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Adaptive Mechanism of Plant Cells Under Low Temperature Stress: An Introduction

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Abstract

Environmental stresses, such as drought, high salinity and low temperature influence the growth of plants and the productivity of crops, among these stresses low temperature plays a critical role. Plants are thought to have common mechanism, not only in terms of the molecular and cellular response, but also in terms of the biochemical and physiological response, for adaptation of these stresses. During cold acclimation, there are compositional, structural and functional changes occurring in the plasma membrane under freezing conditions. Apart from membrane stability, on exposure to such stresses, many genes are induced and function either as cellular protectants of stress-induced damage or as regulator of gene expression and signal transduction.

By using these genes for the genetic engineering of desired crops to produce transgenic may help in sustainable agriculture by increasing the cultivation period of crop even at higher altitude.

Keywords: Cold, membrane stability, osmoprotectants, genes, antifreeze proteins, genetic engineering.

Introduction

A number of abnormal environmental parameters such as drought, salinity, cold, freezing, high temperature, anoxia, high light intensity and nutrient imbalance, collectively called abiotic stresses, negatively affect the processes associated with biomass production and grain yield in plants. With the increasing world population, as predicted to approximately 9 billion by 2050 (Anonymous[1], 2003), there will be a definite shortage of food unless world food production rises by ~ 70% (Guy *et al*[2], 2006). This dramatic demand of sustained food production is needed to be achieved at a time when the world agriculture is facing the challenge of global climate changes. Recent assessments suggest global climate change to increase crop yield at high and mid latitudes and reduce yield at lower latitude (Parry *et al*[3], 2003). It is also considered likely that frost and freezes may become more frequent in some areas and less frequent in other areas creating both windfalls and challenge for agriculture and natural flora and fauna. (Guy *et al*[2], 2006).

Also, cultivable land is less. How is it possible to sustain the already existing agriculture and develop more of it? What about cultivating land where initially it was not possible e.g. high altitude regions, for example, in the steep mountains of Great Himalayas. Though nature has imposed certain limitations at high altitudes: abiotic stresses like cold, frost, radiation, scarcity of water etc., plants cope with potential stress factors by developing stress resistance (Sarad *et al*[4], 2005). This implies all morphological and physiological measures required to inhibit or ameliorate the stress. There are several different mechanisms in the plant kingdom developed for stress resistance but, in principle, three strategies can be differentiated: tolerance towards stress factors, defence against stress factors, and reversion of stress factors by repairing the damage that has occurred. These three types of resistance mechanisms are present constitutively or adaptively. Individually stress resistance depends on specific response range of cell function affected and this can differ greatly in different types of plants (Mohr and Schopfer[-5], 1995). Thus the natural abilities of the plant help them cope with unfavorable environmental conditions (Guy *et al*[2], 2006).

Abiotic stresses

The abiotic stresses affect the plant cell in various aspects, disturbing their normal morphological built up, physiological processes and molecular parameters. Numerous changes occur in plants some of which are built up of reactive oxygen species, changes in plasma membrane structure, up/down regulation of molecular modules, etc.

Abiotic stresses lead to dehydration or osmotic stress through reduced availability of water for vital cellular functions and maintenance of turgor pressure. Stomata closure, reduced supply of CO_2 and slower rate of biochemical reactions during prolonged periods of dehydration, high light intensity, low temperature all lead to high production of reactive oxygen intermediates (ROI) in chloroplasts causing irreversible damage to cell and photo inhibition also. The high influxes and absorption of UV-B radiation affects terrestrial plants through damage to DNA directly or indirectly via formation of free radicals; damage to membranes by peroxidation of unsaturated fatty acids, PS II, phytohormones and even symbiotic relationship of plants with microorganisms. A number of secondary metabolites such as flavonoids, tannins and lignins are increased at elevated levels of UV-B radiation which screen UV-B and protect the cellular components against the UV-B damage (FAO[6], 2001).

Perception of stress

The perception of abiotic stress and signal transduction to switch on adaptive responses are critical steps in determining the survival and reproduction of plants exposed to adverse environments. Plants have stress specific adaptive responses as well as responses which protects the plant from more than one environmental stress. There are multiple stress perception and signaling pathways, some of which are specific but other may cross talk at various steps (Chinnusamy *et al*.[7] 2003). ABA plays a crucial role in the plant response to abiotic stresses like drought, salinity, cold etc. Stress responsive genes have been proposed to be regulated by both ABA-dependent and ABA-independent signaling pathways (Shinozaki and Yamaguchi-Shinozaki[8] 2000, Zhu[9] 2002) (Fig.1). Ultimately the expression of these stress responsive genes leads to various physiological and biochemical changes which make the plant tolerant for that stress.

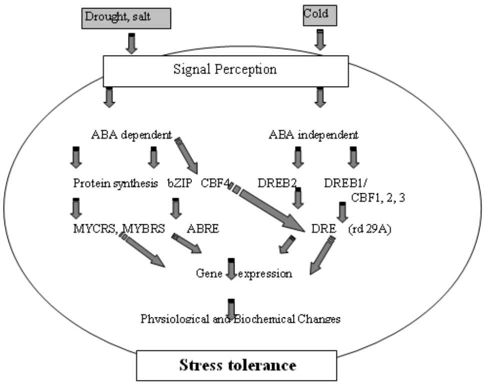

Figure 1: Cellular signal transduction pathways between stress signal perception and gene expression. [Ref.: Guy ,Charles, 1999, "Molecular responses of plants to cold shock and cold acclimation",J. Mol. Microbiol. Biotechnol.,1(2), pp. 231-242].

Low temperature stress: how plants respond to it during acclimation

Low temperature is a major abiotic factor that can damage the plant cell in various ways. Low temperature as such causes frost and chilling which leads to membrane damage and retarded metabolism. It also introduces osmotic stress due to freeze dehydration (Fig.2).

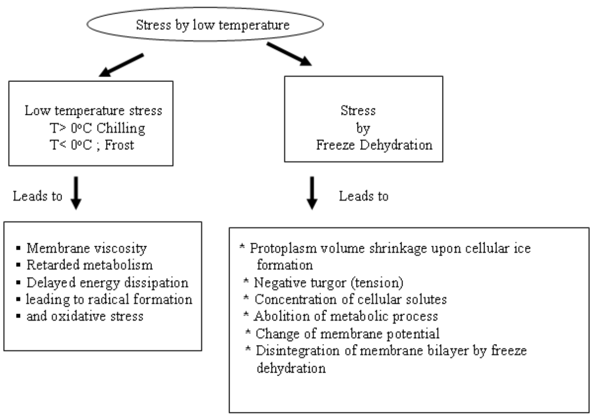

Figure 2: The cold syndrome in plants (Ref.: Beck, E. H.,Hein, R. and Jansen. J., 2004, "Plant resistance to cold stress: Mechanisms and environmental signals triggering frost hardening and dehardening.", J. Biosc., 29, pp.449-459.)

Changes in the plasma membrane

Many temperate plants are able to increase their freezing tolerance when exposed to low temperatures that are non freezing in an adaptive process, which is known as cold acclimation (Nakashima and Yamaguchi-Shinozaki[10], 2006; Van Buskirk and Thomashaw[11],2006; Chinnuswamy *et al*[12], 2006). Through altered gene expression during cold acclimation, there are many seemingly disparate responses occurring in many different aspects, including alterations in membrane composition and accumulation of compatible solutes.

The plasma membrane is considered to be the primary site of injuries because of freeze induced dehydration during freezing injury (Steponokus[13], 1984). It is necessary that plasma membrane increases its cryostability during freeze thaw excursions. During cold acclimation under normal and artificial conditions, there are compositional, structural and functional changes in plasma membrane many, if not all, of which contribute to increased stability of the membrane under freezing conditions (Uemura *et al*[14], 2006). Cold acclimation results in many changes in lipid composition of plasma membrane suggesting that these changes are associated with occurrence of specific freeze induced lesions associated with the plasma membrane (Steponokus et al[15], 1993; Uemura and Steponokus[16], 1999). The most notable changes in lipid composition is an increase in proportion of phospholipids which is observed in a wide range of plants systems from monocot to dicot and from herb to woody plants.

The increase in phospholipids in plasma membrane during cold acclimation occurs at the early stage of cold acclimation when a decrease in cerebrosides occurs gradually throughout the acclimation process. The increase in phospholipids is primarily a result of increase in proportion of unsaturated molecular species of phosphatidyl choline and phosphatidyl ethanolamine, two phospholipids classes in the plasma membrane. In addition there is a decrease in proportion of cerebrosides over a wide range of plants. Also, certain cold responsive plasma membrane proteins affect cold acclimation process to increase freezing tolerance. The outer membrane lipoprotein like protein (lipocalin like protein from Arabidopsis-AtLCN) is a protein that increases substantially during cold acclimation (Kanamura and Uemura[17], 2003) cold induced expression of a lipocalin like protein occurs both in monocots and dicots, suggesting a cryoprotective role of LPCP under freeze induced dehydration. AtLCN seems somehow to change the cryobehaviour of plasma membrane under freeze induced osmotic dehydration such that plasma membrane minimizes the probability of formation of endocytic vesicles. There is possibility that AtLCN may affect cryostability of plasma membrane through an interaction with lipid components of plasma membrane (Uemura *et al*[14], 2006).

Another cold responsive plasma protein ERD14 (Early responsive to dehydration protein 14) seems to affect cryobehaviour of plasma membrane differently, although the mechanism is not clear. It is hypothesized that ERD 14 may minimize the penetration of ice from outside protoplasm by altering characteristics of plasma membrane during freezing. In any case the plasma membrane is the primary site of injury on subjecting plant cells to cold stress. Several genes for change in membrane components are identified which on presence in plant genome may strengthen the cryostability of plasma membrane. Many cold responsive genes have been identified e.g. COR15a, glyceraldehyde-3-phosphotransferase; Dehydrin ERD10,14; glutathione-S-transferase; phospholipase D delta, Rubisco large subunit; carbonic anhydrase etc. (Artus et al[18],1996; Steponokus et al[19] 1998; Uemura et al[16], 2006).

Changes in cytosol

Also changes in cytosol or intracellular compartments affect the cryobehaviour of plasma membrane under freezing conditions. In general, increased concentrations of compatible solutes result in a decrease in extent of freeze induced osmotic dehydration from cells (Levitt *et al*[20], 1980). Some compatible solutes have shown specific cryoprotective effects during freezing. Compatible solutes or osmolytes accumulate in organism in response to osmotic stress. The primary function of compatible solutes is to maintain cell turgor and thus the driving gradient for water uptake. Recent studies indicate that compatible solutes can also act as free radical scavengers or chemical chaperones by directly stabilizing the membranes and/or proteins (Hare *et al*[21], 1998; McNeil *et al*[22], 1999; Bohnert and Shen [23],1999; Diamant *et al*[24], 2001). Compatible solutes may be polyol/ sugars for example mannitol, trehalose, quartenary amines (glycine betaine, dimethylsulfarpropionate) and amino acids like proline. Over expression of compatible solutes in transgenics plants results in stress tolerance (Wang *et al*[25], 2003).

Glycine betaine is widely distributed in plants and protects them from exposure to salt and cold stress. In plants like spinach and barley betaine is synthesized from choline by oxidation of choline to betaine aldehyde and then to betaine. Arabidopsis plants transformed with glycine betaine had enhanced ability to tolerate salt and cold stress. Proline is also known to act as an osmoprotectant and its increased production in transgenic plants has resulted in increased tolerance to drought, a condition associated with cold stress (FAO[6], 2001). Some of the genes associated with osmolytes production are glycine betaine (Chen and Murata[26], 2002), mannitol-1-phosphotransferase (FAO[6], 2001).

Changes in gene expression

A number of genes have been described that respond to cold and osmotic stress in plants (Shinozaki and Yamaguchi-Shinozaki [27], 1997; Thomashow [28], 1999; Zhang *et al*[29], 2004a) and it is thought that these gene products may play important roles for acclimatization of plants. Recently 299 drought inducible genes and 54 cold inducible genes were identified using a cDNA micro array containing ~7000 independent full length Arabidopsis cDNA clones (Seki *et al*[30], 2002, Shinozaki *et al*[31], 2003). Functions of the gene products have been predicted by comparisons of sequence homology with known proteins. Genes induced during osmotic and cold stress conditions are thought to function not only in protecting cells from stress by the production of important metabolic metabolic proteins (functional proteins) but also in the regulation of genes for signal transduction in the stress response(regulatory protein). Examples of stress related regulatory proteins include transcription factors, protein kinases, and enzymes for phosphoinositide turnover and enzymes for synthesis of plant hormone abscisic acid (ABA) (Nakashima and Yamaguchi-Shinozaki[10], 2006).

Cold acclimation involves precise regulation of expression of transcription factors and effector genes collectively called cold regulated (COR) genes (Thomashaw[28],1999; Vishwanathan and Zhu[32], 2002; Xiong *et al*[33], 2002a).Many transcriptional, posttranscriptional and post translational regulators of cold induced expression of COR genes have been identified. Promoters of many of the COR genes contain *cis* elements such as dehydration responsive elements/C-repeat elements (DRE/CRT, A/GCCGAC), absiscic acid (ABA) responsive element (ABRE, PyACGTGGC) and myeloctomatosis (MYC) recognition sequence(CANNTG) (Yamaguchi-Shinozaki and Shinozaki [34], 2005; Zhu [9] , 2002; Chinnuswamy *et al*[12] ,2006).

Plant cells may sense cold stress induced change in membrane fluidity and protein conformation. Cold stress induced rigidification of the plasma membrane at microdomains may lead to actin cytoskeletal rearrangement which may be followed by activation of Ca^{+2} channels and increased cytosolic calcium ion levels triggering the over expression of COR genes during cold acclimation(Orvar et al[35], 2000;Sangwan et al[36],2001). ABA serves as a secondary signal to transducer, at least, in part, cold signals (Chinnuswamy et al[12], 2006). Cold induced reactive oxygen species may activate a mitogen activated protein kinase cascade - cold stress induced CBFs or DRE-binding factors in *Arabidoposis*. The CBF proteins activate the transcription of DRE/CRT cis-element containing COR genes (Liu *et al*[37], 1998; Stockinger *et al*[38], 1997). ICE1 is a constitutively expressed gene localized in nucleus but requiring cold treatment (Chinnuswamy *et al*[7], 2003). ICE1 is a master switch that controls many cold responsive CBF dependant and independent regulons (Chinnuswamy *et al*[7], 2003) (Fig.3). ABA also induces expression of CBF genes but to a significantly lower level than with cold acclimation (Knight *et al*[39], 2004).

Thus there are several regulatory genes that participate in tolerance to cold e.g. CBF 1, CBF3, ICE1, OsMyb4, HOS9, HOS10, RD29A, KIN1, COR15A, COR47A, ADH, etc. (Chinnuswamy *et al*[12], 2006; Vannini *et al*[40], 2004; Zhu *et al*[41], 2004; Chinnuswamy *et al*[42], 2004; and Xiang *et al*[43], 2001).

Table 1: Mechanism, genes and genetically modified plants engineered for abiotic stress tolerance: (Ref.)

Mechanism	Genes	Plant species
Transcription control	CBF/DREB1a	*Arabidopsis thaliana*
		Brassica napus
		Lycopersicon esculentum
	At MYC2, AtMYB2	*A. thaliana*
	ABF3&4	*A. thaliana*
	HSF1&HSF3	*A. thaliana*
	HsfAi	*Lycopersicon esculentum*

		Oryza sativa
	Spl7	*A. thaliana*
	ICE1	*Nicotiana tabacum*
Compatible solutes	P5CS	*A. thaliana*
	ProDH	*N. tabacum*
	IMT1	*N. tabacum*
	Stpd1	*N. tabacum*
Antioxidant and	CuZn- SOD	*Medicago sativa*
detoxification	Mn-SOD or Fe-SOD	*N. tabacum*
		N. tabacum
	GST & GPX	*A. thaliana*
	chyB	*N. tabacum*
	Aldose-aldehyde reductase	*A. thaliana*
LEA-type protein	COR15a	*O. sativa*
	HVA1	*Triticum aestivum*
		A. thaliana
	WCS19	

Freezing in plants

Subzero temperatures prominently cause extracellular water to freeze, a detrimental condition for plants. It is known that freezing tolerant organisms produce various compounds to avoid the effects of cellular dehydration and ice damage. All frost tolerant animal species produce ice nucleators to initiate ice crystal formation extracellularly (Duman *et a*l[44], 1984). No ice formation has been detected within the cells (Duman *et al*[45], 1991). Frost tolerant invertebrates are known to produce AFPs and ice nucleators in their extracellular compartments (Duman *et al*[45], 1991; Murphy[46], 1983). Once ice forms in these organisms, water is drawn from the cells to the growing mass of extracellular ice. To avoid the loss of water, cells of frost tolerant organisms accumulate low molecular weight solutes which increase the osmotic potential of their cells. The solutes also stabilize structure of cell membranes and proteins, thus preserving their capacity to function following a freeze thaw cycle.

Antifreeze proteins

Marilyn Griffith found the first AFP in plants, from winter rye. AFPs have the ability to lower the ice nucleation temperature and to slow the propagation and recrystallization of ice in winter survival. Rye AFPs lower the freezing point of a solution only by 0.3^0 C in vitro, so the primary role within the plant is not to prevent freezing, but rather to modify the growth of ice in a way that improves survival. Thereafter, Wisniewski *et al* [47], in 1999 characterized the first plant dehydrin to show AFP activity, but this was present through cytosol, plastid, and nucleus of xylem and parenchyma cells of bark tissue of peach. Bravo and Griffith[48] in 2005 discovered plant AFPs constitutive expression in the plant species *Deschampsia antartica*. AFPs are now known to be present in teloest fish(fist discovered by Crevel et al[49], 2002), insects, fungi, bacteria and plant species(Moffatt *et al*[50], 2006).Photosynthetic species expressing AFPs include lichens, mosses, lower vascular plant systems(Duman and Olsen[51], 1993) and vascular plant species (Bravo and Griffith[48], 2005, Griffith and Yaish[52], 2004). The use of AFP in food technology, tissue preservation and development of stress tolerant / resistant cultivars remains largely unexploited (Atici and Nalbantoglu[53], 2003).A lot about AFPs is yet to be understood.

Genetic engineering for stress tolerant crops

It is understood that drought, extreme temperature and high salinity are major limiting factors for plant growth and productivity. In their quest to feed the ever-increasing world population agricultural scientists have to contend with these adverse environmental factors. If crops can be redesigned to better cope with abiotic stress, agricultural production can be increased dramatically. With special emphasis to cold stress land initially left unused at high altitudes can be cultivated with crops tolerant to low temperature stress.

The work on genetic engineering of tolerance to abiotic stresses began piecemeal more than a decade back. With the advent of modern biotechnological tools and advancement in understanding of crop abiotic stress mechanism and elucidation of pathways the issue of abiotic stress tolerant crop development can be worked upon. As such many transgenics have been developed e.g. Cortina *et al*[54] in 2005 enhanced tolerance to abiotic stress in tomato by use of yeast trahaloselso-6-phosphate synthase (TPS-1). The transgenics revealed higher starch content and chlorophyll content in relation to wild plants. Prabhavati *et al*[55] in 2002 reported tolerance of transgenic eggplant having *mtl*D gene to NaCl induced salt stress, PEG mediated drought and chilling stress under both *in vivo* and *in vitro* growth conditions. The number of reports will increase on literature survey, the main thing is the gist of all these transgenic events which suggests the use of multiple tolerance mechanisms for one or more abiotic stresses through stepwise or co transformation which will help achieve higher levels of tolerance for commercial exploitation. The QTL mapping of stress tolerance in certain species, comparative mapping and map based cloning in plants may be used to screen genes which function when induced and expressed in response to stress. In context to cold stress tolerance, the mechanism is well understood and many numbers of gene involved directly or indirectly or as a regulating authority are now known. Further molecular understanding of stress perception, signal transduction and transcriptional regulation of abiotic stress responsive genes may help to engineer tolerance for multiple stresses.

Conclusion

Improved tolerance to cold can be achieved by using these genes for the genetic engineering of desired crops by developing transgenic plants that may grow in open at higher altitudes even for a short agricultural period. Even one success story will definitely uplift the socio-economic status of localities apart from making available a fresh supply of consumables e.g. perishable vegetables, which otherwise lose their valuable nutrients during transport.

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Under-standing the mechanisms by which plants perceive and trans-duce these stress signals to initiate adaptive responses is essential for engineering stress-tolerant crop plants. Molecu-lar and biochemical studies suggest that abiotic stress signal-ing in plants involves receptor-coupled phosphorelay, phosphoinositol-induced Ca2+ changes, mitogen-activated protein kinase cascades and transcriptional activation of stress-responsive genes. In addition, protein posttranslational. modications and adapter or scaffold-mediated protein-protein interactions are also important in abiotic stress signal