cereal *VERNALIZATION1* gene is sufficient for transcriptional induction by prolonged cold. PLoS One 6:e29456

Angel A, Song J, Dean C, Howard M (2011) A Polycomb-based switch underlying quantitative epigenetic memory. Nature 476:105–108

Armstrong AF, Badger MR, Day DA, Barthet MM, Smith PMC, Millar AH, Whelan J, Atkin OK (2008) Dynamic changes in the mitochondrial electron transport chain underpinning cold acclimation of leaf respiration. Plant Cell Environ 31:1156–1169

Arora R, Rowland LJ (2011) Physiological research on winter hardiness: deacclimation resistance, reacclimation ability, photoprotection strategies, and a cold acclimation protocol design. Hortscience 46:1070–1078

Axtell MJ, Jan C, Rajagopalan R, Bartel DP (2006) A two-hit trigger for siRNA biogenesis in plants. Cell 127:565–577

Bajwa VS, Shukla MR, Sherif SM, Murch SJ, Saxena PK (2013) Role of melatonin in alleviating cold stress in *Arabidopsis thaliana*. J Pineal Res 56:238–245

Baskar V, Gururani MA, Yu JW, Park SW (2012) Engineering glucosinolates in plants: current knowledge and potential uses. Appl Biochem Biotechnol 168:1694–1717

Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C (2004) Vernalization requires epigenetic silencing of FLC by histone methylation. Nature 427:164–167

Borovskii GB, Stupnikova IV, Antipina AI, Vladimirova SV, Voinikov VK (2002) Accumulation of dehydrin-like proteins in the mitochondria of cereals in response to cold, freezing, drought and ABA treatment. BMC Plant Biol 2:5

Catala R, Lopez-Cobollo R, Castellano MM, Angosto T, Alonso JM, Ecker JR, Salinas J (2014) The *Arabidopsis* 14-3-3 protein RARE COLD INDUCIBLE 1A links low-temperature response and ethylene biosynthesis to regulate freezing tolerance and cold acclimation. Plant Cell 26:3326–3342

Castiglioni P, Warner D, Bensen RJ, Anstrom DC, Harrison J, Stoecker M, Abad M, Kumar G, Salvador S, D'Ordine R, Navarro S, Back S, Fernandes M, Targolli J, Dasgupta S, Bonin C, Luethy MH, Heard JE (2008) Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions. Plant Phyisol 147:446–455

Chen CC, Liang CS, Kao AL, Yang CC (2010) HHP1, a novel signaling component in the cross-talk between the cold and osmotic signaling pathways in *Arabidopsis*. J Exp Bot 61:3305–3320

Chen CC, Simard MJ, Tabara H, Brownell DR, McCollough JA, Mello CC (2005) A member of the polymerase beta nucleotidyltransferase superfamily is required for RNA interference in *C. elegans*. Curr Biol 15:378–383

Chinnusamy V, Ohta M, Kanrar S, Lee BH, Hong X, Agarwal M, Zhu JK (2003) ICE1: a regulator of cold-induced transcriptome and freezing tolerance in *Arabidopsis*. Genes Dev 17:1043–1054

Christie PJ, Alfenito MR, Walbot V (1994) Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways: enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. Planta 194:541–549

Cook D, Fowler S, Fiehn O, Thomashow MF (2004) A prominent role for the CBF cold response pathway in configuring the low-temperature metabolome of *Arabidopsis*. Proc Natl Acad Sci U S A 101:15243–15248

Ding Y, Sheng JP, Li SY, Nie Y, Zhao JH, Zhu Z, Wang ZD, Tang XM (2015) The role of gibberellins in the mitigation of chilling injury in cherry tomato (*Solanum lycopersicum* L.) fruit. Postharvest Biol Technol 101:88–95

Doherty CJ, Van Buskirk HA, Myers SJ, Thomashow MF (2009) Roles for *Arabidopsis* CAMTA transcription factors in cold-regulated gene expression and freezing tolerance. Plant Cell 21:972–984

Dong CH, Agarwal M, Zhang Y, Xie Q, Zhu JK (2006) The negative regulator of plant cold responses, HOS1, is a RING E3 ligase that mediates the ubiquitination and degradation of ICE1. Proc Natl Acad Sci U S A 103:8281–8286

Dong CJ, Li L, Shang QM, Liu XY, Zhang ZG (2014) Endogenous salicylic acid accumulation is required for chilling tolerance in cucumber (*Cucumis sativus* L.) seedlings. Planta 240:687–700

Ergon A, Melby TI, Rognli O (2016) Vernalization requirement and the chromosomal *VRN1*-region can affect freezing tolerance and expression of cold-regulated genes in *Festuca pratensis*. Front Plant Sci 7:207

Fan J, Hu Z, Xie Y, Chan Z, Chen K, Amombo E, Chen L, Fu J (2015) Alleviation of cold damage to photosystem II and metabolisms by melatonin in bermudagrass. Front Plant Sci 6:925

Faust M, Liu D, Millard MM, Stutte GW (1991) Bound versus free water in dormant apple buds – a theory for dormancy. Hortscience 26:887–890

Finnegan EJ, Dennis ES (2007) Vernalization-induced trimethylation of histone H3 lysine 27 at FLC is not maintained in mitotically quiescent cells. Curr Biol 17:1978–1983

Fiorani F, Umbach AL, Siedow JN (2005) The alternative oxidase of plant mitochondria is involved in the acclimation of shoot growth at low temperature. A study of *Arabidopsis* AOX1a transgenic plants. Plant Physiol 139:1795–1805

Fowler S, Thomashow MF (2002) *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell 14:1675–1690

Fujioka S, Yokota T (2003) Biosynthesis and metabolism of brassinosteroids. Annu Rev Plant Biol 54:1371–1464

Ghorbani B, Pakkish Z (2014) Brassinosteroid enhances cold stress tolerance of Washington navel orange (*Citrus sinensis* L.) fruit by regulating antioxidant enzymes during storage. Agric Conspec Sci 79(2):109–114

Gonzalez DH, Welchen E, Attallah CV, Comelli RN, Mufarrege EF (2007) Transcriptional coordination of the biogenesis of the oxidative phosphorylation machinery in plants. Plant J 51:105–116

Guy CL (1990) Cold acclimation and freezing tolerance: role of protein metabolism. Ann Rev Plant Physiol Plant Mol Bio 41:187–223

Hadi F, Gilpin M, Fuller MP (2011) Identification and expression analysis of CBF/DREB1and COR15 genes in mutants of *Brassica oleracea* var. botrytis with enhanced proline production and frost resistance. Plant Physiol Biochem 49:1323–1332

Hara M, Terashima S, Fukaya T, Kuboi T (2003) Enhancement of cold tolerance and inhibition of lipid peroxidation by citrus dehydrin in transgenic tobacco. Planta 217:290–298

Heo JB, Sung S (2011) Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. Science 331:76–79

Hoermiller I, Naegele T, Augustin H, Stutz S, Weckwerth W, Heyer A (2016) Subcellular reprogramming of metabolism during cold acclimation in *Arabidopsis thaliana*. Plant Cell Environ 40:599–560

Houde MH, Dallaire S, N'Dong D, Sarhan F (2004) Overexpression of the acidic dehydrin WCOR410 improves freezing tolerance in transgenic strawberry leaves. Plant Biotechnol J 2:381–387

Hsieh TH, Lee JT, Charng YY, Chan MT (2002) Tomato plants ectopically expressing *Arabidopsis* CBF1 show enhanced resistance to water deficit stress. Plant Phyisol 130:618–626

Hu WH, Xiao YA, Zeng JJ, Hu XH (2010) Photosynthesis, respiration and antioxidant enzymes in pepper leaves under drought and heat stresses. Biol Plant 54:761–765

Hu Y, Jiang L, Wang F, Yu D (2013) Jasmonate regulates the inducer of CBF expression-C-repeat binding factor/DRE binding factor1 cascade and freezing tolerance in *Arabidopsis*. Plant Cell 25:2907–2924

Jablonka E, Raz G (2009) Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. Q Rev Biol 84:131–176

Janska A, Marsik P, Zelenkova S, Ovesna J (2010) Cold stress and acclimation—what is important for metabolic adjustment? Plant Biol 12:395–405

Jeon J, Chulo C, Lee MR, Binh NV, Kim J (2016) *CYTOKININ RESPONSE FACTOR2* (*CRF2*) and *CRF3* regulate lateral root development in response to cold stress in *Arabidopsis*. Plant Cell 28:1828–1843

Jones-Rhoades MW, Bartel DP, Bartel B (2006) MicroRNAS and their regulatory roles in plants. Annu Rev Plant Biol 57:19–53

Kalberer SR, Wisniewski M, Arora R (2006) Deacclimation and reacclimation of cold-hardy plants: current understandings and emerging concepts. Plant Sci 171:3–16

Kaniuga Z, Saczynska V, Miskiewicz E, Garstka M (1999) The fatty acid composition of phosphatidylglycerol and sulfoquinovosyldiacylglycerol of *Zea mays* genotypes differing in chilling susceptibility. J Plant Physiol 154:256–263

Kanaoka MM, Pillittere LJ, Fujii H, Yoshida Y, Bogenschutz NL, Takabayashi J, Zhu JK, Torii KU (2008) SCREAM/ICE1 and SCREAM2 specify three cell-state transitional steps leading to *Arabidopsis* stomatal differentiation. Plant Cell 20:1775–1785

Kaplan F, Kopka J, Haskell DW, Zhao W, Schiller KC, Gatzke N, Sung DY, Guy CL (2004) Exploring the temperature-stress metabolome of *Arabidopsis*. Plant Physiol 136:4159–4168

Kasamo K, Kagita F, Yamanishi H, Sasaki T (1992) Low temperature-induced changes in the thermotropic properties and fatty acid composition of the plasma membrane and tonoplast of cultured rice (*Oryza sativa* L) cells. Plant Cell Physiol 33:609–616

Kasamo K, Noushi I (1987) The role of phospholipids in plasma membrane ATPase activity in *Vigna radiata* L. (mung bean) roots and hypocotyls. Plant Physiol 83:323–328

Kasuga M, Miura S, Shinozaki K, Yamaguchi-Shinozaki K (2004) A combination of the *Arabidopsis DREB1A* gene and stress-inducible *rd29A* promoter improved drought- and low-temperature stress tolerance in tobacco by gene transfer. Plant Cell Physiol 45:346–350

Kasukabe Y, He L, Nada K, Misawa S, Ihara I, Tachibana S (2004) Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana*. Plant Cell Physiol 45:712–722

Khraiwesh B, Zhu JK, Zhu J (2012) Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. Biochim Biophys Acta 1819:137–148

Kim JC, Lee SH, Cheong YH, Yoo CM, Lee SI, Chun HJ, Yun DJ, Hong JC, Lee SY, Lim CO, Cho MJ (2001) A novel cold- inducible zinc finger protein from soybean, SCOF-1, enhances cold tolerance in transgenic plants. Plant J 25:247–259

Kim TW, Wang ZY (2010) Brassinosteroid signal transduction from receptor kinases to transcription factors. Annu Rev Plant Biol 61:681–704

Knight H, Trewavas AJ, Knight MR (1996) Cold calcium signaling in *Arabidopsis* involves two cellular pools and a change in calcium signature after acclimation. Plant Cell 8:489–503

Kojima M, Suzuki H, Ohnishi M, Ito S (1998) Effects of growth temperature on lipids of adzuki bean cells. Phytochemistry 47:1483–1487

Kolesnichenko AV, Pobezhimova TP, Grabelnych OI, Voinikov VK (2002) Stress induced protein CSP 310: a third uncoupling system in plants. Planta 215:279–286

Kovács Z, Simon-Sarkadi L, Szûcs A, Kocsy G (2010) Differential effects of cold, osmotic stress and abscisic acid on polyamine accumulation in wheat. Amino Acids 38:623–631

Kovtun Y, Chiu WL, Tena G, Sheen J (2000) Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proc Natl Acad Sci U S A 97:2940–2945

Krupa Z, Huner NP, Williams JP, Maissan E, James DR (1987) Development at cold-hardening temperatures. The structure and composition of purified rye light harvesting complex II. Plant Physiol 84:19–24

Kumar SV, Wigge PA (2010) H2A.Z-containing nucleosomes mediate the thermosensory response in *Arabidopsis*. Cell 140:136–147

Lee HG, Lee K, Seo PJ (2015) The *Arabidopsis* MYB96 transcription factor plays a role in seed dormancy. Plant Mol Biol 87:371–381

Lee SH, Singh AP, Chung GC, Kim YS, Kong IB (2002) Chilling root temperature causes rapid ultrastructural changes in cortical cells of cucumber (*Cucumis sativus* L.) root tips. J Exp Bot 53:2225–2237

Lee SJ, Kang JY, Park HJ, Kim MD, Bae MS, Choi HI (2010) DREB2C interacts with ABF2, a bZIP protein regulating abscisic acid-responsive gene expression, and its overexpression affects abscisic acid sensitivity. Plant Physiol 153:716–727

Li M, Hada A, Sen P, Olufemi L, Hall MA, Smith BY, Forth S, McKnight JN, Patel A, Bowman GD, Bartholomew B, Wang MD (2015) Dynamic regulation of transcription factors by nucleosome remodeling. Elife. https://doi.org/10.7554/eLife.06249

Lin MK, Belanger H, Lee YJ, Varkonyi-Gasic E, Takoa K, Miura E, Xoconostle-Cazares B, Gendler K, Jorgensen RA, Phinney B, Lough TJ, Lucas WJ (2007) *FLOWERING LOCUS T* protein may act as the long-distance florigenic signal in the cucurbits. Plant Cell 19:488–1506

Liu F, Jensen CR, Andersen MN (2004) Drought stress effect on carbohydrate concentration in soybean leaves and pods during early reproductive development: its implication in altering pod set. Field Crops Res 86:1–13

Liu X, Secombe J (2015) The histone demethylase KDM5 activates gene expression by recognizing chromatin context through its PHD reader motif. Plant Cell Rep 13:2219–2231

Livingston DP, Hincha DK, Heyer AG (2009) Fructan and its relationship to abiotic stress tolerance in plants. Cell Mol Life Sci 66:2007–2023

Lukatkin AS, Brazaityte A, Bobinas C, Duchovskis P (2012) Chilling injury in chilling-sensitive plants: a review. Zemdirbyste-Agriculture 99:111–124

Mayer BF, Ali-Benali MA, Demone J, Bertrand A, Charron JB (2015) Cold acclimation induces distinctive changes in the chromatin state and transcript levels of *COR* genes in *Cannabis sativa* varieties with contrasting cold acclimation capacities. Physiol Plant 155:281–295

Miura K, Furumoto T (2013) Cold signaling and cold response in plants. Int J Mol Sci 14:5312–5337

Miura K, Jin JB, Lee J, Yoo CY, Stirm V, Miura T, Ashworth EN, Bressan RA, Yun DJ, Hasegawa PM (2007) SIZ1-mediated sumoylation of ICE1 controls CBF3/DREB1A expression and freezing tolerance in *Arabidopsis*. Plant Cell 19:1403–1414

Miura K, Lee J, Gong Q, Ma S, Jin JB, Yoo CY, Miura T, Sato A, Bohnert HJ, Hasegawa PM (2011) SIZ1 regulation of phosphate starvation-induced root architecture remodeling involves the control of auxin accumulation. Plant Physiol 155:1000–1012

Murata N, Mohanty PS, Hayashi H, Papageorgiou GC (1992) Glycinebetaine stabilizes the association of extrinsic proteins with the photosynthetic oxygen-evolving complex. FEBS Lett 296:187–189

Murphy C, Wilson JM (1981) Ultrastructural features of chilling-injury in *Episcia reptans*. Plant Cell Environ 4:261–265

Nakashima K, Ito Y, Yamaguchi-Shinozaki K (2009) Transcriptional regulatory networks in response to abiotic stresses in *Arabidopsis* and grasses. Plant Physiol 149:88–95

Nuener G, Xu B, Hacker J (2010) Velocity and pattern of ice propagation and deep supercooling in woody stems of *Castanea sativa, Morus nigra* and *Quercus robur* by IDTA. Tree Physiol 30:1037–1045

Oliveira G, Penuelas J (2005) Effects of winter cold stress on photosynthesis and photochemical efficiency of PSII of Mediterranean *Cistus albidus* L. and *Quercus ilex* L. Plant Ecol 175:179–191

Örvar BL, Sangwan V, Omann F, Dhindsa RS (2000) Early steps in cold sensing by plant cells: the role of actin cytoskeleton and membrane fluidity. Plant J 23:785–794

Paredes M, Quiles MJ (2015) The effects of cold stress on photosynthesis in *Hibiscus* plants. PLoS One 10:e0137472

Parkin KL, Marangoni A, Jackman RL, Yada RY, Stanley DW (1989) Chilling injury: a review of possible mechanism. J Food Biochem 13:1745–4514

Pavangadkar K, Thomashow MF, Triezenberg SJ (2010) Histone dynamics and roles of histone acetyltransferases during cold-induced gene regulation in *Arabidopsis*. Plant Mol Biol 74:183–200

Pearce RS (2001) Plant freezing and damage. Ann Bot 87:417–424

Pramanik MHR, Imai R (2005) Functional identification of a trehalose 6-phosphate phosphatase gene that is involved in transient induction of trehalose biosynthesis during chilling stress in rice. Plant Mol Biol 58:751–762

Ratcliffe OJ, Kumimoto RW, Wong BJ, Riechmann JL (2003) Analysis of the *Arabidopsis MADS AFFECTING FLOWERING* gene family: *MAF2* prevents vernalization by short periods of cold. Plant Cell 15:1159–1169

Ribas-Carbo M, Aroca R, Gonzàlez-Meler MA, Irigoyen JJ, Sánchez-Díaz M (2000) The electron partitioning between the cytochrome and alternative respiratory pathways during chilling recovery in two cultivars of maize differing in chilling sensitivity. Plant Physiol 122:199–204

Rihan HZ, Al-Issawi M, Fuller MP (2017) Advances in physiological and molecular aspects of cold tolerance. J Plant Interact 12:143–157

Rinne PL, Kaikuranta PM, van der Schoot C (2001) The shoot apical meristem restores its symplasmic organization during chilling-induced release from dormancy. Plant J 26:249–264

Rohde A, Bhalerao RP (2007) Plant dormancy in the perennial context. Trends Plant Sci 12:217–223

Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu Rev Plant Biol 57:675–709

Routaboul JM, Fischer S, Browse J (2000) Trienoic fatty acids are required for photosynthesis at low temperatures. Plant Physiol 124:1697–1705

Roy D, Paul A, Roy A, Ghosh R, Ganguly P, Chaudhuri S (2014) Differential acetylation of histone H3 at the regulatory region of OsDREB1b promoter facilitates chromatin remodelling and transcription activation during cold stress. PLoS One 9:e100343

Sakai A, Larcher W (1987) Frost survival of plants: responses and adaptations to freezing stress. Springer-Verlag, Berlin, pp 1–259

Sanghera GS, Wani SH, Hussain W, Singh NB (2011) Engineering cold stress tolerance in crop plants. Curr Genomics 12:30–43

Sangwan V, Foulds I, Singh J, Dhindsa RS (2001) Cold-activation of *Brassica napus* BN115 promoter is mediated by structural changes in the membrane and cytoskeleton, and requires Ca^{2+} influx. Plant J 27:1–12

Sarhan F, Ouellet F, VazquezTello A (1997) The wheat WCS120 gene family. A useful model to understand the molecular genetics of freezing tolerance in cereals. Physiol Plant 101:439–445

Sasse JM (2003) Physiological actions of brassinosteroids: an update. J Plant Growth Regul 22:276–288

Schmitz RJ, Sung S, Amasino RM (2008) Histone arginine methylation is required for vernalization-induced epigenetic silencing of FLC in winter-annual *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 105:411–416

Shan X, Wang X, Yang G, Wu Y, Su S, Li S, Liu H, Yuan Y (2013) Analysis of the DNA methylation of maize (*Zea mays* L.) in response to cold stress based on methylation-sensitive amplified polymorphisms. J Plant Biol 56:32–38

Sheen J (1998) Mutational analysis of protein phosphatase 2C involved in abscisic acid signal transduction in higher plants. Proc Natl Acad Sci U S A 95:975–980

Shibasaki K, Uemura M, Tsurumi S, Rahman A (2009) Auxin response in *Arabidopsis* under cold stress: underlying molecular mechanisms. Plant Cell 21:3823–3838

Shindo C, Aranzana MJ, Lister C, Baxter C, Nicholls C, Nordborg M, Dean C (2005) Role of FRIGIDA and FLOWERING LOCUS C in determining variation in flowering time of *Arabidopsis*. Plant Physiol 138:1163–1173

Spano C, Bottega S, Castiglione MR, Pedranzani H (2017) Antioxidant response to cold stress in two oil plants of the genus *Jatropha*. Plant Soil Environ 63:271. https://doi.org/10.17221/182/2017-PSE

Stockinger EJ, Gilmour SJ, Thomashow MF (1997) *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 U S A 94:1035–1040

Strange A, Li P, Lister C, Anderson J, Warthmann N, Shindo C, Irwin J, Nordborg M, Dean C (2011) Major-effect alleles at relatively few loci underlie distinct vernalization and flowering variation in *Arabidopsis* accessions. PLoS One 6:e1994

Strauss AJ, Krüger GHJ, Strasser RJ, Heerden PDR (2006) Ranking of dark chilling tolerance in soybean genotypes probed by the chlorophyll a fluorescence transient O-J-I-P. Environ Exp Bot 56:147–157

Sugie A, Naydenov N, Mizuno N, Nakamura C, Takumi S (2006) Overexpression of wheat alternative oxidase gene Waox1a alters respiration capacity and response to reactive oxygen species under low temperature in transgenic *Arabidopsis*. Genes Genet Syst 81:349–354

Sung S, Schmitz RJ, Amasino RM (2006) A PHD finger protein involved in both the vernalization and photoperiod pathways in *Arabidopsis*. Genes Dev 20:3244–3248

Svensson AJ, Johansson FI Moller IM, Rasmusson AG (2002) Cold stress decreases the capacity for respiratory NADH oxidation in potato leaves. FEBS Lett 517:79–82

Sweetlove LJ, Lytovchenko A, Morgan M, Nunes-Nesi A, Taylor NL, Baxter CJ, Eickmeier I, Fernie AR (2006) Mitochondrial uncoupling protein is required for efficient photosynthesis. Proc Natl Acad Sci U S A 103:19587–19592

Talts P, Parnik T, Gardeström P, Keerberg O (2004) Respiratory acclimation in *Arabidopsis thaliana* leaves at low temperature. J Plant Physiol 161:573–579

Tang Z, Zhang L, Xu C, Yuan S, Zhang F, Zheng Y, Zhao C (2012) Uncovering small RNA-mediated responses to cold stress in a wheat thermosensitive genic male-sterile line by deep sequencing. Plant Phyisiol 159:721–738

Taylor NL, Heazlewood JL, Millar AH (2011) The *Arabidopsis thaliana* 2-D gel mitochondrial proteome: refining the value of reference maps for assessing protein abundance, contaminants and post-translational modifications. Proteomics 11:1720–1733

Thiebaut F, Grativol C, Carnavale-Bottino M, Rojas CA, Tanurdzic M, Farinelli L, Martienssen RA, Hemerly AS, Ferreira PCG (2012) Computational identification and analysis of novel sugarcane microRNAs. BMC Genomics 13:290

Thomashow MF (1999) Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Physiol Plant Mol Biol 50:571–599

Vella NGF, Joss TV, Roberts TH (2012) Chilling-induced ultrastructural changes to mesophyll cells of *Arabidopsis* grown under short days are almost completely reversible by plant rewarming. Protoplasma 249:1137–1149

Vijayakumar H, Thamilasaran SK, Shanmugam A, Natarajan S, Jung H, Park J, Kim H, Chung M, Nou I (2016) Glutathione transferases superfamily: cold-inducible expression of distinct *GST* genes in *Brassica oleracea*. Int J Mol Sci 17:1211

Whitaker BD (1993) Lipid changes in bell pepper fruit during chilling and rewarming. Hortscience 28:519

Vlachonasios KE, Thomashow MF, Triezenberg SJ (2003) Disruption mutations of *ADA2b* and *GCN5* transcriptional adaptor genes dramatically affect *Arabidopsis* growth, development, and gene expression. Plant Cell 15:626–638

Wan B, Lin Y, Mou T (2007) Expression of rice Ca(2+)-dependent protein kinases (CDPKs) genes under different environmental stresses. FEBS Lett 581:1179–1189

Wani SH, Sandhu JS, Gosal SS (2008) Genetic engineering of crop plants for abiotic stress tolerance. In: Malik CP, Kaur B, Wadhwani C (eds) Advanced topics in plant biotechnology and plant biology. MD Publications, New Delhi, pp 149–183

Wang Q, Liu N, Yang X, Tu L, Zhang X (2016) Small RNA-mediated responses to low- and high-temperature stresses in cotton. Sci Rep 6:35558. https://doi.org/10.1038/srep35558

Wisniewski M, Fuller M, Palta J, Arora R (2008) Ice nucleation, propagation and deep supercooling in woody plants. J Crop Improvement 10(1–2):2004

Wi SJ, Kim WT, Park KY (2006) Overexpression of carnation *S*-adenosylmethionine decarboxylase gene generates a broad spectrum tolerance to abiotic stresses in transgenic tobacco plants. Plant Cell Rep 25:1111–1121

Xiang Y, Huang Y, Xiong L (2007) Characterization of stress-responsive CIPK genes in rice for stress tolerance improvement. Plant Physiol 144:1416–1428

Xin Z, Browse J (1998) Eskimo1 mutants of *Arabidopsis* are constitutively freezing-tolerant. Proc Natl Acad Sci U S A 95:7799–7804

Xiong LZ, Yang YN (2003) Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid-inducible mitogen-activated protein kinase. Plant Cell 15:745–759

Yang T, Chaudhuri S, Yang L, Du L, Poovaiah BW (2012) A calcium/calmodulin-regulated member of the receptor-like kinase family confers cold tolerance in plants. J Biol Chem 285:7119–7126

Yin PH, Wu CC, Lin JC, Chi CW, Wei YH, Lee HC (2010) Somatic mutations of mitochondrial genome in hepatocellular carcinoma. Mitochondrion 10:174–182

Zhang J, Xue B, Gai M, Song S, Jia N, Sun H (2017) Small RNA and transcriptome sequencing reveal a potential miRNA-mediated interaction network that functions during somatic embryogenesis in *Lilium pumilum* DC. Fisch. Front Plant Sci. https://doi.org/10.3389/fpls.2017.00566

Zhao M, Liu W, Xia X, Wang T, Zhang WH (2014) Cold acclimation-induced freezing tolerance of *Medicago truncatula* seedlings is negatively regulated by ethylene. Physiol Plant 152:115–129

Zhou X, Chen S, Wu H, Xu H (2017) Effects of cold stress on the photosynthesis and antioxidant system of *Rhododendron chrysanthemum* Pall. www.preprints.org. doi:https://doi.org/10.20944/preprints201703.0131.v1

Zhu J, Shi H, Lee BH, Damsz B, Cheng S, Stirm V, Zhu JK, Hasegawa PM, Bressan RA (2004) An *Arabidopsis* homeodomain transcription factor gene, HOS9, mediates cold tolerance through a CBF-independent pathway. Proc Natl Acad Sci U S A 101:9873–9878