Role of CBF/DREB Gene Expression in Abiotic Stress Tolerance.

A Review

Amin Ullah Jan1, Fazal Hadi2, Midrarullah1, Ayaz Ahmad3, Khaista Rahman3

1Department of Biotechnology, Faculty of Sciences, Shaheed Benazir Bhutto University, Sheringal Dir Upper, Pakistan.
2Department of Biotechnology, Faculty of Biological Sciences, University of Malakand, Chakdara, 18800, Pakistan
3Department of Botany, Faculty of Biological Sciences, University of Malakand, Chakdara, 18800, Pakistan

Received: May 10, 2017; Accepted: July 28, 2017; Published: September 14, 2017

*Corresponding author: Dr. Ullah Jan A, Department of Biotechnology, Faculty of Sciences, Shaheed Benazir Bhutto University, Sheringal Dir Upper, 18800, Pakistan. Tele: +92-3009079398; E-mail: aminjan@sbbu.edu.pk

Abstract

The population of the world is increasing rapidly and agricultural productions are decreasing due to several abiotic stresses. Plants are sessile organism respond to these stresses with expression of stress related genes. The ability of the plants to adjust an adverse condition has an impact on the survival, distribution and crops production. Various species are incapacitated or killed by abiotic stress and significantly reduced the plant growth and production. The Orthodox method of breeding is not very successful in improving the abiotic stress tolerance inessential crop plants including inter-generic and inter-specific hybridization. The current analyses providing complex transcriptional mechanism that works under abiotic stress. The modifications in gene expression in response to abiotic stresses are followed by escalations of metabolites, which provide protection against the damaging effects of abiotic stress. The adverse effect of stress induced severe cell damage due to dehydration. Stress signal is received by the receptor located at cell membrane and transducer to nucleus. It switches on stress responsive genes and transcription factors for facilitating stress tolerance. Expression of stress related genes are regulated by CBF/DREB (C-repeat binding factor/dehydration-responsive element binding) transcription factors. Several molecular and physiological changes occur during stress which reveals that abiotic stress resistance is more complex and contains more than one pathway. The conclusions concise in the current review have shown latent applied applications for breeding of abiotic stress tolerance in crop appropriate to temperate environmental locations.

Keywords: Abiotic stress; Transcription factor; CBF; Gene

List of Abbreviations

Late Embryogenesis Abundant (LEA)
bZIP (basic leucine zipper)
MYC (myelocytomatosis oncogen),
MYB (myeloblastosis)
CBF (C-repeat binding factor)
DRE (Dehydration responsive elements)

Low Temperature Regulatory Element (LTRE)
AP2 (APETAL2)
ANT (AINTEGUMENTA)
COR (cold regulated)
IRI (Ice re-crystallization inhibition)
ROS (reactive oxygen species)
CAMTA (calmodulin binding transcription activator)
ANT (AINTEGUMENTA)

Introduction

Stress is an altered physiological condition triggered by different factors that lead to interrupt the equilibrium. Strain is any chemical and physical change fashioned by a stress [1]. The term "stress" is used in different meanings, the physiological explanation and appropriate term as replies in diverse situations. The flexibility of metabolism sanctions the response instigation to changes in environment, which alter frequently and are probable over seasonal and daily cycles. Different factor deviation from its optimal condition does not lead to stress. Stress conditions are highly unpredictable fluctuations levied on steady metabolic pathway cause disease, injury or aberrant physiology [2, 3]. Plants are often exposed to several stresses, i.e. biotic and abiotic. The biotic factor includes virus, bacteria, fungi, insects and other living organisms while the abiotic factor contains low temperature, drought, salt, heat, flooding, heavy metal toxicity and oxidative stress. Abiotic stresses are agricultural threats and result in the worsening of environment and lead to crop loss of major crops by more than 50% throughout the world [4]. Abiotic stress leads to morphological, physiological, biochemical and molecular changes that badly affect plant growth [5].
Dehydration Stress

Some stresses show similar effects on plants, such as frost, drought and salt stresses disturb the osmotic homeostasis of the cell and affect the water relations of plant. These stresses cause cellular dehydration and collectively known as dehydration stresses [6]. The dehydration stresses due to their wide range occurrence may cause the most fatal economic losses in agriculture. The world population is increasing rapidly at an alarming rate and production of food is decreasing due to several stresses [7]. The minimization of these damages is a foremost area of concern for plant scientists. It is a difficult or impossible to eradicate or diminish the stresses themselves, it is important to develop stress tolerance or resistance in plants [7]. To maintain plant growth and metabolism, plants must acclimatize to stress environments and use precise tolerance response to stress. Primary stresses such as salinity, drought, heat, cold and secondary stresses such as oxidative stress and osmotic are inter-connected, and cause cells damage. The initial stress signals trigger the transcription factor which stimulates stress responsive mechanisms to re-establish homeostasis and provide protection to the cells [6]. The inadequate response to the signaling and gene activation may finally lead to irreversible changes of cellular homeostasis, destruction of structural, functional protein, membranes and leading to cell death. Plant modification for enhanced tolerance is mostly based on the manipulation of genes that protect and maintain the function and structure of cellular components [7].

Dehydration Stress Responses

Calcium (Ca+) plays vital role in the signal transduction of dehydration stress. In the signal transduction chain, cytoplasmic calcium concentration is very essential components of the cell which stimulate a specific response to the stress [6]. It was proved by a short experiment that thirty percent transcriptome of Arabidopsis counter to water deficiency, salinity and cold treatment in a definite way. The three major stresses (drought, salinity and cold) induced responses only < 5%. Further, this rate is decreased up to < 0.5% after one day, which shows a rising tendency for specific reaction [8]. In the signalling pathways cross talk is an apparent and as known as cross protection, e.g. frost hardening by salt or drought. Transcription factor also works co-operatively and occurrence of different cis-acting element in one promoter exists [6,7]. Dehydration responsive gene’s product can be classified in two groups (1) functional and (2) regulatory protein as shown in Fig. 1. Those genes which encode metabolically active protein, i.e. chaperons, antifreeze proteins leading to the manufacturing of radical scavengers, low molecular osmolytes and stress tolerance compounds. The regulatory genes encode transcription factor, phospholipase C, protein Kinase [6]. The cold pulse expresses the cold regulated genes at the slightly higher level while it strongly induces the dehydrins encodings genes [9]. The dehydrins works like a chaperon that provides stability to proteins, vesicles and membrane structures during abiotic stress condition [10]. Drought or frost causes dehydration, which induces the dehydrins encoding genes. During seed maturation dehydrins are also accumulated which are known as Late Embryogenesis Abundant protein (LEA). Dehydrins are protein having a variable number of molecular masses ranging from 9 to 200 KDa. They are heat tolerant and consist of a high portion of lysine and glycine residues. Dehydrins also present in mosses, vascular plants, ferns, algae and lichens and their function are still not known [7].

Transcription Factors and Abiotic Stress

A transcription factor which belongs to regulatory protein, play crucial role in expression of gene initiation [11, 12]. The transcription factor intermingles with cis-acting elements
which are present in promoter region of several stress related genes. It stimulates cascades of genes and increasing stress resistance. Transcription factors are attractive and powerful tool for the manipulation of stress tolerance and the over expression may lead to either down-regulation or up-regulation of genes. More than 1500 Transcription factors, are convoluted in stress tolerance and regulating responses of abiotic stresses. In Arabidopsis approximately, fifteen hundred (1500) genes encode several transcription factors [13]. Stress related transcription factors are classified into many families, i.e. bZIP (basic leucine zipper), NAC, AP2/ERF, MYC (myelocytomatosis oncogen), MYB (myeloblastosis), zinc-finger, Cys2His2 and WRKY [14].

The unique class protein that plays an important role in abiotic and biotic stress response is the AP2/ERF proteins [15]. The AP2/ERF protein is a large super family, which further classified into three groups, i.e. ERF, RAV and AP 2 [16, 17]. The DREB (Dehydration Responsive Element Binding) also stated as CBF (C-repeat Binding Factor), belonging to ERF (sub-family) and show a vital role in abiotic stress tolerance. DREB protein was further classified into six groups termed A-1, A-2, A-3, A-4 A-5 and A-6. Among these proteins, A-1 and A-2 institutes the two largest groups [18]. The A-1 subgroup consists of (DREB1/CBF genes) and the A-2 subgroup contains (DREB2-like genes).

These two groups are mainly involved in the osmotic and cold stress responsive gene expression. The genome of Arabidopsis consists of six DREB1/CBF genes i.e. DREB1B/CBF1, DREB1A/CBF3, DREB1D/CBF4, DDF1/DREB1F, DREB1C/CBF2 and DDF2/DREB1E [18]. DREB1/CBF transcription factors, mostly found in plants, including Arabidopsis and also exists in plants that are acclimatized to cold, i.e. barley Brassica napus and those that are not acclimatized such as rice and tomato halophytes i.e. Atriplex hortensis and some other plants, such as rye grass and soybean [19-24]. The existence of DREB/CBF pathways in plants has the capacity of cold acclimation [25]. The orthologues of DREB1/CBF have been identified in large number of plants i.e. birch Betula pendula grapes and Eucalyptus [26-28].

Six different signal transduction pathways are found in dehydration (high salinity, drought) stress responses: three are ABA independent and three are ABA dependent. In the ABA dependent pathway, ABRE functions as ABA-responsive element. In ABA-independent pathways, DRE is not only involved in the regulation of drought and salt stress but also of cold stress. CBF/DREB1 is involved in the expression of cold responsive gene while CBF/DREB2 is important Tfs in the expression of drought and salt stress responsive gene. The other ABA-independent pathway is regulated by salt and drought stress but not by cold [29].

![Figure 2: Transcriptional regulatory networks of abiotic stress signals and gene expression.](image-url)
CBF/DREB (C-Repeat Binding/Dehydration Responsive Element Binding Factor)

CBF is a C-repeat (CRT) binding factor which results in the activation of genes with a CRT element in their promoter [30]. In the Arabidopsis plant DRE/CRT which is a cis-acting element controlling the gene expression in dehydration stress, i.e., drought, salinity and cold stress [31]. In plants, a single transcription factor can control so many target genes. This transcription factor binds to cis-acting element in the promoters of the target genes. Such transcription unit is called “regulon” [32]. A regulon is a set of non-adjacent structural genes which are under the control of a common regulatory gene. Regulon structural gene are located on different chromosome or spread over many chromosome [33]. Multiple regulon are present in the cold stress and osmotic stress responsive genes. In Arabidopsis DREB1/CRT binding factor and DREB2 regulon are involve in the expression of stress responsive gene [32].

Efforts are under ways to understand the role of CBFs/DREBs. The Plant can tolerate drought and cold stresses by activating the gene encoding transcription factors. Expression of these genes causes physiological and biochemical changes i.e. increases sugar content, soluble protein, changes in the composition of lipid membranes and proline [34, 30]. Two similar regulatory sequences which are called dehydration responsive elements (DRE; TACCGACAT) and C-repeat (CRT; TGCCCGAC) are present in the promoters of said genes [35]. Many scientists have demonstrated that Arabidopsis promoters COR78, COR15 and COR6.6 genes are induced by low temperature [36]. The regulator element which is basically responsible for this regulation was identified by in RD29A (COR78) promoters [36]. Dehydration Responsive Elements (DRE) are a 9 base pair sequence i.e. TACCGACAT. The DRE (Dehydration Responsive Elements) is 5-bp core sequence, i.e. CCGAC design the C-repeat (CRT) which activate expression of gene in response to drought, low temperature, and high salinity. These elements are also called as Low Temperature Regulatory Element (LTRE) [37].

The first cDNA was isolated by Stockinger et al [44] it encodes a protein that binds the CRT/DRE sequence. The CBF1 transcription factor which binds to the CRT/DRE, has a molecular mass of 24 KDa. It has also an AP2 domain, i.e. a 60 amino acid motif which is mostly present in plant protein along with Arabidopsis ANTSEGUMENATA APETAL2A and TINY [38-40]. Oham and Shinshi [41] also reported that AP2 domain consist of a DNA binding region. In the Arabidopsis, more than 140 proteins having such type of domain. These proteins are divided into five subclasses based on the amino acid sequence of AP2 domain [42].

A recent domain swap study shows that the N-terminal of 115 amino acid are enough to both CBF1 and COR target gene promoter and facilitate binding to CRT/DRE elements. The C-terminal of 98 amino acid is enough for transcriptional activation [43]. Stockinger et al [44] also says that CRT/ DRE sequence of CBF protein bind and turn on the expression of genes in the yeast having CRT/DRE sequence. This report verified that CBF1 is a transcriptional factor which activates CRT/DRE containing genes. In the Arabidopsis, probably it was a regulator of core genes. Jaglo et al [45] presented that in transgenic Arabidopsis, continuously CBF1 over expression resulted in the expression of COR gene, which are under the control of CRT/DRE sequence without in the presence of low temperature. The transcription factor CBF1 is very essential regulator of the cold response. It controls COR gene expression, which leads to improve freezing tolerance. Gilmour et al [46] have demonstrated that CBF1 encoding three closely related transcription factor which belongs to a member of a small gene family. These genes are called CBF1, CBF2 and CBF3 [46]. These genes are also known as DREB1A, DREB1B and DREB1 [47]. These genes are linked on chromosome 4 to direct repeat which are near to molecular markers PG11 and m 600 [47, 48]. These genes are not linked to CRT/DRE control genes i.e. COR15a, COR78, COR6.6 and COR47 which are presents on chromosome 1, 2 and 5 respectively [48].

The function of CBF1, CBF2 and CBF3 are similar, which can activate expression of genes consist of CRT/DRE in their promoter [46]. This was proved by a research on reporter genes in the yeast, which have the CRT/DRE sequence. These results show that these two families are also transcription activator. In the transgenic Arabidopsis plants, the DREB1A/CBF3 over expression enhanced both the drought and freezing tolerance in transgenic plants [47]. Different scientist investigates that transcript for CBF3, CBF2 and CBF1 can accumulate in a proper amount within a few minutes after exposing to low temperature [45, 47, 48] shows highest sensitivity to environmental changes. Orthologs of CBF/DREB1 in Arabidopsis has also found in every higher plants [49, 50]. Recently in the grape a fourth CBF4 gene has been identified. The CBF4 gene expression is low at optimum temperature, but when expose to low temperature (4 °C) it enhance their expression [52].

Many scientists say that only CBF pathway is sufficient to increase the stress tolerance. In transgenic Arabidopsis, the CBF gene constitutive expression induces the expression of CRT-containing genes, which leads to increased drought and freezing resistance without the stimulus [52]. However, in all plants the presence of CBF pathway is not complete; either CBF members are not properly switching on time or for a long time, or smaller-regional CBF The tomato LeCBF1 or Arabidopsis AtCBF3 over expression, in the transgenic Arabidopsis it increased stress tolerance while in the transgenic tomato same effect was not observed. In Arabidopsis a reporter gene-aided and genetic screening was carried out [53]. In the FIERY2 (FRY2) locus seven allelic mutations was reported by [53]. These mutations cause a significant increase in expression of the stress responsive genes through the cis-element CRT/DRE but less affected the non CRT/ DRE type stress responsive genes. It shows that in the regulatory sequence of target genes, the presence of CRT/DRE sequence is essential for the stress responsive genes activation. The over expression of DREB1C, DREB1B or DREB1A in the transgenic Arabidopsis produce dwarfism which is the side effect of these genes [47, 54]. A similar result of dwarfism is also found in genetically modified tomato due to over expression of DREB1B. The dwarf effect can be controlled by the exogenous application of GA3 [55].
For the first time, the discovery of DRE/CRT elements, a reporter gene was used which contains the CBF1 and DRE/CRT elements and it activates reporter gene expression [44]. It shows that protein, which has an EREBP/AP2 binding motif, is a transcription factor [56]. In Arabidopsis the over expression of CBF1, turn on the expression of DRE/CRT regulated COR genes which increase freezing tolerance without the cold stimulus [45]. In Arabidopsis, all of the CBF genes are induced by cold stress and within a few minutes of cold stress, transcript of CBF level is increased [46, 48]. The mechanism of CBF gene, which is induced by low temperature, is still not known [46]. In the plant other cold responsive pathways are also existed which may play a vital role in low temperature [57]. Transcription factor and CBF protein have a role in cold acclimation. Affymetrix Gene Chip technology is used for identification of the core set of cold-responsive genes and to find out target of CBF2 genes or other six transcriptional factors which are regulated with CBF2. A total of 514 genes were placed in the core set of cold responsive gene, among these genes, 302 were unregulated while 212 down regulated. Through Bioinformatics analysis, 84% genes are induced by CBF2 and remaining 8% were regulated by both ZAT12 and CBF2 [57].

The CBF pathway is involved in the cold acclimation of Arabidopsis plant [58]. It consist of CBF3, CBF2 and CBF1 genes [45, 46] which code for transcription factors that can bind to DRE (dehydration responsive element) /C-repeat (CRT) regulatory sequence located in the promoters of COR and other cold related genes [44, 46]. The CBF genes constitutive expression can enhance the CBF regular expression, which leads to cold tolerance in the cell without cold stimulus [54]. The cold stress tolerance was due to the CBF regulon which involve in the synthesis of cryoprotectant-polypeptide i.e. COR15a [59] and compatible salutes accumulation such as Raffinose, proline and sucrose [54]. Response to low temperature, in Arabidopsis about expression of 8000 genes takes place [60] its shows that during cold acclimation a large amount of changes occurs in the transcriptome. About 12% only of these genes are allocated to the regulon of CBF and at least twenty eight percent of the cold-responsive genes exaggerate by the expression of CBF. It accomplishes that turning on of multiple cold regulatory pathways are associated with cold acclimation [60].

**AP2-EREBPs**

AP2 and EREBPs (AP2 stand for APETALA2 and ethylene responsive element binding protein) which are transcription factor in the plants. These transcription factors have a unique feature, that it has the AP2 DNA-binding domain. In the life span of the plant, different important roles can play. In different developmental process it is a key regulator [56]. The AP2 domain is conserved in family of tobacco EREBPs (Ethylene Responsive Element Binding Proteins) [41]. Later on it is also conserved in Arabidopsis AP2 (APETALA2), a gene which play role in the development of flower [39]. Those proteins which consist of an AP2 like domain in different plant species usually having large number of biochemically and genetically AP2 like protein. These include Arabidopsis ANT (AINTEGUMENTA), a functional polypeptide which controls flower development and is very essential for the ovule formation [38]. A gene TINY, which restricts cell proliferation during floral and vegetative organogenesis and CBF AP2 is a 60 amino acid domain present in the protein of transcription fact which can bind to DNA [44].

The AP2 (APETALA2) domain also called as the ERF/AP2 domain (ethylene-responsive element–binding factor). AP2/ERF genes on the bases of AP2 domain presence can be divided into different classes. One class includes protein, which contains two AP2 domains, i.e. Glossy 15 and AINTEGUMENTA (stand for Glossy15 and ANT for AINTEGUMENTA) [38, 39]. The other class includes protein, which encodes one AP2 domain, i.e. TINY, AtEBP and AB14 [41]. The Arabidopsis AP2 gene belongs to family AP2/ERF which are well studied. The AP2 domain are two in number and each domain consist of 68 amino acid with an eighteen subunit of amino acid are core region, which makes an amphipathic a-helix [38]. The AP2 domains are very important for the function of AP2 [40]. Along with AP2, other functional polypeptide which encodes the AP2 domain also studied in the Arabidopsis). In the complete genomic sequence of Arabidopsis, there are 144 AP2/ERF genes are found in the Arabidopsis genome [61]. AP2/ERF genes can be classified into five groups based on their DNA-binding domains similarities [18]. These groups are (1) RAV subfamily having 6 genes (2) ERF subfamily having 65 genes (3) DRE/CFB subfamily having 55 genes (4) AP2 subfamily having 14 genes and (5) other groups having 4 genes [18].

**Structural Characteristics of DREB1/CFB Genes**

All the transcription factors have DNA binding domain, known as DNA binding motif. The DNA binding domain conserved amino acid sequence within a family. The CBF/DEBR protein has conserved DNA binding domain called, AP2/ERF domain. It consists of ~60 amino acids and this domain is deliberated plant-specific [56]. DREB/CFB genes also reported from diverse plant species which containing AP2 domain, including both dicots and monocots. The AP2/ERF domain of three-dimensional analysis shown 3 stranded β-sheets which are connected anti-parallel by α-helix and loops, nearly packed analogous to each other [17, 62]. Amino acids, glutamic acid (at position 19) and Valine (at position 14) are quite conserved in the AP2/ERF domain and play a vital role in binding specificity of dehydration responsive elements (DRE) cis elements [18]. However, some studies on rye, wheat, rice and barley authenticated that E19 is not sealed in DREB1 proteins, and it is substituted by valine [15]. Therefore, it is concluded that E19 may not be very important in the DREB1.
protein, for the recognition of the DNA-binding sequence. Other amino acids that expedite direct contact for the DNA binding activity with DNA are adenine (8), arginine (6), glutamic acid (16), tryptophan (10), tryptophan (27) and arginine (25) [62]. Mostly, these amino acids are found in the transcription factors i.e. DREB1. The domain of AP2 consists of two regions. YRG region (YRG element) is the first one which consists of 20 amino acids. It is rich in the hydrophobic and basic amino acids in the N-terminal stretch. It is suggested to have a role in the activity of DNA-binding, due to its basic nature [63]. The RAYD element is the second region which contains 40 amino acids. Eighteen (18-amino-acid) stretches in the C-terminal region of these RAYD, which is capable of starting an amphipathic alpha helix, and anticipated to arbitrate protein-protein interaction. The binding activity of DREB proteins also regulates by influencing the conformation of the YRG element [63]. Only in the nucleus transcription factor works and very perilous to their function. The entrance of DREB proteins is arbitrated Nuclear Localization Signal (NLS). It is a basic amino acid rich stretch having consent sequence PKRPAGRTK-FRETRHP as NLS. Transcription factor, having no NLSs enter into the nucleus through protein–protein interaction with the transcription factor, that have NLSs. DREB1/ CBF genes retain caboxyl-terminal acidic region. This region is conjectural to be a transcriptional activator motif [44].

Expression Analysis of DREB1/CBF1 Transcription Factors

Expression of CBF/DREB1 genes under abiotic stresses has been explored widely in plant species. Mostly the expression of the stress related gene generally showed a tendency of first increasing and then decreasing. The utmost expression of genes is attained amongst 1 and 4 hours under low stress condition. In Arabidopsis, there are six DREB1/CBF genes, among these; CBF1/DREB1B, CBF3/DREB1A and CBF2/DREB1C were highly expressed under low temperature stress, but not under high-salinity or drought stress [46, 47]. Similarly, CBF genes from various plant species, such as LpCBF3 from ryegrass [23] DREB1 from aloe [64] Ptcbfb from Poncirus trifoliata [65] EguCBF from eucalyptus [67] and OsAP211 from rice [68] showed high level gene expression under cold stress (4°C). It is well notorious that gene expressions of the A-1 group are induced by cold stress, but not by high-salt stress or drought, while the other genes of the A-2 group are delimited by drought and salt [21, 47]. However, recent researches showed some conflicts with veneration to these trends. Several DREB1/ CBFs genes such as MbDREB1 from apple, BrCBF from Chinese cabbage [37], VvDREB1 from Vaccinium vitis-idaea [68] and OsDREB1F from rice [69] reported which are not only responsive to low temperature, but also to drought, high-salt stress and exogenous ABA treatment. The different pathway of signaling in abiotic stress is assumed to intermingle and share some common features that fashioned as potential ‘node’ for crassstalk. These CBF/DREB1 genes may act as a node connecting or cross point of several pathways and concurrently regulate drought, cold, ABA pathways and salt stress. Interestingly, OsDREB1B exhibited high level of expression in low temperature stress, but high temperature was also induced [70]. In different plant species, the majority of CBFs gene upregulated by cold stresses and accomplishes the vital role of cold adaptation. Some genes such as OsDREB1C from Oryza sativa, CrCBF from Catharanthus roseus are established to be continuously expressed under low temperature stress [71]. Mostly CBFs showed fast and robust response to abiotic stresses. Within 10 min at 4°C the AtDREB1A is induced and levels of transcript of MdDREB1 and EguCBF are measureable within 30 and 15 minutes respectively, under low temperature condition [72]. ZmDREB1A, OsDREB1A/CBF3 and EguCBF1 are induced by cold stress within a period of 60 min, 40 min, 30 min, respectively [70].

In limited plant species, some information is available in tissue-specific gene expression. Earlier studies shown that CBF/DREB1 is expressed in all organs and tissues. However, the kinetics is different in different tissues. The CrCBF gene is expressed at high levels in callus tissues and hairy root as compared to root and leaf tissues [71]. Similarly, OsDREB1F gene expression was higher in callus and panicles as compared to other tissues. The PbCBFb gene was continuously expressed under cold stress and expression of gene was higher in stem and leaf and markedly increased by root. The MdDREB1 gene expression was maximum in mature organs as compared to young tissues [72].

Regulation of DREB1/CBF Genes

The regulatory network of CBF genes is well-studied in Arabidopsis. The transcription is regulated through regulatory proteins, requisite to cis-acting elements situated upstream genes. Promoter analysis of high salinity, cold and drought stress inducible genes RD29A/COR78/LT178 in Arabidopsis showed a 9 base pair conserved sequence (TACCGACAT). The conserved sequence consists of Dehydration Responsive Elements (DRE). It is mostly found in promoter region of many cold and drought stress inducible genes [29]. The cis-acting elements, named Low-Temperature Responsive Element (LTRE) and C-repeat (CRT) both consisting an A/GCCGAC motif. It customs the core of Dehydration Responsive Elements (DRE) sequence [29, 44]. CRT/DRE and ABA-responsive element (ABRE) are the major cis-acting elements which function in ABA-independent and ABA-dependent gene expression, respectively, under adverse stress conditions. The protein of DREB1/CBF contains the conserved AP2 DNA binding domain, which exactly drags to the CRT/DRE sequences and triggers the transcripts of genes motivated by the CRT/DRE sequence. Three DREB1/CBF proteins in Arabidopsis, are encoded by genes that present on chromosome 4 in sequence of DREB1B/CBF1, DREB1C/CBF2 and DREB1A/CBF3 [46]. These DREB1 proteins are the major transcription factor involved in the expression of cold inducible genes. In contrast, the expression of the DREB2A and DREB2B genes, are induced by high-salinity and dehydration stresses but it is not induced by cold stress [17, 73]. Moreover ICE1 (inducer of CBF expression), DRE/CRT cis-acting element, MYB15 and HOS1 (high expression of somatically responsive genes) are direct regulators of CBF/DREB1. The transcription activator of CBF3 is ICE1 and continuously expressed and binds on the CBF3 promoter precisely to the MYC recognition sequences (CANNTG) and thereby enhanced the expression of CBF3/DREB1A genes [74]. ICE1 mutant lines are malfunctioning in the cold regulated expression of CBF3 and COR.

genes, which significantly reduced in chilling and low temperature tolerance in Arabidopsis [75]. Transgenic lines continuously over expressing the ICE1 did not enhance the expression of CBF3 at high temperatures, but exhibited up regulation of the CBF3 and its target gene expression at low temperatures. Exposing of a plant to chilling stress, alteration of either associated protein or ICE would sanction ICE to bind to the prompter of CBF and to enhanced CBF3 transcription [74]. The expression of CBF3 and CBF2 is negatively regulated by HOS1. The mutant plant of HOS1 showed super stimulation of CBFs and related genes [74]. Ring-finger protein is encoded by it which is confined in the cytoplasm at optimum temperature, upon cold stress accumulates in the nucleus and facilitates the degradation and ubiquitination of ICE1 [76]. The functioning of ICE1 is negatively regulated by HOS1 in low temperature adaptation. MYB15 is the next regulator of CBF/DREB gene expression [77]. The mutant line of ICE1 increased elevated levels of MYB15 transcript as compared to wild-type. It suggested that ICE1 is a negative regulator of MYB15 [75]. The expression of CBF/DREB1 is also moderately meticulous by the DREB1/CBFFactors themselves [78]. All these studies recommend that all three proteins (DREB1B, DREB1A and DREB1C) function in a regulatory cascade to modulate expression of CBF/DREB1 genes to control plant responses to low temperatures [78].

**Cold Acclimation and CBF/DREB Genes in Plants**

In some plants, when expose to low temperature, i.e. non-freezing leads to increase the freezing tolerance and this process is called as cold acclimation [79]. Variation is present in response to low temperature in different plants, even within an organ of different tissue response to low temperature in a different way and expresses different genes [80]. To understand the molecular and biochemical basis of low temperature, an extensive research work was carried out on acclimation. When the plant exposes to low temperature a variety of changes occur in the cell during cold acclimation response, i.e. protein level, alteration in lipids and carbohydrate composition. As the cell detects the cold stimulus, calcium ion influx start from extracellular space to cell cytosol and thus initiates cell signalling pathway [81] followed by downstream gene regulation. This process activates the transducer followed by inducing expression of CBF, and finally downstream COR genes (cold regulated) expression takes place [82]. Though the cold acclimation molecular mechanism is still not well known [83].

Once it was known by the investigators that cold acclimation cause changes in the gene expression, this discovery open a new era for the scientist to characterize and identify cold responsive genes [80]. The research on expression of cold-regulated gene in Arabidopsis, CBF/DREB1 sequence which is a transcription factor is discovered. These TFs plays a vital role in the cold acclimation [58]. Some gene, which response to cold stress continues their high level of expression till cold stress is removed while some genes express only transiently [23]. These genes are switched on; as the cell detects cold stimulus [58]. During cold stress freezing injury occurs due to cellular dehydration. The ice formation causes dehydration stress, which may lead to damage of cellular membrane [84]. The 2nd group of gene expression reduce the injury of dehydration by up-regulation of COR genes (cold-regulated). The IRI genes (Ice re-crystallization inhibition) which minimized the ice crystal and regulation of respiration and photosynthesis related genes which is very essential and increase low temperature tolerance [83]. Cold acclimation which produces cold tolerance is not constant, but variable and quickly lost whenever, plants are returned to the normal environment [85]. Moderate to high light condition are required for a successful cold acclimation. If plants are not exposed to light properly, it may cause photo-inhibition and formation of ROS (reactive oxygen species) [85].

The potential of the plants to acclimate cold stress is multigenic trait and different biochemical and physiological changing occur during cold acclimation (Fig. 4). The most prominent changes, i.e. reduction in water content and the growth of the plant [86] (Levitt, 1980), changes occurs in the lipid composition of the membrane, increase ABA level, increases in the antioxidant and the production and accumulation of osmolytes i.e. polyols, soluble sugar, betaine [87]. The primary target sites of freezing injury are the cell membranes. To develop cold tolerance changes in membranes are critically important. Changes in the ultra-structure of the plasma membrane in Arabidopsis occur within six hours of the cold acclimation [88]. During cold acclimation, variation in the lipid composition of membranes is correlated with membrane cryostability [89].

**CBF Cold Response Pathway**

CBF/DREB1 are cold responsive genes which are activated transiently by low temperature and the expression of these genes regulate the COR gene expression [46]. In the Arabidopsis three different CBF/DREB1 genes were discovered i.e. CBF2/DREB1C, CBF1/DREB1B and CBF3/DREB1A [58]. In Arabidopsis the CBF1 over expression give freezing tolerance. In transgenic plant, the induction of CBF/DREB1s genes shows the activation of downstream cold responsive genes and improves the salinity, drought and freezing tolerance [45]. The orthologues of CBF/DREB1 in the Arabidopsis is also found in other plant species and it is concluded that during cold stress, the CBF transcription factor is highly conserved in the plant kingdom. The expressions of CBF/DREB1 genes are related to changes in the temperature. As lower the temperature, the CBF transcription will be high. The CBF gene expression become desensitized at low temperature and high temperature is required for the re-sensitization of CBF genes [90]. The expression of CBF genes can be repressed by either their downstream target gene’s product or their own product and thus expression of these genes are tightly controlled [75]. CBF2/DREB1C gene expressions negatively control the CBF3/DREB1A and CBF1/DREB1B [78]. Transcript level of CBF genes, i.e. CBF1, CBF2 and CBF3 are increasing within the fifteen minute treatment of cold stress. After cold stress the CBF genes are induced which arbitrate cold-related genes (COR genes) at about three hours [9]. The COR gene expression induces by the CBF1 and bZIP (CBF1 is ABA-independent and zip is ABA dependent pathways) [91]. The eskl which is freezing tolerant mutant accumulate high level of proline but the COR gene expression does not occur continuously [9]. The presence of ICE, which is a transcription factor and acts
Role of CBF/DREB Gene Expression in Abiotic Stress Tolerance. A Review


at the CBF/DREB promoters. When the plant exposes to low temperature the ICE transcription factor switch on the expression of CBF/DREB genes which leads to induction of CBF regulon (Fig. 5) and thus freezing tolerance are increases [58].

Chinnusamy et al [75] find out Inducer of CBF Expression1 (ICE1) which is an upstream transcription factor in the Arabidopsis by using the PCBF3: LUC bioluminescent genetic screen. He says that dominant ICE1 mutation can block the CBF3 expression and reduces the expression of CBF target functional polypeptides. The mutant ICE1 shows weak low temperature tolerance and cold acclimation. The continuous ICE1 over expression increases the CBFs expression and COR genes are expressed constitutively. For activation of CBF gene, cold treatments are required. So it is concluded that ICE1 can control many CBF dependent regulon of cold responsive genes and may work as a master switch. Another transcription factor, i.e. bHLH which work like ICE1 involve in the regulation of CBF1 or CBF2 [92]. In the future regulon biotechnology will be a new hope for abiotic stress tolerance in plants and can contribute to sustainable food production [92].

Function of Regulon

In plants, the basic function of CBF regulon is to provide protection to the cell against freezing and other dehydration stresses [58]. Those genes which control under the CRT/DRE are six in number, i.e. COR6.6/KIN2, KIN1, COR47/RD17, COR15a, ERD10 and COR78/RD29a [58, 59]. COR15a gene over expression increases the cold tolerance by 1 to 2°C in non-acclimated plants [58]. These genes decrease the tendency of membrane to form detrimental hexagonal II phase lipids upon free-dehydration [59]. The CBF over expression in plants enhances the level of total sugars and proline [35]. Under cold acclimation different plant increase the level of proline and total sugars, which leads to play a significant role in the cold tolerance [58].

Calcium Role in CBF Regulon Pathway

How CBF genes and cold signal is perceived and regulated, the exact mechanism is still not well understood [93].

Calcium is an essential nutrient for the plants and involve in the cold acclimation. When the plant exposed to cold stress, a sudden increase occurs in the free calcium level in the cytoplasm. This calcium comes from extra and intracellular store and induces the CBF regulon [94]. The proof of the relationship between the cold switching of CBF and calcium signaling pathway was possible with the innovation of CAMTA (Calmodulin binding transcription activator) protein, which can bind to the regulatory sequence in the CBF promoter, which perform a very essential role in the regulation of CBF regulon and cold resistance [95]. Calmodulin is a protein which bind to Ca⁺ and those proteins which bind to calmodulin binding transcription factors are called CAMTA protein. This complex plays direct role in inducing cytosolic Ca⁺ signal into downstream regulation of gene expression [93, 95]. When plants are cold acclimated, the Ca⁺ influx and the peak level are altered. The amount of Ca⁺ is reduced and prolong in their length. Thus the expression of low temperature responsive gene takes place, i.e. CBF and COR target genes and it shows that Ca⁺ is a 2nd messenger [90]. The using of some chemical agent which minimized the cold induce influx of calcium, the COR gene expression are also slowing down. When such chemical is used which can enhance the calcium influx at high temperature? The expression of COR genes is induced [90].

Regulation of CBF Pathway

In the CBF gene promoters, DRE/CRT sequences are present, which shows that these genes are not controlled by auto-regulation [46]. The CBF expressions by low temperature controlling factors are identified. One example is the Inducer of CBF Expression (ICE) which is identified through mutational screening. On the other side, ICE1 shows a slight effect on the

Figure 4: Cold acclimation induces changes in cellular processes. Different responses are observed while exposing plants to low non-freezing temperatures.
accumulation of CBF2 transcript, which are induced at low temperature. It shows that variation is present in the switching mechanism of CBF/DREB1 family [75]. The ICE1 over expression increases the expression of the CBF Regulon and enhance the low temperature tolerance in genetically modified plants [76]. DEAD box RNA helicase is encoded by LOS4 which plays an important role in the expression of CBF genes. In the mutant plants, i.e. los4-1 the CBFs expression, their target genes which are located downstream and cold acclimation are impair [96]. The los4-1 plants are very susceptible to low temperature when is treated with cold in the darkness. This is due CBF2 impair expression in los4-1 plants, while in wild type Arabidopsis plant express CBF2 when treated with cold in the darkness [96].

The promoter region of the CBF2/DREB1c, CBF1/DREB1b and CBF3/DREB1a are responsive to cold stress and ICE1 which is a transcription factor (Inducer of CBF Expression 1) playing a vital role in the expression of CBF [75]. When Arabidopsis plants are suddenly transferred from normal temperature to low temperature (20 to 4°C), leads to rapid CBF transcript accumulation [97]. At three hours the CBF transcript is reaching to their peak, then significant reduction occur [98]. The CBF transcript levels are affected by the intensity of the cold shock. When a plant transfers from 20-10°C, after two hours the CBF level is less as compare to plant which are transferred from 20 - 4°C. Similarly CBF expression is high in plants which are transferred from 20 to -5°C as compare to 20 to 4°C [98]. For the CBF induction the threshold temperature is 14°C. At this temperature the transcript of CBF is detectable [98]. When the temperature has dropped below the threshold level, the CBF expressions are increasing along with the COR15a [98]. Cold acclimation in plant takes place at 4°C for 14 days. When a plant is transferred to 0°C or -50°C the CBF level are also increasing. If a plant is constantly exposed to low temperature, then CBF expression are decreases [90].

### Half Life Span of CBF Transcript

At high temperature the CBF transcripts have a very short life span i.e. seven minutes at high temperature. As the plants are transferred from low temperature to high temperature the CBF gene promoters suddenly inactive, i.e. within a few minutes. After 90 minutes of the transferring to high temperature, no transcript will be found [98].

### Conclusion

Abiotic stress is the major ecological stresses which limit crop production and affects all cellular function of the plants. The stress signal is transduced through signal transduction pathways. The foremost components are reactive oxygen species, protein kinase, calcium, lipid signaling cascades and protein phosphatase. The stress signal leads to regulation of effector genes and transcription factors which are collectively called stress regulated genes. Effector genes which encode proteins under this type containate embryogenesis abundant proteins, chaperones, antifreeze proteins, osmotin, mRNA binding proteins, osmolyte biosynthetic enzyme such as water channel proteins, proline, proline transporters, sugar, detoxification enzymes, ferritin, proteinase inhibitors and lipid transfer proteins. The TFs which are involved in stress response are inducers of C-repeat binding factor expression. The use of the transcription factors and concern genes in genetic alteration of agricultural crops can increase stress tolerance.

---

**Figure 5:** CBF cold acclimation pathway (Thomashow, 2001).
Conflict of Interest

There is no conflict of interest.

References

Role of CBF/DREB Gene Expression in Abiotic Stress Tolerance. A Review


60. Fowler S, Thomashow MF. Arabidopsis transcriptome pro-filing indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell. 2005;17(4):1675-1690.


