



# Signal transduction during cold stress in plants

Amolkumar U. Solanke and Arun K. Sharma

Department of Plant Molecular Biology, University of Delhi, South Campus, New Delhi – 110 021

## ABSTRACT

Cold stress signal transduction is a complex process. Many physiological changes like tissue break down and senescence occur due to cold stress. Low temperature is initially perceived by plasma membrane either due to change in membrane fluidity or with the help of sensors like  $\text{Ca}^{2+}$  permeable channels, histidine kinases, receptor kinases and phospholipases. Subsequently, cytoskeleton reorganization and cytosolic  $\text{Ca}^{2+}$  influx takes place. Increase in cytosolic  $\text{Ca}^{2+}$  is sensed by CDPKs, phosphatase and MAPKs, which transduce the signals to switch on transcriptional cascades. Photosynthetic apparatus have also been thought to be responsible for low temperature perception and signal transduction. Many cold induced pathways are activated to protect plants from deleterious effects of cold stress, but till date, most studied pathway is ICE-CBF-COR signaling pathway. However, the importance of CBF independent pathways in cold acclimation is supported by few *Arabidopsis* mutants' studies. Cold stress signaling has certain pathways common with other abiotic and biotic stress signaling which suggest cross-talks among these. Most of the economically important crops are sensitive to low temperature, but very few studies are available on cold susceptible crop plants. Therefore, it is necessary to understand signal transducing components from model plants and utilize that knowledge to improve survival of cold sensitive crop plants at low temperature. [Physiol. Mol. Biol. Plants 2008; 14(1&2) : 69-79] E-mail : arun@genomeindia.org

**Key words :** Calcium; CBF; cold; reactive oxygen species; signal transduction

Nature nurtures all living beings by providing suitable resources like air, light, water, temperature and soil. Optimum level of these factors helps the organisms to grow in healthy condition. Adversities in these environmental conditions compel the organisms to change their habitats. How do sessile plants manage during adverse conditions? As a consequence of their sessile nature, plants have developed ability to adapt to external, often unfavorable environmental conditions. Cold is one of the most common environmental factors that limit the geographical distribution and growing season of many plant species (Guy, 1990). On the basis of survival ability in cold stress, plants can be divided into tolerant and susceptible. The plants growing in tropical or subtropical regions are susceptible to low temperature whereas plants growing in temperate region have ability to survive even at  $-30\text{ }^{\circ}\text{C}$ , through the process of cold acclimation. By definition cold acclimation is the process, by which plants are able to adjust their metabolism to cold and increase freezing tolerance in response to low non-freezing temperature. The objective of this review is to understand the signal transduction process from cold

tolerant plants, mostly *Arabidopsis* and compare various features of this plant with cold-susceptible plants. Most of the economically important crop plants like rice, maize, tomato, soybean, cotton and fruit crops like banana, papaya, mango, grapes and orange are "chilling sensitive" often adversely affecting their quality and productivity. Understanding the importance of different signal transducing components will help to manipulate signaling pathway in susceptible plants to make them survive at low temperature. Therefore, it is necessary to understand it step by step, from stimulus to perception and from perception to early signal transduction which further eventually leads to dramatic alteration like change in plasma membrane fluidity, actin cytoskeleton rearrangement, cytosolic  $\text{Ca}^{2+}$  influx, protein phosphorylation, alteration in gene activity, increase in new protective gene products formation and secondary metabolism.

## Physiological changes due to low temperature

The effect of cold stress depends on the degree of severity and the time of exposure. The seedling stage of plants is most sensitive to chilling. The potential chilling symptoms are: surface lesion, a water-soaked appearance

**Correspondence and Reprint requests :** Arun K. Sharma

of the tissue, discoloration, desiccation, tissue break down, accelerated senescence, ethylene production, shortened shelf life and faster decay due to leakage of plant metabolites (Sharma *et al.*, 2005). Exposure of plants to temperature ranging from 0 °C to 10 °C causes chilling stress whereas exposure to temperature below 0 °C causes freezing stress. Chilling stress decreases the membrane fluidity due to fatty acid unsaturation in membrane lipids, changes composition and ratios of lipids to proteins in cell membrane (Wang *et al.*, 2006). Low non-freezing temperature also causes dehydration, mainly due to reduction in water uptake by roots and an impediment to close stomata. At freezing temperature, membrane damage is caused by severe cellular dehydration, associated with ice formation. The accumulation of ice in the intracellular spaces causes the physical disruption of cells and tissues. Depending on the freezing temperature and the degree of cellular dehydration, different forms of membrane damages can occur, including expansion-induced lysis, lamellar-to-hexagonal-II phase transition and fracture jump lesion. Freezing temperature also causes protein denaturation and precipitation of solutes as a result of freezing induced dehydration (Uemura *et al.*, 1995; Salinas, 2002). Both low and freezing temperatures result in the oxidative stress due to generation of reactive oxygen species (ROS). These ROS disequilibrate the electron transfer reactions and disturb accompanying biochemical reactions. Thus generation of ROS leads to cellular injury, which ultimately leads to death of plant due to damage of photosystem II reaction centre and membrane lipids (Prasad *et al.*, 1994; Suzuki and Mittler, 2006). Plants exposed to low or freezing temperatures confront a plethora of dangerous circumstances. Susceptible plants are unable to survive in such deleterious condition; however the tolerant plants are able to counter this deadly situation by triggering cold acclimation response, which is switched on by a systematic intracellular signaling cascade.

#### Cellular changes during low temperature stress

The very first step in cold signaling cascade is the perception of the low temperature. It was demonstrated that a very brief exposure to low temperature results in the onset of a cold signaling pathway and a continuous exposure of low temperature is not necessary (Henriksson and Trewavas, 2003). Temperature sensing in plants is dependent on the cooling rate,  $dT/dt$ , which means stepwise decrease in temperature rather than absolute temperature  $T$ . In *Arabidopsis*, roots were found to be sensitive to cooling rates of less than 0.01 °C/s  $dT/dt$

(Minorsky and Spanswick, 1989; Plieth *et al.*, 1999). Various changes brought about by low temperature are described in following sections.

*a. Changes in membrane fluidity* : Membrane fluidity changes instantly as a result of low temperature. It undergoes qualitative as well as quantitative modification in its lipid composition under cold stress (Wang *et al.*, 2006). The degree of fatty acid unsaturation and the content of phospholipids increases during cold acclimation. Therefore it is thought that membrane rigidification might play a major role in cold perception. This change in membrane rigidity is perceived by membrane proteins and they act as primary sensors or regulators (Mikami and Murata, 2003; Los and Murata, 2004). Many experimental evidences proved that membrane is the primary site of cold perception. Mutation in a gene encoding fatty acid desaturase, in a strain of *Synechocystis* exhibited plasma membrane rigidification even at room temperature and increased expression of number of cold inducible genes (Inaba *et al.*, 2003). In alfalfa cell suspension culture, benzyl alcohol (BA), a membrane fluidizer, prevented the expression of cold induced genes and the  $Ca^{2+}$  influx at 4 °C, but these events were induced at 25 °C, when dimethylsulfoxide was used as membrane rigidifier (Orvar *et al.*, 2000; Sangwan *et al.*, 2002). Cold activation of *Brassica napus BN115* promoter was strongly inhibited by BA, and induced at 25 °C by dimethylsulfoxide (Sangwan *et al.*, 2001). Voultier *et al.* (2006) also suggested the role of membrane rigidification in cold perception and signal transduction process. They analyzed diacylglycerol kinase (DAGK) activation, which is very early event occurring within seconds of cold exposure.

*b. Cytoskeletal reorganization* : Rearrangement of the actin cytoskeleton occurs downstream of the changes in membrane fluidity but up-stream of  $Ca^{2+}$  influx (Orvar *et al.*, 2000; Sangwan *et al.*, 2002). In alfalfa cell suspension culture, the induction of cold acclimation specific gene (*cas30*) and  $Ca^{2+}$  influx at 4 °C were prevented by jasplakinolide (JK), an actin microfilament stabilizer, but induced at 25 °C by the actin microfilament destabilizer cytochalasin D (CD). This suggests the role of actin cytoskeleton reorganization in cold signaling (Orvar *et al.*, 2000). It was also emphasized that the cytoskeleton may be a platform for several other physiological functions involved in cold acclimation, such as protein trafficking and modulation of activities of protein kinases/phosphatases that may or may not be dependent on  $Ca^{2+}$  influx (Orvar *et al.*, 2000).

### Cold sensors

Very little is known about cold sensors in plants. There are some potential low temperature sensors suggested by different researchers, but as yet there is no direct proof for either of them. Many authors emphasized the role of membrane based proteins as the sensors for detection of conformational changes in microdomain of membrane. Thus membrane located proteins sense the transition of the physical phase from the liquid-crystalline to gel state (Murata and Los, 1997; Los and Murata, 2004). Some putative sensors of cold are described here:

*a. Ca<sup>2+</sup> permeable channels* : Cold stress induces transient Ca<sup>2+</sup> influx into the cytoplasm. Therefore, calcium permeable channels responsible for this Ca<sup>2+</sup> influx are considered as sensors for low temperature (Monroy and Dindsa, 1995). The activation of calcium channels by cold is thought to be the result of physical alterations in cellular structure. Minorsky (1989) also proposed that the cold induced cytosolic Ca<sup>2+</sup> response may be the sensor of cold.

*b. Histidine kinases* : The two-component histidine kinases are the strongly advocated low temperature sensors in plants. The experimental evidence suggests that the cyanobacterium histidine kinase Hik33 and Hik19 (Suzuki *et al.*, 2000) and the *Bacillus subtilis* histidine kinases DesK (Aguilar *et al.*, 2001) are temperature sensors and regulate desaturase gene expression in response to low temperature. Several putative two-component histidine kinases are identified in *Arabidopsis* (Urao *et al.*, 2000a, b) out of these *AtHK1*, has been found to be upregulated by low temperature, high salinity and dehydration. *AtHK1* protein senses these stresses and transduces the signal to the nucleus through a phosphorylation cascade (Urao *et al.*, 1999).

*c. Receptor kinases* : Receptor like kinases (RLKs) are other putative low temperature sensors. They have membrane spanning domains, which function in transmitting extracellular signals into intracellular target molecules. In *Arabidopsis*, one member of this family, *RPK1* was shown to induce its expression by cold, dehydration, high salt and ABA (Hong *et al.*, 1997).

*d. Phospholipases* : Changes in the membrane phospholipids metabolism are implicated in cold response signaling. *Phospholipase C* and *D* are accumulated as early as 15 seconds after cold treatment (Ruelland *et al.*, 2002). It increases the production of phosphatidic acid (PtdOH) by hydrolyzing membrane phospholipids, which is proposed as membrane based secondary messenger molecule. Phospholipase D

anchors the microtubules to “plasma membrane” so its activation can lead to conformational change in the cytoskeleton (Gardiner *et al.*, 2001; Dhonukshe *et al.*, 2003). It further leads to actin filaments rearrangement, thus probably activating stretch-induced Ca<sup>2+</sup> channels.

*e. Photosynthetic apparatus* : It is proposed that in low temperature, photosynthetic apparatus itself functions as a sensor for imbalance between the light energy absorbed through photochemistry versus the energy utilized through metabolism. Thus energy imbalance is sensed through alteration in the excitation pressure of photosystem II, which results into reduction in photosynthesis. It has been presumed that the redox signal, which is generated from the plastoquinone pool, regulates chloroplast and nuclear photosynthetic gene expression (Fey *et al.*, 2005; Ensminger *et al.*, 2006). Thus redox state of the photosynthetic apparatus can act as a low temperature sensor (Huner *et al.*, 1998; Ensminger *et al.*, 2006), which further interacts with other processes responsible for cold acclimation (Gray *et al.*, 1997).

### Cold signal transducers in plants

The elevation in cytosolic Ca<sup>2+</sup> from extracellular spaces is an early event in the response to cold. This cytosolic Ca<sup>2+</sup> is suggested as an important second messenger in low temperature signal transduction and developing cold acclimation. A positive correlation between cold-induced Ca<sup>2+</sup> influx and accumulation of cold-induced transcripts has been shown for alfalfa (Monroy and Dhidsa, 1995; Reddy and Reddy, 2004) and *Arabidopsis* (Henriksson and Trewavas, 2003). Knight and coworkers used transgenic approach to demonstrate transient increase in cytosolic Ca<sup>2+</sup> in response to cold. They developed transgenics of *Arabidopsis* and tobacco expressing the calcium-sensitive luminescent protein aequorin and demonstrated a rise in cytosolic Ca<sup>2+</sup> concentration in response to low temperature (Knight *et al.*, 1991). Different chelators and channel blockers were also used to show the cytosolic Ca<sup>2+</sup> influx and its role as second messenger in response to cold in alfalfa (Monroy *et al.*, 1993; Knight *et al.*, 1996) and *Arabidopsis* (Tahtiharju *et al.*, 1997). Along with cold, cytosolic Ca<sup>2+</sup> influx also observed in other environmental stresses, which suggest that, there is a specific signature of the cytosolic Ca<sup>2+</sup> for different stresses. Ca<sup>2+</sup> signature can be described as the characteristics of the time course of changes in cytosolic Ca<sup>2+</sup>. Their sites of appearance in cells are used by the plant to identify the type and intensity of the stimulus (Evans *et al.*, 2001; Plieth, 2005). Thus the precise kinetics, magnitude and cellular source of stimulus-induced cytosolic Ca<sup>2+</sup> influx (calcium signature) have

been proposed to encode information regarding particular stimulus and to determine the specific end response (Lecourieux *et al.*, 2006). Various abiotic stress response use  $\text{Ca}^{2+}$  from different subcellular sources, including the extracellular compartment, vacuole and mitochondria (Knight *et al.*, 1996; Subbaiah *et al.*, 1998). It is also observed that effective  $\text{Ca}^{2+}$  signature is produced only in particular tissue or organs. During cold stress cytosolic  $\text{Ca}^{2+}$  influx occurs in whole plant, in contrast to drought, where it is present only in roots (Knight and Knight, 2000).

The elevation in cytosolic  $\text{Ca}^{2+}$  transmits primary signal through  $\text{Ca}^{2+}$  regulated proteins called  $\text{Ca}^{2+}$  sensors and changes phosphorylation status of different proteins (Monroy *et al.*, 1993). The major  $\text{Ca}^{2+}$  sensors in plants are calmodulin (CaM), CaM domain-containing protein kinases (CDPKs), calcineurin B-like proteins (CBLs) and CBL-interacting protein kinases (CIPKs). These  $\text{Ca}^{2+}$  sensors are categorized into “sensor relay” and “responders”. Sensor relay bind to  $\text{Ca}^{2+}$  and undergo conformational changes that regulate the gene expression e.g., calmodulin (CaM) and calcineurin B-like proteins (CBLs). Responders, like protein kinase and phospholipase have effector domains through which they relay the message to their downstream targets (Reddy and Reddy, 2004; Klimecka and Muszynska, 2007). Thus elevation of cytosolic  $\text{Ca}^{2+}$  alone or in association with calmodulin could activate  $\text{Ca}^{2+}$  dependent protein kinases. Different  $\text{Ca}^{2+}$  signatures are distinguished by different  $\text{Ca}^{2+}$  binding proteins and protein kinases and decoding of these signals causes changes in gene expression leading to appropriate physiological responses (Yang and Poovaiah, 2003; Sathyanarayanan and Poovaiah, 2004). Some of the components of calcium signaling are described in the following sections.

*a. CDPKs* : Many CDPKs are found to be upregulated by cold in different plants. In alfalfa cell suspension culture, Monroy and coworkers utilized W7, an antagonist of CDPKs and CaM, which inhibited the capacity of the culture to cold acclimate (Monroy *et al.*, 1993). In same study, H7, a protein kinase inhibitor, inhibited the capacity of cell culture to cold acclimate upto 50%. Similar results were obtained by Tahtiharju *et al.* (1997) working with *Arabidopsis*. Kim *et al.* (2003) showed the role of *CIPK3* in cold signaling via changes in expression pattern of *RD29A* (*Responsive to desiccation 29A*), *KIN1* (*cold-inducible1*) and *KIN2* (*cold-inducible2*) genes in *Arabidopsis*. They proposed that *CIPK3* is located upstream of transcription factors and downstream of the  $\text{Ca}^{2+}$  signal. The over-expression

of *OsCIPK03*, *OsCIPK12* and *OsCIPK15* confers significantly improved tolerance to cold, drought and salt stress in rice (Xiang *et al.*, 2007). In tobacco, a calmodulin gene *NpCaM1* was found to be upregulated by cold (van der Luit *et al.*, 1999). The comprehensive analysis of CDPKs in rice shows that *OsCPK4*, *OsCPK5* and *OsCPK13* (*OsCDPK7*) are unregulated in response to cold (Ray *et al.*, 2007). The overexpression of *OsCDPK7* in rice resulted in improvement of cold, salt and drought stress tolerance (Saijo *et al.*, 2001). In another study, the overexpression of *OsCDPK13* and calreticulin interacting protein (CRTintP1) conferred cold tolerance to rice (Komatsu *et al.*, 2007). A direct involvement of protein kinase C dependent phosphorylation in cold stress in *Brassica juncea* was shown by Deswal *et al.* (2004).

*b. Phosphatases* : Protein phosphatases (PP2A and 2B) also act as  $\text{Ca}^{2+}$  sensor. Monroy *et al.* (1998) showed that low temperature provoked a rapid decrease in PP2A activity, and this inhibition was mediated by cold-induced  $\text{Ca}^{2+}$  influx and occurred at a post-transcriptional level. The cytoplasmic PP2A has been shown to inactivate MAP kinases, receptor protein kinases, and CDPKs in different systems (Salinas, 2002). They speculated that phosphatases acted either indirectly via inactivation of protein kinases or directly via regulating trans-acting factors. Thus, it can be concluded that when plants are exposed to low temperature, the signal is sensed and transduced into an increase in cytosolic  $\text{Ca}^{2+}$ , which in turn inhibits protein phosphatase 2A (PP2A) activity and activates a series of phosphorylation switches involving CDPKs and low temperature regulated gene expression. The gene of CBL family, *AtCBL1*, was highly up-regulated by cold and drought in *Arabidopsis* (Kulda *et al.*, 1999). Its over-expression led to enhanced tolerance to salt and drought stress but reduced tolerance to freezing stress, suggesting its role as a negative regulator of cold response in *Arabidopsis* (Cheong *et al.*, 2003).

*c. MAPKs* : Mitogen activated protein kinase cascade is also involved in cold stress signaling. MAPKs perform their function as a part of protein kinase modules, which is mainly composed of MAPKs, MAPKKs and MAPKKKs. Cold associated increase in mRNA levels of MAPKs has been observed in response to high salt, drought, wounding and pathogen attack (Widmann *et al.*, 1999; Nakagami *et al.*, 2005). It has been reported that *AtPK6* and *AtPK19* are upregulated in response to low temperature (Mizoguchi *et al.*, 1995). Other two genes *AtMPK3* (MAPK) and *AtMPKK1* (MAPKKK) are also transcriptionally induced by cold (Mizoguchi *et al.*, 1996).

In accordance with a role of *AtMPK3* in cold acclimation, a closely related alfalfa homologue, *MMK4*, is also upregulated upon cold stress (Jonak *et al.*, 1996). A stress activated MAP kinase (SAMK) was also upregulated in alfalfa in response to cold (Sangwan *et al.*, 2002). The reactive oxygen species, which is integral part of cold stress, have been shown to activate a MAPK cascade in *Arabidopsis*. This cascade includes *ANP1* (a MAPKKK), *AtMPK3* and *AtMPK6* and its positive regulator, nucleoside diphosphate kinase 2 (Kovtun *et al.*, 2000; Moon *et al.*, 2003). Homologue of *ANP1* from tobacco, *NPK1* functions in cold signal transduction cascade and influences the auxin signal transduction (Kovtun *et al.*, 1998) and plant development (Jin *et al.*, 2002). *NPK1* acts as a positive regulator of cell death associated with plant immunity and disease (del Pozo *et al.*, 2004). The overexpression of *NPK1* also enhances freezing and drought tolerance in transgenic maize (Shou *et al.*, 2004a, b). This suggests that stress tolerance not only involves cold-responsive mechanism but also the basic machinery of the plant.

*d. Reactive Oxygen Species (ROS)* : Cold stress results into an oxidative burst due to the generation of active oxygen species like superoxide, hydrogen peroxide and hydroxyl radicals. These reactive oxygen species induce ROS scavengers and other protective mechanisms, as well as damaging agents contributing to injury in the plants (Prasad *et al.*, 1994). The mechanism by which plants are able to sense the oxidative burst in response to cold is still unknown. But some reviews have elaborated the role of ROS in stress signal transduction (Mittler, 2002; Mittler *et al.*, 2004; Suzuki and Mittler, 2006).

### Transcriptional cascade

The low temperature signal is perceived by membrane and transduced by different transduction components results in stimulation of transcription of several genes. Numerous transcription factors that facilitate cold signaling and control expression of cold regulon have been identified in *Arabidopsis* (Shinozaki *et al.*, 2003; Gilmour *et al.*, 2004; Chinnusamy *et al.*, 2006; Yamaguchi-Shinozaki and Shinozaki, 2006, Zhu *et al.*, 2007) and homolog of these factors have been reported in other plants also (Jaglo *et al.*, 2001; Zhang *et al.*, 2004).

*a. CBF pathway* : The linking components between signal transduction and activation of transcription factors are yet to be revealed. But, present information support that *ICE1* (*Inducer of CBF Expression 1*) a MYC-like transcription factor is the upstream component

in cold transcription cascade (Chinnusamy *et al.*, 2003). Zhu and coworkers suggested that in *Arabidopsis*, *ICE1* get phosphorylated in response to cold treatment and therefore transcription cascades that are directed by *ICE1* and *ICE1*-like bHLH proteins are probably activated by the cold sensing and signaling pathway via protein phosphorylation (Zhu *et al.*, 2007). Recent advances suggest that the *HOS1* (high expression of osmotically responsive genes), a RING-type ubiquitin E3 ligase negatively regulates the function of *ICE1* at low temperature by ubiquitination-mediated degradation (Dong *et al.*, 2006), but the SUMO E3 ligase *SIZ1*-mediated sumoylation represses the polyubiquitination that leads to an increase in its stability at low temperature (Miura *et al.*, 2007). Moreover, it was observed that at normal growth temperature, *HOS1* resides in the cytoplasm, but it is shifted to nucleus upon cold treatment, suggesting its role in relay of cold signal to the nucleus (Lee *et al.*, 2001). An R2R3-type MYB transcription factor, *AtMYB15*, was also found to be physically interact with *ICE1* (Agarwal *et al.*, 2006). Phosphorylation and *SIZ1*-mediated SUMO conjugation/deconjugation of *ICE1* are the key processes that finally activate *ICE1* to bind to its target genes. Active *ICE1* binds to *MYC cis*-elements (CANNTG) in the *CBF3* (C-repeat Binding Factor) promoter to induce its expression (Chinnusamy *et al.*, 2003). There are three *CBF* genes present in *Arabidopsis* genome in tandem and are induced by low temperature (Stockinger *et al.*, 1997; Medina *et al.*, 1999). These are the most characterized transcription factors which directly or through other transcription factor activate cold regulon (Liu *et al.*, 1998; Thomashow, 1999; Yamaguchi-Shinozaki and Shinozaki, 2006). Protein products of these *CBF* genes bind to core CRT/DRE (C-repeat/Dehydration Responsive Element) *cis*-element (A/GCCGAC), that are present in promoters of target genes through their AP2/ERF DNA binding domains (Yamaguchi-Shinozaki and Shinozaki, 1993, 1994, 2005; Thomashow, 1999). Low temperature transiently induces the expression of all *CBF* transcription factors within minutes (Vogel *et al.*, 2005). Overexpression of each *CBF* gene constitutively induces *CBF* regulon and enhances freezing tolerance in transgenic plants (Jaglo-Ottosen, 1998; Gilmour *et al.*, 2000, 2004). A study using loss of function mutant *cbf2*, the gene encoding *CBF2*, suggests that *CBF2* is the negative regulator of *CBF1* and *CBF3* (Novillo *et al.*, 2004), where as Chinnusamy *et al.* (2003, 2006) suggested that *CBF3* acts as negative regulator of *CBF2*. In recent years, many microarray experiments have been carried out for identifying genes that are present in *CBF* regulon (Gilmour *et al.*, 2004; Vogel *et al.*, 2005). The genes

assigned to this regulon encode proteins that fall into a wide range of functional categories like transcription factor, signal transduction pathway components, biosynthetic proteins, cryoprotectants and other stress related proteins as well as a huge number of genes encoding proteins of unknown function (Thomashow, 1998; Fowler and Thomashow, 2002; Maruyama *et al.*, 2004; Vogel *et al.*, 2005). Many proteins from this regulon have been previously shown to contribute to increased freezing tolerance. Cryoprotectant protein, COR15a, an enzyme involved in regulating proline levels (P5CS2) and galactinol synthase, the enzyme that catalyzes the first step in the synthesis of raffinose are few of such proteins (van Buskirk and Thomashow, 2006). Several genes from CBF regulon encode transcription factors such as RAP2.1, RAP2.6 and ZAT10. This suggests that in cold acclimated plant like *Arabidopsis* ICE1-CBF-COR pathway plays an important role.

*b. CBF independent pathways* : Apart from CBF, there are some other pathways which contribute to cold tolerance in *Arabidopsis*. *ZAT12* is a transcription factor, regulon of which is well characterized and has been shown to be activated by ROS (Davletova *et al.*, 2005; Vogel *et al.*, 2005). Other transcription factors like *HOS9*, a putative homeodomain transcription factor (Zhu *et al.*, 2004) and *HOS10*, a putative R2R3-type MYB transcription factor (Zhu *et al.*, 2005) also confer cold acclimation to *Arabidopsis*, via CBF independent pathways. Recently identified gene for *eskimo1* mutant, *ESK1* also acts in a manner independent of CBF pathway and provides tolerance to *Arabidopsis* plants in cold stress via set of genes regulated by salt and osmotic stress and ABA treatment (Xin *et al.*, 1998, 2007). This shows that along with ICE-CBF-COR pathway, other transcriptional networks are simultaneously activated for cold acclimation.

*c. Transcriptional activators* : In addition to the transcription factors, which directly bind to the *cis*-elements in the promoter regions of the stress responsive genes, transcriptional activation also needs some additional cofactors. These cofactors play an important role in determining the levels of gene expression. In *Arabidopsis*, CBF1-mediated transcription requires the transcriptional adaptor ADA and the histone acetyltransferase GCN5 (Stockinger *et al.*, 2001). The mutation in either of them can affect the low temperature regulation of COR genes. It has been proposed that mutation in *Arabidopsis sfr6* may fall in this category, because in *sfr6* mutants expression of some COR genes get affected without any change in expression pattern of CBF genes (Knight *et al.*, 1999).

### Comparison of cold signaling in tolerant and susceptible plants

Although the mechanism of cold perception, signal transduction and activation of cold-responsive machinery is yet to be revealed completely, a lot is known in model plant like *Arabidopsis* (Zhu *et al.*, 2007) with reverse genetic tools developed by Ishitani and coworkers (1997) and available mutants. However, it is yet to be found out if this signaling cascade is similar in other cold tolerant and susceptible plant species. Different response of tolerant and susceptible plants to cold stress may be due to inactivation of some signaling molecules or transcription factors during long period of evolution, or may be due to loss of function of regulatory molecules acting downstream to signaling components. This could be possible due to mutation in genes or their promoter regions, or due to loss of some important genes responsible for cold acclimation. One study of Poaceae family by Sarhan *et al.* (1997) with cold tolerant species wheat and rye; and cold susceptible species rice and maize, has revealed that all cereals contain homologues of low temperature regulated wheat genes in their genomes. But gene expression in response to cold is found only in the tolerant cereals (Sarhan and Danyluk, 1998). This suggests changes in promoter activity of top ranking components of signal transduction or transcription factors in susceptible plants. In another study, the comparison of the CBF regulon in *Arabidopsis* and a cold susceptible tomato plants again supported this view (Zhang *et al.*, 2004). As discussed earlier, in *Arabidopsis*, CBF pathway actively participates in cold acclimation, but unlike *Arabidopsis*, tomato cannot cold acclimate. It was shown that all three CBF genes are present in tomato in tandem like that of *Arabidopsis*, out of which only the expression of *LeCBF1* gene gets up-regulated in response to cold in tomato. The constitutive overexpression of either *LeCBF1* or *AtCBF3* in transgenic tomato plants did not improve freezing tolerance. Gene expression study using microarray identified only four genes, which were induced 2.5 fold or more in the *LeCBF1* or *AtCBF3* over-expressing plants (Zhang *et al.*, 2004), whereas there are more than 500 genes (Vogel *et al.*, 2005) and 250 metabolites (Cook *et al.*, 2004) in *Arabidopsis* CBF regulon. This indicates that CBF regulon changed in tomato in due period. But this conclusion is subject to change if complete set of cDNA data of tomato used for microarray experiment.

### Cross-talk with other stresses

Many reviews are available on cross-talk among different abiotic stresses (Knight and Knight, 2001; Zhu, 2001; Xiong *et al.*, 2002; Chinnusamy *et al.*, 2004; Beck *et al.*,

2007; Vij and Tyagi, 2007). Dehydration is the common physiological disorder during cold, drought and salt stress (Beck *et al.*, 2007). Decrease in turgour pressure due to freezing is known to induce biosynthesis of plant stress hormone ABA. In cold stress, ABA dependent pathway plays very little but significant role (Gusta *et al.*, 2005). As discussed before, generation of reactive oxygen species occurs in almost all abiotic stresses. At every node of signaling, from  $\text{Ca}^{2+}$  influx, signal transducers (CDPKs and MAPKs) to transcription factors, cross-talk is possible (Knight and Knight, 2001). So it is necessary to consider cold signaling as a network, in which much more overlap between its branches is possible.

### CONCLUSION AND PERSPECTIVES

Current review covered the recent advances in low temperature signal transduction and activation of molecular response in plants. On the basis of growth under cold stress, plants are divided into tolerant and susceptible types. Physiological changes in plants can be categorized into changes due to chilling and changes due to freezing. The very first organelle to perceive low temperature change is plasma membrane. Changes in plasma membrane are sensed by different sensor proteins and result in cytoskeleton reorganization, which finally leads to cytosolic  $\text{Ca}^{2+}$  influx. Calcium signal transduction further transduces these signals to nucleus by different kinases and phosphatases and switched on a complete transcriptional cascade. Thus the reprogramming of gene expression in response to low temperature results in the accumulation, not only of protective proteins but also of hundreds or more of metabolites, some of which are known to have protective response. To gain a better understanding of different signaling components, their functions and how these signals communicate with others, it is important to develop novel methods of analysis. A single effort of developing mutant population of *Arabidopsis* plants over-expressing *luciferase* gene under the control of cold and dehydration inducible *CBF3* promoter throws light on cold tolerance mechanism. Development of mutants using transposon tagging and T-DNA insertion lines will help to analyze the function of identified genes. The availability of the complete genome sequence of cold acclimated plant *Arabidopsis* and cold susceptible plant like rice has enabled the use of strategies like genome-wide expression profiling to identify genes associated with cold acclimation or genes responsible for hazardous effect in sensitive plants. Comparative analysis of cold-tolerant to cold-susceptible species is now possible due

to the advances in sequencing information from several species. In summary, a complete understanding on how cold acclimation response is activated in tolerant plants, with the help of transgenic approach will help to manipulate susceptible crop plants and increase agricultural productivity.

### REFERENCES

- Agarwal, M., Hao, Y., Kapoor, A., Dong, C.H., Fujii, H., Zheng, X. and Zhu, J.K. (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.
- Aguilar, P.S., Hernandez-Arriaga, A.M., Cybulski, L.E., Erazo, A.C. and de Mendoza, D. (2001). Molecular basis of thermosensing: a two-component signal transduction thermometer in *Bacillus subtilis*. *EMBO J.*, 20: 1681-1691.
- Beck, E.H., Fetting, S., Knake, C., Hartig, K. and Bhattarai, T. (2007). Specific and unspecific responses of plants to cold and drought stress. *J. Biosci.*, 32: 501-510.
- Cheong, Y.H., Kim, K.N., Pandey, G.K., Gupta, R., Grant, J.J. and Luan, S. (2003). CBL1, a calcium sensor that differentially regulates salt, drought, and cold responses in *Arabidopsis*. *Plant Cell*, 15: 1833-1845.
- Chinnusamy, V., Ohta, M., Kanrar, S., Lee, B.H., Hong, X., Agarwal, M. and Zhu, J.K. (2003). ICE1: a regulator of cold-induced transcriptome and freezing tolerance in *Arabidopsis*. *Genes Dev.*, 17: 1043-1054.
- Chinnusamy, V., Schumaker, K. and Zhu, J.K. (2004). Molecular genetic perspectives on cross-talk and specificity in abiotic stress signalling in plants. *J. Exp. Bot.*, 55: 225-236.
- Chinnusamy, V., Zhu, J. and Zhu, J.K. (2006). Gene regulation during cold acclimation in plants. *Physiol. Plant.*, 126: 52-61.
- Cook, D., Fowler, S., Fiehn, O. and Thomashow, M.F. (2004). A prominent role for the CBF cold response pathway in configuring the low-temperature metabolome of *Arabidopsis*. *Proc. Natl. Acad. Sci. USA*, 101: 15243-15248.
- Davletova, S., Schlauch, K., Coutu, J. and Mittler, R. (2005). The zinc-finger protein Zat12 plays a central role in reactive oxygen and abiotic stress signaling in *Arabidopsis*. *Plant Physiol.*, 139: 847-856.
- del Pozo, O., Pedley, K.F. and Martin, G.B. (2004). MAPKKKalpha is a positive regulator of cell death associated with both plant immunity and disease. *EMBO J.*, 23: 3072-3082.
- Deswal, R., Chowdhary, G.K. and Sopory, S.K. (2004). Purification and characterization of a PMA-stimulated kinase and identification of PMA-induced phosphorylation of a polypeptide that is dephosphorylated by low temperature in *Brassica juncea*. *Biochem. Biophys. Res. Commun.*, 322: 420-427.

- Dhonukshe, P., Laxalt, A.M., Goedhart, J., Gadella, T.W. and Munnik, T. (2003). Phospholipase D activation correlates with microtubule reorganization in living plant cells. *Plant Cell*, 15: 2666-2679.
- Dong, C.H., Agarwal, M., Zhang, Y., Xie, Q. and Zhu, J.K. (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. USA*, 103: 8281-8286.
- Ensminger, I., Busch, F. and Huner, N.P.A. (2006). Photostasis and cold acclimation: sensing low temperature through photosynthesis. *Physiol. Plant.*, 126: 28-44.
- Evans, N.H., McAinsh, M.R. and Hetherington, A.M. (2001). Calcium oscillations in higher plants. *Curr. Plant Biol.*, 4: 415-420.
- Fey, V., Wagner, R., Brautigam, K. and Pfannschmidt, T. (2005). Photosynthetic redox control of nuclear gene expression. *J. Exp. Bot.*, 56: 1491-1498.
- Fowler, S. and Thomashow, M.F. (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.
- Gardiner, J.C., Harper, J.D., Weerakoon, N.D., Collings, D.A., Ritchie, S., Gilroy, S., Cyr, R.J. and Marc, J. (2001). A 90-kD phospholipase D from tobacco binds to microtubules and the plasma membrane. *Plant Cell*, 13: 2143-2158.
- Gilmour, S.J., Fowler, S.G. and Thomashow, M.F. (2004). *Arabidopsis* transcriptional activators CBF1, CBF2, and CBF3 have matching functional activities. *Plant Mol. Biol.*, 54: 767-781.
- Gilmour, S.J., Sebolt, A.M., Salazar, M.P., Everard, J.D. and Thomashow, M.F. (2000). Overexpression of the *Arabidopsis* CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation. *Plant Physiol.*, 124: 1854-1865.
- Gray, G.R., Chauvin, L.P., Sarhan, F. and Huner, N. (1997). Cold acclimation and freezing tolerance (A complex interaction of light and temperature). *Plant Physiol.*, 114: 467-474.
- Gusta, L.V., Trischuk, R. and Weiser, C.J. (2005). Plant cold acclimation: The role of abscisic acid. *J. Plant Growth Regul.*, 24: 308-318.
- Guy, C.L. (1990). Cold acclimation and freezing stress tolerance: Role of protein metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 41: 187-223.
- Henriksson, K.N. and Trewavas, A.J. (2003). The effect of short-term low-temperature treatments on gene expression in *Arabidopsis* correlates with changes in intracellular Ca<sup>2+</sup> levels. *Plant, Cell Environ.*, 26: 485-496.
- Hong, S.W., Jon, J.H., Kwak, J.M. and Nam, H.G. (1997). Identification of a receptor-like protein kinase gene rapidly induced by abscisic acid, dehydration, high salt, and cold treatments in *Arabidopsis thaliana*. *Plant Physiol.*, 113: 1203-1212.
- Huner, N.P.A., Oquist, G. and Sarhan, F. (1998). Energy balance and acclimation to light and cold. *Trends Plant Sci.*, 3: 224-230.
- Inaba, M., Suzuki, I., Szalontai, B., Kanasaki, Y., Los, D.A., Hayashi, H. and Murata, N. (2003). Gene-engineered rigidification of membrane lipids enhances the cold inducibility of gene expression in *synechocystis*. *J. Biol. Chem.*, 278: 12191-12198.
- Ishitani, M., Xiong, L., Stevenson, B. and Zhu, J.K. (1997). Genetic analysis of osmotic and cold stress signal transduction in *Arabidopsis*: interactions and convergence of abscisic acid-dependent and abscisic acid-independent pathways. *Plant Cell*, 9: 1935-1949.
- Jaglo-Ottosen, K.R., Gilmour, S.J., Zarka, D.G., Schabenberger, O. and Thomashow, M.F. (1998). *Arabidopsis* CBF1 overexpression induces COR genes and enhances freezing tolerance. *Science*, 280: 104-106.
- Jaglo, K.R., Kleff, S., Amundsen, K.L., Zhang, X., Haake, V., Zhang, J.Z., Deits, T. and Thomashow, M.F. (2001). Components of the *Arabidopsis* C-repeat/dehydration-responsive element binding factor cold-response pathway are conserved in *Brassica napus* and other plant species. *Plant Physiol.*, 127: 910-917.
- Jin, H., Axtell, M.J., Dahlbeck, D., Ekwenna, O., Zhang, S., Staskawicz, B. and Baker, B. (2002). NPK1, an MEKK1-like mitogen-activated protein kinase kinase, regulates innate immunity and development in plants. *Dev. Cell*, 3: 291-297.
- Jonak, C., Kiegerl, S., Ligterink, W., Barker, P.J., Huskisson, N.S. and Hirt, H. (1996). Stress signaling in plants: a mitogen-activated protein kinase pathway is activated by cold and drought. *Proc. Natl. Acad. Sci. USA*, 93: 11274-11279.
- Kim, K.N., Cheong, Y.H., Grant, J.J., Pandey, G.K. and Luan, S. (2003). CIPK3, a calcium sensor-associated protein kinase that regulates abscisic acid and cold signal transduction in *Arabidopsis*. *Plant Cell*, 15: 411-423.
- Klimecka, M. and Muszynska, G. (2007). Structure and functions of plant calcium-dependent protein kinases. *Acta Biochim. Pol.*, 54: 219-233.
- Knight, H. and Knight, M.R. (2000). Imaging spatial and cellular characteristics of low temperature calcium signature after cold acclimation in *Arabidopsis*. *J. Exp. Bot.*, 51: 1679-1686.
- Knight, H. and Knight, M.R. (2001). Abiotic stress signalling pathways: specificity and cross-talk. *Trends Plant Sci.*, 6: 262-267.
- Knight, H., Trewavas, A.J. and Knight, M.R. (1996). Cold calcium signaling in *Arabidopsis* involves two cellular pools and a change in calcium signature after acclimation. *Plant Cell*, 8: 489-503.
- Knight, H., Veale, E.L., Warren, G.J. and Knight, M.R. (1999). The *sfr6* mutation in *Arabidopsis* suppresses low-temperature induction of genes dependent on the CRT/DRE sequence motif. *Plant Cell*, 11: 875-886.
- Knight, M.R., Campbell, A.K., Smith, S.M. and Trewavas, A.J. (1991). Transgenic plant aequorin reports the effects of touch and cold-shock and elicitors on cytoplasmic calcium. *Nature*, 352: 524-526.



- Komatsu, S., Yang, G., Khan, M., Onodera, H., Toki, S. and Yamaguchi, M. (2007). Over-expression of calcium-dependent protein kinase 13 and calreticulin interacting protein 1 confers cold tolerance on rice plants. *Mol. Genet. Genomics*, 277: 713-723.
- Kovtun, Y., Chiu, W.L., Tena, G. and Sheen, J. (2000). Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. *Proc. Natl. Acad. Sci. USA*, 97: 2940-2945.
- Kovtun, Y., Chiu, W.L., Zeng, W. and Sheen, J. (1998). Suppression of auxin signal transduction by a MAPK cascade in higher plants. *Nature*, 395: 716-720.
- Kudla, J., Xu, Q., Harter, K., Gruissem, W. and Luan, S. (1999). Genes for calcineurin B-like proteins in *Arabidopsis* are differentially regulated by stress signals. *Proc. Natl. Acad. Sci. USA*, 96: 4718-4723.
- Lecourieux, D., Ranjeva, R. and Pugin, A. (2006). Calcium in plant defence-signalling pathways. *New Phytol.*, 171: 249-269.
- Lee, H., Xiong, L., Gong, Z., Ishitani, M., Stevenson, B. and Zhu, J.K. (2001). The *Arabidopsis* *HOS1* gene negatively regulates cold signal transduction and encodes a RING finger protein that displays cold-regulated nucleocytoplasmic partitioning. *Genes Dev.*, 15: 912-924.
- Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K. and Shinozaki, K. (1998). Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *Plant Cell*, 10: 1391-1406.
- Los, D.A. and Murata, N. (2004). Membrane fluidity and its roles in the perception of environmental signals. *Biochim. Biophys. Acta.*, 1666: 142-157.
- Maruyama, K., Sakuma, Y., Kasuga, M., Ito, Y., Seki, M., Goda, H., Shimada, Y., Yoshida, S., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2004). Identification of cold-inducible downstream genes of the *Arabidopsis* DREB1A/CBF3 transcriptional factor using two microarray systems. *Plant J.*, 38: 982-993.
- Medina, J., Bargues, M., Terol, J., Perez-Alonso, M. and Salinas, J. (1999). The *Arabidopsis* *CBF* gene family is composed of three genes encoding AP2 domain-containing proteins whose expression is regulated by low temperature but not by abscisic acid or dehydration. *Plant Physiol.*, 119: 463-470.
- Mikami, K. and Murata, N. (2003). Membrane fluidity and the perception of environmental signals in cyanobacteria and plants. *Prog. Lipid. Res.*, 42: 527-543.
- Minorsky, P.V. (1989). Temperature sensing by plants: A review and hypothesis. *Plant Cell Environ.*, 12: 119-135.
- Minorsky, P.V. and Spanswick, R.M. (1989). Electrophysiological evidence for calcium in temperature sensing by roots of cucumber seedlings. *Plant Cell Environ.*, 12: 137-143.
- Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.*, 7: 405-410.
- Mittler, R., Vanderauwera, S., Gollery, M. and Van Breusegem, F. (2004). Reactive oxygen gene network of plants. *Trends Plant Sci.*, 9: 490-498.
- Miura, K., Jin, J.B., Lee, J., Yoo, C.Y., Stirm, V., Miura, T., Ashworth, E.N., Bressan, R.A., Yun, D.J. and Hasegawa, P.M. (2007). SIZ1-mediated sumoylation of ICE1 controls CBF3/DREB1A expression and freezing tolerance in *Arabidopsis*. *Plant Cell*, 19: 1403-1414.
- Mizoguchi, T., Hayashida, N., Yamaguchi-Shinozaki, K., Kamada, H. and Shinozaki, K. (1995). Two genes that encode ribosomal-protein S6 kinase homologs are induced by cold or salinity stress in *Arabidopsis thaliana*. *FEBS Lett.*, 358: 199-204.
- Mizoguchi, T., Irie, K., Hirayama, T., Hayashida, N., Yamaguchi-Shinozaki, K., Matsumoto, K. and Shinozaki, K. (1996). A gene encoding a mitogen-activated protein kinase kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA*, 93: 765-769.
- Monroy, A.F., Castonguay, Y., Laberge, S., Sarhan, F., Vezina, L.P. and Dhindsa, R.S. (1993). A new cold-induced alfalfa gene is associated with enhanced hardening at subzero temperature. *Plant Physiol.*, 102: 873-879.
- Monroy, A.F. and Dhindsa, R.S. (1995). Low-temperature signal transduction: induction of cold acclimation-specific genes of alfalfa by calcium at 25 degrees C. *Plant Cell*, 7: 321-331.
- Monroy, A.F., Sangwan, V. and Dhindsa, R.S. (1998). Low temperature signal transduction during cold acclimation: protein phosphatase 2A as an early target for cold-inactivation. *Plant J.*, 13: 653-660.
- Moon, H., Lee, B., Choi, G., Shin, D., Prasad, D.T., Lee, O., Kwak, S.S., Kim, D.H., Nam, J., Bahk, J., Hong, J.C., Lee, S.Y., Cho, M.J., Lim, C.O. and Yun, D.J. (2003). NDP kinase 2 interacts with two oxidative stress-activated MAPKs to regulate cellular redox state and enhances multiple stress tolerance in transgenic plants. *Proc. Natl. Acad. Sci. USA*, 100: 358-363.
- Murata, N. and Los, D.A. (1997). Membrane Fluidity and Temperature Perception. *Plant Physiol.*, 115: 875-879.
- Nakagami, H., Pitzschke, A. and Hirt, H. (2005). Emerging MAP kinase pathways in plant stress signalling. *Trends Plant Sci.*, 10: 339-346.
- Novillo, F., Alonso, J.M., Ecker, J.R. and Salinas, J. (2004). CBF2/DREB1C is a negative regulator of CBF1/DREB1B and CBF3/DREB1A expression and plays a central role in stress tolerance in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA*, 101: 3985-3990.
- Orvar, B.L., Sangwan, V., Omann, F. and Dhindsa, R.S. (2000). Early steps in cold sensing by plant cells: the role of actin cytoskeleton and membrane fluidity. *Plant J.*, 23: 785-794.
- Plieth, C. (2005). Calcium: Just another regulator in the machinery of life? *Ann. Bot. (Lond.)*, 96: 1-8.

- Plieth, C., Hansen, U.P., Knight, H. and Knight, M.R. (1999). Temperature sensing by plants: the primary characteristics of signal perception and calcium response. *Plant J.*, 18: 491-497.
- Prasad, T.K., Anderson, M.D., Martin, B.A. and Stewart, C.R. (1994). Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. *Plant Cell*, 6: 65-74.
- Ray, S., Agarwal, P., Arora, R., Kapoor, S. and Tyagi, A.K. (2007). Expression analysis of calcium-dependent protein kinase gene family during reproductive development and abiotic stress conditions in rice (*Oryza sativa* L. ssp. *indica*). *Mol. Genet. Genomics*, (DOI 10.1007/s00438-007-0267-4)
- Reddy, V.S. and Reddy, A.S. (2004). Proteomics of calcium-signaling components in plants. *Phytochemistry*, 65: 1745-1776.
- Ruelland, E., Cantrel, C., Gawer, M., Kader, J.C. and Zachowski, A. (2002). Activation of phospholipases C and D is an early response to a cold exposure in *Arabidopsis* suspension cells. *Plant Physiol.*, 130: 999-1007.
- Saijo, Y., Kinoshita, N., Ishiyama, K., Hata, S., Kyojuka, J., Hayakawa, T., Nakamura, T., Shimamoto, K., Yamaya, T. and Izui, K. (2001). A Ca<sup>2+</sup>-dependent protein kinase that endows rice plants with cold- and salt-stress tolerance functions in vascular bundles. *Plant Cell Physiol.*, 42: 1228-1233.
- Salinas, J. (2002) Molecular mechanisms of signal transduction in cold acclimation. In : *Plant Signal Transduction* (Eds. Scheel D. and Wasternack C.) Oxford University Press, pp. 116-139.
- Sangwan, V., Foulds, I., Singh, J. and Dhindsa, R.S. (2001). Cold-activation of *Brassica napus* *BN115* promoter is mediated by structural changes in membranes and cytoskeleton, and requires Ca<sup>2+</sup> influx. *Plant J.*, 27: 1-12.
- Sangwan, V., Orvar, B.L., Beyerly, J., Hirt, H. and Dhindsa, R.S. (2002). Opposite changes in membrane fluidity mimic cold and heat stress activation of distinct plant MAP kinase pathways. *Plant J.*, 31: 629-638.
- Sarhan, F. and Danyluk, J. (1998). Engineering cold-tolerant crops-throwing the master switch. *Trends Plant Sci.*, 3: 289-290.
- Sarhan, S., Hitchcock, J.M., Grauffel, C.A. and Wettstein, J.G. (1997). Comparative antipsychotic profiles of neurotensin and a related systemically active peptide agonist. *Peptides*, 18: 1223-1227.
- Sathyanarayanan, P.V. and Poovaiah, B.W. (2004). Decoding Ca<sup>2+</sup> signals in plants. *CRC Crit. Rev. Plant Sci.*, 23: 1-11.
- Sharma, P., Sharma, N. and Deswal, R. (2005). The molecular biology of the low-temperature response in plants. *BioEssays*, 27: 1048-1059.
- Shinozaki, K., Yamaguchi-Shinozaki, K. and Seki, M. (2003). Regulatory network of gene expression in the drought and cold stress responses. *Curr. Opin. Plant Biol.*, 6: 410-417.
- Shou, H., Bordallo, P., Fan, J.B., Yeakley, J.M., Bibikova, M., Sheen, J. and Wang, K. (2004a). Expression of an active tobacco mitogen-activated protein kinase kinase enhances freezing tolerance in transgenic maize. *Proc. Natl. Acad. Sci. USA*, 101: 3298-3303.
- Shou, H., Bordallo, P. and Wang, K. (2004b). Expression of the *Nicotiana* protein kinase (NPK1) enhanced drought tolerance in transgenic maize. *J. Exp. Bot.*, 55: 1013-1019.
- Stockinger, E.J., Gilmour, S.J. and Thomashow, M.F. (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. USA*, 94: 1035-1040.
- Stockinger, E.J., Mao, Y., Regier, M.K., Triezenberg, S.J. and Thomashow, M.F. (2001). Transcriptional adaptor and histone acetyltransferase proteins in *Arabidopsis* and their interactions with CBF1, a transcriptional activator involved in cold-regulated gene expression. *Nucleic Acids Res.*, 29: 1524-1533.
- Subbaiah, C.C., Bush, D.S. and Sachs, M.M. (1998). Mitochondrial contribution to the anoxic Ca<sup>2+</sup> signal in maize suspension-cultured cells. *Plant Physiol.*, 118: 759-771.
- Suzuki, I., Los, D.A. and Murata, N. (2000). Perception and transduction of low-temperature signals to induce desaturation of fatty acids. *Biochem. Soc. Trans.*, 28: 628-630.
- Suzuki, N. and Mittler, R. (2006). Reactive oxygen species and temperature stresses: A delicate balance between signaling and destruction. *Physiol. Plant.*, 126: 45-51.
- Tahtiharju, S., Sangwan, V., Monroy, A.F., Dhindsa, R.S. and Borg, M. (1997). The induction of kin genes in cold-acclimating *Arabidopsis thaliana*. Evidence of a role for calcium. *Planta*, 203: 442-447.
- Thomashow, M.F. (1998). Role of cold-responsive genes in plant freezing tolerance. *Plant Physiol.*, 118: 1-7.
- Thomashow, M.F. (1999). Plant cold acclimation: Freezing tolerance genes and regulatory mechanisms. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 50: 571-599.
- Uemura, M., Joseph, R.A. and Steponkus, P.L. (1995). Cold acclimation of *Arabidopsis thaliana* (Effect on plasma membrane lipid composition and freeze-induced lesions). *Plant Physiol.*, 109: 15-30.
- Urao, T., Miyata, S., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2000a). Possible His to Asp phosphorelay signaling in an *Arabidopsis* two-component system. *FEBS Lett.*, 478: 227-232.
- Urao, T., Yakubov, B., Satoh, R., Yamaguchi-Shinozaki, K., Seki, M., Hirayama, T. and Shinozaki, K. (1999). A transmembrane hybrid-type histidine kinase in *Arabidopsis* functions as an osmosensor. *Plant Cell*, 11: 1743-1754.
- Urao, T., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2000b). Two-component systems in plant signal transduction. *Trends Plant Sci.*, 5: 67-74.

- Van Buskirk, H.A. and Thomashow, M.F. (2006). *Arabidopsis* transcription factors regulating cold acclimation. *Physiol. Plant.*, 126: 72-80.
- van der Luit, A.H., Olivari, C., Haley, A., Knight, M.R. and Trewas, A.J. (1999). Distinct calcium signaling pathways regulate calmodulin gene expression in tobacco. *Plant Physiol.*, 121: 705-714.
- Vaultier, M.N., Cantrel, C., Vergnolle, C., Justin, A.M., Demandre, C., Benhassaine-Kesri, G., Cicek, D., Zachowski, A. and Ruelland, E. (2006). Desaturase mutants reveal that membrane rigidification acts as a cold perception mechanism upstream of the diacylglycerol kinase pathway in *Arabidopsis* cells. *FEBS Lett.*, 580: 4218-4223.
- Vij, S. and Tyagi, A.K. (2007). Emerging trends in the functional genomics of the abiotic stress response in crop plants. *Plant Biotechnol. J.*, 5: 361-380.
- Vogel, J.T., Zarka, D.G., Van Buskirk, H.A., Fowler, S.G. and Thomashow, M.F. (2005). Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of *Arabidopsis*. *Plant J.*, 41: 195-211.
- Wang, X., Li, W., Li, M. and Welti, R. (2006). Profiling lipid changes in plant response to low temperatures. *Physiol. Plant.*, 126: 90-96.
- Widmann, C., Gibson, S., Jarpe, M.B. and Johnson, G.L. (1999). Mitogen-Activated Protein Kinase: Conservation of a three-kinase module from yeast to human. *Physiol. Review*, 79: 143-180.
- Xiang, Y., Huang, Y. and Xiong, L. (2007). Characterization of stress-responsive *CIPK* genes in rice for stress tolerance improvement. *Plant Physiol.*, 144: 1416-1428.
- Xin, Z. and Browse, J. (1998). *Eskimo1* mutants of *Arabidopsis* are constitutively freezing-tolerant. *Proc. Natl. Acad. Sci. USA*, 95: 7799-7804.
- Xin, Z., Mandaokar, A., Chen, J., Last, R.L. and Browse, J. (2007). *Arabidopsis ESK1* encodes a novel regulator of freezing tolerance. *Plant J.*, 49: 786-799.
- Xiong, L., Schumaker, K.S. and Zhu, J.K. (2002). Cell signaling during cold, drought, and salt stress. *Plant Cell*, 14 Suppl: S165-183.
- Yamaguchi-Shinozaki, K. and Shinozaki, K. (1993). Characterization of the expression of a desiccation-responsive *rd29* gene of *Arabidopsis thaliana* and analysis of its promoter in transgenic plants. *Mol. Gen. Genet.*, 236: 331-340.
- Yamaguchi-Shinozaki, K. and Shinozaki, K. (1994). A novel *cis*-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. *Plant Cell*, 6: 251-264.
- Yamaguchi-Shinozaki, K. and Shinozaki, K. (2005). Organization of *cis*-acting regulatory elements in osmotic- and cold-stress-responsive promoters. *Trends Plant Sci.*, 10: 88-94.
- Yamaguchi-Shinozaki, K. and Shinozaki, K. (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.*, 57: 781-803.
- Yang, T. and Poovaiah, B.W. (2003). Calcium/calmodulin-mediated signal network in plants. *Trends Plant Sci.*, 8: 505-512.
- Zhang, X., Fowler, S.G., Cheng, H., Lou, Y., Rhee, S.Y., Stockinger, E.J. and Thomashow, M.F. (2004). Freezing-sensitive tomato has a functional CBF cold response pathway, but a CBF regulon that differs from that of freezing-tolerant *Arabidopsis*. *Plant J.*, 39: 905-919.
- Zhu, J., Dong, C.H. and Zhu, J.K. (2007). Interplay between cold-responsive gene regulation, metabolism and RNA processing during plant cold acclimation. *Curr. Opin. Plant Biol.*, 10: 290-295.
- Zhu, J., Shi, H., Lee, B.H., Damsz, B., Cheng, S., Stirm, V., Zhu, J.K., Hasegawa, P.M. and Bressan, R.A. (2004). An *Arabidopsis* homeodomain transcription factor gene, *HOS9*, mediates cold tolerance through a CBF-independent pathway. *Proc. Natl. Acad. Sci. USA*, 101: 9873-9878.
- Zhu, J., Verslues, P.E., Zheng, X., Lee, B.H., Zhan, X., Manabe, Y., Sokolchik, I., Zhu, Y., Dong, C.H., Zhu, J.K., Hasegawa, P.M. and Bressan, R.A. (2005). *HOS10* encodes an R2R3-type MYB transcription factor essential for cold acclimation in plants. *Proc. Natl. Acad. Sci. USA*, 102: 9966-9971.
- Zhu, J.K. (2001). Cell signaling under salt, water and cold stresses. *Curr. Opin. Plant Biol.*, 4: 401-406.