CHAPTER 2

ROLE OF TRANSCRIPTION FACTORS IN ABIOTIC STRESS MANAGEMENT

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Abstract

Plants are recurrently confronted to a wide range of environmental conditions, viz availability of water (drought and flooding), temperature (high and low) and salinity which strongly affect plant growth and development. Severity of these conditions (abiotic stresses) adversely affect different biochemical and physiological mechanisms associated with plant responses and adaptation to stress: photosynthetic mechanisms, e.g. stomatal control of CO_2 diffusion, photosystem II repair, ribulose bisphosphate carboxylase/oxygenase (Rubisco) activity and scavenging of reactive oxygen species (ROS), are very prone to damage, and photosynthetic efficiency is greatly decreased. Plants respond to these environmental challenges through a number of homeostatic mechanisms that maintain the water balance and the integrity of tissues. Responses, adaptations and mitigation necessitate differential gene expression, which is regulated by specific transcription factors (TFs). TFs are proteins with a DNA binding domain that binds to the *cis*-acting elements present in the promoter of a target gene. They induce (activators) or repress (repressors) the activity of the RNA polymerase, thus regulating gene expression. Plant genome ascribes approximately 7% of their coding sequence to transcription factors that proves the complexity of transcriptional regulation. Interaction of transcription factors with *cis*-element in the promoter regions of various stress related genes upregulate the expression of many downstream genes. Abiotic stress mainly induce two group of gens according their protein products first group, whose coding products directly confer to plant cells the resistance to environmental stress such as late embryogenesis abundant (LEA)

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protein, anti-freezing protein, osmotic regulatory protein, enzymes for synthesizing betaine, proline and other osmo-regulators and second group of genes controlled by a certain type of TF is known as a regulon. At least four different regulons can be identified, two ABA independent (1) the CBF (C-repeat or CRT-binding factors)/DREB (dehydration response element- DRE-binding factors) regulon; (2) the NAC (NAM, ATAF and CUC) and ZF-HD (zinc-finger homeodomain) regulon; and two ABA dependent; (3) the AREB/ABF (ABA-responsive element-binding protein/ ABA-binding factor) regulon; and (4) the MYC (myelocytomatosis oncogene)/MYB (myeloblastosis oncogene) regulon. As per the signaling cascade pathways these transcription factors play vital role to plants adaptation and mitigation strategy towards abiotic stress.

Key Words: Transcription factors, photosynthesis, abiotic stress, signaling, cold, drought, salinity, LEA Protein, ABA, MYC/MYB, AREB/ABF, CBF/DREB.

2.1 Introduction:

World population is increasing at an alarming rate and is expected to reach about six billion by the end of year 2050. On the other hand food productivity is decreasing due to the effect of various abiotic stresses; therefore minimizing these losses is a major area of concern for all nations to cope with the increasing food requirements (Mahajan and Tuteja, 2005.). Abiotic stresses reduce crop yield worldwide by more than 50% (Bray *et al.*, 2000). Susceptibility and tolerance to these stresses affects multiple stages of plant development. Plant stress tolerance involves changes at cellular, physiological and molecular levels. Stress tolerance at molecular levels includes perception, signal transduction, gene expression and ultimately metabolic changes in the plant. The early events of plant adaptation to environmental stresses are the stress signal perception and subsequent signal transduction through either ABA-dependent or ABA-independent pathways which lead to the activation of various physiological and metabolic responses (Agarwal and Jha, 2010). Abscisic acid (ABA) is produced under water deficit conditions and plays an important role in the tolerance of plants to abiotic stress. Hundreds of genes are thought to be involved in abiotic stresses (Seki *et al.*, 2002).

Drought stress retards plant growth due to decline in photosynthesis and non-availability of nutrients as soil dries. Similarly, salinity leads to physiological dryness. Chilling and freezing temperatures can also cause osmotic stress (Chinnusamy *et al.*, 2004). Plants respond to these conditions with an array of morphological, physiological, biochemical and molecular changes, which enable plants to survive and reproduce. When a plant is

subjected to abiotic stresses, an assortment of genes with diverse functions are induced or repressed. Analysing and elucidating the function of these genes is very critical for further understanding of the molecular mechanisms governing plant abiotic stress tolerance, and this may help in genetic manipulation of crops for enhanced stress tolerance (Shinozaki and Yamaguchi-Shinozaki2007; Lata and Prasad 2011).

2.2 Stress Signalling Pathways: An Overview

Stress is perceived by the receptors present on the membrane of the plant cells (Fig. 1), the signal is then transduced downstream, which results in the generation of second messengers including calcium, reactive oxygen species (ROS) and inositol phosphates. These second messengers such as inositol phosphates further modulate the intracellular calcium level. This change in cytosolic Ca^{2+} level is sensed by calcium binding proteins, also known as Ca^{2+} sensors. As the name suggest these sensors change their conformation in a calcium dependent manner. These sensory proteins then interact with their respective interacting partners often initiating a phosphorylation cascade and target the major stress responsive genes or the transcription factors (e.g. CBF/DREB, ABF, bZIP, MYC/MYB)
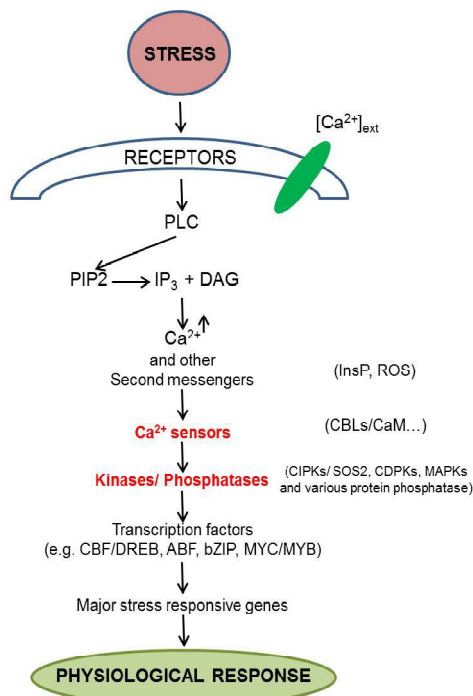

Figure 1. Signal transduction pathway in response to abiotic stress signalling (Mahajan S. and Tuteja N., 2005)

controlling these genes. The products of these stress responsive genes ultimately lead to plant adaptation and help the plant to survive and surpass the unfavourable conditions. Thus, plant responds to stresses as individual cells and synergistically as a whole organism. Stress induced changes in gene expression in turn may participate in the generation of hormones like ABA, salicylic acid and ethylene. These molecules may amplify the initial signal and initiate a second round of signalling that may follow the same pathway or use altogether different components of signalling pathway.

Table 1. Consequences of abiotic stress and plant responses

Stress	Consequences	Plant Responses
Drought	Inability to water transport to leaves leads to photosynthesis declines.	Leaf rolling and other morphological adaptations. Stomata closure reduces evaporative transpiration induced by ABA. Accumulation of metabolites, consequently lower internal water potential and water attracting.
Flooding and submergence	Generates anoxic or micro aerobic conditions interfering with mitochondrial respiration.	Development of cavities mostly in the roots that facilitate the exchange of oxygen and ethylene between shoot and root (aerenchyma).
Salinity	Salt accumulation can modify plant cell plasma membrane lipid and protein composition, cause ion imbalance and hyperosmotic stress and eventually disturb normal growth and development.	Premature senescence of adult leaves, and thus a reduction in the photosynthetic area available to support continued growth.
Chilling and cold stress	Biochemical reactions proceed at slower rate, photosynthesis proceeds, carbon dioxide fixation lags, leading to oxygen radical damage. Indeed, freezing lead to ice crystal formation that can disrupt cells membranes.	Cessation of growth in adaptable species may be overcome by changes in metabolism. Ice crystal formation can be preventing by osmolyte accumulation and synthesis of hydrophilic proteins.

(Loredana *et al.*, 2011)

What is Stress? Stress in physical terms is defined as mechanical force per unit area applied to an object. In response to the applied stress, an object undergoes a change in the dimension, which is also known as strain. As plants are sessile, it is tough to measure the exact force exerted by stresses

and therefore in biological terms it is difficult to define stress. A biological condition, which may be stress for one plant may be optimum for another plant. The most practical definition of a biological stress is an adverse force or a condition, which inhibits the normal functioning and well-being of a biological system such as plants.

2.3 Transcription Factors

TFs are proteins with a DNA binding domain that binds to the *cis*-acting elements present in the promoter of a target gene. They induce (activators) or repress (repressors) the activity of the RNA polymerase, thus regulating gene expression. TFs can be grouped into families according to their DNA-binding domain. The presence or absence of transcription factors, activators and suppressors regulating transcription of target genes often involves a whole cascade of signalling events determined by tissue type, developmental stage or environmental condition (Saibo *et al*., 2009).

Cis-acting elements:

DNA sequences in the vicinity of the structural portion of a gene that is required for gene expression. As we know several genes seem to be transcribed co-ordinately. For example, the different members of a storage protein or photosynthetic protein family are expressed at the same time in development. These genes have sequence modules in common that control the coordinate regulation. These modules are called **response elements** (Wang, 2005). These elements are a class of *cis*-acting elements.

Features of Response Elements:

- contain short consensus sequences
- not fixed in location but usually within 200 bp upstream of the transcription start site
- a single element is usually sufficient to confer a regulatory response
- can be located in a promoter or an enhancer
- assumed that a specific protein binds to the element and the presence of that protein is developmentally regulated

2.3.1 Abiotic Stress-Inducible Genes

Plant response to abiotic stress involves many genes and biochemical-molecular mechanisms. The analysis of the functions of stress-inducible genes is an important tool not only to understand the molecular mechanisms of stress tolerance and the responses of higher plants, but

also to improve the stress tolerance of crops by gene manipulation (Avni Öktem *et al*., 2008). Many drought-inducible genes are also induced by salt stress and cold, which suggests the existence of similar mechanisms of stress responses.

Environmental stress-inducible genes can be mainly divided into two groups in terms of their protein products:

- One type of genes, whose coding products directly confer to plant cells the resistance to environmental stress such as late embryogenesis abundant (LEA) protein, anti-freezing protein, osmotic regulatory protein, enzymes for synthesizing betaine, proline and other osmoregulator;

- The other groups of genes, whose coding products play an important role in regulating gene expression and signal transduction such as the transcriptional elements. A group of genes controlled by a certain type of TF is known as a **regulon**. At least four different regulons can be identified, two ABA independent (1 and 2) and two ABA dependent (3 and 4): (1) the CBF (C-repeat or CRT-binding factors)/DREB (dehydration response element- DRE-binding factors) regulon; (2) the NAC (NAM, ATAF and CUC) and ZF-HD (zinc-finger homeodomain) regulon; (3) the AREB/ ABF (ABA-responsive element-binding protein/ ABA-binding factor) regulon; and (4) the MYC (myelocytomatosis oncogene)/ MYB (myeloblastosis oncogene) regulon (Saibo *et al*., 2009).

2.3.2 Transcriptional Factors Involved in Abiotic Stress Response

Plant genome ascribes approximately 7% of their coding sequence to transcription factors that proves the complexity of transcriptional regulation (Udvardi *et al*., 2007). Interaction of transcription factors with cis-element in the promoter regions of various stress related genes upregulate the expression of many downstream genes. (Agarwal and Jha, 2010). Transcriptome data in *Arabidopsis* and in numerous other plants suggesting that stress susceptibility or tolerance is controlled by an extremely intricate gene regulatory network (Umezawa *et al*., 2006) (Fig.2).

2.3.3 Transcriptional Factor Involved in Response to Drought Stress

i. The CBF/DREB regulon:

This regulon is conserved throughout the plant kingdom (Dubouzet *et al*., 2003). Yamaguchi-Shinozaki and Shinozaki in 1994, identified a novel *cis*-acting element that, in addition to the ABA-responsive element
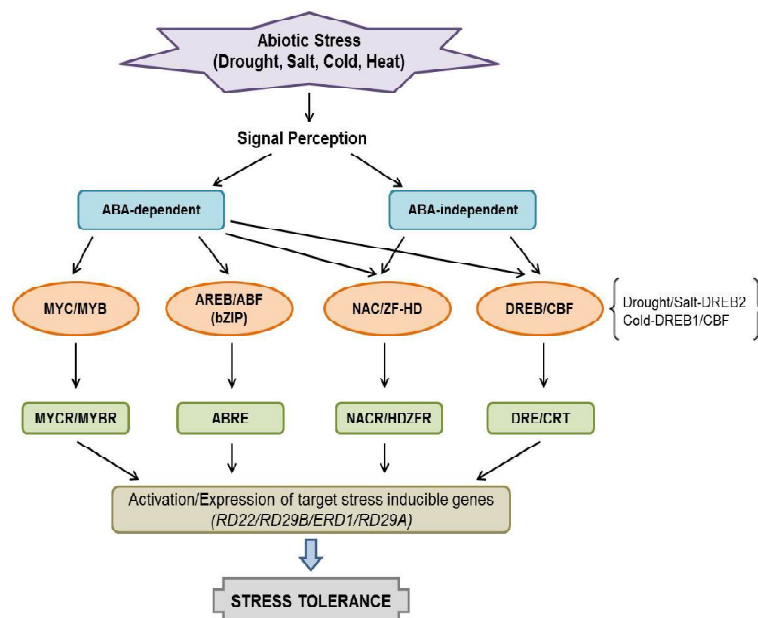
Figure 2. A schematic representation of transcriptional regulatory networks of transcription factors involved in abiotic-stress-responses. *Cis-acting* **elements are shown in boxes; Transcription factors are shown in ellipses; and target stress inducible genes are shown in long rectangular box at the bottom.** *RD*-**response to dehydration,** *ERD*-**early responsive to dehydration stress (Umezawa** *et al.,* **2006)**

(ABRE), is also present in the promoter of the RESPONSIVE TO DEHYDRATION 29A (RD29A), a gene induced by drought, high salinity and cold. This new element was named C-repeat/dehydration-responsive element (CRT/DRE) and characterized as ABA independent.

The DREB TFs could be divided into two groups: DREB1, involved signal transduction pathways under low temperature; DREB2, involved in signal transduction pathways under dehydration. They belong to the ethylene responsive element binding factors (ERF) family of TFs. ERF proteins are a sub-family of the AP2/ethylene responsive element binding protein (EREBP) TFs that is distinctive to plants. The DREB2 genes are constitutively expressed (under normal and stress conditions), although their target genes (e.g. RD29A, RD29B, RD17 and LEA14) are only induced upon dehydration. This indicates that DREB2 factors are activated through post-translational modifications in order to regulate downstream genes (Sakuma *et al.*, 2006). In 1999, Kasuga *et al.* observed that the over-expression of CBF/DREB1 genes in Arabidopsis resulted in plants with improved survival rates when exposed to salt, drought and low temperatures. This improved tolerance was correlated with both altered relative abundance
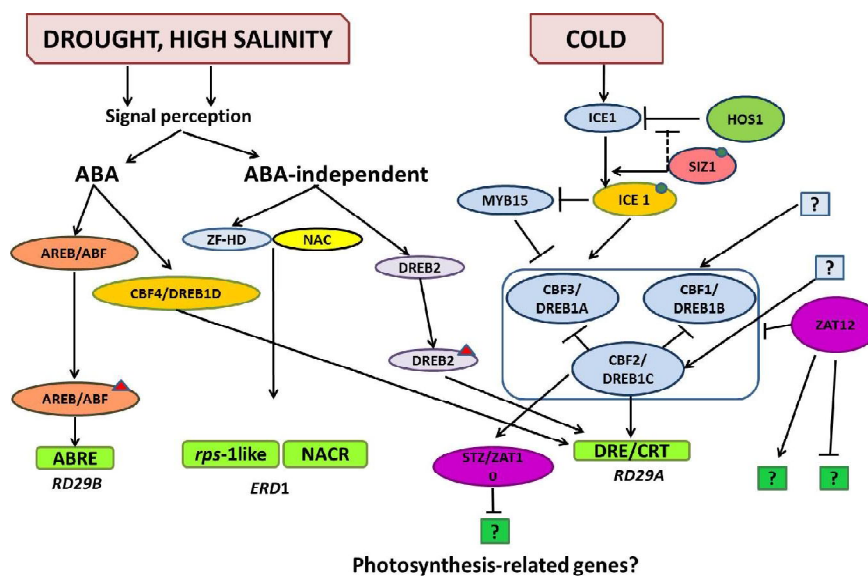
Figure 3. Transcriptional network of abiotic stress responses: Transcription factors are shown in ovals. Transcription factor-modifying enzymes are shown in circles. The small triangles correspond to post-translational modifications. Blue squares with question marks represent putative MYC ICE1-like transcription factors that may activate CBF1/DREB1B and CBF2/DREB1C. The green boxes represent the *cis*-elements present in stress-responsive genes. The green boxes with question marks represent putative *cis*-elements on the promoter of stress-responsive genes. The black dot corresponds to the sumoylation modification by SIZ1 of the ICE1 transcription factor. The dashed black line from SIZ1 to HOS1 represents competition for binding places on the ICE1 transcription factor. SIZ1 blocks the access of HOS1 to the ubiquitination sites on the ICE1. CBF4/DREB1D is a DRE cis-element binding factor that is ABA dependent. (Loredana *et al*, 2011)

of transcripts encoding proteins associated with stress adaptation and increased sugar contents (Gilmour *et al*., 2004). When CBF/ DREB1 genes from Arabidopsis were over-expressed in other plants, the result was similar to that in Arabidopsis (Hsieh *et al*., 2002a; Pellegrineschi *et al*., 2004), revealing a conserved signalling and response mechanism even between dicots and monocots. Various studies have demonstrated that improved stress tolerance by over-expression of CBF/DREB1 genes is associated with sustained photochemical efficiency and photosynthetic capacity as compared with wild-type plants (Savitch *et al*., 2005; Oh *et al*., 2007).

ii. The NAC and ZF-HD regulon:

One of the largest family of plant specific transcription factor NAC (NAM, ATAF and CUC), have been reported to enhance tolerance against various stresses, such as drought, high salinity and cold, in a number of

plants. NAC was derived from names of the first three described proteins containing the DNA-binding domain, namely NAM (no apical meristem), ATAF1-2 and CUC2 (cup-shaped cotyledon) (Souer *et al*., 1996; Aida *et al*., 1997). The Most NAC proteins contain a variable C-terminal domain, a highly conserved N-terminal DNA-binding domain and a nuclear localization signal sequence. The first NAC gene isolated was NAM from petunia (Souer *et al*., 1996). NACs can regulate abiotic stress responses through both ABA-dependent and ABA independent pathways.

The core motif of this *cis*-acting element is CATGTG and the three NAC proteins (ANAC019, ANAC055 and ANAC072) that bind to it were regulating several stress-inducible genes (Tran *et al*., 2004). Nakashima *et al*. (1997) observed that EARLY RESPONSIVE TO DEHYDRATION STRESS 1 (ERD1) gene transcripts accumulated before any increase of ABA in response to dehydration and high salinity, suggesting the presence of an ABA-independent pathway. Promoter analysis of ERD1 revealed TFs belonging to the NAC family and zinc finger homeodomain (ZF-HD) as essential to the activation of the ERD1 gene (Tran *et al*., 2007). However, Tran *et al*., (2004) found that over-expression of NAC genes in Arabidopsis enhanced drought tolerance without activation of the ERD1 gene, suggesting that other interacting factors may be necessary to control the expression of ERD1 under stress conditions.

Recently, the STRESS-RESPONSIVE NAC1 (SNAC1) gene was isolated from an upland rice variety and over-expressed in low land rice ('Nipponbare'; Hu *et al*., 2006). SNAC1 encodes a NAM, ATAF and CUC (NAC) TF with transactivation activity and is induced by drought, predominantly in guard cells. When compared with the wild type, rice plants over-expressing SNAC1 showed drought tolerance at anthesis and increased drought and salt tolerance at the vegetative stage. The increased drought tolerance may be in part due to the reduced transpiration rate (increased stomatal closure) and to an increased ABA sensitivity. Interestingly, the photosynthesis rate was not significantly affected by the over-expression of the SNAC1 gene. It is claimed that usually rice leaves may function with more open stomata than necessary to have a normal photosynthetic rate. The strong induction of SNAC1 gene expression by drought in guard cells suggests an effect in stomatal closure. In addition, the over-expression of SNAC1 upregulates a rice R2R3-MYB gene (UGS5) with NAC recognition site in its promoter region. However, the relationship between SNAC1 and the TFs implicated in stomatal closure is not known. This connection needs to be investigated further to understand the regulatory mechanisms underlying stomatal movement under drought stress. SNAC1

also induced the expression of genes encoding proteins related to both osmotic adjustment (such as a sorbitol transporter and exoglucanase) and stability of cell membranes, which can be related to the stress response (Saibo *et al*., 2009).

iii. The AREB/ABF regulon:

Transgenic plants either over expressing key enzymes in ABA biosynthesis (e.g. 9-cis-epoxycarotenoid dioxygenase; NCED) or mutated ABA degrading enzymes (e.g. cytochrome P450 CYP707A family member) have enhanced drought tolerance. The ABRE (ABA-responsive element) motif is a cis-acting element present in the ABA-responsive genes. ABA-dependent gene induction during water deficit is controlled by at least five different classes of TFs. The ABA response element (ABRE) with the consensus ACGTGG/TC is bound by basic Leucine Zipper Domain (bZIP-type) TFs and activates ABA-dependent gene expression (Choi *et al*., 2000). Activation of some of these TFs, such as AREB1 and AREB2, require a posttranslational modification for their maximum activation (Uno *et al*., 2000). This post-translational modification is probably an ABA-dependent phosphorylation. A family of protein kinases, the Snf1-related kinases family, has been implicated in the ABA signal transduction pathway. Members of this family (SnRK2) play an important role in controlling stomatal closure and are activated by drought, salinity and ABA (Mustilli *et al*., 2002; Yoshida *et al*., 2002).

iv. The MYB/MYC regulon:

The MYB family of proteins is large, functionally diverse and represented in all eukaryotes. Both MYC/MYB transcription factors participate in the ABA-dependent pathway for the upregulation of the abiotic stress responsive genes. The DNA-binding domain of plant MYB proteins usually consist of two imperfect repeats of about 50 residues (R2, R3). Most of the drought-inducible genes studied to date are also induced by ABA. It appears that drought stress triggers the production of ABA, which, in turn, induces various genes. The ABA-induced gene expression of *rd22* and *AtADH1* was enhanced. The promoter region of Responsive to Dehydration 22 (RD22) contains MYC (CANNTG) and MYB (C/TAACNA/G) *cis*-element recognition sites. MYC and MYB TFs only accumulate after an increase of ABA concentration. Microarray analysis of the transgenic plants overexpressing both AtMYC2 and AtMYB2 cDNAs revealed that several ABA inducible genes also are upregulated in the transgenic plants. These results indicated that both AtMYC2 and AtMYB2 proteins function as transcriptional activators in ABA-inducible gene

expression under drought stress in plants.

2.3.4 Transcriptional Factor Involved in Response to Flooding Stress

Flooding is a major issue for plant survival in many regions of the world. Flooding is an example of excess of water, which primarily results in reduced oxygen supply to the roots. Reduced O_2 results in the malfunctioning of critical root functions including limited nutrient uptake and respiration. To understand how plants respond and/or adapt to flooding, one must focus on the root system and its surrounding environment, historically the least studied compartment in plant science. Because flooding will initially affect the underground part of a plant, the stress must initially be sensed by the roots. Soil water logging must be perceived to trigger a cellular signal transduction pathway leading to physiological and morphological changes. In this way, the physical stress (flooding) must be converted into a biochemical response. Undoubtedly several rhizospheric changes may serve such a role (Fig. 4).
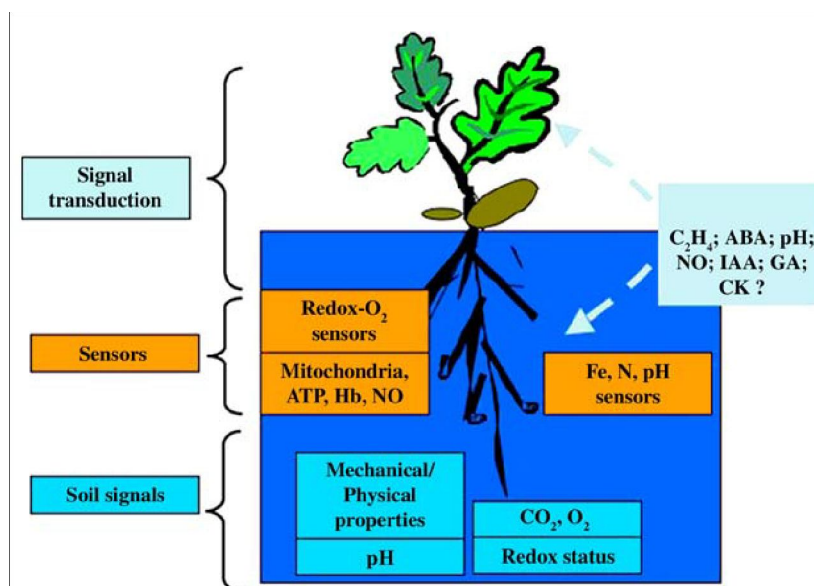

Figure 4. Schematic diagram showing the potential soil signals during flooding, the likely sensory mechanisms and the plant signalling network linked to plant responses. ABA, abscisic acid; C_2H_4, ethylene; CK, cytokinin; GA, gibberellic acid; Hb, hemoglobin; IAA, auxin; NO, nitric oxide; SA, salicylic acid. (Dat *et al.*, 2004)

During flooding, concentration of ABA was increased (Newman and Smith, 1991). In addition, exogenous ABA applications increased anoxia tolerance in maize and *Arabidopsis* (Ellis *et al*., 1999), and transcript levels of AtbZIP50, AtMYB2 were transiently increased in anoxia treated

Arabidopsis root cultures (Klok *et al*., 2002, Dennis *et al*., 2000). It has been observed recently in Arabidopsis and rice that the expression of several transcription factors, such as ethylene response-binding proteins, AP2 domain, leucine zipper, zinc finger and WRKY factors, increases in response to various regimes of oxygen deprivation. (Loreti *et al.,* 2005, Lasanthi-Kudahettige *et al*., 2007). Some TFs, particularly, five hypoxia-induced TFs (At4g29190; LBD41, At3g02550; HRE1, At1g72360; At1g69570; At5g66980) from different TF families [Zinc Finger, Ligand Binding Domain (LBD) or Lateral Organ Boundary Domain, ERF, DNA binding with one finger (DOF), ARF] active in flooding stress are able to regulate the expression of hypoxia responsive genes.

Accumulation of ROS is a common consequence of biotic and abiotic stresses, including oxygen deprivation. There is evidence of redox-sensitive TFs, at least one of which might be involved in the adaptive response to low oxygen. ZAT12, a putative zinc finger-containing TF, is recognized as a component in the oxidative stress response signalling network of Arabidopsis, promotes expression of other TFs and the upregulation of cytosolic ascorbate peroxidase 1, a key enzyme in the removal of H_2O_2.

2.3.5 Transcriptional Factor Involved in Response to Salinity Stress

Excess amount of salt in the soil adversely affects plant growth and development. Nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity (Zhu, 2001). Processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by high salt concentration, ultimately causing diminished economic yield and also quality of produce. During salt and drought stress the ABA level was increased which is mainly achieved by the induction of genes coding for enzymes that catalyze ABA biosynthetic reaction figure 5 (Xiong *et al*., 2002).

ABA is synthesized from a C40 precursor â-carotene via the oxidative cleavage of neoxanthin and a two-step conversion of xanthoxin to ABA via ABA-aldehyde. Environmental stress such as drought, salt and, to a lesser extent, cold stimulates the biosynthesis and accumulation of ABA by activating genes coding for ABA biosynthetic enzymes. Stress activation of ABA biosynthetic genes is probably mediated by a Ca^{2+}-dependent phosphorelay cascade, as shown at left. In addition, ABA can feedback stimulates the expression of ABA biosynthetic genes, also likely through a Ca^{2+}-dependent phosphoprotein cascade. Also indicated is the
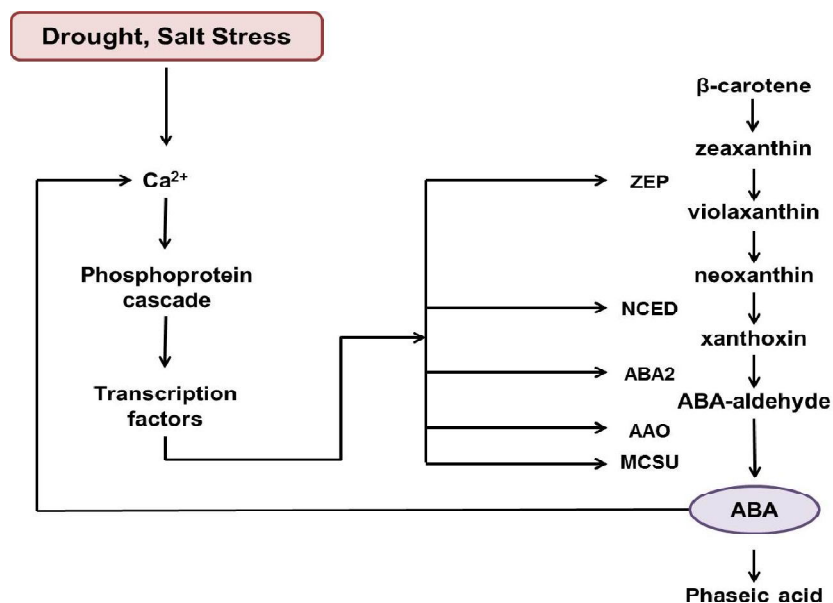
Figure 5. Pathway and Regulation of ABA Biosynthesis

breakdown of ABA to phaseic acid. AAO, ABA-aldehyde oxidase; MCSU, molybdenum cofactor sulfurase; NCED, 9-*cis*-epoxycarotenoid dioxygenase; ZEP, zeaxanthin epoxidase (Xiong *et al*., 2002).

In general, high NaCl concentrations affect plant physiology and metabolism at different levels (water deficit, ion toxicity, nutrient imbalance, and oxidative stress), and at least two main responses can be expected:

1. During initial exposure to salinity, plants experience water stress, which in turn reduces leaf expansion.

2. During long-term exposure to salinity, plants experience ionic stress, which can lead to premature senescence of adult leaves, and thus a reduction in the photosynthetic area available to support continued growth.

Two classes of stress-responsive genes:

- Early response genes
- Delayed response genes

The early response genes encode transcription factors that activate downstream delayed response genes.

Gene expression profiling using cDNA microarrays or gene chips has identified many more genes that are regulated by salt stress (Seki *et al*., 2001). A group of such genes represented by *RD29A* (also known as *COR78/LTI78*). The promoters of this group of genes contain both the ABRE and the DRE/CRT (Yamaguchi-Shinozaki and Shinozaki, 1994; Stockinger *et al*., 1997). Transcription factors DREB2A and DREB2B are activated by osmotic stress and may confer osmotic stress induction of target stress-responsive genes (Liu *et al*., 1998). The ability of the CBF/ DREB1 transcription factors to activate the DRE/CRT class of stress-responsive genes was further demonstrated by the observation that over expression or enhanced inducible expression of CBF/DREB1 could activate the target genes. Over expression also increased tolerance of the transgenic plants to salt stress (Kasuga *et al*., 1999), suggesting that regulation of the *CBF/DREB1* class of genes in plants is important for the development of stress tolerance. Moreover, basic region leucine zipper (bZIP) proteins contain a DNA binding domain rich in basic residues that bind to an ACGT core sequence. One bZIP subfamily has been linked genetically to an ABA response: ABI5 and its homologs, binding factors (ABFs/AREBs). ABRE binding factors (ABFs)/ABA-responsive element binding (AREBs) proteins respond at the transcriptional and post-transcriptional level to dehydration and salt stress.

A large number of genes from a variety of biochemical pathways participate in responses conferring salt tolerance. These pathways include notably those involved in:

- signal transduction;

- carbon metabolism and energy production;

- oxidative stress protection;

- uptake, exclusion, transport and compartmentalization of sodium ions;

- modifications of structural components of cell walls and membranes.

Several genes have been identified as functional components in the plant response to salt stress, including those encoding detoxifying enzymes like glutathione peroxidase ((Roxas *et al*., 1997), Na^+/H^+ antiporter AtNHX1 (Apse *et al*., 1999), osmolytes such as glycine-betaine and LEA (late embryogenesis abundant protein) (Xu *et al*., 1996), flavoprotein AtHAL3 (Espinosa-Ruiz *et al*., 1999), signal mediator Ca2+/calmodulin-

dependent protein phosphatase (Pardo *et al*., 1998) and transcription factor Alfin1 (Bastola *et al*., 1998). Analysis of complete transcriptomes suggest that systems like synthesis of osmolytes and ion transporters and regulation of transcriptional and translational machineries have distinct roles in salt-stress response. In particular, induction of transcripts of specific TFs, RNA-binding proteins, ribosomal genes and translation initiation and elongation factors has been reported to be important during salt stress (Sahi *et al*., 2006).
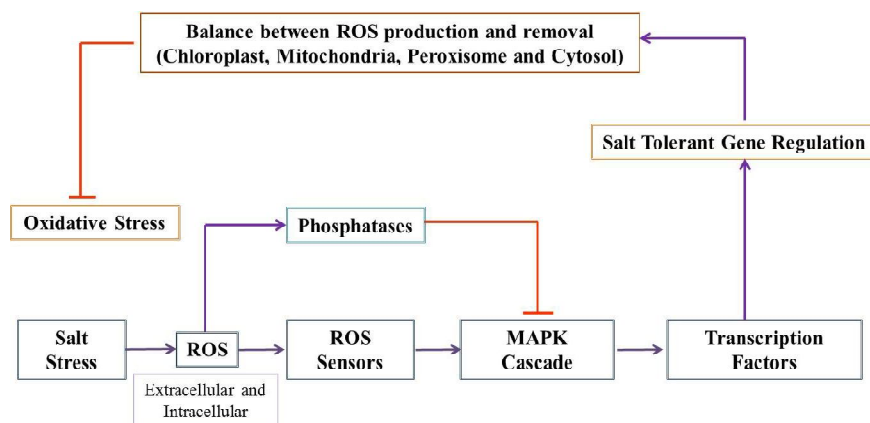

Figure 6. ROS signal transduction pathway under salt stress (Loredana *et al*, 2011).

The toxic effect of NaCl on plants was mediated by ROS-reactive oxygen species (Attia *et al*., 2008). ROS are predominantly generated in the chloroplast by direct transfer of excitation energy from chlorophyll to produce singlet oxygen, and to some extent in mitochondria. ROS interact non-specifically with many cellular components and thus, triggering peroxidative reactions which are responsible for causing significant damage to proteins, lipids, and nucleic acids. Living organisms evolved antioxidant defense systems, comprised of enzymatic and non-enzymatic components to cope with ROS and to maintain redox homeostasis. Major nonenzymatic antioxidants include ascorbate (vitamin C) and glutathione in plants, although tocopherol (vitamin E), flavonoids, alkaloids, and carotenoids can also act as antioxidants. These ROS can also influence the ROS induced MAPK signal pathway through inhibition of phosphatases or downstream transcription factors (Mittler *et al*., 2004) (Fig.6).

2.4 Chilling and Cold Stress: Gene Regulation and Transcriptional Factor

Cold stress is a major environmental factor that limits the agricultural productivity of plants in hilly areas (Xin and Browse, 2001).

Plants respond with changes in their pattern of gene expression and protein products when exposed to low temperatures. However, expression of a variety of genes is induced by different stresses in diverse plants (Sanghera *et al*., 2011). Plants differ in their tolerance to chilling (0-15ºC) and freezing (< 0ºC) temperatures. In general, plants from temperate climatic regions are considered to be chilling tolerant with variable degree, and can increase their freezing tolerance by being exposed to chilling, non-freezing temperatures, a process known as cold acclimation (Levitt, 1980), which is associated with biochemical and physiological changes (Shinozaki and Yamaguchi-Shinozaki, 1996; Thomashow, 1998; Gilmour *et al*., 2000) and ultimately showed marked changes in gene expression, biomembrane lipid composition, and small molecule accumulation.

The CBF/DREB regulon:

CCGAC is the core motif of this cis-acting element and the TFs that bind to it were named CRT-binding factors or DRE-binding proteins 1 (CBF/DREB1) (Gilmour *et al*., 1998; Liu *et al*., 1998). CBF/DREB1 gene expression is quickly and transiently induced by cold stress, and in turn CBF/DREB1 TFs activate the expression of several other genes (e.g. encoding proteins involved in production of osmo-protectants and antioxidants). Interestingly, it was shown that the low temperature induction of the CBF1–CBF3 genes is gated by the circadian clock, suggesting that the regulation of these genes has aspects in common with the regulation of Arabidopsis CAB genes (Fowler *et al*., 2002). CBFs regulate the expression of genes involved in phosphoinositide metabolism, transcription, osmolyte biosynthesis, ROS detoxification, membrane transport, hormone metabolism and signalling and many others with known or presumed cellular protective functions. In *Arabidopsis*, MYB15 is involved in cold-regulation of *CBF* genes and in the development of freezing tolerance. Cold regulation of *CBF3* involves an upstream bHLH-type transcription factor, ICE1. ICE1 binds to the Myc recognition sequences in the *CBF3* promoter. Apart from Myc recognition sequences, *CBF* promoters also have Myb recognition sequences. ICE1 is constitutively expressed and localized in the nucleus, but it induces expression of CBFs only under cold stress. This suggests that cold stress-induced post-translational modification is necessary for ICE1 to activate downstream genes in plants. Overexpression of *MYB15* results in reduced expression of *CBF* genes whereas its loss-of-function leads to increased expression of *CBF* genes in the cold. The *myb15* mutant plants show increased tolerance to freezing stress whereas its overexpression reduces freezing tolerance. The results suggested that MYB15 is part of a complex network of transcription factors

controlling the expression of *CBFs* and other genes in response to cold stress (Chinnusamy *et al*., 2003).

2.5 Post Translational Regulation of Transcription Factor

Controlled proteolysis of transcriptional regulators has an important role in shaping the cold-responsive transcriptome in plants, as evidenced from studies on HOS1 (High Expression of Osmotically Responsive Gene 1). The Arabidopsis hos1 mutation causes super-induction of CBFs and its downstream target genes under cold stress (Lee, 2001). Two important post-translational protein modifications:

i) Ubiquitination: It is mediated by HOS1. *HOS1* gene encodes for a RING finger ubiquitin E3 ligase that physically interacts with ICE1 and mediates the ubiquitination of ICE1 to regulate negatively the expression of ICE1 target genes and is thus critical for the de-sensitization of plant cells to cold stress (Dong *et al*., 2006).

ii) Sumoylation: It is induced by SUMO (Small Ubiquitin-related Modifier) proteins that are conjugated to proteins substrates in a process dependent on SUMO E3 ligases. Sumoylation might protect target proteins from proteasomal degradation preventing the ubiqiutination (Ulrich, 2005).

2.6 Conclusions

Abiotic stresses are serious threats to crop productivity and may upsurge the harshness under climate change scenario. Amidst, plant genes are regulated in response to abiotic stresses and their gene products function in stress response. Such genetic systems are thought to be very important in increasing tolerance of plants to abiotic stresses as well as in management for successful crop cultivation. The key players of molecular mechanism elucidating that mediate the complex stress-responses in plants system is an important step to develop improved variety of stress tolerant crops. Understanding the effects of different types of abiotic stress is a rapidly emerging domain in the area of plant research to develop better, stress tolerant plants. Information about the transcription factors, transcription factor binding sites, function annotation of proteins coded by genes expressed during abiotic stress (for example: drought, cold, salinity, abscisic acid, and (flood) oxidative stress) response will provide better understanding of this phenomenon. TFs in plants which regulate expression of various stress-responsive genes, generally in an ABA-independent manner through binding to DRE/CRT *cis*-elements and help plants to sustain single or multiplicative effects of different abiotic stresses. Genetic modification of plants using DREB1/CBF genes anchored with either

constitutive or stress-responsive or tissue-specific and stage specific promoters can help in enhancing tolerance of crop plants against abiotic stresses. However, functional analysis of DREB1/CBF's target genes and DRE/CRT *cis*-elements in detail can give better understanding of the molecular basis of stress tolerance. There is still only fragmentary knowledge of abiotic-stress signalling pathways. Functional analysis of DRE-binding factors will provide more information on the complex regulatory networks that are involved in the responses to abiotic stresses and future work should help delineate the different signalling pathways and their cross-talk during adaptation of plants to drought and other stresses. Although, engineering crop plants with DREB transcription factors has resulted in improved stress tolerance unlike the previous reports of limited tolerance with single genes, further efforts are needed to increase stress tolerance and improve plants ability to reduce yield losses under drought and saline conditions.

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