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The Role of Transcription Factors in Wheat Under Different Abiotic Stresses

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1. Introduction

Abiotic stresses such as drought, salinity and low temperature adversely affect the growth and productivity of plants. The development of stress-tolerant crops will be essential for agriculture in the many regions in the world that are prone to such stresses [48].

Wheat (*Triticum aestivum* L.) is one of the four major cereals in the world. As one of the most important agricultural crops, wheat is a staple food crop for a large portion of the world's population [83]. It is grown under both rain-fed and irrigated cultivation and thus under conditions subjected to many environmental stresses [68]. Unfortunately, its production is severely affected by adverse environmental stresses. Therefore, the identification and functional study of stress responsive genes will elucidate the molecular mechanisms of the plant stress response and tolerance, and will ultimately lead to improvement of stress tolerance in wheat [58, 83]. Abiotic stresses such as drought and high salinity lead to wide range of biochemical, physiological and morphological, responses in plants in the process of adaptation to these adverse conditions. These adaptations require a large number of changes in gene expression. Many of the differentially expressed gene products protect plant cells from damage, such as dehydrins, enzymes for the synthesis of osmolytes and enzymes for the removal of reactive oxygen species (ROS) [3]. The production of these functional proteins is widely regulated by specific transcription factors [58, 65].

Transcription factors (TFs) are considered to be the most important regulators that control genes and gene clusters [49]. Many families of transcription factors have been demonstrated to play a role in stress responses in plants. Among them, the bZIP [69], WRKY [43], AP2 [64], NAC [78] and C2H2 zinc finger [31] families comprise a high proportion of abiotic stress-responsive members [58]. One TF gene can control the expression of a broad range of target genes through binding to the specific cis-acting element in the promoters of these genes, also

referred to as regulon [49]. Transcription factors (TFs) provide a possibility for plants to overcome and respond to biotic and abiotic stresses and are also involved in modulating developmental processes [45, 83].

Until now, several major regulons involved in response to abiotic stress have been identified in *Arabidopsis*. Recent studies have demonstrated that DREB1/CBF, DREB2, AREB/ABF, and NAC regulons have important functions in response to abiotic stresses in rice [49]. Significant advances have been made in recent years towards identifying regulatory genes involved in stress responses which confer abiotic stress tolerance in plants [18].

In this review, we provide an overview of the functions of different TF family members with particular emphasis on the role of bZIP, bHLH, WRKY, MYB, and NAC TFs and their involvement in abiotic stress responses in wheat.

2. bZIP transcription factors

Basic region/leucine zipper (bZIP) TFs possess a basic region that binds DNA and a leucine zipper dimerization motif. The bZIP domain comprises two structural features located on a contiguous α -helix: a basic region of about 16 amino acid residues with a nuclear localization signal, an invariant N-x7-R/K motif to contact the DNA as well as a heptad repeat of leucines or other bulky hydrophobic amino acids located exactly nine amino acids towards the C-terminus, to create an amphipathic helix. When binding to DNA, two subunits adhere through interactions of the hydrophobic sides of their helices, which create a superimposing coiled-coil structure (zipper). The capability to form homo- and heterodimers is governed by the electrostatic attraction and repulsion of polar residues adjacent to the hydrophobic interaction surface of the helices. Proteins with bZIP domains are present in all eukaryotes analyzed to date and bZIP proteins typically bind to DNA sequences with an ACGT core. Plant bZIPs bind to the A-box (TACGTA), C-box (GACGTC) and G-box (CACGTG), but there are also reports of nonpalindromic binding sites for bZIPs [24]. Based on the sequence similarities of common domains, 75 bZIP protein members have been divided into ten subgroups in *Arabidopsis* [24, 35]. In plants, bZIP transcription factors present a divergent family of TFs which regulate processes including light and stress signaling, seed maturation, pathogen defense, and flower development [24, 59].

The plant hormone abscisic acid (ABA) plays an essential role in maturation and germination in seeds, as well as mediating adaptive responses to abiotic environmental stresses. ABA induces the expression of many genes, including late-embryogenesis-abundant (LEA) genes. *HVA1* is one of the LEA genes whose expression is affected by ABA. Analysis of the interplay between ABA and TaABF1 as a bZIP factor in the aleurone cells of imbibing wheat grains by Keyser [32] indicated that the two are not additive in their induction of the *HVA1* promoter. It has been shown that TaABF1 may undergo an ABA-induced posttranslational modification. However, the lack of synergism between ABA and TaABF1 overexpression in *HVA1* induction does not support this conclusion. These findings indicate that the branch of ABA signaling leading to *HVA1* is more complex [32].

Kobayashi et al. [35] isolated a wheat *lip19* (encoding bZIP-type transcription factors) homologue, *Wlip19* and analyzed its expression in response to cold stress. *Wlip19* expression was stimulated by low temperature in seedlings and was higher in a freezing-tolerant wheat cultivar than in a freezing-sensitive variety. *Wlip19* expression was also activated by drought and exogenous ABA treatment. Heterologous expression of *Wlip19* in tobacco has showed a significant increase in abiotic stress tolerance, especially freezing tolerance.

It was indicated that WLIP19 acts as a transcriptional regulator of Cor/Lea genes in the development of abiotic stress tolerance by enhancement of expression of four wheat Cor/Lea genes, *Wdhn13*, *Wrab17*, *Wrab18*, and *Wrab19*, in wheat callus and tobacco plants. Furthermore, direct protein–protein interactions between WLIP19 and another bZIP-type transcription factor in wheat, the OBF1 homologue TaOBF1, was observed, implying that this interaction is conserved in cereals [35].

Expression analysis of a group of bZIP candidate genes in long term salinity into contrasting cultivars of wheat by reverse northern blot showed that *bZIP1* (CN011839) was up-regulated in a susceptible variety (Chinese Spring) and down-regulated in a tolerant cultivar (Mahouti) during salt stress. Sequence analysis by BLASTX showed that this gene's protein has two homologues in *Arabidopsis* (AtZIP56, E value=1e⁻²⁰) and wheat (TaABF, E value=6e⁻⁵). The results of published work showed that *TaABF* mRNA accumulates together with *PKABA1* mRNA (an ABA-induced protein kinase) during wheat grain maturation and dormancy acquisition and *TaABF* transcripts increase transiently during imbibitions of dormant grains. In contrast to *PKABA1* mRNA, *TaABF* transcripts are seed specific and were not markedly produced in vegetative tissues in response to ABA application or abiotic stress [29, 59].

HY5, another bZIP1 homologue from the H group of *Arabidopsis* bZIPs, is involved in photomorphogenesis regulation. The necessary TF for response to a broad spectrum of wavelengths of light acts as a positive regulator in photomorphogenesis by regulating the expression of downstream genes in response to a light signal. Interestingly, *HY5* integrates both hormone and light signaling pathways. In *hy5* mutants, the expression of hundreds of genes is affected by UV-B or blue light [7]. Another affected bZIP by salt stress in tolerant genotype was bZIP5 (CV765814) from group I bZIPs. The analysis of group I genes from several species indicates that they might play a role in vascular development [24, 59].

3. bHLH transcription factors

Basic helix-loop-helix (bHLH) proteins comprise a group of diverse transcription factors with highly diverse functions and are present in both plants and animals. The bHLH domain, the characteristic of this family, consists of about 60 amino acids with two functionally distinct regions. The basic region at the N-terminal end of the domain is required for DNA binding while the C-terminal HLH region functions as a dimerization domain. These TFs in plants, act as transcriptional regulators required for phytochrome signaling, anthocyanin biosynthesis, fruit dehiscence, carpel, and epidermal development, as well as for stress response. However, the biological function of most members of this gene family in plant has not yet been elucidated [37].

Gene expression analysis by reverse northern blot has shown that two selected candidate wheat bHLHs (*bHLH2*: CA599618 and *bHLH3*: CJ685625) are affected by salt stress in a tolerant wheat cultivar. The BLASTx results showed that both have a homologue in wheat, *bHLH94* (E value= $5e^{-85}$ for *bHLH2* and E value= $5e^{-102}$ for *bHLH3*). AtAIB was another homologue for *bHLH3* from *Arabidopsis* involved in the regulation of ABA signaling in *Arabidopsis* and plays a role in drought tolerance and ABA treatment response [37, 59]. The high homology (E value= $2e^{-51}$) between these orthologues and the result of reverse northern blot hybridizations in that research indicate that these two bHLH gene may have an important function in tolerance to salt stress in wheat [59].

4. WRKY transcription factors

WRKY transcription factors have been studied in plants extensively in the last two decades. First Ishiguro and Nakamura [23] identified a WRKY protein in sweet potato (*Ipomoea batatas*); since then many other members of this TF family have been cloned and functionally characterized in plants, including wild oats (*Avena fatua*) [62], parsley (*Petroselinum crispum*) [63], tobacco (*Nicotiana tabacum*) [8, 19, 34, 61], *Arabidopsis thaliana* [12], potato (*Solanum tuberosum*) [5, 14], orchardgrass (*Dactylis glomerata*) [2], winter bittersweet nightshade (*Solanum dulcamara*) [22], desert legume (*Retama raetam*) [55], barley (*Hordeum vulgare*) [66], rice (*Oryza sativa*) [41], cotton (*Gossypium arboreum*) [77], and coconut (*Cocos nucifera*) [44]. More recently, WRKY family TFs were also identified in lower plants including ferns (*Ceratopteris richardii*), mosses (*Physcomitrella patens*) [4], a slime mold (*Dictyostelium discoideum*) and the protist (*Giardia lamblia*) [81, 73].

A WRKY domain of about 60 amino acids is a characteristic of WRKY proteins. This domain comprises the absolutely conserved sequence WRKYGQK followed by a zinc finger motif. The WRKY domain binds to the W box ([T][T]TGAC[C/T]) of target gene promoters to modulate transcription [10, 73]. It should be mentioned that in spite of the strong conservation of their DNA-binding domain, the overall structures of WRKY TFs are highly divergent. WRKY TF family members are grouped into three distinct groups based on the number and type of the WRKY domains which might also reflect their different functions [59]. WRKY TFs with two WRKY domains belong to group I and members of group II and group III possess one WRKY domain. Group I and group II have a C2H2 zinc finger motif, while in group III, the WRKY domain contains a C2HC motif. WRKY TFs can then be further classified into different subgroups based on their phylogenetic clades. The WRKY family is one of the TF families for which the regulatory role in biotic and abiotic stresses has been demonstrated in plants. These include infection of bacteria, fungi, oomycetes and viruses, treatment with salicylic acid (SA) or H_2O_2 , mechanical stimulation, drought, cold, wounding, high-salinity and UV radiation.

Most WRKY TFs of group III play a role in plant defense signaling pathways. Some members of the WRKY family may have key functions in plant development, such as embryo development, fruit maturation, tannin synthesis in the seed coat, maturation of root cells, morphogenesis of trichomes, senescence, and dormancy. Furthermore, some of WRKY family

members have a role in hormone signaling such as OsWRKY71 and OsWRKY51 which were ABA-inducible and could repress GA signaling transduction in aleurone cells.

Wu et al. [73] obtained sequences for 15 wheat cDNAs encoding putative WRKY proteins. Phylogenetic analysis showed that the 15 WRKY genes classified to three major WRKY groups and expression analysis revealed that most genes were highly expressed in leaves. A few of them such as *TaWRKY10* are expressed in the crown intensively and several genes are strongly up-regulated during the senescence of leaves. Eight isolated genes were responsive to high or low temperature, NaCl or PEG (polyethylene glycol) treatment. In addition, differential expression was also measured between wheat hybrids and its parents, and some genes were more responsive to PEG treatment in the hybrid. The authors concluded that the differential expression of these WRKY genes in the hybrid might contribute to heterosis by improving the stress tolerance in hybrids [73].

Orthologous genes are subjected to similar transcriptional regulation by orthologous TFs, suggesting that the terminal stages of signal transduction pathways leading to defense are conserved, implying a fundamental role of pathogenesis-related genes, such as PR4 genes in plant defense. This suggests that diversification between monocot and dicot plants has most likely occurred after the differentiation of WRKY functions. Proietti et al. [56] reported the ability of TaWRKY78 to bind to a W-box-containing region of the *wPR4e* promoter. Transient expression assays of *TaWRKY78* and *AtWRKY20* showed that both TFs are able to recognize the cognate cis-acting elements present in the *wPR4e* and *AtHEL* promoters [56].

Expression analysis by reverse northern blot hybridizations of a group of putative wheat WRKYs showed that WRKY1 (CN009320) and WRKY2 (CJ873146) were up-regulated in a stress-tolerant genotype. AtWRKY75 (E value=3e⁻⁴²) is a homologue from *Arabidopsis* for WRKY1 which is up-regulated in response to phosphorous deficit stress [15, 17, 59]. This gene also acts as positive regulator in defense responses to pathogens. Functional characterization of the WRKY2 homologue in *Arabidopsis*, AtWRKY33 (E value=4e⁻¹⁸), showed that its expression in response to salt, mannitol (simulated drought) treatment and cold stress in shoots and roots increased but this gene was down-regulated during heat stress. It also appears that its expression is independent of SOS signaling and only partly dependent on ABA signaling, but forms part of plant responses to microbial infections [27, 40, 59].

5. MYB transcription factors

MYB TFs form one of the largest transcription factor families in plants. More than 200 MYB proteins are encoded in genomes of *Arabidopsis* and rice. MYB TFs contain one to four imperfect repeats [50–53 amino acids) in their DNA-binding domain (MYB domain) near to the N-terminus and are classified into four subfamilies [58, 83].

According to the number of repeat(s) in the MYB domain: 4R-MYB has four repeats, 3R-MYB (R1R2R3-MYB) has three consecutive repeats, R2R3-MYB has two repeats, and the MYB-related type usually, but not always, has a single repeat [16, 28, 61]. Typically, the MYB repeat

is 50–53 amino acids in length and contains three regularly-distributed tryptophan (or phenylalanine) residues, which can together form a hydrophobic core. Each MYB repeat forms three α -helices: the two that are located at the C-terminus adopt a variation of the helix–turn–helix (HLH) conformation that recognizes and binds to the DNA major groove at the specific recognition site such as C/TAACG/TG [51, 52].

Since the first plant MYB gene, *C1*, was isolated in *Zea mays* [54], research concerning different aspects of the MYB gene family, including gene number, sequence characterization, evolution, and potential functions, has been widely conducted in plants [9, 16, 72]. So far, large numbers of MYB genes have been identified in different plant species, comprising 204 members in *Arabidopsis*, 218 members in rice, 279 members in grapevine, 197 members in poplar, and 180 members in *Brachypodium* [9, 70, 72].

MYB proteins are involved in many significant physiological and biochemical processes, including the regulation of primary and secondary metabolism, the control of cell development and the cell cycle, the participation in defense and response to various biotic and abiotic stresses, and hormone synthesis and signal transduction [16, 83].

Extensive studies of the MYB gene family in various plant species have provided a better understanding of this gene family; however, little is known about this gene family in bread wheat [83].

We previously analyzed the expression levels of ten MYB TF genes from wheat (*Triticum aestivum*) in two recombinant inbred lines contrasting in their salt tolerance in response to salt or drought stress via quantitative RT-PCR [58]. A potential new MYB gene (*TaMYBsdu1*) was significantly up-regulated in leaves and roots of wheat plants subjected to long-term drought stress. Furthermore, *TaMYBsdu1* showed higher transcript abundance in the salt-tolerant genotype than in the susceptible genotype under salt stress. These data suggested that *TaMYBsdu1* is a potentially important regulator for wheat adaptation to both salt and drought stresses [58].

In other work, two putative MYB genes, *MYB2* (DQ353858.1) and *MYB3* (CJ920766) were up-regulated in a tolerant variety (Mahouti) under salt stress conditions but down-regulated in the susceptible cultivar (Chinese Spring), *MYB2*. Sequence analysis with the BLASTx and Plant Gene Ontology assignment showed that *MYB2* is a part of *TaMYB1* (E value=6e⁻¹⁵). The results of a study by Lee et al. [36] show that *TaMYB1* is involved in abiotic stresses responses in wheat. The expression of this gene increases during oxygen deficiency (flooding), PEG treatment (drought) and salt increases, especially in roots. In addition, its transcript gradually increases in starting ABA and PEG treatments [36]. In research conducted by Mott and Wang [46] on comparative transcriptome analysis of salt-tolerant wheat germplasm lines using wheat genome arrays, it was found that *TaMYB1* was one of the up-regulated genes with 34 times higher expression levels under stress condition relative to the control. Functional analysis of the *MYB2* homologue in *Arabidopsis*, *AtMYB44* (E value=1e⁻⁵⁹), showed that this gene was up-regulated in response to drought, salt, cold and ABA treatments, especially in stomata guard cells and vascular tissue. Transgenic plants overexpressing this gene showed more tolerance to mentioned stresses compared to wide-type plants [28]. Homology analysis of *MYB3* (a

member of R2R3MYB) has shown that there is a high homology between this gene and *AtMYB59* in *Arabidopsis* (E value= $4e^{-60}$). It has been shown that *AtMYB59* expression increases in response to phytohormones including jasmonic acid, SA, gibberellic acid and ethylene, especially in leaf and stem tissues [38, 39, 59]. But its expression level in roots and inflorescences was lower than in other organs, showing its role in hormonal signal pathways in response to biotic stresses and plant defense against pathogen attacks [38, 39, 59].

Full-length cDNA is an important resource for isolating the functional genes in wheat. Recently, Zhang et al. [83] analyzed a group of MYB genes that respond to one or more stress treatments. They isolated 60 full-length cDNA sequences encoding wheat MYB proteins. A phylogenetic tree with wheat, rice, and *Arabidopsis* MYB proteins was constructed to examine their evolutionary relationships and the putative functions of wheat MYB proteins based on *Arabidopsis* MYB proteins with known functions. Tissue-specific analysis and abiotic stress response expression profiles were carried out to find potential genes that participate in the stress signal transduction pathway, including the analysis of transgenic *Arabidopsis* plants expressing the MYB gene, *TaMYB32* [83].

Recently, Qin et al. [56] identified a new R2R3-type MYB transcription factor gene, *TaMYB33*, from wheat (*T. aestivum*). This gene was induced by ABA, NaCl, and PEG treatments, and its promoter sequence contains the putative ABRE, MYB and other abiotic stress-related cis-elements. Ectopic over-expression of this gene in *Arabidopsis* significantly enhanced its tolerance to drought and NaCl treatments, but not to LiCl and KCl stresses. The expression of two genes, *AtP5CS* (involved in proline synthesis) and *AtZAT12* (a C2H2 zinc finger transcription factor that is involved in regulating ascorbate peroxidase expression), was induced in the *TaMYB33*-expressing transgenic *Arabidopsis* lines. This suggests that *TaMYB33* promotes the ability for ROS scavenging and osmotic pressure balance reconstruction. *TaMYB33* over-expression lines displayed up-regulation of *AtAAO3* along with down-regulation of *AtABF3* and *AtABI1*, indicating that ABA synthesis was elevated while its signaling was constrained. The authors concluded that *TaMYB33* enhances salt and drought tolerance partially via an improved ability for ROS detoxification and osmotic balance reconstruction [57].

TaMYB56 (on chromosomes 3B and 3D) in wheat was identified as a cold stress-related gene by Zhang et al. [82]. The expression levels of *TaMYB56-B* and *TaMYB56-D* were strongly induced by cold stress, but slightly induced by salt stress in wheat. Detailed characterization of the *Arabidopsis* transgenic plants that overexpressed *TaMYB56-B* revealed that *TaMYB56-B* is possibly involved in the responses of plants to freezing and salt stresses. The expression of some cold stress-responsive genes, such as *DREB1A/CBF3* and *COR15a*, were found to be elevated in the *TaMYB56-B*-overexpressing *Arabidopsis* plants compared to wild-type [82].

TaMYB3R1 is another MYB gene which has been shown to be potentially involved in wheat response to drought, salt and cold stress. Cai et al. [6] cloned *TaMYB3R1* from wheat (*T. aestivum*). *TaMYB3R1* amino acid sequence shares high identity to other plant MYB3R proteins. Subcellular localization experiments in onion epidermal cells proved that *TaMYB3R1* was present in the nucleus. Trans-activation assays in yeast cells confirmed that *TaMYB3R1* was a TF that required the C-terminal region to activate the expression of reporter gene. DNA-binding tests showed the MSA cis-element-binding activity of *TaMYB3R1*. *TaMYB3R1*

expression was induced following ABA treatment and gradually increased expression until 72 h after salt or cold treatment. In contrast, PEG treatment lead to an early expression peak at 6 h after treatment, and then gradually decreased [6].

Zhang et al. [84] identified *TaMYB32* as a salt stress-related gene, during the bulk sequencing of full length cDNAs in wheat (*T. aestivum*). The sequences of *TaMYB32* were cloned from different varieties of hexaploid wheat and its diploid ancestors. Sequence analysis indicated that two types of sequences existed in the diploid ancestors and four in the hexaploid wheat. One of the sequences was identical in both diploid and hexaploid wheat. This implied that *TaMYB32* was conserved during the evolution of wheat. The genomic *TaMYB32* sequences proved to be non-intron genes after comparing with their cDNA sequences. *TaMYB32* was mapped onto the homoeologous group 6 of wheat using the electronic mapping strategy, and two copies of the gene were found in each genome of hexaploid wheat. Homologous analysis found that *TaMYB32* had a similarity with some R2R3-MYB proteins from rice (*Oryza sativa* L.) and maize (*Zea mays* L.) as high as 72.4% and 73.7%, respectively. The expression of *TaMYB32* in roots, stems, leaves, pistils, and anthers in wheat, was induced by salt stress [84].

6. NAC transcription factors

The first sequenced cDNA encoding a NAC protein was the *RESPONSIVE TO DEHYDRATION 26 (RD26)* gene in *Arabidopsis* [80]. The NAC domain was characterized based on consensus sequences from *Petunia* NAM and *Arabidopsis* ATAF1/2 and CUC2 proteins [1]. Many NAC TFs, including *Arabidopsis* CUC2, play important roles in plant development. Some NAC genes mediate viral resistance [48], while others are up-regulated during wounding and bacterial infection [11].

NAC domains mediate transcriptional regulation of various biological processes by forming a helix-turn-helix structure that specifically binds to the target DNA [1]. NAC TFs are quite diverse in their C-terminal sequences which possess either activation or repression activity. More than 100 NAC genes have so far been identified in *Arabidopsis* and rice which can be categorized into six major groups. Phylogenetic analyses suggest that these were already present in an ancient moss lineage. NAC TFs play a range of important roles during plant development and abiotic stress responses [48]. Many plant growth and developmental processes are regulated by NAC TFs, including shoot apical meristem formation, lateral root development, senescence, cell wall development, and secondary metabolism. A large number of NAC TFs are also differentially expressed in responses to abiotic and biotic stresses [74] and transgenic *Arabidopsis* and rice plants overexpressing stress-responsive NAC genes have displayed improved drought tolerance. These studies indicate that stress-responsive NAC transcription factors have important roles for the control of abiotic stress tolerance and that their overexpression can improve stress tolerance via biotechnological approaches [48].

Interestingly, rice plants overexpressing *OsNAC6* possessed enhanced tolerance to abiotic (dehydration, high salinity) as well as biotic stresses (blast disease) [47]. The *Arabidopsis* NAC TF, ATAF2, is induced by salicylic acid (SA) and methyl jasmonate (MeJA) treatments, and is

also differentially expressed following wound stress response [22]. The potato *StNAC* gene shows induced expression in responses to *Phytophthora infestans* infection and wounding treatment [23]. Barley plants with the *HvNAC6* gene knocked down show penetration resistance in epidermal cells when inoculated with virulent isolates of *Blumeria graminis* f. sp. *hordei* [25]. Overexpression of rice *OsNAC4* resulted in hypersensitive response (HR) cell death; and in the *OsNAC4* knocked down lines, HR cell death was markedly decreased in response to the avirulent bacterial strain (*Acidovorax avenae* N1141) [67]. Therefore, it seems that plant NAC TFs play multiple roles in defense responses to pathogen attack as well as exogenous stimuli [74].

Although these transcription factors can bind to the same core NAC recognition sequence, recent reports have shown that the different NAC TFs have different functions in plant development. In addition, NAC proteins can form homo- or hetero-dimers. Stress-responsive NAC TFs can be used for improving stress tolerance in transgenic plants, although the mode of action appears complex in plants. Recent reports support the notion of substantial crosstalk between plant growth and stress responses. In rice, Kikuchi et al. [33] characterized the molecular properties of eight NAC genes (*OsNAC1* to *OsNAC8*).

In contrast to *Arabidopsis*, the NAC regulon may have additional roles in monocot plants. Important future tasks will, therefore, lie in the comparative analysis of gene expression patterns and the identification of their target genes to determine the function of these genes in plant development and tolerance to abiotic and biotic stresses [49].

Xia et al. [74] reported the full-length cDNA sequence of a novel wheat (*T. aestivum*) NAC TF, *TaNAC8*, (using *in silico* cloning, reverse transcription PCR and 3' rapid amplification of cDNA ends PCR methods). *TaNAC8* shows strong homology to rice *OsNAC8* with an N-terminal NAC domain and a trans-membrane helices motif in the C-terminus. Yeast one hybrid assays confirmed that *TaNAC8*'s C-terminal region acted as transcriptional activator. Inoculation of wheat with an incompatible isolate of the stripe rust pathogen *Puccinia striiformis* f. sp. *tritici* or treatments with MeJA or ethylene led to increased *TaNAC8* transcription in leaves 24 h post inoculation/treatment. However, SA and ABA had no significant effect on gene expression. Abiotic stress treatments, including high salinity, PEG and low-temperature, also induced *TaNAC8* expression, suggesting that *TaNAC8* may function as a transcriptional activator involved in wheat defense responses to both abiotic and biotic stresses [74].

Mao et al. [42] obtained a fragment of *TaNAC2* from suppression subtractive cDNA libraries of wheat treated with PEG, and its full-length cDNA was obtained by screening a full-length wheat cDNA library. Gene expression profiling indicated that *TaNAC2* was involved in response to drought, salt, cold, and ABA treatment. Overexpression of *TaNAC2* in *Arabidopsis* resulted in enhanced tolerances to drought, salt, and freezing stresses which coincided with enhanced expression of abiotic stress-response genes and several physiological indices [42].

TaNAC4 encodes another NAC TF in wheat high homology with rice *OsNAC4* [73]. Functional analysis using onion epidermal cells and yeast one-hybrid assays confirmed that *TaNAC4* functions as a transcriptional activator. *TaNAC4* expression was induced in wheat leaves by infection with stripe rust, and also by MeJA, ABA and ethylene treatments. However, SA had no

obvious effect on *TaNAC4* expression. Similar to *TaNAC8*, abiotic stresses such as high salinity, wounding, and low-temperature also induced *TaNAC4* expression, suggesting a role of *TaNAC4* as a transcriptional activator during biotic and abiotic stresses responses in wheat [75].

Rahaie et al. [59] have shown that *NAC67* (BU672229), a putative member of the NAC family was up-regulated during salt stress treatment. The encoded protein has a close homologue in wheat (*TaNAC69*, E value= $2e^{-151}$) [59, 78]. Xue et al. [78] demonstrated the role of *TaNAC69* in response to abiotic stresses including drought, cold and ABA treatments. Expression analysis of three highly homologous *TaNAC69* genes showed that these were up-regulated during the above-mentioned stresses, especially drought stress. Besides their up-regulation by drought, *TaNAC69* genes were expressed at high levels in the root under unstressed conditions. This suggests that *TaNAC69* genes are not just involved in drought stress, but may also be required in normal cellular activities of roots [78]. Over-expression of *TaNAC69* in transgenic wheat leads to enhanced dehydration tolerance and improvement of water use efficiency [79]. *AtNAC2* is also a *NAC67* homologue in *Arabidopsis* which is involved in salinity stress, ABA, ACC and NAA treatment in *Arabidopsis*, but *AtNAC2* induction by salt stress requires the ethylene and auxin signaling pathways. It has been shown that the expression level of *AtNAC2* in roots and flowers has been higher than in other tested tissues [20, 59].

7. Enhanced abiotic stress resistance by genetic manipulation of a transcription factor linked to crop yield improvement in the field

In the past decade numerous transgenic plant studies have demonstrated that the improvement of abiotic stress resistance can be achieved by genetic manipulation of transcription factors. However, many resistant transgenic lines with constitutive over-expression of a transcription factor exhibit a slower rate of growth under non-stress conditions. Field trials have also shown that some transgenes tend to have a negative effect on grain yield under normal growth conditions [76]. This phenomenon can theoretically result from the following two causes: (i) genes that are induced during stress generally have a negative impact on the growth and yield, and (ii) the energetic cost of the stress-related metabolite accumulation due to over-expression of a transcription factor. Therefore, the expression of a transcription factor needs to be tailored to meet the requirement for plant stress adaptation if the crop yield is concerned. Any reduction of crop yield under normal growth conditions could potentially override a marked yield advantage under stress.

The expression of a transcription factor can be tailored to stress adaptation by using a stress-inducible promoter. For example, transgenic *Arabidopsis* plants carrying a drought inducible promoter-driven *DREB2A* gene exhibit the improved drought resistance with no significant difference in growth rate under normal growth conditions [64]. Other aspects for consideration of minimizing the negative impact of transgene expression on growth and yield include the appropriate expression level of the transgene and cell specificity. Recently, a root-specific promoter has been used for driving expression of drought-upregulated transcription factors for engineering drought tolerance [26, 60]. Most interestingly, a number of transcription factors

have been shown to improve crop yield under field conditions when they are over-expressed in transgenic plants (Table 1). These studies clearly demonstrate that genetic manipulation of stress-responsive transcription factors has potential for improvement of crop yield in the future, including wheat.

| Gene description | Host | Expression mode | Acquired traits | Reference |
|---|-------|--------------------------------------|---|-----------|
| Rice SNAC1 (NAC) | Rice | Constitutive OE | Improved spikelet fertility under drought and reduced transpiration | 21 |
| Maize NF-YB2 (NF-YB) | Maize | Constitutive OE | Less wilting, delayed senescence, higher photosynthesis rate and improved yield under drought | 50 |
| ZAT10 (C ₂ H ₂ zinc finger) | rice | Drought-inducible or constitutive OE | Improved spikelet fertility and grain yield per plant under drought | 76 |
| CBF3 (AP2) | rice | Drought-inducible OE | Improved spikelet fertility and grain yield per plant under drought | 76 |
| Rice AP37 (AP2) | Rice | Constitutive OE | Enhanced drought resistance and grain yield under severe drought conditions | 53 |
| Rice NAC10 (AP2) | Rice | Root-specific OE | Enhanced drought resistance and grain yield under both normal and drought conditions | 26 |
| Rice NAC9 (AP2) | Rice | Root-specific OE | Enhanced drought resistance and grain yield under both normal and drought conditions | 60 |

OE = over-expression

Table 1. Transgenic crops with over-expression of a transcription factor improve yield under field conditions

8. Conclusion and prospective

Abiotic stresses such as drought, salinity and low temperature adversely affect the growth and productivity of plants. Successful breeding of stress-tolerant varieties will be vital to ensure food supply in areas that are prone to such stresses. Recent advances towards identifying potential abiotic stress tolerance genes have been made. Many TFs and other regulatory genes involved in stress responses have been identified, giving rise to the idea that plants have developed flexible molecular and cellular response mechanisms to respond to various abiotic stresses. bZIP, WRKY, bHLH, MYB and NAC transcription factors represent the major groups of regulatory genes of which some members are found to be involved in abiotic stress responses in plants. To date, the functions of a number of abiotic stress-responsive transcription factor genes have been studied in many different species, including wheat. Recent studies have

indicated that certain stress-induced TF genes play significant roles in wheat stress tolerance. These studies enhance our understanding of the mechanisms of responses and tolerance to abiotic stress in wheat. Also, it provides us a collection of suitable candidate genes for over- or under-expression studies in transgenic wheat aiming to achieve increased abiotic stress tolerance.

In the future, a systems biology approach using reverse genetics, functional genomics and proteomics, as well as metabonomics during various developmental stages and stress conditions will provide us with critical information to elucidate the function of the different stress-responsive TFs and their relationship in transcriptional control in wheat.

In the years ahead, the verification of abiotic stress tolerance and agronomic traits of transgenic wheat utilizing stress-responsive TF genes should be evaluated under harsh field conditions over several years. It can be expected that with increases in climatic variations, more robust cultivars that withstand a wide variety of stresses will be superior over those that are high yielding under optimal conditions. To this end, it will be necessary to clarify the differential function of the individual stress-responsive TF genes from different families of TFs for the control of abiotic stress tolerance and other biological processes including biotic stress tolerance, growth regulation, senescence and yield in order to fully utilize the potential of transcription factors.

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