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# Role of cold responsive gene (C*OR*), late embryogenesis abundant (LEA) and anti freeze proteins (AFPs) in chilling stress tolerance

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**Abstract**
Plants feel stress under high as well as low temperature exposure. At lower temperatures, stress tolerance can be induced by exposure to reduced temperature and is known as chilling tolerance or cold acclimation. Chilling tolerance is the ability of a plant to tolerate low temperatures (0–15 ◦C) without injury or damage. Both cold acclimation and chilling tolerance involve an array of biochemical, molecular and metabolic processes. Plants adjust their metabolism that gets altered due to rise or fall of temperature by synthesizing stress-responsive metabolites particularly include soluble sugars, amino acids, organic acids, polyamines and lipids, Antifreeze proteins (AFPs) and Cold stress-induced genes known as cold-responsive genes (*COR*). Most of such genes encoding polypeptides are homolog's of late embryogenesis abundant proteins (LEA) and the polypeptides that are synthesized during the late embryogenesis phase. These late embryogenesis abundant-like proteins are mainly hydrophilic and have relatively simple amino-acid composition. The expression of cold responsive genes has been shown to be critical for both chilling tolerance and cold acclimation in plants. In this short review we have summarized the cellular and physiological changes occurring during chilling and role of COR, AFP in tolerance to chilling stress.

**Keywords:** Chilling stress, tolerance, biochemical, *COR*, LEA, acclimation

## Introduction

More than half of the 350 000 plant species on Earth are grown in the tropics and subtropics. Some of plants are sensitive at temperatures which are above the freezing point of tissues, but lower than 15°C (chilling temperatures) (Levitt 1980) [25] Chilling injury is damage to chilling-sensitive plant species during storage at temperatures above the freezing point of tissues, but lowers than 15°C. *Chilling-sensitive plants* are the plants sensitive to chilling and damaged at chilling temperatures. The ability of plants in a vegetative state to survive the action of chilling temperatures without harm to the future growth and development is called cold resistance. In turn, chilling-sensitive plants are sensitive to chilling and after prolonged storage in these temperatures external symptoms of injury are developed and death of the organism occurs. (Raison and Lyons 1986) [32] Plants such as *rice, maize, tomato, cucumber, cotton, soybeans*, etc., introduced in the higher latitudes have not acquired substantial resistance to chilling, despite the long history of cultivation in temperate regions (Thomashow 1994) [35]. Chilling temperatures effects on plants in temperate climates lead to a reduction or complete crop failure due to either direct damage or delayed maturation. No visible change or damage could be observed with a narrow drop of temperature in chilling sensitive plants. Still it can cause decrease in productivity. In South and South-East Asia, high-yielding varieties of rice are not grown in areas of more than 7 million hectares, where they may be exposed to chilling temperatures. (Browse and Xin 2001) [4] Obviously, the problem of plant resistance to chilling temperatures, which often occur in spring and autumn in many countries, is important for practical plant breeding.

### Cellular and physiological changes caused by chilling in sensitive plants

In chilling sensitive plants cold temperatures causes multiple disorganizations in ultra-structure of cell (Kratsch and Wise 2000) [23]. It causes damage to cell membrane system, cells compartmentation, plasmalemma swelling and rupture (Beck, Heim *et al*. 2004) [3] damage to endoplasmic reticulum and associated membrane system (Marangoni, Palma *et al*. 1996) [27] also leads to damage of Golgi apparatus. Mitochondria swelling and degeneration matrix enlightenment and a decrease in number of cristae, leading to a reduced oxidative phosphorylation (Ju, Xing *et al*. 2009) [19].

Chilling stress causes morphological and structural changes in chloroplasts, destruction of chloroplasts membranes, disintegration of grains, reduction of ribosome number and the accumulation of lipid bodies, and the disappearance of starch grains (Kratsch and Wise 2000) [23]. One of the important regulators of cellular response to chilling is membrane fluidity. Plants cannot avoid changing temperature as they are immobile. But they have sophisticated and well developed mechanisms which lead them to tolerate and acclimatize the changing environmental conditions like unfavorable temperatures. Lower temperature cause a number of alterations in cellular components like composition of glycerolipids and fatty acid unsaturation. (Lynch and Thompson 1982) [26] The positional redistribution of saturated (Cossins 1994) [5] and unsaturated fatty acids within lipid molecules changes in the protein composition (Thompson and Nozawa 1984) [37].

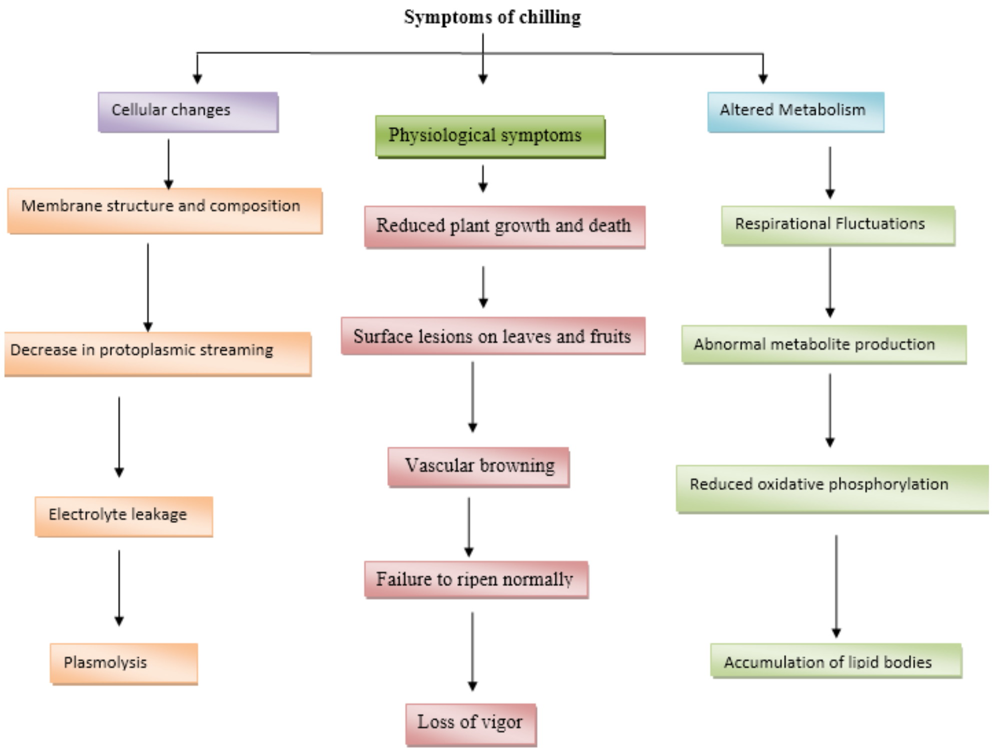


**Fig 1**

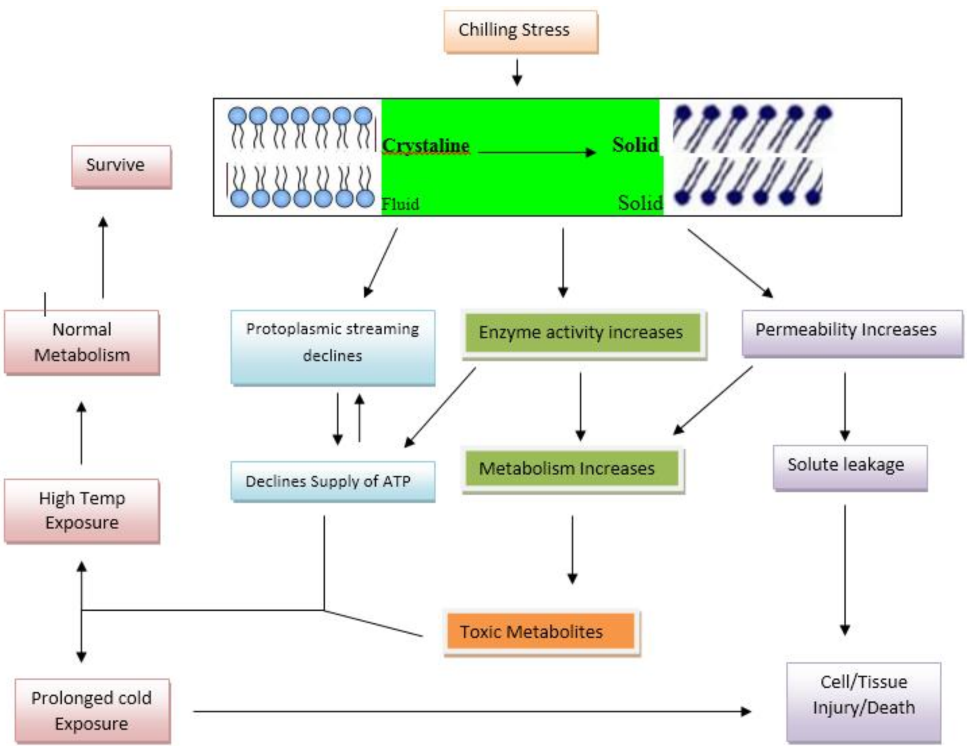


**Fig 2**

This figure/ flowchart give insights about chilling injury symptoms in sensitive plants. Primary site of injury in plants is its membrane. Once membrane is exposed to chilling temperature/injury it leads to cascade of cellular events. If a plant faces chilling for small period of time, it can be transitory and plant may survive once temperature gets normal again. However if it is prolonged exposure plant will develop injury in cells, tissues and it will exhibit necrosis and it may die (Fikret Yasar *et al*. 2018) [9].

**Role of Cold-responsive genes (*COR*)/ late in embryogenesis (LEA) Proteins**

Various genes have been proven to be induced during cold acclimation. Among which many encode proteins which contribute to chilling (cold) tolerance. However newly discovered Arabidopsis *COR6.6*, *COR15a*, and *COR78* polypeptides or homologs of LEA proteins such as Arabidopsis *COR4* encode new polypeptides extremely hydrophilic amino acid sequence (Thomashow 1994) [35]

COR15am a mature 9.4kD polypeptide having amino acid sequence rich in Ala, Lys, Glu, and Asp making 60% of protein is highly hydrophilic. Similarly cold responsive gene in barley *HVA1* encodes a 22kD polypeptide rich in Ala, Thr, & Lys making more than 50% of protein which is extremely hydrophilic. (Hong, Barg *et al*. 1992) [16] Studies have revealed various components which play a role in cold response in Arabidopsis, in Arabidopsis three transcriptions factors CBF1/DREB1B, CBF2/DREB1C and CBF3/DREB1A, play a role in respond to low temperatures. CBF1∼3 belongs to the AP2/ERF transcription factor family (Thomashow, Gilmour *et al*. 2001) [36] and the three *CBF* genes are tandemly clustered in an 8.7 kb region in chromosome 4. Three *CBF* genes have high sequence similarity, are paralogous and are induces during cold/chilling temperature. These recognize and bind to cis-elements in the promoters of cold-responsive (*COR*) genes.(Medina, Catalá *et al*. 2011) [29] *CBFs* play main role in determining the cold tolerance of plants.(Shi, Ding *et al*. 2014; Park, Lee *et al*. 2015) [33, 30] In all *CBFs, CBF1*∼3 play a major role in cold acclimation.(Zhao, Zhang *et al*. 2016) [41] Constitutive over expression of each *CBF* in plants resuls in enhancement to the cold stress tolerance various other factors such as ethylene.(Shi, Ding *et al*. 2014) [33] abscisic acid (*ABA*)(Lee and Seo 2015) directly or indirectly may contribute to chilling tolerance, while as *CBFs* are the central components in the complex cold responsive network. Recent studies revealed that CBFs played an essential role in cold acclimation among the three *CBF* the *CBF2* was more important than other *CBF1* and *CBF3* in Arabidopsis. (Zhao, Zhang *et al*. 2016) [41]

Late embryogenesis abundant (LEA) proteins are hyper-hydrophilic and intrinsically disordered, which can remain soluble after boiling and freeze–thaw treatments. (Artus, Uemura *et al*. 1996) [2] These proteins are accumulated in seeds during the late stage of embryogenesis and in vegetative tissues under stress conditions such as cold, drought and high salinity. Thus they have a protective role during dehydration (Thomashow, Gilmour *et al*. 2001) [36].

LEA has been classified into various groups on basis of amino acid sequence similarity (Wise 2003) LEA*25*, a group 4 LEA protein from tomato (*Solanum lycopersicum*), may increase tolerance against freezing when expressed in *Saccharomyces cerevisiae*. Overexpression of barley (*Hordeum vulgare*) *HVA1* in wheat (*Triticum aestivum*) and rice (*Oryza sativa*) confers high drought tolerance. (Xu, Duan *et al*. 1996) [39] Biochemical analyses have shown that several LEA proteins have properties which function as cryoprotectants in vitro. LEA proteins are not present in plants only but LEA-like proteins, termed as hydrophilins, exist in a wide range of organisms including *Escherichia coli*, yeast and invertebrates. (Gal, Glazer *et al*. 2004) [10] (Kikawada, Nakahara *et al*. 2006) [21]

## Role of Plant Antifreeze proteins (AFPs)

Plants like other animals (certain fishes, insects) synthesize AFPs in response to cold acclimation. (Hoshino, Odaira *et al*. 1999) [17] (Antikainen and Griffith 1997) [1] These way by which these proteins act is called thermal hysteresis, it is the temperature at which ice is formed but do not affect the melting point of the solution. Theses affect the shape of formation of ice crystals when temperature decreases (they inhibit the coalescing of small ice crystals into large ice crystals). Almost 20-30 species including monocots and dicots shows thermal hysteresis in cell sap. (Antikainen and Griffith 1997) [1] (Griffith, Antikainen *et al*. 1997) [1] AFPs are accumulated in the apoplastic fluid during cold acclimation. These AFPs have been related to pathogenesis-related proteins (PR), an intriguing situation given that winter cereals reported to be more resistant to fungal diseases after cold acclimation.(Griffith, Antikainen *et al*. 1997) [1] N-terminal amino acids sequencing established that two of the AFPs are endochitinase-like proteins, two areb-1,3-glucanase-like proteins, and two are thaumatin-like proteins.(Antikainen and Griffith 1997) [1] One of the rye AFPs has been biochemically purified they have also shown to have both endochitinase and antifreeze activity (the protein alters the shape of ice crystals and has a low level of thermal hysteresis activity). On the other hand a purified endochitinase from tobacco, a freezing-sensitive plant, was found to be devoid of antifreeze activity.

## Role of antifreeze proteins in cold stress

The physiological and biochemical roles of antifreeze proteins (AFPs) are important to protect the plant tissues from mechanical stress caused by ice formation. Antifreeze activity is present in overwintering plants in response to exposure to low temperatures and only in plants that tolerate the presence of ice in their tissues. Antifreeze activity is shown by different plant parts, including seeds, stems, crowns, bark, branches, buds, petioles, leaf blades, flowers, berries, roots, rhizomes and tubers. First reported in plants in 1992, the antifreeze activity has noted in many overwintering vascular plants, including ferns, gymnosperms, and monocot and dicot angiosperms (Griffith, Ala *et al*. 1992; Duman and Olsen 1993; Doucet, Byass *et al*. 2000; Zamani, Sturrock *et al*. 2003) [12, 8, 7, 40]

During winter as temperatures drops below 0C, ice formation is typically initiated initially in extracellular spaces of plant tissues and finally in intercellular spaces, xylem vessels and tracheids. Freezing injury usually occurs when intracellular water is lost growing extracellular ice resulting in cellular dehydration. The injury is also caused when plants are frozen for prolonged periods which results in a spontaneous process of recrystallization of ice wherein ice crystals coalesce to minimize their surface area (Knight, Wen *et al*. 1995) [22] Moreover, plant pathogens which thrive under low temperature conditions and have optimal growth temperatures below 20C, prosper under snow cover (Gaudet, Laroche *et al*. 2000) [11] (Snider, Hsiang *et al*. 2000) [34]. The freezing injury caused due to intercellular ice formation is lethal and results in the loss of semi permeability of the plasma membrane. However Freezing-tolerant plants have evolved mechanisms that allow them to avoid intracellular ice formation and thus many perennial and biennial plants survive winter without injury. These plants require a period of acclimation to cold temperature to develop freezing tolerance which involves the accumulation of proteins whose synthesis increases in response to low temperature. Some of these proteins are intracellular, including dehydrins, proteins involved in carbohydrate metabolism, 14-3-3 proteins, kinase regulators, and anti freeze are secreted into the apoplast that inhibit the growth of extracellular ice and pathogenic fungi. These plant AFPs are unusual proteins that have multiple, hydrophilic ice-binding domains that appear to function as inhibitors of ice recrystallization and ice nucleation and could enhance winter survival by slowing freezing processes (Griffith and Yaish 2004) [14].

**Role of antifreeze proteins in freezing in plants**

The overwintering plants exhibit freezing tolerance which helps them avoid the freeze injury during winter. The lower limit of freezing tolerance of a plant population is measured as LT50, which depicts the lethal temperature for 50% of the individuals. In case of a individual plant, LT50 is often determined as the loss of 50% of the electrolytes from plant tissues after freezing. (Marentes, Griffith *et al*. 1993) [1] The overwintering plants get acclimated to low temperatures in autumn and acquire freezing tolerance during which the LT50 becomes progressively low. Based on This LT50 and changes in it, plants are often selected for increased freezing tolerance in breeding programmes for cold stress tolerance. Although AFPs accumulate in the apoplast of winter cereals during cold acclimation as the plants acquire freezing tolerance (Marentes, Griffith *et al*. 1993) [1], their direct influence on cold tolerance has been approached in several ways. Increasing the AFP content of canola (*Brassica napus)* leaves by vacuum-infiltrating a solution of winter flounder type I AFP into the apoplast lowered their freezing temperature by 1.88C (Cutler, Saleem *et al*. 1989) [6], whereas extracting AFPs from cold-acclimated winter *rye* leaves did not had any effect on the freezing temperature but did increase injury after freezing and thawing(Marentes, Griffith *et al*. 1993) [1] Winter rye AFPs had no effect on protoplast survival after freezing and thawing, but did lower the LT50 of non acclimated winter *rye* suspension cells by 2.58C (Pihakaski-Maunsbach, Tamminen *et al*. 2003) [31]. Because winter *rye* AFPs are associated with the outer surfaces of cell walls, they can reduce freezing injury in intact cells by inhibiting the propagation of ice through the cell wall or by binding to extracellular ice and slowing its growth (Pihakaski-Maunsbach, Tamminen *et al*. 2003; Griffith and Yaish 2004) [31, 14]

**Plant transformation with genes encoding antifreeze proteins**

The transformation of agriculturally important crop plants by transferring single genes encoding antifreeze proteins to freezing-sensitive plants lowered their freezing temperatures by 18C. This will enhance the yields by improving the freezing tolerance of an overwintering crop or by increasing the survival of freezing sensitive crop plants following light frosts in colder climates (Hightower, Baden *et al*. 1991) [15]. Further, AFPs could improve the quality by increasing the shelf life of frozen foods by inhibiting the recrystallization of ice if the AFPs are targeted to accumulate in fruits and vegetables before harvest (Hightower, Baden *et al*. 1991) [15] (Kenward, Brandle *et al*. 1999) [20]. Presently plants such as *tobacco*, *tomato*, *potato* and *Arabidopsis*, have been used to study the effect of expressing cDNAs encoding AFPs originally isolated from fish, insects or plants. When plants transformed with genes encoding fish, insect or plant AFPs were cooled in the presence of an ice nucleator, there was no change in LT50. However, in the absence of an ice nucleator, plants transformed with genes encoding AFPs super cool by 1–38C more than wild type (Kenward, Brandle *et al*. 1999) [20] (Huang, Nicodemus *et al*. 2002) [13]. Although the CaMV 35S promoter is used to achieve constitutive gene expression in all plants transformed to produce AFPs, cold acclimation still has an effect on the accumulation and/or activity of the AFPs

**Summary and conclusion**

Chilling sensitive plants species show symptoms like growth retardation, chlorosis and necrosis, this leads to death of a plant. But cold tolerant species grow under low temperature conditions. Plants adjust their metabolism that gets altered due to rise or fall of temperature by producing stress responsive metabolites particularly include soluble sugars, amino acids, organic acids, polyamines and lipids, Antifreeze proteins (AFPs), Cold stress-induced genes (COR). Most of such genes encoding polypeptides are homolog's of late embryogenesis abundant proteins (LEA) and the polypeptides that are synthesized during the late embryogenesis phase. Development of high yielding cultivars to enhance high productivity under low temperature is a need in temperate regions of the world. Studying the physiological, metabolic, and molecular aspects of cold stress tolerance in different crops can explore the gene regulation process and mechanism. Genetic engineering can be reliable for the development of new cold stress tolerant varieties. New gene modifying /editing techniques like gene pyramiding and CRISPR/cas9 can be utilized for developing such high yielding and chilling resistant crops. QTL mapping, whole genome sequencing and functional genomics of various crops have already provided insights about the mechanism of cold tolerance and acclimation.

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