Recent Trends in Biotechnology To Improve Cold Stress Tolerance in Horticultural Crops

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Abstract: **The changes in the pattern of gene expression and putative proteins synthesis are according to their response with environment when exposed to low temperatures. Thus, the adaptation mechanism of plants and their survival are strictly governed by the mode of the transcriptional changes happened within nucleic acids under adverse climatic conditions. In other cases, plant species of tropical or subtropical origin are severely affected by the temperatures just above the freezing point and displaying various symptoms of chilling injury and even died at final stage. But the same physiological fate is not observed in chilling tolerant species and they are able to grow under such extremities. Conventional breeding methods like inter-specific or inter-generic hybridization techniques have several limitations in reshaping the cold stress tolerance mechanisms in valuable horticultural crop plants. Recent advancement in whole genome editing/ sequencing, mutational response and plant analysis for transgenic development provide us in-depth knowledge of the complex transcriptional mechanism that mainly carried out under cold stress. Various inducible genes are hyperactive at adverse environmental condition and have the possibility to isolate from wild plant species. Most of them are playing key role in cold stress tolerance and the expression of few of them is regulated by C-repeat binding factor/ dehydration-responsive element binding transcription factors. Such defense mechanisms reveal that the cold resistance is more complex than perceived and involves through different pathways. These findings abridged in this review and shown potential practical applications for introducing cold tolerance in agricultural crops for domesticating them in temperate regions.**

Keywords: **Abiotic, Biotechnology, Cold, RNA, Stress Transcription, Tolerance.**

I. INTRODUCTION

The natural environment for plants is composed of a complex set of abiotic stresses and biotic stresses. Abiotic stress is known as the adverse influence of non-living factors beyond the routine variation range on the living organisms in a specific environment. This stress exposure results in changes in reactive oxygen species and reactive nitrogen species. Thereby, modifies the redox ratio within the cell that lead to alteration in interaction between molecular pathways, enzyme action and expressional regulation of genes. Abiotic stress is the reason of fall out of approx. 50% production of edible crops. If we consider the increasing rate of human population in consistent manner with the proportionate deviation of agricultural productivity, the time is not so far when sustainable food security will be the most critical issue among any types of burning problems that human being facing in this earth. Among all severe stresses plants are suffering, cold stress is considered as the prime matter of headache which need to be minimize to uplift the agricultural production [1].

The ultimate fate of cold stress in crop plants lead to poor quality of germination, seedlings with stunted growth, various critical symptoms in leaves like chlorosis, retarded growth, frost injury due to damage of membrane and even constant wilting make the whole plant tissues dead (Necrosis). The stress related signal is initially captured by specific receptor followed by transduction of same signal at final fire point to switch on the specific cold-responsive genes and transcription factors; as a result the instantly and previously synthesized functional proteins activated and protect the plants from chilling damage. Therefore understanding the exact mechanisms of cold stress tolerance governed by various biochemical pathways could flash-on the knowledge and make a way out to destroy the extreme cold susceptibility in most of the agricultural crop plants.

With the fast fall down of the yield capacity of agricultural crops due to cold stress, conventional breeding techniques could not mitigate the ever increasing food demand by implying long term inter-specific or inter-generic hybridization methods. A bunch of potential molecular techniques like full genome profiling/ sequencing, mutational and transgenic plant analyses are improving day by day. These techniques widen the clear visibility of the complex transcriptional mechanism that some of the temperate plants developed to disseminate a tolerance barrier against cold stress. These types of such genetic behavioral response to cold temperatures create new challenges to scientists that how the same genes function can be fit on the susceptible plants by employing latest biotechnological approaches. The present chapter focus not only on potential credibility of such kind of approaches that applied for last two decades but also some promising technologies already implied in few cases like RNAi technology, gene knock-out and knock-down and in latest genome editing for making a world having healthy crop plant ecosystem with less abiotic stresses [2].

II. COLDSTRESS: A HIGHLY EFFECTUAL ABIOTIC STRESS

Plant shows variable degree of tolerance towards lower temperature according to their geographical location, developmental stage and even involved plant organ. Unlike to tropical and subtropical region specific plants, the temperate plants show adaptive mode in facing low to chilling temperature, a resultant of a phenomenon called as ‘cold acclimation’ i.e. a non–constitutive, but inducible response of plants for short term adaptation against low temperature. Initial research was based around the possible mechanism for cold response. It was found that not one, but many biochemical, physiological factors are involved in this process.

For instance, alteration in cellular membrane lipid content against low temperature was evidentially proved. Sucrose and proline had been found to have cryo-protective role in some plants. Genetic analysis indicated that there is existence of cold stress inducible many, not a single one, inter-dependent genes, which upon induction, give a cumulative action, and also the involvement of transcriptional factor regulation [3]. The underlying hypothesis was that the increase in freezing tolerance that occurred with cold acclimation was likely to include the action of genes that were induced in response to low temperature. In particular, a low temperature regulatory pathway, the CBF cold-response pathway, was discovered and shown to participate in cold acclimation [4]. The gain of stress tolerance in plants is a very complex mechanism, given that a single stress-tolerance gene has multiple effects on plant-stress tolerance. Additionally, plant organs also have diverse sensitivity to cold stress, and the stress responses might vary at different time points of developmental stages in plants. Hence, the mechanisms of acclimation to low temperature stress are various; however these require the plant to be pre-exposed to periods of cold, to be able to trigger adequate responses to subsequent exposures. If the temperatures are gradually lowered the plant will gain hardness, which is a usual way to acquire tolerance to cold stress. At this stage, signalling cascades and transcriptional control take place [5]. Xiong et al., 2003 stated that plant adaptation to cold and drought is to a great extent dependent on transcriptional factors regulation [6]. Thanks to transcriptomic studies for the expression of some transcription factors with additional ways to tolerate cold stress that are more related to membrane stability; an increase in the degree of fatty acid unsaturation helps maintain the stability of the membrane and give tolerance to cold. For example Hayashi (1997) also stated that other factors will play significant roles in the acclimation of transgenic plants and this example only proves that fatty acids are an important contributor to cold tolerance [7].

Another mechanism to gain low temperature tolerance is to enter a dormant stage. At this stage, plants stop metabolic processes of growth [8], until the period of low temperature concludes. In dormancy, plants reduce their energy use and start to accumulate sugars as osmo-protectant. For example, experiments led by Gupta (1993) demonstrated that an increase in plant frost tolerance of 1°C is considered to be significant; due to the highly complex nature of plant adaptation to low temperature [9]. Given these points, there are many ongoing approaches to the development of new traits for tolerance and to gain a more complete understanding of cold acclimation. Thanks to the new technological resources with many valuable improvements for sustainable food production can be attained. Under freezing conditions, ice crystals are built in the intercellular organelles, causing significant water losses in the cells. Ice crystals within the cell are lethal.

SECONDARYMETABOLITESAND COLD STRESS RESISTANCE

A comparative metabolite analysis in *Arabidopsis* by indicated that heat and cold stress regulation might also depend on the accumulation of other temperature-regulator metabolites, such as sucrose, proline, monosaccharides (glucose, fructose), raffinose, galactinol and myo-inositol [10].

ARABIDOPSIS CBF COLD-RESPONSE PATHWAY

The Arabidopsis CBF cold-response pathway was discovered through investigations of cold-regulated gene expression. A number of earlier studies demonstrated that changes in gene expression occur in Arabidopsis in response to low temperature. These genes were given various designations including COR (cold-regulated), LTI (low-temperature-induced), KIN (cold-induced) and RD (responsive to dehydration). Within 2–4 hours of transferring plants to low temperature, the transcript levels for these genes were found to increase dramatically and remain elevated for weeks if plants were kept at low temperature. When plants were returned to warm temperatures, the transcript levels for these genes were shown to quickly (within hours) decrease to the levels found in non-acclimated plants. This cold adaptation involves a certain types of genes, generally known as cold-regulated genes or COR genes. Transcription factor (CBF1) inducing the expression of many COR genes. Cold stress tolerance in transgenic Arabidopsis plants that has constitutive CBF1 gene expression is similar to that of cold-adapted non-transgenic control plants [11].

Gene/enzyme	**Source / host species**	**Transgenic plant**	**Referenc**
CuCOR19	Citrus	Tobacco	(11)
Rare cold-inducible gene(RCI3)	Arabidopsis	Arabidopsis	(14)
Myeloblastosis binding factor (Osmyb4)	Rice	Arabidopsis	(15)
Cu/Zn superoxide dismutase	Pea	Tobacco	(9)
Mn-superoxide dismutase	Tobacco	Maize	(10)
Fe-superoxide dismutase	Tobacco	Arabidopsis	(16)
Glutathione S-transferase/ peroxidase (GST/GPX)	Tobacco	Tobacco	(17)
Glutathione peroxidase (GPX)	Chlamydomonas	Tobacco	(18)
Inducer of CBF expression1 (ICE1)	Arabidopsis	Arabidopsis	(12)
OSISAP1 (Zinc-finger protein)	Rice	Arabidopsis	(19)
Soybean cold-inducible factor1	Soybean	Tobacco	(20)
Dehydration responsive element binding factor 1 (CBF-1)	Arabidopsis	Tomato	(21)
Dehydration-responsive element binding protein (DREB1A)	Arabidopsis	Tobacco	(22)
Glycerol-3-phosphate acyltransferase (*GPAT*)	Arabidopsis	Tobacco	(23)
Glycerol-3-phosphate acyltransferase (*GPAT*)	-	Rice	(24)

Glycerol-3-phosphate acyltransferase (*GPAT*)	Arabidopsis an Spinach	-	(25)
Glycerol-3-phosphate acyltransferase (*GPAT*)	Squash	Tobacco	(26)
Chloroplast ω-3 fatty acid desaturase (*fad7*)	Arabidopsis	Tobacco	(27)
Acyl-lipid Δ9 desaturase (*des9*)	*S. vulcanus*	Tobacco	(28)

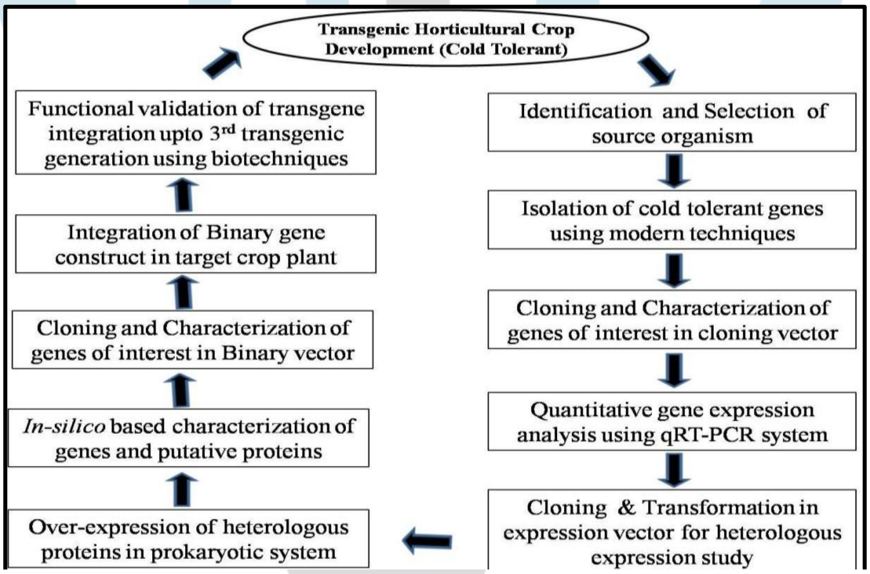
OTECHNOLOGICAL APPROACHES TO DEVELOP COLD_STRESS RESISTANCE IN AGRICULTURAL CROPS

Transcription factor and cold resistance:(TFs) which confer chilling and freezing tolerance to plants had been revealed. For instance, cold stress initiates the expression of ABA (Abscisic acid) growth regulators, CBF (C-repeat Binding Factor) and DREB (Dehydration responsive element binding), which are important transcription factors that activate many downstream genes to help stand cold temperatures [12]. A number of cold stress responsive transcription factors and noble genes have been identified and isolated from different animal and plant sources and integrated into model and cultivated crop plants to develop chilling stress resistance transgenic plants (Table 1).

Table 1: Cold stress resistant successful transgenic crops

Whole genome study and cold resistance: Transcriptional profiling is used to analyze RNA-binding proteins which modulate the function of other regulatory factors. A good example of this approach is the whole genome analysis of RNA binding proteins of *A. thaliana*. In this analysis the authors confirmed the expression of glycine rich RNA-binding proteins (GR-RBPs) and small RNA-binding proteins (S-RBPs) which stabilize the cell wall after stress stimuli. Essentially, the conformation of RNA secondary structures is affected by cold stress, and consequently gene and protein expression are altered enabling enzymatic equilibrium to be re-established. The aforementioned studies clearly show the complexity of responses to cold stress [29].

Bioprospecting of genes for cold stress tolerance: Harnessing of promising genes or allele from extremely cold or frost tolerant organisms followed by enrichment of such genes through cloning and characterization make a way out to perfectly introduce them into horticultural crops prone to cold injury by means of biotechnological approaches. The whole process is not a single step event and needs validation of experimental success by using various advanced techniques. From the beginning of bioprospecting of valuable genes and upto the development of transgenic fruit or ornamental plants resistant to cold stress can be drawn out through a layout diagram (Fig. 1).



Recent development of recombinant DNA technology execute a number of strategies that smoothen the gene transfer in efficient way and as an outcome, we successfully obtained a number of transgenic crop species with magnificent tolerance capacity. Several genes inherently tolerant to freezing stress have been isolated and characterized but their mode of expression could be differ under critical conditions when they have transferred to plant system [1]. It is obvious that the phenomenon of cold stress response in a plant is purely controlled by polygenic trait but there is no such scientific background established on their complex network functions under cold stress conditions [2]. Therefore it is imperative to understand molecular mechanism of every cold responsive gene, allele or transcription factor and how they are interacting with signal molecules, expressing at transcription level followed by translating to synthesize functional protein which finally block the cold wave entering into the cell system. Side by side it also essential to take care on accurate functional validation of the harnessing gene when it is going through *in-vitro* multiplication, transcription and functional protein translation with the help of various biotechnological tools and techniques. Bioprospecting of some important genes from plant and animal origin followed by integration of such valuable genes in model plant systems like arabidopsis and tobacco was also reported and a few of them are given here. A study was undertaken on the functional behavior of ACD5 ceramide kinase gene under cold stress condition in Arabidopsis. The gene expression and plant growth pattern were examined in cold stressed and unstressed plant by using TLC and in vivo metabolic radio-labeling techniques. Theses lead to the formation of ceramide phosphates (Cer-P) in stressed plant and that strongly impaired with acd5 in mutant Arabidopsis [3].

Transformation of a cold stress reactive gene encoding chloroplast w^3 fatty acid desaturase in tobacco followed by its over-expression lead to cold tolerance at 1 ºC up to 7 days was achieved [4]. Beside these, selection of genes or transcription factors with unique mechanism of chilling tolerance were also taken under consideration that finally depending on which crop plant they are going to be integrated. Some of the most promising factors still identified and isolated are genes have function of synthesizing osmo-protectants and some enzymes involved in modifying late embryogenesis abundant (LEA) protein, membrane lipids and detoxifying enzymes [5]. If we short out the genes with cold tolerance property have been harvested from different plant and animal resources till date and transformed only into horticultural crop plants, moderate number of reports comes in front from existing literature and that are precisely presented in Table 1.

CONCLUSION

Recognition of wild type alleles that encodes important agronomic traits will sub sequentially help in conservation of rapidly deteriorating gene pool of cultivated crops and fast natural habitat extinction due to exploding anthropological reason. Studies of genetic diversity among cultivars, wild accessions and ecotypes of cereals are useful for discovery of novel QTLs and alleles which can be further exploited in the programmes of chilling-tolerance improvement in cereals. Populations of wild species frequently harbour high intra-species variation for tolerance traits which is extinct in the modern cultivars. This provides a chance to engineer the transcriptome of crops for obtaining chilling tolerance at global scale. Thus the current study provides essential information to further understand and gain insights into the genetic basis of differential adaptation of plants to adverse chilling stress.

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