Transcriptomic and proteomic mechanisms underlying cold tolerance in plants

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Abstract

Abiotic stress is one of the major challenges facing crop production globally. Abiotic stress resulting from low temperature is a major limitation to crop production, especially in the temperate regions of the world. Cold stress not only influence crop development and reduce yields, but also curtail the efficient distribution of agricultural products worldwide. An understanding of the molecular mechanisms underlying cold stress tolerance is important for the development of strategies to manage crop loss and improve yield. In this review, we explore the major molecular mechanisms involved in plant cold tolerance, including recent discoveries on interrelated gene networks and regulatory mechanisms for cold stress adaptation in crops. Further, we highlight the role of proteomics in the discovery of proteins involved in key signaling pathways, including late embryogenesis-abundant proteins, antifreeze proteins, cold-regulated proteins, heat shock proteins, and pathogenesis-related proteins. The role of these proteins, and their relative abundance in physiological-biochemical reactions, are discussed and key candidate proteins for plant genetic enhancement are suggested.

Keywords: molecular mechanisms; proteomics; physiological-biochemical reactions; plant cold stress, regulatory mechanisms.

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Abbreviations: ABA - abscisic acid; ABRE - ABA-responsive element; ABF - ABRE binding factor; AGC - ascorbate-glutathione cycle; AP2/ERF - APETALA2 and ethylene-responsive element-binding factor; AREB - ABA-responsive element binding factor; APX - ascorbate peroxidase; bHLH - basic-helix-loop-helix; bZIP - basic leucine zipper; CaM - calmodulin; CAMTA3 - calmodulin-binding transcription activator 3; CAT - catalase; CBF - C-repeat binding factor; CBF/DREB1 - C-repeat binding factor/dehydration-responsive element binding protein 1; CBL - calcineurin B-like proteins; AFGPs - antifreeze glycoproteins; AFPs - antifreeze proteins; CE1 - coupling element 1; CE3 - coupling element 3; CIPKs - cross-protein kinases; COR - cold-regulated; CRT - C-repeat; CSPs - cold shock proteins; CYP - cytochrome P450; Cys - conserved cysteine; DRE - dehydration-responsive element; DREB1 - dehydration-responsive element binding protein 1; DRIP1 - DREB2A-interacting protein 1; DRIP2 - DREB2A-interacting protein 2; ERD10 - early reaction to dehydration 10; GOLGAS - golgin subfamily A member 5; GR - glutathione reductase; GSH - glutathione; GSSG - glutathione disulfide; HSPs - heat shock proteins; ICE - inducer of CBF expression; IRI - ice recrystallization; JA - jasmonate; LEA - late embryogenesis abundant; MaMYC2 - Musa acuminata- Myelocytomatosis 2; miRNAs - microRNAs; NADPH - nicotinamide adenine dinucleotide phosphate; NF-Y - nuclear factors-Y; ObTLPI - Ocimum basilicum thiamatin-like protein; P5CR - pyrroline-5-carboxylate reductase; P5CS - Δ1-pyrroline-5-carboxylate synthetase; POD - peroxidase; PR - pathogen-related; RAD- Radialis; RDR - ribonucleoside-diphosphate reductase; RNAi - RNA interference; ROS - reactive oxygen species; siRNAs - small interfering RNAs; SOD - superoxide dismutase; TFs - transcriptional factors; TH - thermal hysteresis; VRN-1 - vernalization gene; ZNF - Zinc-finger.

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Introduction

Drought, salinity, heat, and cold are major sources of abiotic stress that have a direct influence on the development and production of plants (Mboup et al. 2012, Hasanuzzaman et al. 2019, Jamshidi Goharrizi et al. 2020a,c,d, Chen et al. 2021). Cold stress, which is defined as chilling stress (< 20 °C) (Sales et al. 2017) or freezing stress (< 0 °C), is one of the most dangerous stressors that higher plants face (Theocharis et al. 2012, Zhou et al. 2017). Cold stress greatly limits the spatial distribution of plants and impacts plant growth (Mickelbart et al. 2015, Guo et al. 2017, Liu and Zhou 2018, Shi et al. 2018).

Plants native to temperate climates can survive freezing conditions after being subjected to non-freezing temperatures for a certain length of time, a phenomenon known as cold acclimation (Chinnusamy et al. 2007). During early spring and winter, plants that grow in temperate climates are more resistant to seasonal variations in temperature and can withstand cold stress. On the other hand, plants in the tropics and subtropics are not subject to regular cold conditions and lack the ability to acclimate. Plants that are susceptible to chilling and incapable of cold acclimation include many economically important agronomic and horticultural crops such as rice, maize, soybean, potato, cotton, and tomato (Ritonga and Chen 2020). Interestingly, some tropical species such as Santalum album have a wide temperature tolerance (4.5 to 38 °C) and can withstand chilling (Zhang et al. 2017a).

Many plant species, including Arabidopsis, winter wheat, and barley lack the ability to acclimate under freezing temperatures (Zhao et al. 2015). Even for temperate species, such as the Betula utilis, that dominates cold environments; seasonal weather patterns resulting from monsoon winds can cause susceptibility to temperature regimes during the growing season (Pandey et al. 2018). The biochemical mechanisms of response and adaptation to cold stress can vary in the duration of exposure to low-temperature extremes. For example, in tea plants, early periods of cold stress are marked by elevated activity of biological processes, cellular components, and molecular functions. In contrast, the latter stages of cold stress are characterized by enhanced metabolism of amino acids (glutamate and aspartate), nucleotide sugars, as well as accelerated protein (e.g. alanine) export (Hao et al. 2018).

In recent years, transcriptome profiling, proteomics, and studies of cold-resistant mutants have increased our knowledge regarding the genes, signaling pathways, and processes related to cold acclimation (Janda et al. 2014). Transcriptome studies in the model species Arabidopsis thaliana first identified multiple genes involved in response to cold stress (Nordin et al. 1991, Welin et al. 1995, Medina et al. 1999). Transcriptome approaches have been applied to study cold stress responses in Juglans regia and Pinus koraiensis, for the development of a gene co-expression network in maize, and also for researching wheat flowering and shade-avoidance pathways (Trapnell et al. 2010, Da Maia et al. 2017, Zhang et al. 2017b). Proteomics approaches have been employed to study the cold tolerance of many plants, including Chorispora bungeana, Oryza sativa, Musa nana, Buchloe dactyloides, and desert woody plants; desert shrub (Ammpoipantthus mongolicus) and desert poplar (Populus euphratica) (Yue et al. 2010, Kurdrd et al. 2011, Zhao et al. 2013, Wang et al. 2017). Collectively, these studies have revealed the complexity of the mechanisms involved in cold stress adaptation in plants (Tolosa and Zhang 2020).

In this review, we provide a detailed overview of the transcriptomic and proteomic profiles of plants under cold stress.

Transcriptomic profile under cold stress

Cold acclimation can be achieved by either up- or down-regulation of a specific set of genes (Hannah et al. 2005). In this phenomenon, multiple gene expression pathways and gene networks of cold-induced genes (regulons) are stimulated (Thomashow 2001). However, it should be noted that these gene networks also mediate response to other abiotic stresses (Thomashow 1999, Seki et al. 2003, Zhao and Zhu 2016, Jamshidi Goharrizi et al. 2020b,e, Nazari et al. 2019) through a common signal transduction pathway (Heidarvand and Amiri 2010).

The signaling pathway starts with low-temperature perception by cells (sensing), which activates signal transduction mechanisms (Denesik 2007). During the acclimation process in response to cold stress, free Ca²⁺ rapidly increases in the plant cell cytoplasm, thus likely plays an important role in signal initiation (Eckardt 2009). Indeed, transient cytosolic calcium ([Ca²⁺][cyt]) elevation is a ubiquitous denominator of the signaling network when plants are subjected to every known stress (abiotic and biotic) (Eckardt 2009). These stress-induced [Ca²⁺]cyt elevations differ in frequency, shape, and magnitude, depending on the severity of the stress as well as the type of stress experienced (Bose et al. 2011). Rapid calcium inflow is facilitated by physical alterations in the cell membrane resulting from modified lipid stoichiometry (Knight and Knight 2000). For example, cytosolic Ca²⁺ can accumulate in the cell within 5 - 10 s of exposure to cold stress (Miura and Furutomo 2013). Elevated cytosolic Ca²⁺ induces the C-repeat Binding Factor (CBF), an Apetela 2 domain-containing transcriptional factor that regulates many cold-induced genes (Gopal and Chanakya 2012). Furthermore, Ca²⁺ influx acts as a sensor for protein activation and accumulation during cold stress (Jenks and Wood 2009). In plants, three major categories of Ca²⁺-dependent proteins have been identified (Jenks and Wood 2009): Ca²⁺-dependent protein kinases, calmodulin (CaM), and calcineurin B-like proteins (CBL). Calmodulin is a Ca²⁺ cytosolic sensor, which is highly conserved among eukaryotes (Rudd and Franklin-Tong 2001). On the other hand, calmodulin genes regulate calcium fluxes. Calcium-bound calmodulin proteins interact with an element of the promoter of the CBF2 gene, which controls the CBF regulon and freezing resistance (Doherty et al. 2009). Moreover, CAMTA3 (Calmodulin-binding transcription activator 3) (also called SR1) has a major function in the transformation of cytosolic Ca²⁺ signals caused by low temperatures into...
downstream gene expression regulation (Eckardt 2009). In addition to calmodulin, CBL proteins and their cross-protein kinases (CIPKs) can form functional sets that transmit downstream signals to membrane effectors helping in their adaptation to adverse environmental conditions (Shabala et al. 2021). In response to abiotic stress (e.g., cold and salinity), Ca\(^{2+}\) is also involved in oxidative stress signaling and detoxification of reactive oxygen species (ROS) (Tyystjärvi 2013). Briefly, an oxidative burst (the transient, rapid generation and accumulation of ROS even at a low level) caused by abiotic stresses elevates the cytoplasmic influx of Ca\(^{2+}\) (Rao et al. 2006). Afterwards, NADPH (nicotinamide adenine dinucleotide phosphate) oxidase is activated by Ca\(^{2+}\) to generate ROS by yielding O\(^{2-}\), which is then transformed to H\(_2\)O\(_2\) by superoxide dismutase (SOD). Thus, ROS development is related to Ca\(^{2+}\) influx whose levels are mediated by the activation of Ca\(^{2+}\) channels in the plasma membrane by the content of ROS (Kwak 2003). The function of various proteins regulating the expression of particular protein- or non-related genes is modulated by an interaction of Ca\(^{2+}\) and ROS in the nucleus (Rao et al. 2006). Moreover, there is evidence that protein phosphorylation requires calcium in response to cold temperatures (Monroy et al. 1998). In fact, cold treatment greatly reduces the calcium-dependent activity of protein phosphatase, and on the other hand, low calcium is assumed to limit the phosphorylation potential of phosphatase 2A in response to cold stress (Monroy et al. 1998).

Other key genes, such as kinases, and transcription factors play an important role in activating signaling cascades for modulating responses to abiotic stresses including cold, salinity, and drought (Rao et al. 2006). For example, in the ascorbate-glutathione cycle (AGC), glutathione has a substantial function in the regulation of hydrogen peroxide content in plants (Kocsy et al. 2001). Deposition of H\(_2\)O\(_2\) typically results from the reduction of oxygen to superoxide radical (O\(^{2-}\)) by extra electrons originating from the photosynthetic and respiratory electron transfer chains (Kocsy et al. 2001). The O\(^{2-}\) is then transformed to H\(_2\)O\(_2\) by SOD. Therefore, the generation of the oxidized form of glutathione (GSH) from its reduced form (glutathione disulfide: GSSG), provides plants with an important mechanism for eliminating excess activity of H\(_2\)O\(_2\). Generally, high content of GSH and NADPH-dependent glutathione reductase (GR) are found in cold-stressed (0 - 15 °C) plants and are a reliable indicator of response to cold stress (Kocsy et al. 2001). In addition, the GSH/GSSG ratio and alterations in the content of H\(_2\)O\(_2\) have been reported to play a crucial function during cold acclimation by modulating the redox status of cells. The observation of many defensive genes having antioxidant-responsive regions or GSSG-binding elements in their regulatory zones has confirmed this hypothesis (Kocsy et al. 2001).

In both plants and animals, the role of micro-RNAs (miRNAs) and small interfering RNAs (siRNAs) in the suppression of gene expression has been proven (Ghildiyal and Zamore 2009). Using bioinformatics tools, numerous miRNAs in multiple plant species have been identified, cloned, and sequenced (Zhou et al. 2008, Zhang et al. 2009, Lv et al. 2010, Chen et al. 2012). However, little information is available regarding the miRNA-controlled target genes in cold stress conditions (Miura and Furumoto 2013). Table 1 summarizes the major cold stress-responsive genes.

**CBF pathway for cold stress response**

The C-repeat Binding Factor (CBF) transcriptional factors play an important function in cold stress adaptation. CBF regulatory genes have been reported across major agronomic and horticultural crops and natural herbaceous and woody plants (Sanghera et al. 2011, Mizoi et al. 2012). Extensive research in Arabidopsis has led to the discovery of a family of CBF/DREB1 (C-repeat binding factor/dehydration-responsive element binding protein 1) transcription factors involved in cold acclimation (Gilmour et al. 1998). In the promoters of cold and dehydration-responsive genes, these transcription factors interact with specific regulatory regions. These sequences are C-repeats (CRT: TGGCCCGAC) and elements which are dehydration-responsive (DRE: TACCCGACAT). They include a core 5-bp sequence (CCGAC), which is highly conserved and functions to regulate the transcription of genes involved in response to drought, salinity, and cold stress (Baker et al. 1994, Yamaguchi-Shinozaki and Shinozaki 1994, Gao et al. 2007). The expression of cold-responsive genes (COR genes) is thus induced by CBF, hence indicating its pivotal role in the cold tolerance of plants (Mizoi et al. 2012).

The function of the CBF/DREB1 pathway under cold stress was verified in soybean (Yamasaki and Randall 2016); however, evaluations have shown that the downstream genes in this pathway are inefficient in cold response. This inefficiency leads to the inability of soybean to significantly acclimate to freezing/cold stress, which could result from insufficient amounts of GmCBF/DREB1 transcripts and/or proteins, as well as deficiency in relevant cofactors or the presence of negatively acting promoters (Yamasaki and Randall 2016). On the other hand, in Arabidopsis, three distinct cold-responsive CBF/DREB1 genes have been reported and defined as: CBF/DREB1B, CBF2/DREB1C, and CBF3/DREB1A (Thomashow 2001). More recently, Monroe et al. (2016) analyzed CBF genes among 477 wild accessions of Arabidopsis and reported that variation in CBF sequences is closely correlated with winter temperatures. Gery et al. (2011) used RNA interference (RNAi) to target the expression of three CBF genes across eight accessions of Arabidopsis thaliana and reported that observed polymorphisms were correlated with freezing tolerance. In addition, a close co-regulation between CBF1 and CBF3 was observed in the study of the interaction between the different forms of CBFs, with the expression of CBF2 and CBF1 or CBF3 being influenced by the genetic background in which the RNAi constructs were expressed (Gery et al. 2011). Although three strongly linked CBF genes have been identified in Arabidopsis, an expansion of this gene family has taken place in wheat and some other cereals (Pearce et al. 2013), so that in each
of the three hexaploid wheat genomes, there are at least 15 CBF genes (Badawi et al. 2007). An over-expression study of CBF/DREB1 in transgenic plants revealed that about 12% of COR genes in Arabidopsis are regulated by CBF/DREB1. In contrast, no clear target was observed among the three CBF orthologs. In warm temperatures, the expression of these genes in transgenic plants triggers the expression of downstream COR genes, increasing the cold resistance of these plants (Liu et al. 1998, Kasuga et al. 1999).

In many plant species, the CBF/DREB1 pathway has been identified and described, suggesting that CBF transcriptional pathway under cold stress in the plant kingdom is strongly conserved (Jaglo et al. 2001).

The expression of CBFs/DREBs has been shown to be associated with temperature variations, suggesting the lower the temperature, the greater the expression of these genes. However, temperature-shift experiments have shown that the cold response becomes desensitized at a particular low-temperature point, thus requiring an interval of warm temperature to reset (Zarka et al. 2003). This phenomenon implies the presence of a low-temperature ‘thermometer’ and a high-temperature ‘transducer’ signal that controls the expressions of CBFs (Zarka et al. 2003). For instance, Gilmour et al. (1998) showed that a transcription factor, referred to as inducer of CBF expression (ICE), is expressed in warm temperatures, and ICE recognizes DNA-regulatory elements.

### Table 1. A list of differentially expressed genes in response to cold stress.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Family</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FtbHLH2</td>
<td>bHLH</td>
<td>Fagopyrum tataricum</td>
<td>(Yao et al. 2018)</td>
</tr>
<tr>
<td>BpUVR8</td>
<td>UVR</td>
<td>Betula platyphylla</td>
<td>(Zhao et al. 2016)</td>
</tr>
<tr>
<td>FAD2-3</td>
<td>FAD2</td>
<td>Gossypium hirsutum</td>
<td>(Kargiotidou et al. 2008)</td>
</tr>
<tr>
<td>FAD2-4</td>
<td>FAD2</td>
<td>Gossypium hirsutum</td>
<td>(Kargiotidou et al. 2008)</td>
</tr>
<tr>
<td>FAD8</td>
<td>FAD</td>
<td>Arabidopsis thaliana</td>
<td>(Matsuda et al. 2005)</td>
</tr>
<tr>
<td>Sb08g007310</td>
<td>GST</td>
<td>Sorghum bicolor</td>
<td>(Ortiz et al. 2017)</td>
</tr>
<tr>
<td>Sb06g018220</td>
<td>ZEP</td>
<td>Sorghum bicolor</td>
<td>(Hu et al. 2015)</td>
</tr>
<tr>
<td>AtGRXS17</td>
<td>Trx</td>
<td>Arabidopsis thaliana</td>
<td>(Hu et al. 2015)</td>
</tr>
<tr>
<td>AtCBF3</td>
<td>AP2/ERF</td>
<td>Arabidopsis thaliana</td>
<td>(Yao et al. 2018)</td>
</tr>
<tr>
<td>VaERF080</td>
<td>AP2/ERF</td>
<td>Vitis amurensis</td>
<td>(Yao et al. 2018)</td>
</tr>
<tr>
<td>VaERF087</td>
<td>AP2/ERF</td>
<td>Vitis amurensis</td>
<td>(Yao et al. 2018)</td>
</tr>
<tr>
<td>SiDHN</td>
<td>DHN</td>
<td>Saussurea involucrata</td>
<td>(Yao et al. 2018)</td>
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<td>OsGH3-2</td>
<td>GH3</td>
<td>Oryza sativa</td>
<td>(Guo et al. 2017)</td>
</tr>
<tr>
<td>MYBS3</td>
<td>MYB</td>
<td>Oryza sativa</td>
<td>(Du et al. 2012)</td>
</tr>
<tr>
<td>RDM4</td>
<td>-</td>
<td>Arabidopsis thaliana</td>
<td>(Mickelbart et al. 2015)</td>
</tr>
<tr>
<td>OsMADS57</td>
<td>MIKC</td>
<td>Oryza sativa</td>
<td>(Chan et al. 2016)</td>
</tr>
<tr>
<td>GHDBRE1</td>
<td>DREB</td>
<td>Gossypium</td>
<td>(Arora et al. 2007)</td>
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<td>PUB25/26</td>
<td>U-box E3 ligases</td>
<td>Arabidopsis thaliana</td>
<td>(Wang et al. 2019b)</td>
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<td>(Cao et al. 2019)</td>
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<td>(Cao et al. 2019)</td>
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<td>AQP</td>
<td>Arabidopsis thaliana</td>
<td>(Xu et al. 2020)</td>
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<tr>
<td>MaPPIP2-7</td>
<td>AQP</td>
<td>Musa acuminate</td>
<td>(Xu et al. 2020)</td>
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<td>CsCPKs</td>
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<td>Camellia sinensis</td>
<td>(Ding et al. 2019)</td>
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<tr>
<td>COR413</td>
<td>COR</td>
<td>Camellia sinensis</td>
<td>(Guo et al. 2019)</td>
</tr>
<tr>
<td>SET, JmJc</td>
<td>HKMTases, HDMases</td>
<td>Saussurea involucrata, Brassica rapa</td>
<td>(Liu et al. 2019a)</td>
</tr>
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<td>TaTPS11</td>
<td>TPS</td>
<td>Triticum aestivum</td>
<td>(Liu et al. 2019b)</td>
</tr>
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<td>TaSMT1, TaSMT2</td>
<td>SMT</td>
<td>Triticum aestivum</td>
<td>(Valitova et al. 2019)</td>
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<td>RC11A, RC11B</td>
<td>14-3-3 proteins</td>
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<td>(Visconti et al. 2019)</td>
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<td>MdMYB108L</td>
<td>MYB</td>
<td>Malus domestica</td>
<td>(Wang et al. 2019c)</td>
</tr>
<tr>
<td>MdHys</td>
<td>bZIP</td>
<td>Malus domestica</td>
<td>(Wang et al. 2019c)</td>
</tr>
<tr>
<td>CsLEA</td>
<td>LEA</td>
<td>Camellia sinensis</td>
<td>(Wang et al. 2019a)</td>
</tr>
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<td>DlICE1</td>
<td>bHLH</td>
<td>Dimocarpus longan</td>
<td>(Yang et al. 2019)</td>
</tr>
<tr>
<td>ZjICE1</td>
<td>bHLH</td>
<td>Zosysia japonica</td>
<td>(Zuo et al. 2019)</td>
</tr>
<tr>
<td>VvCBF</td>
<td>DREB</td>
<td>Vitis vinifera</td>
<td>(Rubio and Pérez 2019)</td>
</tr>
<tr>
<td>ArGLR1.2ArGLR1.3</td>
<td>GLR</td>
<td>Arabidopsis thaliana</td>
<td>(Zheng et al. 2018)</td>
</tr>
<tr>
<td>STCH4/ REIL2</td>
<td>Zinc-finger proteins (ZNFs)</td>
<td>Arabidopsis thaliana</td>
<td>(Schmidt et al. 2013)</td>
</tr>
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</table>
called ‘ICE boxes’, in CBF promoters. Indeed, ICE1, a nuclear gene encoding an MYC-like bHLH protein, plays an important role in controlling certain CBF genes (Chinnusamy et al. 2003). In numerous plant species, the function of the ICE1-CBF/DREB1 network in enhancing cold resistance has been evaluated. In wheat, two ICE1 homologues, TaICE141 and TaICE187, up-regulated CBF group IV genes (Badawi et al. 2008). The presence of CBF/DREB homologs and their functions in enhancing the cold resistance of rice plants have been documented in several studies (Ito et al. 2006, Zhang et al. 2009). For example, expression of OsICE1 and OsICE2 in rice is caused by cold stress, resulting in OsDREB1B, OsHsfA3 and OsTPP1B up-regulation (Nakamura et al. 2011). Zhao et al. (2013) reported that through the expression of Musa acuminate-Myeloctomatosis 2 (MaMYC2), the chilling resistance of banana plants (Musa acuminate) was enhanced by jasmonate (JA). However, MaMYC2 also interacts with MaICE1, activating the CBF-dependent pathway for cold-tolerance signaling. Soltész et al. (2013) indicated that in the cold resistance mechanism of wheat, TaCBF14 and TaCBF15 play important roles. In addition, these genes have been documented to enhance cold resistance of transgenic plants by various degrees compared to normal plants. Both apples and peaches have at least five CBF genes that show a spectrum of expression patterns in response to low temperatures (Wisniewski et al. 2014). On the other hand, up-regulation of ICE2, an ICE1 homolog, increases cold resistance and CBF/DREB1 expression in Arabidopsis (Fursova 2009). This indicates that the over-expression of CBF/DREB1 via ICE1 could result in chromatin remodeling. However, besides ICE proteins, other proteins like LOS4, HOS1, and LOS1 show positive regulation of CBF expression (Matthew and Jenks 2005).

The DNA-regulatory element CRT/DRE in the COR gene promoter is recognized by CBF proteins. By this mechanism, CBF proteins regulate the expression of the COR genes (Baker et al. 1994, Yamaguchi-Shinozaki and Shinozaki 1994). Three CBF genes found in tandem on chromosome 4 of Arabidopsis encode proteins with masses of approximately 24 kDa. These proteins share about 90 % amino acid sequence identity and have a conserved DNA-binding motif with almost 60 APETALA2 and ethylene-responsive element-binding factor (AP2/ERF) domain-designated amino acids (Riechmann and Meyerowitz 1998). In contrast, 144 AP2/ERF genes have been identified in Arabidopsis (Riechmann and Meyerowitz 2000) and categorized into 5 subgroups according to their DNA-binding domain similarities: AP2 subfamily (14 genes), RAV subfamily (6 genes), DREB/CFB subfamily (55 genes), ERF subfamily (65 genes), and a 5th group consisting of 4 genes (Sakuma et al. 2002). Increased expression of COR genes by activation of CRT/DRE elements in their CBF protein promoters is known as a part of the CBF regulon. Extensive expression profiling studies of CBF genes have recorded and assigned 109 genes to the CBF regulon (Fowler and Thomashow 2002, Vogel et al. 2005), and divided CBF regulon-assigned genes into four groups based on the nature of the encoded proteins (Matthew and Jenks 2005). The first main group contains more than 50 % of proteins with uncertain functions; the second group is comprised of cryoprotective proteins that include COR proteins, the third group consists of regulating proteins, while the fourth consists of biosynthetic proteins (Matthew and Jenks 2005). For these groups, COR genes are of particular importance as they encode highly hydrophilic proteins such as dehydrins and LEA proteins (Close 1997). It is proposed that LEA proteins are necessary for membrane stabilization and avoidance of protein aggregation (Hundertmark and Hincha 2008). Most functions of COR proteins, however, are only speculative. Several studies indicate that the COR protein, one of the most extensively researched COR proteins, helps the stabilization of cell membranes against freezing disruption (Lin and Thomashow 1992, Artus et al. 1996, Steponkus et al. 1998). In addition, COR proteins have been shown to reduce membranes’ propensity to construct hexagonal-II structures, a damaging non-bilayer structure owing to cellular dehydration associated with freezing (Thomashow 1999). Moreover, COR proteins play a role in shielding other proteins from in vitro freeze-thaw inactivation (Bravo et al. 2003, Hará et al. 2003), as well as functioning as dehydration buffers, and protease inhibitors (Bray 1993). In addition, studies have shown that up-regulation of COR15a in transgenic Arabidopsis (non-acclimated plants) increased chloroplast freezing resistance by around 2 °C (Artus et al. 1996). Besides COR genes, the expression of heat shock proteins can be caused by low-temperature stress (Timperio et al. 2008). Studies have also shown that bacterial cold shock proteins (CSPs) increase stress adjustment in plants such as maize and rice (Castiglioni et al. 2008, Guddimalli et al. 2021). Cold resistance in transgenic Arabidopsis increased with the expression of bacterial CSPs (Karlsen and Imai 2003, Nakamura et al. 2008). Different PR (pathogen-related) proteins including PR1, PR2, PR5, PR10, PR11, and PR14 have also been shown to be up-regulated by exposure to low temperature (Seo et al. 2008).

A possible link exists between cold adjustment processes and other physiological pathways in plants. As an example, an association between low-temperature adjustment and vernalization has been reported in temperate cereals since both processes involve exposure to low non-freezing temperature (Dhillon et al. 2010). In addition, it was found that the major vernalization gene (VRN-1) plays an important role in decreasing the cold acclimation potential of plants (Fowler and Limin 2004). On the other hand, CBF gene mRNA is comparatively decreased once the vrn-1 allele-carrying winter genotype is vernalized versus non-vernalized plants (Stockinger et al. 2007). In wheat, Fr-1 and Fr-2, have been reported to be the primary loci regulating low-temperature tolerance (Vágújfalvi et al. 2000). It has been documented that the Fr-1 locus is closely related to the Vrn-1 locus that is well recognized to be the primary regulator of the transition from vegetative to reproductive meristem under cold stress (Laudencia-Chingcuano et al. 2011). Moreover, it has been confirmed that Vrn-1 and Fr-1 are genetically linked (Båga et al. 2007). The pleiotropic features of the Vrn-1 locus explain much of the associated temperature and winter resistance habit of temperate cereals (Dhillon et al. 2007).
et al. 2010).

In reference to the FR-2 locus, evidence shows that it contains a known group of CBFs that play a crucial function in increasing the expression of COR genes in various plant species (Campoli et al. 2009, Knox et al. 2010). Increases in metabolites are followed by changes in gene expression as a reaction to low-temperature stress, some of which have been shown to play a protective role against low-temperature stress damage (Sanghera et al. 2011). However, when experiencing low-temperature stress, plants encounter alterations in their gene expression patterns and protein products, and the modification of CBF genes has demonstrated utility in breeding for cold-resistant crops adapted to moderate environments (Sanghera et al. 2011).

In summary, whole genomes and transcriptomes can provide detailed knowledge about the physiological condition of plant species in a given situation; however, global transcriptions are not always associated with translated proteins. Furthermore, transcript analysis does not screen certain important post-translational changes. In this regard, proteomic research has the ability to offer a detailed picture of plant stress responses at the protein level. Plants use specific signaling and transcriptome control to acclimate to cold stress. An inducer of dehydration responsive elements or C-repeat binding factor expression-1 and dehydration-responsive elements or C-repeat binding factors are two transcription factors that play important functions in the control of cold-responsive genes and plant cold resistance. These genes and transcription factors could be targeted to enhance cold resistance and productivity in agricultural crops through genetic modification.

**Proteomic profile of cold stress**

Cold stress triggers the expression of a significant number of protein-encoding genes that contribute to plant chilling tolerance (Hughes and Dunn 1996). The cold response proteins, including LEA, antifreeze, cold-regulated, and dehydrins, are encoded by these stress-inducible genes. Under cold stress, they have a crucial function in plant adaptation.

**LEA proteins** are water-soluble proteins that accumulate during seed desiccation and under different stresses such as low temperature, drought, or salinity (Liu et al. 2016). These proteins act as a dehydration buffer, sequestering ions and stabilizing chromatin structures, membranes, and other proteins, thus defending the cells from desiccation and low temperature (Amara et al. 2013, Battaglia and Covarrubias 2013, Yang et al. 2015). They stop the aggregation of proteins and are essential for membrane stability throughout cold conditions (Hundertmark and Hincha 2008). For example, the overexpression of the citrus LEA gene CuCOR19 increases the chilling tolerance of transgenic tobacco plants (Hara et al. 2003). In barley, the LEA group III protein (LEA D7 protein) encoding by the HVAI gene showed up-regulation following exposure to cold stress (Thomashow 1998). On the other hand, the expression of LEA D113 encoding by the tomato LE25 gene in yeast cells improved their freezing tolerance (Thomashow 1998). Over-expression of transcriptional factors (TFs) such as DREB1A and DREB2A has been found to contribute to the increased aggregation of various enzymes and kinases besides LEA proteins, and ultimately to cold adaptation in Arabidopsis (Maruyama et al. 2009). In addition, increased deposition of Cor15am, an LEA-related protein in Arabidopsis, has been found to attenuate stromal protein aggregation under various stress conditions (Nakayama et al. 2007).

Dehydrins are a category of heat stable, membrane stabilizing LEA proteins and denaturation defense proteins during cytoplasm dehydration (Hara 2010). They are hydrophilic proteins containing polar amino acids and glycine, distinguished by the presence in the cell of a K-segment and lysine-rich amino acid motif that acts as chaperones or emulsifiers (Kosová et al. 2007). A predicted amphiphilic a-helix domain causing an interaction between hydrophilic and hydrophobic dehydrins is characterized by the inclusion of the K-segment (lysine-rich amino acid sequence). These dehydrins stick and mask with a coherent water layer with the intracellular molecules, preventing their coagulation during desiccation/dehydration (Xu et al. 2014). In addition to different developmental mechanisms, dehydrins are major dehydration-inducible proteins aggregated and often caused by cold stress (Hanin et al. 2011, Shi et al. 2016). They function as molecular chaperones that avoid the disaggregation of denatured proteins (partly) and interact by electrostatic force with phospholipid vesicles (Miura and Furumoto 2013, Graether and Boddington 2014). The primary dehydrins that are induced in plants during cold stress are LTI30 and COR47 (Kosová et al. 2007). Several dehydrins have been documented to increase during cold stress including Y2SK4 in apple (Garcia-Bañuelos et al. 2009) and PpDHN1 in peach (Artlip et al. 2016). These, and other dehydrins such as PCA60 in peach (Wisniewski et al. 1999), DN5 in rye, WCOR410 in wheat, and CuCOR19 in citrus (Miura and Furumoto 2013), function as antifreeze proteins in response to low temperature by avoiding intracellular ice formation. In addition, wheat dehydrin DHN5, which has a pleiotropic effect on stress responses in Arabidopsis, either changes the expression of abiotic stress-responsive genes (LEA, RD29B, RAB18, and LTI30) or controls the protein-related defense response genes expression. More importantly, DHN5 also controls the signaling of jasmonate (JA) by downregulating the negative regulator (jasmonate-ZIM domain) gene expression (Hanin et al. 2011). Cold tolerance proteome research in Trifolium pratense has recently revealed the existence of dehydrins as the most notable improvement in proteome composition in cold-acclimated crowns (Bertrand et al. 2016). Increased amount of the wheat dehydrin WCS19 in transgenic Arabidopsis enhances the resistance to cold stress (N'Dong et al. 2002), while an increased wheat dehydrin WCO410 in transgenic strawberry leaves increases cold resistance of the strawberry plant (Houde et al. 2004).

In addition, some dehydrin and Radialis (RAD)
homologous proteins have been recognized in the plasma membrane following cold stress. RAD23 is thought to be active in proteolysis due to proteasome 26S (Chen and Madura 2002), indicating the role of RAD23 for increased cryostability in response to cold stress in plasma membrane reorganization. In addition, the ERD10 (early reaction to dehydration 10) and ERD14 proteins, which are from the dehydrin-type acidic SK family and mimic COR47, associate with phospholipid vesicles and function like molecular chaperones (Miura and Furumoto 2013). On the other hand, the presence of phosphorylated ERD14 with a calcium-binding is found in the cytosol near the plasmalemma. The phosphorylated and calcium-bound ERD14 can act like a sugar chaperone or ionic buffer in response to low temperature, in a similar manner to calnexin or calreticulin binding Ca<sup>2+</sup> in the endoplasmic reticulum (Kosová et al. 2007).

**Antifreeze proteins** (AFPs) are one of the significant types of proteins that confer cold resistance to overwintering plants, i.e. cold acclimated plants. They include a category of binding proteins that inhibit further development of ice crystals (Gupta and Deswal 2012). Although first isolated as macromolecules from Antarctic fish having the capacity to prevent blood ice development during cold stresses, these proteins are also recognized in plants, fungi, bacteria, vertebrates, and invertebrates (Wen et al. 2016). Conversion of the water in the extracellular space into ice occurs at freezing temperatures, i.e. below 0 °C, since the extracellular fluid shows a higher freezing temperature relative to the intracellular fluid (Atici and Nalbantoglu 2003). In extracellular space, AFPs are able to adsorb these ice crystals, inhibit their development, and reduce the freezing temperature (Atici and Nalbantoglu 2003, Griffith et al. 2005). This protein family protects plants from freezing stress by displaying two distinct properties: thermal hysteresis (TH) and inhibition of ice recrystallization (IRI). In TH, AFPs attach to the ice crystals and avoid the aggregation of water molecules. AFPs decrease the cell sap’s freezing temperature as well as freezing point and avoid the development of ice crystals in plants, if it is frozen outside (Zachariassen and Kristiansen 2000). Moreover, AFPs block IRI, as well as the development of tiny ice crystals into larger ones, as these phenomena can cause physical injury and rupture of the plasma membrane, as well as cell death. With the exception of *Prunus persica* and *Forsythia suspensa*, which contain intracellular AFPs, AFPs extracted from eleven other plants are largely apoplastic, suggesting their function in inhibiting intracellular ice nucleators (Simpson et al. 2005, Gupta and Deswal 2014). In addition, glycoproteins (AFGPS) offer antifreeze protection in plants. AFGPs have been extracted from numerous plants, including *Daucus carota, Loliun perenne,* and *Hippophae rhamnoides* (Sidebottom et al. 2000, Pudney et al. 2003, Gupta and Deswal 2012). In AFGPs, the sugar moiety has a key function in attaching and stopping the growth of ice crystals (Gupta and Deswal 2014). On the other hand, different studies have indicated that different hormones such as JA and ethylene influence the antifreeze function. An increased antifreeze activity was seen in *Secale cereale* under ACC (an ethylene precursor) application, suggesting the function of ethylene in regulating antifreeze activity.

Characterization of AFP revealed that they consist of a zinc finger motif available in WRKY proteins, a class of TFs that govern the aggregation of proteins associated with pathogenesis in plants (Griffith et al. 2005). AFP has a unique function in that it has 10 consecutive 13-mer repeats at the C-terminus, which is a typical animal antifreeze protein characteristic (Huang and Duman 2002). Interestingly, AFPs’ constitutive activities have not been recorded to date, however, studies have shown that the outputs of AFP genes (mRNA and protein) accumulate during cold adaptation (Griffith and Yaish 2004). Thermal hysteresis and ice recrystallization suppression in transgenic plants containing an AFP gene was observed in overwintering plants (Griffith and Yaish 2004). In addition, the AFPS demonstrated antifreeze behavior in rye by modifying the structure of ice crystals and showing very high thermal hysteresis activity (Thomashow 1998). PR-proteins like class I and class II chitinases, β-1,3-glucanase, and thaumatin-like proteins are homologous to AFPS (Hon et al. 1995). These proteins can inhibit intracellular ice production by suppressing the intercellular ice recrystallization in apoplastic areas, because extracellular freezing induces cell dehydration (Janská et al. 2010).

**Cold regulated (COR) proteins** are water soluble and have a key function toward plant low-temperature resistance (Kazuoka and Oeda 1992). Not only in cold acclimation but also in chilling resistance, COR accumulation in plants has been found to be significant (Miura and Furumoto 2013). Dehydrins encoding by COR6.6, COR15a, COR47, and COR78/RD29A are caused by cold stress in *Arabidopsis* and other plants (Thomashow 1998). By reducing the hexagonal II phase lipids synthesis, COR15a aggregation prevents membrane damage and improves the freezing resistance of plants (Uemura and Steponkus 1997). Moreover, to reduce freezing damages, COR15a is able to form enzyme-binding oligomers in *Arabidopsis* and avoid chloroplast stromal protein aggregation (Nakayama et al. 2007). The COR15 aggregation detected in *Citrus paradise* enhances chilling resistance in mature fruits (Porat et al. 2002). Furthermore, increased COR14b aggregation enhances the freezing resistance of barley (Crosatti et al. 2008). Numerous heat-stable COR proteins from cold-adjusted spinach were isolated by Kauzuoka and Oeda (1992), but there were no similar proteins in non-adjusted spinach. In contrast, during cold acclimation, functional chloroplasts are necessary, because up-regulation of transcription factor CBF1-3 in mutant results in down-regulation of the downstream genes, COR47, COR15a, and COR78, in comparison with wild type (Kindgren et al. 2015). Increased accumulation of COR protein in the chloroplast, such as COR15a, has been found to improve the chloroplasts’ chilling resistance in non-acclimated plants by almost 2 °C (Artus et al. 1996). CuCOR19 enhances the resistance to stress via clearing free radicals created during low temperature
conditions in *Citrus unshiu*. Moreover, this study has demonstrated that free radical-coring action of dehydrogen can stop lipid peroxidation and enhance cold tolerance (Hara et al. 2003). It has been reported that CuCOR19 is a protein rich in histidine, glycine, and lysine and it can scavenge peroxidyl and hydroxyl radicals (Hara et al. 2004). Another histidine-rich protein has been documented in *Citrus unshiu* namely metal-binding CuCOR15, which has a strong antioxidant capability. Because free metal ions serve like effective catalytic compounds to produce radicals in cells, by binding these free metal ions under low-temperature conditions, CuCOR15 stops oxidation of proteins and peroxidation of lipids (Hara et al. 2005). Furthermore, WCOR14 and WCOR15 are chloroplastic COR proteins that are correlated with cold resistance in wheat, while as WCS120 and WCOR410 were found to be strongly increased under low temperature conditions in *Arabidopsis* and other plants (Fowler et al. 2001, Takumi and Nakamura 2005, Shimamura et al. 2006). WCS120 protects plants against low temperature by accumulation in the meristematic tissue; and its maintenance is extremely necessary for the low-temperature maintenance of the entire plant (Kosová et al. 2007). On the other hand, WCOR410 protects plant cells against freezing damage by accumulating near the plasma membrane (Xu et al. 2014).

**Heat shock proteins (HSPs):** These proteins mediate response to low-temperature conditions and are a significant part of the early response to cold stress (Timperio et al. 2008, Jannmohammadi et al. 2015). They are molecular chaperones that inhibit the accumulation of denatured proteins and prevent membrane degradation under cold stress by being highly cryo-protective (Renaut et al. 2006, Timperio et al. 2008). Five large HSP families are recognized: HSP40, HSP70, HSP90, HSP100, and another tiny HSP based on their molecular mass (Gupta et al. 2010). HSP40 or J-protein (J-domain containing protein) as a part of HSP70, enhances the affinity of HSP70 to different substrates in stressed plant cells (Kampinga and Craig 2010). The HSP70, which represents the highly conserved family of HSPs, inhibits misfolding of proteins and ensures accurate folding of proteins under stress conditions (Kosakovska et al. 2008, Park and Seo 2015). In response to environmental stress, HSP90 is the most frequent cytosolic HSP. The HSP90 recruits different proteins, like tubulin, actin, calmodulin, receptor proteins and kinases, and reacts with several other co-chaperones, like tetratricopeptide repeat to control different cellular processes under cold stress (Park and Seo 2015). By resolving non-stable protein aggregations and destroying weakened polypeptides, HSP100 retains the functional integrity of multiple essential polypeptides (Gupta et al. 2010). Moreover, small HSPs stabilize protein conformation in conjunction with other HSPs by blocking the assembly of faulty folded or denatured proteins and facilitating the denaturation of disaggregated proteins under low-temperature stress (Gupta et al. 2010). Small HSPs play a vital function in plant adjustment to low temperature by promoting refolding of HSP70- and HSP100-directed proteins (Sun et al. 2002, Mogk et al. 2003, Jannmohammadi et al. 2015). This collective evidence indicates the important role of HSPs for cold acclimation in plants (An et al. 2016). For example, in *Manihot esculenta*, in response to dehydration, HSPs simplify protein folding, block cellular injury, and enhance plant stress resistance (Fu et al. 2016). Similarly, different HSPs have been reported to be over-expressed in leaves of winter barley under low temperatures (Janská et al. 2011). Small HSPs (class I and II) are reported to accumulate in seeds throughout mid-maturation and have been found to be extremely common in dry seeds, indicating their function in resistance to desiccation (Hara 2010).

**Pathogenesis-related (PR) proteins** induced by pathological conditions are a large group of proteins that are classified in 17 families (Almeida-Silva and Venancio 2022). The increased expression of certain PR proteins, like PR-2 (β-1,3-glucanase), PR-3, PR-4, PR-5 (thiamatin-like protein), PR-8, PR-10, PR-11 (chitinase), and PR-14 (lipid transport protein), can also be caused by low temperature stress (Janská et al. 2010). Among these, the overwintering monocots can synthesize thiamatin-like protein, β-1,3-glucanase, and chitinases, which have antifreeze action that inhibit intracellular ice formation and prevent ice recrystallization in the apoplastic space (Yu et al. 2001, Griffith and Yaish 2004, Renaut et al. 2006). In winter rye, maize, wheat, and bermudagrass frost causes chitinase and thiamatin-like protein aggregation (Yu et al. 2001). Low temperature triggers the initiation of endogenous ethylene development in winter rye, which results in the expression of the *AFP* gene and aggregation of thiamatin-like proteins, chitinases and glucanases (Yu et al. 2001). Proteins with a low molecular mass containing conserved cysteine (Cys) residues, are thiamatin-like proteins. These Cys residues regulate intra-molecular disulfide bonds and give stability to proteins. Misra et al. (2016) demonstrated that ObTLP1 supplies resistance to abiotic stress in transgenic *Arabidopsis* while in *Ocimum basilicum* thiamatin-like protein (ObTLP1) can function as the target of various hormone signaling mechanisms in regulating reaction of plants to abiotic stress. However, there is no definite biological role of thiamatin-like proteins (Misra et al. 2016). In overwintering grasses, glucanases and chitinases with the capacity to attach to ice crystals enhance cold tolerance (Hon et al. 1995). Glucanases, along with chitinases adsorbed on the ice crystal surface, have a combined impact, enhancing the ice crystal potency alteration and growth suppression (Hon et al. 1995, Nakamura et al. 2008). There is an elevated accumulation of PR proteins in the leaf apoplast of winter rye during acclimation to low temperature (Hon et al. 1995). Two PR-10 proteins (ZmPR-10 and ZmPR-10.1) are known to be induced by various stresses, including cold stress (Jain et al. 2012). Interestingly, a protein related to pathogenesis (PR1b1) was found to cause disease tolerance in tomato fruit in response to cold stress (Goyal et al. 2016). Furthermore, extensive genomic analysis of the PR-1 gene family and its involvement in defense response in *Musa* spp. revealed its potential for conferring resistance to multiple stresses in novel banana varieties.
(Anuradha et al. 2022).

Protein profiles of numerous plant species in response to low-temperature conditions, in particular, have sparked a lot of scientific interest, culminating in the discovery of differentially expressed proteins that have greatly enhanced our understanding of the cold response. These data shed new light on the reaction of plants against low-temperature conditions and could pave a way for further studies into the molecular processes that decide how plant cells react to environmental change. Nonetheless, it should be stated that a considerable proportion of the discovered proteins to-date remained largely unexplained in terms of probable function. This is due in part to lack of high-quality protein-coding gene annotations for several plant species. The experimental confirmation of these unannotated proteins would help close the knowledge gap between protein profile of stress-induced molecules and annotated proteins would help close the knowledge gap between protein profile of stress-induced molecules and their actual function. Such information could facilitate isolation of potential candidates involved in cold stress adaptation via plant genetic engineering.

Conclusions

In this review, the molecular concepts underlying plant response to low temperatures were introduced. Moreover, the significant function of cold acclimation and the ensuing molecular alterations were discussed. This review highlighted cold-regulated genes and the mechanisms that govern their regulation. In addition, the important role of CBF genes in the overexpression of COR proteins were discussed. The beneficial role of various molecular mechanisms in conferring resistance to low temperatures in plants has been shown by recent studies. Proteomics offers an important platform for understanding the molecular processes in various plants adapted to cold stress. The latest proteomics research revealing protein-amount response to cold stress have been examined here, with special focus to changes at organ and organelle levels. There are numerous classes of proteins that accumulate during low-temperature stress, such as dehydrins, antifreeze proteins, and late embryogenesis abundant proteins. These proteins have roles in various processes, such as free radical scavenging, ice crystal inhibition, ion binding, and antifreeze functions during plant exposure to cold stress. Even with these advancements, more comprehensive molecular methods are urgently required to decipher the structural predictions based on genome sequence data and to explore protein modifications and their correlations with cold-resistant genotypes.

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