

Abiotic Stress Tolerance in Crop Plants: With Respect to NAC Transcription Factor

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Abstract

Natural factors such as salinity, drought and high temperatures are the main cause of low productivity of crop plants worldwide. Plants respond to such extremities through various mechanisms. Thus an insight into the various complex mechanisms can broaden our understanding and can be a remedy to the obscurity of abiotic stress tolerance in plants. Many workers all over the world have tried to unveil the gene networks responsible for abiotic stress tolerance. After the discovery of transcription factors, it was clear that abiotic stress tolerance is modulated by numerous genes which get up regulated during stress conditions. TFs are therefore good candidates for molecular genetics to improve crop tolerance to abiotic stress because of their role as major regulators of clusters of genes. Thus these collective efforts and results of collaborative studies will positively contribute to sustainable food production in the world and will help to prevent global-scale environmental damage that results from abiotic stress. In this review NAC transcription factor genes utilized for development of transgenic abiotic stress tolerant plants and will be discussed

Keywords: Transcription factors; Abiotic stress; ROS; NAC; DREB; ARF; Protein

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Abiotic Stresses: Abiotic stresses such as drought, temperature, and salinity are location specific, displaying inner variation in occurrence, intensity and duration, and generally cause reduced crop productivity. There is a necessary concern for food security in developing countries including India for the following reasons: (i) The food demand is increasing for the ever growing population; (ii) declining productivity in past high productivity regions, often described as 'Green Revolution' fatigue; and (iii) increasing susceptibility of agriculture to potential climate change. Climatic change is becoming a large concern towards humanity in coming decades. Agriculture also produces significant effects on climate change as possible contributor of greenhouse gases to the atmosphere and as an industry that is highly sensitive to climatic changes. Climate is significant in the distribution, productivity and security of food.

There should be a realization that climate is both a resource to be managed wisely and a hazard to be dealt with. Thus a portfolio of assets to prepare for climate change is needed which is a major cause abiotic aberration. Of all the parameters, abiotic environmental stresses contribute most significantly to the reduction in potential yield (Flowers & Yeo, 1995). To deal with scarce good quality water

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resources, agronomic research activity has focused on the optimization of water use for irrigation and on the use of alternative sources of waters. Breeders take a relatively traditional approach. They grow and cross varieties then evaluate how the progenies vary in their ability to deal with stresses.

The best-adapted plants will then be selected for growing in fields exposed to stresses. Biotechnologists, meanwhile, have taken advantage of recent advances in functional genomics and biotechnology to genetically engineer crops which can give better yield than the unmodified ones in adverse conditions (Katerji, *et al.* 2003; Katerji, *et al.* 2004; Manavalan, *et al.* 2009; Umezawa, *et al.* 2006). The genetic basis of stress tolerance is complex and not well understood although many attempts are being made (Yadav, *et al.* 2013). The ability of plants to adapt and/or acclimate to different environments is directly or indirectly related with the plasticity and resilience of photosynthesis, in combination with other processes, determining plant growth and development, namely reproduction (Chaves, *et al.* 2011). The most striking feature of plant adaptation to abiotic stresses is the activation of multiple responses involving complex gene interactions and crosstalk with many molecular pathways (Basu 2012; Umezawa, *et al.* 2006). Abiotic stresses elicit complex cellular responses that have been elucidated by progresses made in exploring and understanding plant abiotic responses at the whole-plant, physiological, biochemical, cellular and molecular levels (Grover 2001). At the molecular level this involves induction of stress-responsive and stress-tolerance genes (Matsui, *et al.* 2008), often mediated by the phytohormone abscisic acid (ABA). ABA is referred to as the plant stress hormone because, in addition to its role in development, it plays a key role in responses to abiotic stress factors by regulating stomatal closure to optimize transpiration, and by triggering the activation of many stress-related genes (Cutler, *et al.* 2008) (Finkelstein, *et al.* 2002).

Several genes are activated in response to abiotic stresses at the transcriptional level, and their products are contemplated to provide stress tolerance by the production of vital metabolic proteins and also in regulating the downstream genes (Kavar, *et al.* 2007). Extensive transcriptome analyses have divulged that these gene products can largely be classified into two groups (Bohnert, *et al.* 2001; Seki, *et al.* 2002; Fowler and Thomashow 2002), First group [Figure 1] comprises of genes that encode for proteins that defend the cells from the effects of water-deficit. These genes mainly include those that regulate the accumulation of compatible solutes (enzymes for osmolyte biosynthesis like proline, betaine, sugars, etc.); passive and active transport systems across membranes (water channel proteins and membrane transporters); and protection and stabilization of cell structures from damage by ROS (the detoxification enzymes such as glutathione S-transferase, catalase, superoxide dismutase, ascorbate peroxidase, etc.); fatty acid metabolism enzymes, proteinase inhibitors, ferritin and lipid-transfer proteins; and other proteins for the protection of macromolecules (LEA protein, osmotin, chaperons, etc.). Another group of genes stimulated by abiotic stresses includes regulatory proteins that further regulate the stress signal transduction and alter gene expression and hence possibly function in stress response. They comprise several transcription factors (TFs) emphasizing the role of various transcriptional regulatory mechanisms in the stress signal transduction pathways; protein kinases (MAP kinase, CDP kinase, receptor protein kinase, etc.); protein phosphatases and proteinases implicated in the regulation of stress signaling and gene expression (Seki, *et al.* 2003; Shinozaki and Yamaguchi- Shinozaki, 2007).

As we know that classical tools of genetics and plant breeding have already confirmed that abiotic stress tolerance in plants is multi-genic and quantitative. Hence, it is difficult to manipulate abiotic-stress-related pathways using modern molecular genetics techniques. Introduction of a single gene, encoding functional proteins like LEA proteins, antifreeze proteins, and molecular chaperons, would confer some degree of tolerance but do not give sustained tolerance to most of the abiotic stresses. However, during the evolutionary process, plants have devised some intense molecular mechanisms in order to survive under the extreme environmental conditions. In this way, another category of genes namely genes encoding regulatory proteins have emerged. Such genes play important roles in survival of plants under stress situation by acting as a switch of sets of downstream stress-responsive genes.

Recent advances in biotechnology have dramatically changed our capabilities for gene discovery and functional genomics. For the first time, we can now obtain a holistic “snapshot” of a cell with transcript, protein and metabolite profiling. In plant genomes approximately 7% of the coding sequences are assigned to transcription factors (TFs) (Udvardi, *et al.* 2007), and many of these are

immediate-early abiotic stress-responsive genes (Kilian., *et al.* 2012). [Figure 2] represents the key factors responsible for stress tolerance. The Abiotic stress (Cold, drought, salinity and heat) induces subsequent signal transduction through either ABA-dependent or ABA-independent pathways, leading to the activation of various physiological and metabolic responses leading to activation of NAC Genes thus subsequent production of NAC mRNA and finally after translation production of NAC TF protein. These TFs interact with the conserved “stress recognition sequences” present as cis-acting elements upstream the promoter of the concerned gene, thereby activating the gene responsible for stress tolerance. Manipulation of these genes should be the major target of attempts to produce plants with enhanced multiple stress tolerance. Furthermore, the potential NAC genes which confer multiple abiotic stress tolerance in model plant species must be tested in crop plants and greater emphasis should be placed on the field evaluation of the transgenic crops harboring these genes, especially focusing on the productive success.

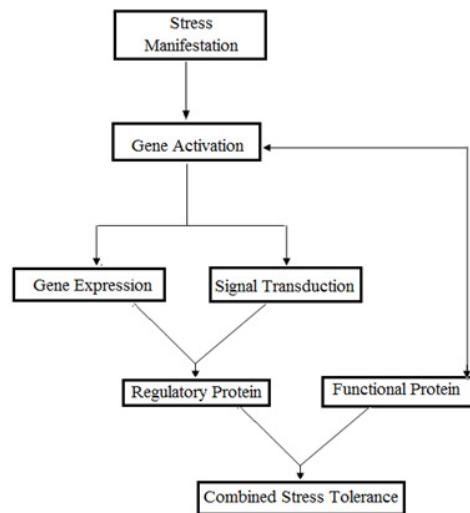


Figure 1: Diagrammatic representation of stress tolerance by two groups of Proteins and their combined action.

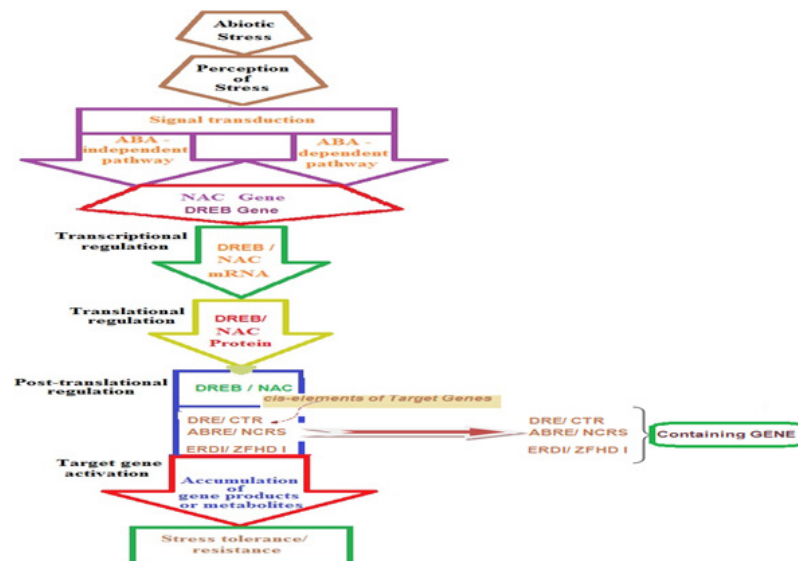


Figure 2: Schematic diagram of NAC TF as key components in transcriptional regulatory networks during abiotic stress.

Structure and Function of TF with reference to NAC: The expression of inducible genes in crops is controlled by specific proteins known as transcription factors at the transcriptional level. A typical transcription factor usually contains a DNA-binding domain, a transcription regulation domain, a dimerization site and a nuclear localization domain. These functional domains define the characteristic, localization and regulatory role of a transcription factor. Transcription factors recognize and bind to specific cis-acting elements or interact with other proteins, and then activate or repress the transcription of target genes by their functional domains. In recent years, elucidation on the structure and function of transcription factors has become an important subject in plant molecular biology. Thus all TFs have a DNA-binding domain containing a short peptide region, called the DNA-binding motif. The NAC domain (for *Petunia hybrida* (*Petunia*) NAM and for *Arabidopsis* ATAF1, ATAF2, and CUC2) is an N-terminal module of ~160 amino acids, the NAC domain is accompanied by diverse C-terminal transcriptional activation domains. The NAC domain has been shown to be a DNA-binding domain (DBD) and a dimerization domain (www.ebi.ac.uk/interpro/entry/IPR003441). Regulation of transcription often works through binding of regulatory proteins to the cis-acting elements located upstream of functional genes. The C-terminal parts of NAC proteins are highly diverse and do not contain any known protein domains (Ooka, *et al.* 2003).

However, sequence analysis does reveal five *Arabidopsis* NAC genes that appear to encode two NAC domains in tandem (At1g60280, At1g60300, At1g60340, At1g60350 and At1g60380) as well as sequences predicted to contain transmembrane regions or nuclear import and/or export signals. Elucidation of the molecular functions of NAC proteins began with the report of two NAC proteins being able to activate a cauliflower mosaic virus (CaMV) 35S promoter construct in yeast (Xie, *et al.* 2000). Since then, three NAC proteins from *Arabidopsis* (NAC1, AtNAM and ANAC019) and several from *Brassica napus* have been shown to bind the CaMV 35S promoter (Xie, *et al.* 2000; Hegedus, *et al.* 2003; Ernst, *et al.* 2004; Duval, *et al.* 2002). Recently, three ANAC proteins were shown to bind a fragment of the ERD1 (EARLY RESPONSIVE TO DEHYDRATION STRESS 1) promoter. Substitution analysis identified the sequence CACG as the core DNA motif recognized by the ANAC proteins in the promoter fragment (Tran, *et al.* 2004). The DNA-binding ability has been assigned to the NAC domain (Hegedus, *et al.* 2003; Ernst, *et al.* 2004; Duval, *et al.* 2002), but the mode of DNA recognition by the NAC domain is still unknown.

Bioengineering for Improving Stress Tolerance: Engineering plants for overexpression of genes encoding for transcriptional factors are identified as an effective method for generating abiotic stress-tolerant plants. Gene expression studies using constitutive promoters provide very little biological information compared with the use of inducible promoters or cell type-specific promoters. The choice of promoters can significantly affect the results from a transgenic manipulation; basic molecular biology research has established that expression of a given gene is governed by the promoter sequence present mostly at 5' end of gene. The promoter sequences determine the strength of the expression (i.e. the extent to which the gene will be expressed) as well as the preciseness of the quadrants to which the gene will be expressed (temporal and/or spatial). For instance, regulation of any gene for example Heat shock (HS) gene is mediated by a core DNA sequence called heat shock element (HSE), located in the promoter region of the hs gene which upon stimulation by stress condition transcribes certain proteins called as transcription factors which act as cis-acting element in enhancing the promoter activity thereby increasing the gene expression of the concerned gene.

Thus abiotic stress tolerant crops could be engineered by [1] successful fine-tuning of the stress response by engineering novel regulatory targets; [2] proper knowledge of posttranslational modifications which regulate plant growth performance under stress; [3] overexpression of miRNAs or their targets; [4] maintaining hormone homeostasis to avoid pleiotropic effects under stress; and [5] applying plant synthetic biology approaches to improve genetic engineering strategies (Abogadallah 2010); thus this could be achieved by altering the gene expression by altering the transcription factors, which is more or less like epigenetic modifications as there is no alteration of gene sequence. The tight regulation and fine tuning of TF genes during plant stress contribute to the foundation of complex signaling web and the important roles of NAC proteins are controlling gene expression through interacting with cis-elements, which are nothing but conserved sequence present upstream the promoter, which in turn is being induced by the respective TF involved as shown in example with rice [Figure 3] where the cis-acting elements(like NACR, DRE/CRT, ABRE, CE) and the signaling kinases that phosphorylate the concerned TFs and the carry out the gene expression of the Abiotic stress related genes.

Several recent reviews have addressed the role of specific TF families in abiotic stress responses which elucidates the possible mechanism to combat stress condition. In this review, the focus will be on selected TFs i.e., NAC from several large plant TF families, which have been shown to play significant roles in responses to the important abiotic stress factors, including drought, high salinity, high osmolarity, threshold temperatures and the chemical abiotic stress factor ABA.



Figure 3: Transcriptional regulatory networks of the cis-elements NAC transcription factors involved in abiotic stress-induced gene expression.

Adapted from Nuruzzaman., *et al.* 2013 PMC3759801

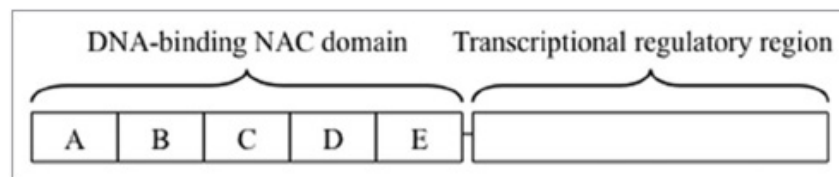
Engineering for transcription factors: Management of single gene level, for stress tolerance is not so affective; efforts were made to raise transgenic plants for stress inducible transcription factors: as a transcription factor regulates many genes. It is a possibility that many stress responsive genes, may share a common transcription factor. Various transcription factors belonging to the families of DREB, NAC, MYB, MYC, Cys2His2 zinc finger, bZIP, AP2/ERF and WRKY are known to be involved in abiotic stress tolerance. They bind to the promoter and/or regulatory elements of genes responsive to stress. Member (s) of different groups may be involved in a single response, and members of the same group may also be responsible for different kind of stress responses. Many transgenic tolerant to abiotic stresses have been produced through genetic engineering of gene (s) for transcription factors. The plant specific transcription factor group NAC (NAM, ATAF1/2, and CUC2) is required for its role in plant development and stress response. Transgenic rice plants overexpressing *SNAC1* (stress responsive NAC1) showed enhanced salinity and drought tolerance (Hu., *et al.* 2006). Similarly, rice plants overexpressing *SNAC2* (a rice NAC transcription factor group), exhibit higher salinity tolerance (Hu., *et al.* 2008). Overexpression of *OsNAC5* in rice and Arabidopsis ectopic expression in enhanced salinity and drought tolerance, while knockdown of this gene in rice by RNAi lead to salt susceptibility (Song., *et al.* 2011). Likewise overexpression of *ONAC045* encoding for NAC transcription factor gene in rice enhanced salinity and drought tolerance (Zheng., *et al.* 2009).

Genes responsible for Abiotic stress tolerance: Some of the transcription factors known to be responsible for abiotic stress tolerance and have been used for development of transgenic plants against various extreme environmental stresses are described below: There has been a continuous and tremendous research on transcriptional control of abiotic stress in model and crop plants. So to enumerate all that work is out of the scope of this review. More emphasis will be given on work done on drought, salinity and cold tolerance in regard to transcription factors especially family.

NAC (NAM ATAF and CUC) Transcription Factors: The NAC acronym is derived from three genes that were initially discovered to contain a particular domain (the NAC domain): NAM (for no apical meristem), ATAF1 and -2, and CUC2 (for cup-shaped cotyledon) (Souer, *et al.* 1996; Aida, *et al.* 1997). The NAC genes constitute one of the largest families of plant-specific TFs and are present in a wide range of species. Extensive investigation aided by the availability of several complete plant genomic sequences has identified 117 NAC genes in Arabidopsis, 151 in rice, 79 in grape, 26 in citrus, 163 in poplar, and 152 each in soybean and tobacco (Rushton, *et al.* 2008; Hu, *et al.* 2010; Nuruzzaman, *et al.* 2010, 2012a; Le., *et al.* 2011).

NAC Transcription Factors and their DNA Binding Structure: In Arabidopsis the DNA binding site of stress-inducible NAC TFs, the NAC recognition sequence (NACRS) containing the CACG core motif, has been identified by intensive analysis of the promoter of the drought inducible EARLY RESPONSE TO DEHYDRATION1 (ERD1) gene, which encodes a protein with homology to the ATP-binding subunit of the Clp ATP dependent protease from Escherichia coli. (Tran, *et al.* 2004; Simpson, *et al.* 2003). Molecular analysis of the ERD1 promoter demonstrated that ERD1 expression during dehydration mainly depends upon the integrity of both the "CAC TAA ATT GTC AC" ZFHDRS and the "ANN NNN TCN NNN NNN ACA CGC ATG T" NACRS sequences. (Tran, *et al.* 2004; Tran, *et al.* 2007), The CACG core motif is underlined. The rice drought-inducible ONAC TFs bind to a similar NACRS found in rice genome, demonstrating that the NACRS might be conserved across the plant kingdom at least for stress-inducible NAC TFs. (Hu, *et al.* 2006; Fang, *et al.* 2008). In addition, other sequences have also been reported as NAC binding sites. The Arabidopsis NAC1 protein has been shown to bind to a 21-bp segment (CTG ACG TAA GGG ATG ACG CAC) within the 35S -90 promoter fragment (Xie, *et al.* 2000). Independently; Duval, *et al.* 2002 demonstrated that purified AtNAM recombinant protein protected the region of the CaMV 35S promoter between -70 and -76, which is located in the 21-bp segment (AGGGATG). On the other hand, *CBNAC* TF of Arabidopsis bound specifically in vitro and in vivo to *CBNACBS*. Thus the NAC TFs contain a highly conserved N-terminal DNA-binding NAC domain and a variable transcriptional regulation C-terminal domain (Olsen, *et al.* 2005; Ernst, *et al.* 2004).

The presence of NAC domain in the NAC TFs was first revealed by database searches with the sequences of the cloned NAM, ATAF and CUC2 (Olsen, *et al.* 2005; Ooka, *et al.* 2004). Typically, the NAC domain is ~150 amino acids in length, and crystal structure of the NAC domain of an Arabidopsis NAC protein, the *ANAC* (ABA responsive NAC), was determined (Ernst, *et al.* 2004; Valliyodan, *et al.*).



NAC TF Structure: The N-terminal DNA-binding Domain of NAC consists of five subdomain (A-E). C-terminal acts as transcriptional regulatory sequence which interacts with stress responsive sequences either as activators or repressors of the gene

NACs and their role in abiotic stress Response through Transgenic Development: NAC transcription factors play important roles in plant growth, development and stress responses (Hao, *et al.* 2011) The NAC gene family encodes one of the largest families of plant specific transcription factors and is absent in other eukaryotes (Gao, *et al.* 2007). Rice and *Arabidopsis* genomes contain 75 and 105 putative NAC genes, respectively For example, 33 NAC genes changed significantly in *Arabidopsis* under salt treatment (Jiang and Deyholos, 2006), 38 NAC genes were involved in response to drought in soybean (Le., *et al.* 2011), 40 NAC genes responded to drought or

salt stress in rice (Fang, *et al.* 2008), 32 NAC genes responded to at least two kinds of treatments in *Chrysanthemum lavandulifolium* (Huang, *et al.* 2012). It appears that a significant proportion of NAC genes function in stress response according to the expression data from genome-wide transcriptome analyses in many plants. The role of NAC gene family in abiotic stresses was discovered in *Arabidopsis*.

Three NAC genes were induced under salt and/or drought stress. Overexpression of three genes *ANACO19*, *ANACO55*, *ANACO72*, greatly enhanced drought tolerance in *Arabidopsis* (Tran, *et al.* 2004). Multiple rice transcription factors, including a NAC gene were induced in the early stages of salt stress (Chao, *et al.* 2005) *OsNAC6*, a member of *ATAF* family, was also induced by cold, salt, drought and abscisic acid (ABA) (Ohnishi, *et al.* 2005). Overexpression of stress responsive gene *SNAC1* (stress-responsive *NAC1*) significantly enhances drought resistance in transgenic rice (22-34% higher seed setting than control) in the field under severe drought stress conditions at the reproductive stage while showing no phenotypic changes or yield penalty. The other NAC Family *SNAC3-OE* transgenic plants exhibited significantly lower levels of H_2O_2 , malondialdehyde (MDA), and relative electrolyte leakage than the wild-type control under heat stress conditions, implying that *SNAC3* may confer stress tolerance by modulating reactive oxygen species (ROS) homeostasis. Quantitative PCR experiments showed that the expression of a large number of ROS-scavenging genes was dramatically increased in the *SNAC3-OE* plants, but significantly decreased in the *SNAC3-RNAi* transgenic plants (Xiong, *et al.* 2015).

The transgenic rice also shows significantly improved drought resistance and salt tolerance at the vegetative stage. Plant TFs of different families, e.g., NAC, bHLH, MYB, bZIP, WRKY, ZF, and AP2/ERF also have significant degrees of ID, which can play a functional role in interactions with other regulatory proteins, as exemplified by the interaction between the C-terminal domain of HvNAC013 and Radical Induced Cell Death1 (RCD1) (Kjaersgaard, *et al.* 2011; Kragelund, *et al.* 2012). Compared with wild type, the transgenic rice were more sensitive to abscisic acid and lost water more slowly by closing more stomatal pores, yet displayed no significant difference in the rate of photosynthesis. DNA chip analysis revealed that a large number of stress related genes were up-regulated in the *SNAC1*-overexpressing rice plants. *SNAC1* holds promising utility in improving drought and salinity tolerance in rice (Hu, *et al.* 2006).

Similarly several NAC genes are found to be induced by drought, high salinity, and cold stresses. *OsNAC6/SNAC2* encodes a NAC transcription factor in rice (Hu, *et al.* 2008). Expression of *OsNAC6/SNAC2* is induced by cold, drought and high salinity. Transgenic rice plants overexpressing *OsNAC6* showed enhanced tolerance to dehydration and high salinity stresses, although the plants showed growth retardation and low yield under the non-stress condition. This low yield was increased in transgenic rice plants driven by a stress-inducible promoter. These results indicate *OsNAC6* functions as a transcriptional activator in abiotic stress responses (Chung, *et al.* 2009). In a similar experiment *OsNAC5* expression was induced by dehydration, cold, ABA, and MeJA treatments. The growth of rice plants overexpressing *OsNAC5* was comparable to that of control plants under non-stress conditions. The *OsNAC5* over expressers exhibited enhanced tolerance to high salinity. In another study it was reported that RNA interference (RNAi) transgenic rice plants with reduced *OsNAC5* expression exhibited a reduced amount of tolerance to abiotic stresses than control plants, whereas overexpression of *OsNAC5* improved abiotic stress tolerance. It was also revealed that accumulation of compatible solutes such as proline and soluble sugars was absolutely linked with *OsNAC5* expression level (Song, *et al.* 2011). A recent report revealed that *GmNAC11* acts as a transcriptional activator, whereas *GmNAC20* functions as a mild repressor; however, the C-terminal end of *GmNAC20* has transcriptional activation activity.

Over-expression of *GmNAC20* increased salt and freezing tolerance in transgenic *Arabidopsis* plants; however, over-expression only improved salt tolerance. Over-expression of *GmNAC20* also enhanced lateral root formation (Hao, *et al.* 2011). Thus the above study revealed that *GmNAC20* may regulate stress tolerance through activation of the *DREB/CBF-COR* pathway, and may control lateral root development by altering auxin signaling related genes. Recently, an attempt was made to introduce *NAC1* gene from *E. coli* into tobacco through *Agrobacterium* mediated transformation (Ramegowda, *et al.* 2012). In another experiment, overexpression of *TaNAC2* resulted in enhanced tolerances to drought, salt, and freezing stresses in *Arabidopsis*, which were simultaneously demonstrated by enhanced expression of abiotic stress-response genes and several physiological indices (Mao, *et al.* 2012). The transgenic plants resulted in increased tolerance towards abiotic stresses.

The above results thus indicate that *NAC* genes also play an important role in the transcriptional complex of abiotic stress responses in plants not only this *NAC* also plays important role in senescence, Overexpression of *AtNAP* [58], *ORE1* (Kim., *et al.* 2009; Balazadeh., *et al.* 2010), *ORS1* (Balazadeh., *et al.* 2011), *ANAC016* (Kim., *et al.* 2013), and *ATAF1* (Garapati., *et al.* 2015) resulted in precocious senescence, whereas blocking the function of these genes delayed senescence suggesting that they function as nonredundant positive regulators of senescence in *Arabidopsis* so *NAC* transcription factors are extensively implicated in the senescence process as judged from global gene expression analysis which show their diversity of function (Skriver., *et al.* 2015). So understanding the molecular mechanisms of *NAC* TFs networks integrating multiple stress responses will be essential for the development of broad-spectrum stress tolerant crop plants that can better cope with environmental challenges in future climates (Shao., *et al.* 2015).

Gene	Species	Targeted Trait	References
<i>AhNAC2</i>	<i>Arachis pintoi</i>	Salinity Stress	Liu., <i>et al.</i> 2011
<i>ATAF1</i>	<i>Arabidopsis thaliana</i>	Drought Stress	Lu., <i>et al.</i> 2007
<i>AtNAC2</i>	<i>Arabidopsis thaliana</i>	Salinity Stress	He., <i>et al.</i> 2005
<i>AtNAC019</i>	<i>Arabidopsis thaliana</i>	Drought and Salt Stress	Lu., <i>et al.</i> 2007
<i>AtNAC055</i>	<i>Arabidopsis thaliana</i>	Drought and Salt Stress	He., <i>et al.</i> 2005
<i>AtNAC072</i>	<i>Arabidopsis thaliana</i>	Drought and Salt Stress	Tran., <i>et al.</i> 2004
<i>ANAC013</i>	<i>Arabidopsis thaliana</i>	Drought and Salt Stress	Clercq., <i>et al.</i> 2013
<i>ONAC063</i>	<i>Arabidopsis thaliana</i>	High Salinity and Osmotic Stress	Yokotani., <i>et al.</i> 2009
<i>TaNAC2</i>	<i>Arabidopsis thaliana</i>	Drought and Salt Stress	Mao., <i>et al.</i> 2012
<i>TaNAC29</i>	<i>Arabidopsis thaliana</i>	Salt, drought, Senescence Process	Huang., <i>et al.</i> 2015
<i>NTL6, SnRK2.8</i>	<i>Arabidopsis thaliana</i>	Drought Stress	Kim., <i>et al.</i> 2012
<i>CsNAM</i>	<i>Camellia sinensis</i>	Drought, Osmoticum, Salt and Heat Stress	Paul., <i>et al.</i> 2012
<i>CarNAC5</i>	<i>Cicer arietinum</i>	Drought and Salt Stress	Yu., <i>et al.</i> 2015
<i>CarNAC5</i>	<i>Cicer arietinum</i>	Drought and Heat Stress	Peng., <i>et al.</i> 2009
<i>GhNAC3</i>	<i>Gossypium hirsutum</i>	Cold Stress	Meng., <i>et al.</i> 2009
<i>GhNAC4</i>	<i>Gossypium hirsutum</i>	Drought Cold and Salt Stress	Meng., <i>et al.</i> 2009
<i>GhNAC5</i>	<i>Gossypium hirsutum</i>	Cold and Drought Stress	Meng., <i>et al.</i> 2009
<i>GhNAC6</i>	<i>Gossypium hirsutum</i>	Cold, Drought and Salt Stress	Meng., <i>et al.</i> 2009
<i>SNAC1</i>	<i>Gossypium hirsutum</i>	Drought and salinity Stress	Liu., <i>et al.</i> 2014
<i>GmNAC11, GmNAC20</i>	<i>Glycine max</i>	Drought and salinity Stress	Hao., <i>et al.</i> 2011
<i>GmNAC11</i>	<i>Glycine max</i>	Salinity Stress	Hao., <i>et al.</i> 2011
<i>HvNAC6</i>	<i>Hordeum vulgare</i>	ABA mediated defense responses	Chen., <i>et al.</i> 2013
<i>TaNAC2a</i>	<i>Nicotiana tabacum</i>	Drought Stress	Tang., <i>et al.</i> 2012
<i>OsNAC5/ Os11g08210</i>	<i>Oryza sativa</i>	ABA, salt, cold tolerance, grain filling Stress	Sperotto., <i>et al.</i> 2009
<i>OsNAC9</i>	<i>Oryza sativa</i>	Drought Stress	Kim., <i>et al.</i> 2012
<i>OsNAC10/Os11g03300</i>	<i>Oryza sativa</i>	Root, panicle, drought, salt, ABA	Jeong., <i>et al.</i> 2010
<i>OsOAT, SNAC2</i>	<i>Oryza sativa</i>	Drought and oxidative stress tolerance	You., <i>et al.</i> 2013
<i>OsEREBP1</i>	<i>Oryza sativa</i>	Abiotic and biotic stress	Jisha., <i>et al.</i> 2015
<i>SNAC1</i>	<i>Oryza sativa</i>	Cold, Drought, and Salt Stress	Hu., <i>et al.</i> 2006
<i>SNAC3</i>	<i>Oryza sativa</i>	Drought and Salt Stress	Fang., <i>et al.</i> 2015
<i>OsNAC6</i>	<i>Oryza sativa</i>	Drought, Salt Stress	Nakahsima., <i>et al.</i> 2007
<i>OsNAP</i>	<i>Oryza sativa</i>	Salinity, drought and low temperature Stress	Chen., <i>et al.</i> 2014

ATNAC3	<i>Solanum lycopersicum</i>	Drought and Salt Stress	Abdallat., <i>et al.</i> 2015
SINAC4	<i>Solanum Lycopersicum</i>	Drought and Salt Stress	Zhu., <i>et al.</i> 2014
SiNAC	<i>Setaria italica</i>	Drought and Salt Stress	Puranik., <i>et al.</i> 2011
TaNAC4	<i>Triticum aestivum</i>	Cold, Salt, and Wounding Stress	Xia., <i>et al.</i> 2010
SNAC1	<i>Triticum aestivum</i>	Drought and salinity Stress	Yu., <i>et al.</i> 2013
<i>TaNAC4a, TaNAC6</i>	<i>Triticum aestivum</i>	Dehydration, salinity and low temperature Stress	Tang., <i>et al.</i> 2012
<i>ZmSNAC1</i>	<i>Zea. mays</i>	Low temperature, Drought and Salinity Stress	Lu., <i>et al.</i> 2012

Table 1: Transgenic plants developed for Abiotic stress tolerance by over-expression of NAC genes.

Conclusion

We also are aware of the fact that this stress tolerance cannot be achieved only by single gene as the abiotic stress is a polygenic trait, so for constitutive expression of these genes can be achieved by strong constitutive promoter but it has its own problems of energy loss and deleterious effects but if we can control gene expression a well regulated expression of stress-induced transcription factors, which would regulate the expression of a large no of concerned downstream genes. Considerable information through transcriptome profiling strategies has been gained about NAC TFs since the discovery of them, but the research in this area is still in its initial phase. Genome-wide identification and expression profiling has undoubtedly open new avenues for describing the key features of TFs. As a result, our current understandings of the regulatory functions of the TFs in various plant species will be definitely accelerated. In particular, the stress responsive TFs can be used as promising candidates for generation of stress tolerant transgenic plants possessing high productivity under adverse conditions. But there are still some problems to be solved as some studies have shown. Firstly, the constitutive overexpression of TF genes occasionally may lead to negative effects in transgenic plants such as dwarfing, late flowering and lower yields. Secondly, the transgenic plants overexpressing genes may occasionally have antagonistic responses to different stresses. Thirdly, very few of transgenic plants overexpressing NAC TFs genes were evaluated in the field trials so far.

Thus as everyone knows, the prime aims for plant stress research is to provide targets for the improvement of stress tolerance in crop plants. With the unforeseen changes in climatic conditions will lead to more complex stress environment in the fields, we will face new hurdles in creating the multiple stress-tolerant crops. Targeting transcription factors are safe as it is just like epigenetic switching on or off of the gene expressing machinery. Future research should focus on additional approaches including: 1) better knowledge of the complex network of genes and the resultant metabolic and physiological changes during stress tolerance; 2) Better understanding of the molecular determinants that simultaneously regulate responses to stress and plant developmental processes; 3) combining different approaches with multiple gene engineering to target diverse stress response mechanisms and obtain the desired traits and effects; 4) optimizing TF technologies through reducing growth abnormalities and enhancing the introduced trait to withstand stress, thus taking advantage of available high throughput tools such as transcriptomics, proteomics and Metabolomics. Finally, the collective and cooperative efforts of plant molecular biologists, physiologists, and breeders are required to generate stress-tolerant grasses through genetic engineering. It is hoped that, in the future, these collective efforts and results of collaborative studies will positively contribute to sustainable food production in the world and will help to prevent global-scale environmental damage that results from abiotic stress.

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