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## Chapter

# Regulatory Network in Plant under Abiotic Stress

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## Abstract

Abiotic stress is one of the primary causes of crop yield loss worldwide; it contributes to a nearly 50% reduction in crop production. Anti-oxidants are produced in plants to scavenge ROS that causes cellular damage during abiotic stress. The plant stress response is a complex mechanism that involves protein initiation pathways, abscisic acid signaling, transcriptional and post-transcriptional modifications. Abiotic stress-inducible genes are divided into two groups based on protein products, one is for signal transduction and other is for expressing resistance. Transcriptional factors bind to the promoter of the target gene at specific DNA sequences thus regulating the gene expression, so different kinds of transcriptional factors known as regulons are involved in regulation of genes during drought, salinity and cold stress.

**Keywords:** abiotic stress, transcriptional factors, signalling, genes

## 1. Introduction

Abiotic stress is one of the main reasons for crop yield loss worldwide, almost more than 50% reduction in crop yield is due to abiotic stress. Plant are sessile in nature and environmental conditions constantly affect the plant. When these conditions are extreme and rapid, plants face stress conditions. But stresses are not necessarily a problem because plants have defense mechanisms to reduce or avoid the damage caused by these stresses.

For salinity and drought, it is very easy to distinguish the primary stress signals from the secondary stress signals, which is caused by too much salt or too little water. Primary signals caused by the drought which is hyperosmotic stress. Drought is often called as osmotic stress because hypo-osmotic condition does not harm for the cell. Salt stress cause both the osmotic and ionic effects on cells. The secondary effects of salinity and drought cause the oxidative damage to cell like damage to cellular proteins, lipids, nucleic acid and dysfunction of metabolites. Salinity and drought have overlapping and unique stress signals. Both cause the hyperosmotic signals which causes the phyto-hormone abscisic acid accumulation [1].

Abiotic stress such as high temperature, low temperature, high light intensity, heavy metals, osmotic stress and the number of herbicides and toxic elements leads to a high amount of reactive oxygen species (ROS) productions that cause cellular damage and reduce the amount of photosynthesis in the plant (**Table 1**). The plant produces the antioxidants that remove the ROS but sometimes in extreme stress

Stress	Plant response	Effects of stress on plant
Drought stress	Stomata closure, leaf rolling, accumulation of metabolites	Reduction in photosynthesis due to inability of water transport to leaf
Heat stress	Protein stability support survival, activation of protein repair systems	High evaporation, water deficit, plant death due to enzyme turnover
Cold stress	Synthesis of hydrophilic protein and accumulation of osmolytes to prevent ice crystal formation	Slow rate of biochemical reactions, photosynthesis, leading to oxygen radical damage and CO <sub>2</sub> fixation lags. Formation of ice crystal which disrupt the cell membrane

**Table 1.**  
*Effects of abiotic stress and response of plant.*

conditions amount of ROS is so much high that antioxidants are unable to remove them. So a high amount of ROS in the cell leads to cell damage that reduces the various metabolic processes of the plant. To repair these damages, plant cells have a complex defense system, including the antioxidant stress-related defense genes that cause the changes in plant cell machinery.

Stress-responsive pathways in plants are not linear, they are complex that involved multiple pathways and specific tissues and cellular compartments [2]. When plants face stress, at the initial stress stages, the activation of protein signaling pathways and activation of Ca<sup>+</sup> act as the initial sensor that leads to the expression of stress-responsive genes and physiological changes [3]. Accumulation of abscisic acid (ABA) in cell plays a crucial role in transduction pathways, and stress signaling and activate many defense responses [4].

It is known that abiotic stress, through regulation of protein turnover and gene expression, changes the abundance of many proteins and mRNAs, showing that transcriptional and post-transcriptional gene regulation plays a very important role in cellular function adaptation to environmental changes. Recent advances in genomics, metabolomics and proteomics have provided a way to study the gene regulatory network system which consists of inducible genes, expression programming and regulatory elements [5]. Through genetic studies, scientists revealed that stress-related attributes are quantitative trait loci, so the selection of these traits is difficult.

The plant produces various metabolic proteins such as osmoprotectants, regulatory proteins that play role in signal transduction pathways like kinases and transcriptional factors in responses to abiotic stress. Transcriptional gene regulation is controlled by transcriptional factor binding sites (TFBS) and a network of transcriptional factors [6].

Transcriptional factors are proteins in nature that have DNA binding domains that bind to cis-acting elements that are present in the target gen promoter. TFs repress (repressor) or induce (activators) the RNA polymerase activity that leads to the expression of gene regulation. TFs are grouped into families based on their DNA binding domain [7]. The absence and presence of TFs (activators or repressors) lead to the regulation of gene transcription that involved the whole signaling cascade events specified by plant developmental stage, environmental condition and tissue type [8].

Abiotic stress-inducible genes are divided into two groups based on protein products. The first group of genes, proteins of these genes play role in the regulation of signal transduction such as TFs and gene expression, another group of genes whose proteins directly confer resistance in plant stress against environmental stress such

as anti-freezing protein, late embryogenesis abundant (LEA), enzymes for synthesis of proline, betaine and osmoregulators. Four different regulons have been identified, two regulons are ABA dependent (1 and 2) and two are ABA independent (3 and 4): (1) AREB (ABA-responsive element-binding protein) /ABF regulons (ABA-binding factor); (2) MYC/MYB (myelocytomatosis oncogene/myeloblastosis oncogene) regulons; (3) CBF/DREB regulons (4) ZF-HD (zinc-finger homeodomain) and NAC (ATAF, CUC and NAM) regulons.

Our knowledge related to molecular mechanisms involved in plant defense against abiotic stress is limited but many numbers of genes have been identified in recent years that are involved in these responses. These genes can be induced by stimuli and proteins that confer resistance to abiotic conditions.

The book chapter aims to report recent advances in abiotic stress-responsive mechanisms and describe the regulation of gene expression.

## **2. Abiotic stress-inducible genes**

Many genes are involved in plant response to abiotic stress. The study of the function of abiotic stress-inducible genes is an important tool to study the molecular mechanisms of stress tolerance and plant responses but it also helps scientists to improve the stress tolerance in crop plants by manipulation of genes. Hundreds of genes have been identified that play role in abiotic stress responses [9]. Most of the drought-inducible genes are induced by cold stress and salinity stress.

These genes are divided into 3 groups: (1) in this group, genes product directly protect the cell against stresses e.g. heat shock proteins (HSPs), chaperones, osmoprotectants, LEA proteins, detoxification of enzymes, antifreeze proteins and free radical scavengers [10]; (2) in this group, genes that involved in signaling cascade and transcriptional control e.g. Calcium-dependent protein kinase (CDPK) and Mitogen-activated protein kinase (MAPK), phospholipases and transcriptional factors [11]; (3) genes that are involved in ion and water uptake and transport via ion transporter and aquaporin [12].

## **3. Transcriptional factors genes**

Plant growth and developmental processes are affected by environmental stress. These stress are salinity, drought and high temperature etc. Human activities cause adverse effects on the atmosphere that leads to an increase in conc. of O<sub>3</sub> in the troposphere and this causes oxidative stress, oxidative stress cause the destruction of various important proteins and cells, and a reduction in crop yield.

Susceptibility or tolerance is a very complex phenomenon because stress can occur at any plant developmental stage and mostly more than one stress affects the plant [13]. Abiotic stress responses involved the production of metabolic proteins such as proteins which are involved in the production of regulatory proteins and osmoprotectants that play role in signal transduction e.g. transcription factors or kinases.

Regulation of responses needs proteins that operate in signal transduction pathways, e.g. transcription factors. These factors bind to the promoter of the target gene at specific DNA sequences thus regulating the gene expression. This type of regulation is known as regulons. Four different regulons have been identified that are active in plants during abiotic stress. Dehydration responsive element binding protein /C-repeat binding factor (DREB1/CBF), DREB2 regulons play important role in

ABA-independent gene expression, but in ABA-dependent gene expression regulons ABA-responsive element binding protein/ABREF binding factor (AREB/ABF) [14]. Some other regulons like NAC (No apical meristem) and MYB/MYC (Myeloblastosis-Myelosytomatosis) are also involved in gene expression of the abiotic stress-responsive gene. TF OsNAC6 is a NAC-type in produced by the plant during abiotic stress especially cold, salinity and drought. In Rice, over-expression of OsNAC6 during abiotic stress is studied by using microarray analysis [15]. Transcription factors can be the target for genetic engineering for the development of abiotic stress-resistant crop plants.

#### 4. Transcription factor for drought stress

The plant genome controls the regulation of TF under water deficit conditions. By using the microarray technique in *Arabidopsis thaliana*, also known as the model plant, many genes have been identified that are repressed or induced in response to a condition that leads to cellular drought stress [16]. The drought genes have been divided into four groups, transcriptional regulations, protection of cellular structures, cellular metabolism and signal transduction. Almost six different classes of TFs have been identified that participate in gene repression or induction under drought conditions. Multiple treatments that mimic drought stress, induce NAC domain and Homeobox domain containing TFs. Adaptation of plants to water stress is promoted by the accumulation of proteins which have structural and metabolic functions. Late embryogenesis abundant (*Lea*) gene play role in plant protection. In desiccating seeds, *Lea* genes are expressed. *Lea* genes produce a hydrophilic protein that protects the plant membranes and proteins because of its chaperons-like function. In rice plants, these hydrophilic proteins are identified that protect the cell membrane from injury under drought stress [17].

#### 5. Transcriptional factors in drought stress

In wheat genome microarray analysis was performed, and the result of the analysis showed that in 300 unique ESTs (expressed sequence tag), 18% of genes were down-regulated and 30% of genes were up-regulated under water stress [18]. Bray [19] reported the 130 genes in *A. thaliana* that were up-regulated under water stress. Under water stress, these genes are involved in cellular response by detoxification, signaling events and other functions.

##### 5.1 Salinity stress

Large world cultivated areas are salt-affected. Salt stress affects plant growth, development, metabolic processes and physiological processes that lead to a reduction in crop yield [20]. Plant water relations are also affected by salt stress. Salinity creates ionic stress by the accumulation of  $\text{Cl}^-$  and  $\text{Na}^+$  ions in the cell. Homeostasis of some other initial ions e.g.  $\text{Ca}^{++}$ ,  $\text{NO}^{-3}$  and  $\text{K}^+$ . The plant cell membranes are also affected by salt stress. It affects the lipid and proteins of the cell membrane which leads to cell injury because of hyperosmotic stress and ion imbalance. These changes in plants cause disturbance in normal plant development and growth [21, 22]. High NaCl concentration in the root zone affects the plant metabolism and physiology at

various levels (oxidative stress, water deficit, nutrient imbalance and ion toxicity [23]. At the initial stages of salinity stress, the plant experienced drought stress which causes a reduction in leaf expansion. When exposed to long-term salinity stress, ionic stress occurs in the plant that causes the premature senescence of leaves so that the photosynthetic area is reduced.

The salt-responsive genes are classified into two groups: 1) early responsive genes and 2) delay responsive genes [24]. The early responsive genes are induced transiently and quickly while the late responsive genes are activated slowly and the expression of these genes is constant. Early responsive genes produce proteins that are transcription factors. These transcription factors are involved in the activation of downstream late response genes [1]. When microarray expression profile, a T-DNA insertion knockout mutant of AtNHX1 and rescued line (NHX1::nhx1) exposed to both long (1 and 2 weeks) and short (12 h and 48 h) duration of salt stress were studied in wild type plant, 147 transcripts were found which showed both significant effects of AtNHX1 and salt responsiveness.

A large number of genes have been identified that are involved in responses conferring salt tolerance. As functional components, many genes are identified in plant response under stress conditions, including genes that code for an enzyme that has a role in detoxification like glutathione peroxidase [25], osmolytes such as LEA and glycine-betaine [26], AtNHX1 Na<sup>+</sup>/K<sup>+</sup> antiporter [27], Alfin1 TF [28] and AtHAL3 flavoprotein [29]. The complete study of transcriptomes showed that synthesis of ion transporter and osmolytes and regulation of translational and transcriptional machinery have important roles in salinity stress response. Induction of various transcripts that encode for RNA-binding protein, specific TFs, elongation factors, ribosomal genes and translation initiation is reported and these transcripts are important during salinity stress [30].

Some stress-specific consensus sequences are identified in the promoter of genes, these regions help in activating or repressing these gene transcriptions, TF should be located in the nucleus that binds with DNA and interacts with the basal transcription apparatus. TFs that play role in stress responses are DRE-related binding factors, putative Zn-finger proteins, leucine zipper DNA binding protein, AP2/EREBP, myb proteins and bZIP/HD-ZIPs [31], Interact with osmotic regulated genes promoter [32]. AP2/ERF protein domain has CBF or DREB proteins that bind with DRE (dehydration response elements) or C-repeats. DRE/C-repeat promoter sequence and DREB/CBF factors in stress-activated genes control the expression of salt-responsive genes. In Arabidopsis, various stress-inducible genes e.g. Kin1, Cor6.6, rd29A and Cor15a are the target of DREB/CBFs and contain DRE/C-repeat sequences in the promoter region.

Basic leucine zipper protein consists of a DNA binding domain that binds to the ACGT core sequence of the target region. One subfamily of bZIP has been identified that is linked with ABA response ABRE binding factors (ABFs/AREBs), ABI5 and its homologs. Under salt stress when plants go under dehydration ABRE binding factors are transcribed by the plant cell [33].

Some regulatory intermediates such as SOS3 (calcium-binding protein), SOS2 (Suc nonfermenting -like) kinase, mitogen-activated protein kinase and Calcium-dependent protein kinases [34]. Salt tolerance and ion homeostasis are regulated by a single pathway which includes SOS1, SOS3 and SOS2, their functions are calcium-dependent. SOS1 encode the protein (plasma membrane Na<sup>+</sup>/H<sup>+</sup> antiporter) which play important role in Na<sup>+</sup> extrusion [35]. This antiporter makes a component which increases the Ca<sup>++</sup> and reversible phosphorylation [36].

Several studies reported that oxidative stress and reactive oxygen species (ROS) may be mediating the toxic effects of NaCl in legumes and vascular plants [37]. ROS are produced in plant chloroplast by direct excitation energy transfer from chlorophyll to produce the singlet oxygen or in Mehler reaction by the reduction of univalent oxygen at photosystem 1 and in mitochondria. ROS can interact with many cellular components and trigger the peroxidation reaction which leads to significant damage to cellular lipids, proteins and nucleic acids. Plant have an antioxidant defense system that helps in the detoxification of ROS and maintain redox homeostasis. This system consists of enzymes and non-enzyme components which maintain the level of ROS in the plant cells. Major non-enzymatic antioxidants present in plants are ascorbic acid and glutathione, some others are tocopherol, alkaloids flavonoids and carotenoids.

## **5.2 Cold or chilling stress**

Cold stress inhibits the expression of the full genetic potential of the crop plants which leads to a reduction in metabolic reaction and indirectly through the oxidative, cold-induced osmotic (chilling induced prevent the water uptake and freezing induced leads to cell dehydration), and other stresses. Cold stress has 2 types: 1) chilling stress at  $<20^{\circ}\text{C}$  and freezing stress at  $<0^{\circ}\text{C}$ . These low temperatures cause adverse effect on plant cells and growth.

Chilling and cold stress leads to slow the biochemical process such as effect on enzyme and membrane transport. It also leads to the formation of ice crystals which causes the cell membrane system disruption [38]. A large number of genes studies have been used for the identification of genes that respond to cold stress in Arabidopsis. Results of the studies showed that under cold stress, mRNA levels of many genes that are important for plant growth and development altered [39].

The cold induction of genes involved in lipid signaling, calcium signaling or encoding receptor-like protein kinases are affected by the *ice1* mutation. TFs that bind with DRE/CRT is known as DREB2 and DREB1/CTR-binding factors. AP<sub>2</sub>/ERF family of transcription factors increases under cold stress. This family have CBFs which bind to the promoter region of COR genes and activate the expression of the genes. CBFs regulate the expression of many genes which are involved in ROS detoxification, transcription, phosphoinositide metabolism, membrane transport, signaling, osmolytes and hormone metabolism [40].

The first cDNA isolated from Arabidopsis, encodes for the DRE binding proteins (DREB1A and DREB2A) reported in scientific study [41] after that many *DREB* genes were isolated from many plants. In barley and wheat, the number of *CBF* homologs have been identified and mapped to low temperate QTLs, and *Fr-2* chromosomal region [42]. Results of the scientific research showed that DREB1/*CBF* regulons are ubiquitous in nature within higher plants. Expression of *DREB1* genes was investigated in many crop plants under different abiotic stresses. It was concluded that *AtDREB1* gene expression is induced by only cold stress, not due to dehydration or salinity [43]. Another gene *CBF* gene expression was studied and concluded that this gene also expressed under sold stress and its mRNA was detected after 30 minutes in the plant when exposed to  $4^{\circ}\text{C}$  and showed the maximum expression at 1 hour [44]. Two cold-responsive TFs of genes RAP2.1 and RAP2.7 can sub-regulate the *CBF* region. This results showed by the microarray analysis when performed on transgenic Arabidopsis ectopically expressing the CBFs. CBFs can regulate the expression of some COR genes that create the cold resistance in plants.

The expression of ZAT10 can be induced by the CBFs which can down regulate the expression of COR genes. Phosphorylation and sumoylation which is induced by cold stress can activate the constitutive expressed ICE1. Activated ICE1 can induced the transcription of reprime *MYP15* and *CBFs*. MYB15 and ZAT12 can negatively regulate the expression of *CBFs*. HOS1 mediate the proteolysis and ubiquitination of ICE1, so that it negatively regulate the CBF regulons [45]. Small ubiquitin-related Modifier proteins (SUMO) can induced the sumoylation. Sumoylation protect the ubiquitination of the proyein because it protect the protein from the proteosomal degeradation [46].

## Key findings

Late embryogenesis abundant (Lea) gene play role in plant protection during drought and cold stress, Lea genes produce a hydrophilic protein. In rice plants, these hydrophilic proteins are identified that protect the cell membrane from injury under drought stress. Under salt stress when plants go under dehydration, ABRE binding factors are transcribed by the plant cell which protect the plant against salinity. A large number of genes studies have been used for the identification of genes that respond to cold stress in Arabidopsis. Results of the studies showed that under cold stress, mRNA levels of many genes that are important for plant growth and development altered.

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