

# Plant tolerance to drought and salinity: stress regulating transcription factors and their functional significance in the cellular transcriptional network

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**Abstract** Understanding the responses of plants to the major environmental stressors drought and salt is an important topic for the biotechnological application of functional mechanisms of stress adaptation. Here, we review recent discoveries on regulatory systems that link sensing and signaling of these environmental cues focusing on the integrative function of transcription activators. Key components that control and modulate stress adaptive pathways include transcription factors (TFs) ranging from bZIP, AP2/ERF, and MYB proteins to general TFs. Recent studies indicate that molecular dynamics as specific homodimerizations and heterodimerizations as well as modular flexibility and posttranslational modifications determine the functional specificity of TFs in environmental adaptation. Function of central regulators as NAC, WRKY, and zinc finger proteins may be modulated by mechanisms as small RNA (miRNA)-mediated posttranscriptional silencing and reactive oxygen species signaling. In addition to the key function of hub factors of stress tolerance within hierarchical regulatory networks, epigenetic processes as DNA methylation and posttranslational modifications of histones highly influence the efficiency of stress-induced gene expression. Comprehensive elucidation of dynamic coordination of drought and salt responsive TFs in interacting pathways and their specific integration in the cellular network of stress adaptation will provide new

opportunities for the engineering of plant tolerance to these environmental stressors.

**Keywords** Drought · Epigenetics · Transcription factor · RNAi · Salt tolerance · *Arabidopsis*

## Introduction

A major challenge for current agricultural biotechnology is to satisfy an ever increasing demand in food production facing a constantly increasing world population that will reach more than 9 billion in 2050 (Godfray et al. 2010; Tester and Langridge 2010). This growing demand for food is paralleled by dramatic losses of arable land due to increasing severity of soil destruction by abiotic environmental conditions. Thus, drought and salinity are the two major environmental factors that adversely affect plant growth and development and have a crucial impact on agricultural productivity and yields. Drought due to shortage of water is critical for crop production in large agronomic areas worldwide and it is usually coped with extensive irrigations. Although earth is rich in water, most water resources are highly salinized whereas high quality fresh water that is suitable for irrigation is extremely limited. Accordingly, not only drought but also soil salinity becomes increasingly an agricultural problem due to extensive spreading of agricultural practices as irrigation (Flowers 2004) and it urgently requires the breeding of crops with increased water use efficiency and salt tolerance.

Exposure of plants to excess salt causes ion imbalance and ion toxicity-induced imbalances in metabolism. Another component of salinity is hyperosmotic stress that results in water deficit in a comparable way to drought-induced water deficit. Plants basically counteract the

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negative effects of salinity and drought by activation of biochemical responses that include (1) the synthesis and accumulation of osmolytes, (2) maintaining the intracellular ion homeostasis, and (3) scavenging of reactive oxygen species (ROS) generated as a secondary effect of drought (Flowers 2004; Ashraf and Akram 2009).

Plant engineering strategies for cellular and metabolic reprogramming to increase the efficiency of plant adaptive processes may either focus on (1) conferring stress tolerance by directly re-programming ion transport processes and primary metabolism or (2) by modulating signaling and regulatory pathways of the adaptive mechanisms. The second approach seems to be more perspective because it is likely that signaling and regulatory factors orchestrate as key signaling components the transcriptional and translational control of group (1) adaptive mechanisms (Diédhiou et al. 2008; Popova et al. 2008). Accordingly, molecular reprogramming to enhance stress tolerance of plants would probably require the genetic engineering of a single or a few master regulators of adaptation instead of modulating numerous metabolic and cellular adaptive mechanisms.

However, although several plant stress signaling components have been dissected in detail the knowledge on integration of regulatory mechanisms in stress signaling cascades and on key regulators is still limited, although knowledge on the regulating key factors of stress adaptation is highly necessary for biotechnological engineering of stress tolerance. In this review, we focus on recent advances in transcription factor (TF)-based engineering of increased drought and salt adaptation. Putative integrations and links of TFs in stress adaptive signaling networks coordinating the endogenous programs of environmental adaptation will be highlighted. Accordingly, for this review TFs out of the wider range of all stress inducible TFs were selected so that we do not comprehensively cover all stress-related factors. Major criterion for this selection was a putative potential of the TFs in controlling sub-regulons of stress-adaptive cellular mechanisms within the hierarchical transcriptional network that will be discussed in the review.

### Arabidopsis and related model species: learning from species with different natural stress tolerance

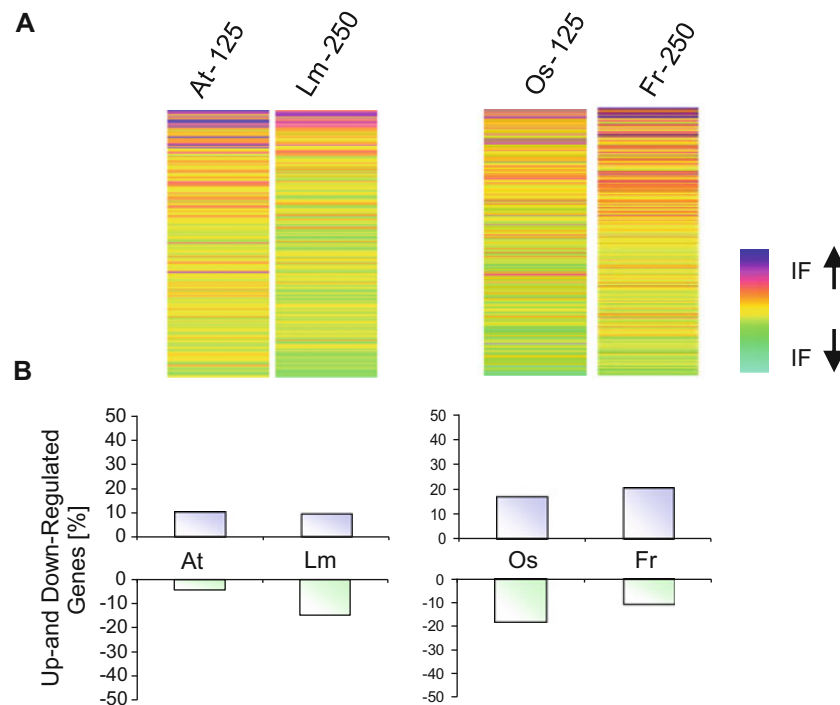
Traditional breeding attempts for sustainable agricultural use of dry and salinized soils have been clearly facilitated and stimulated by the wealth of knowledge of genomics and transcriptomics data available from the model species *Arabidopsis* (*Arabidopsis thaliana*) and rice (*Oryza sativa*). Linear general frameworks of plant drought and salt adaptation have been established that were mainly based on systematic and comprehensive mutant analyses.

Thus, it is now accepted that changes in membrane integrity and modulation of lipid synthesis are key factors in the primary sensing of drought and salt (Kader and Lindberg 2010). Secondary, osmotic stress-induced signaling involves changes in plasma membrane  $H^+$ -ATPase and  $Ca^{2+}$ -ATPase activities that trigger concerted changes of  $Ca^{2+}$  influx, cytoplasmic pH, and apoplastic production of ROS (Beffagna et al. 2005). In addition, osmotic stress-induced  $Ca^{2+}$  fluxes are linked to abscisic acid (ABA), and calcium-responsive protein kinases act as key regulators in drought and salinity-induced signaling cascades (Diédhiou et al. 2008). As convergent down-stream elements of transcriptional activation, many genes that are responsive to drought and to salinity belong to the ABA-responsive element (ABRE) and dehydration-responsive element/C-repeat element (DRE/CRT) regulons (Yamaguchi-Shinozaki and Shinozaki 2005).

Despite this knowledge derived from the model plants *Arabidopsis* and rice, the applicability of these data for biotechnological engineering of increased drought and salt tolerance is clearly limited. Comprehensive comparisons of the salt inducible transcriptomes of the salt-sensitive species *Arabidopsis* and rice and, for example, transcriptional data of the closely related salt-tolerant model species *Lobularia maritima* (Brassicaceae) and *Festuca rubra* ssp. *litoralis* (Poaceae) show extensive differences in salt responsive expressional regulations (Popova et al. 2008; Diédhiou et al. 2009b; Fig. 1). In contrast to salt excluding and avoiding halophyte models as *Thellungiella halophila* with a very limited number of salt responsive transcripts, the salt-accumulating and -detoxifying halophytes *L. maritima* and *F. rubra* ssp. *litoralis* allowed identification of a wide range of transcripts with different salt responsive regulation in the salt-sensitive and salt-tolerant species (Volkov et al. 2003; Taji et al. 2004; Popova et al. 2008; Diédhiou et al. 2009b). In addition, transgenic modulation of regulatory and signaling elements in *Arabidopsis* and rice according to the pattern in the halophytes *L. maritima* and *F. rubra* ssp. *litoralis* successfully activated stress adaptation in the sensitive model species (Diédhiou et al. 2008; Yang et al. 2009). Accordingly, understanding of stress-induced signaling complexity in stress-sensitive model species has to be complemented by comparisons with naturally tolerant species for a systematic identification of key regulators of stress tolerance with the potential of biotechnological application.

### bZIP TFs and their role in conferring stress tolerance to plants

Research on salt and drought regulatory TFs has mainly focused on single factors and linear pathways. Emerging



**Fig. 1 a** Hierarchical clustering of signal ratios (salt-treated plants/control plants) of transcript levels monitored by array hybridization with leaf cDNA obtained from *A. thaliana* stressed with 125 mM NaCl for 6 h (At-125), the Arabidopsis-related halophyte *Lobularia maritima* (Brassicaceae) stressed with 250 mM NaCl for 6 h (Lm-250), rice stressed with 125 mM NaCl for 6 h (Os-125), the rice-related halophyte *Festuca rubra* ssp. *litoralis* (Poaceae) stressed with 250 mM NaCl for 6 h (Fr-250) (IF induction factor, arrow up

upregulation of gene expression, arrow down downregulation of gene expression). One vertical line represents each one homologous gene in *Arabidopsis* and *L. maritima* and in rice and *F. rubra* ssp. *litoralis*. **b** Comparison of the number of up-regulated and down-regulated salt-responsive genes in *Arabidopsis* and *L. maritima* and in rice and *F. rubra* ssp. *litoralis* (the data were modified from Popova et al. 2008; Diédhiou et al. 2009a)

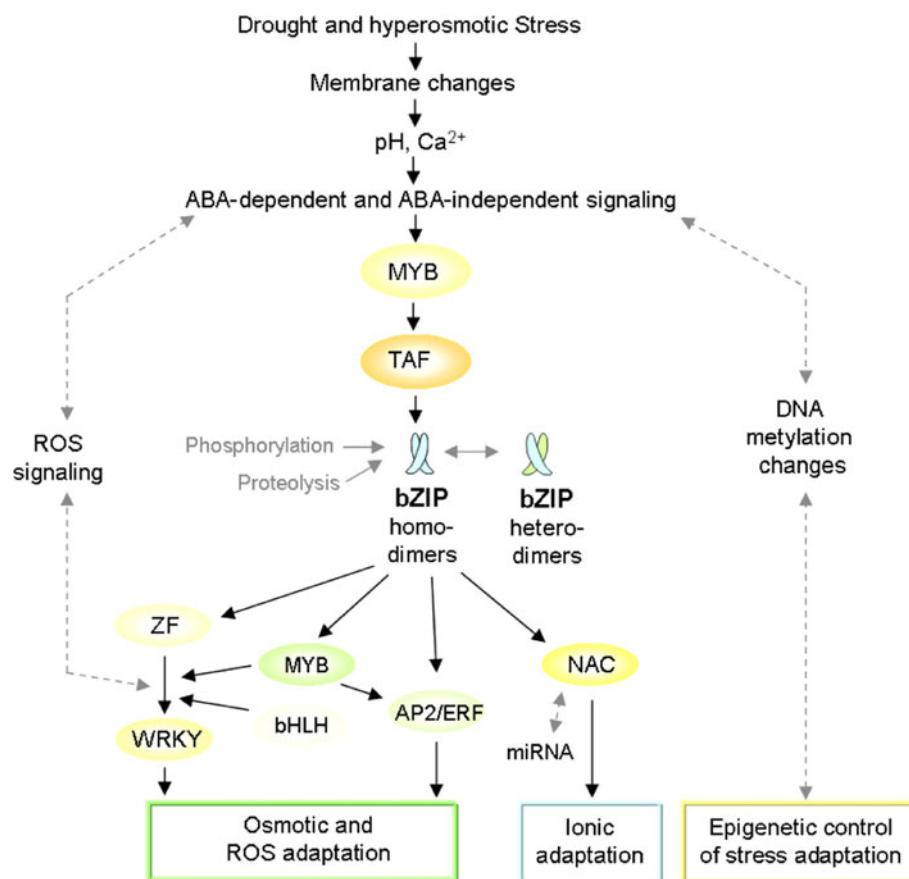
findings increasingly suggest, however, integration of the TFs in dynamic network hubs as well as interaction and competition of pathways manifesting complexity of molecular links in stress adaptation.

The emerging view of the salt- and drought-signaling network unequivocally supports a key and integrative function of members of the bZIP TFs in these regulatory networks (Fig. 2) and the potential of these factors to confer enhanced stress tolerance has been demonstrated repeatedly. A key regulator of salt stress adaptation, the group F bZIP TF bZIP24, was identified by differential screening of salt-inducible transcripts in *A. thaliana* and a halophytic *Arabidopsis*-relative model species (Yang et al. 2009). Expressional regulation of *bZIP24* was different with induced transcription in the salt-sensitive and transcriptional repression in the halotolerant species, and RNAi-mediated repression of the factor conferred increased salt tolerance to *Arabidopsis*. The improved tolerance was mediated by stimulated transcription of a wide range of stress-inducible genes that are e.g. involved in cytoplasmic ion homeostasis, osmotic adjustment, as well as in plant growth and development demonstrating a central function of bZIP24 in salt tolerance by regulating

multiple mechanisms that are essential for stress adaptation (Yang et al. 2009). Next to bZIP24 and its function in salt adaptation, group A bZIP factors AREB1, AREB2, and ABF3 have a key regulatory role in ABA signaling under drought stress. Thus, *A. thaliana areb1 areb2 abf3* triple knock out mutants had increased tolerance to ABA and reduced drought tolerance (Yoshida et al. 2010). In addition, in other species as rice and tomato transgenic modification of group A bZIP TFs modified the tolerance of plants to water deficit and to salt stress (Amir Hossain et al. 2009; Hsieh et al. 2010) strongly suggesting trans-species potential of these factors for increasing stress tolerance.

From animal systems dynamic coordinations of numerous bZIP controlled signal transduction pathways by molecular re-organization and by posttranslational mechanisms are well-known (Jindra et al. 2004; Miller 2009). Thus, specific homodimerizations and heterodimerizations within the class of bZIP TFs as well as modular flexibility of the interacting proteins and posttranslational modifications might determine the functional specificity of bZIP factors in cellular transcription networks (Miller 2009). Excitingly, evidences for involvement of homologous mechanisms in signaling hubs in plant systems are just now

**Fig. 2** Model of signaling pathways and regulatory transcription factors involved in plant adaptation to drought and salt



emerging. As an example, the three factors AREB1, AREB2, and ABF3 can form homodimers and heterodimers as well as interact with a SnRK2 protein kinase suggesting ABA-dependent phosphorylation of the proteins (Yoshida et al. 2010). As another example for the function of bZIP factors in salt adaptation in *A. thaliana*, salt stress induced proteolytic processing and translocation of the group B factor AtbZIP17 to the nucleus followed by transcriptional up-regulation of salt-responsive transcripts (Liu et al. 2007). The group F factor AtbZIP24 shows salt-inducible subcellular re-targeting to the nucleus and formation of homodimers suggesting that molecular dynamics of bZIP factors could mediate new signaling connections within the complex cellular signaling network (Yang et al. 2009). In contrast to the homodimerization of bZIP24, specific heterodimerization was shown for the salt-responsive group S AtbZIP1 with group C bZIP TFs (Weltmeier et al. 2009). In conclusion, it might be hypothesized that specific homodimerizations and heterodimerizations as well as posttranslational modifications (e.g. phosphorylations) might determine the functional specificity of bZIP factors in the cellular transcription networks of drought and salt adaptation. Interestingly, transgenic over-expression of rice SnRK2-type SAPK4 in rice regulated ion and ROS homeostasis under salt stress

supporting the hypothesis of key functions of SnRK kinases in the intracellular signaling cascades of osmotic adaptation thus further supporting key modulatory function of posttranslational phosphorylations in diverse plant systems that might, e.g. target bZIP factors (Diédhiou et al. 2008; Fig. 2).

Recently, it was recognized that general TFs might also have an important role in stress-responsive transcription. Thus, the TBP-associated factor AtTAF10 has a specific and key function in plant salt and osmotic stress adaptation by regulating accumulation of Na<sup>+</sup> and proline (Gao et al. 2006). This functional overlap to bZIP24 (Yang et al. 2009) strongly suggests linked regulation and cofunctions of bZIP proteins and TAFs within the complex drought and salt signaling network—a hypothesis that awaits further clarification (Fig. 2).

### The role of WRKY TFs and Cys2/His2 zinc finger proteins in the regulation of adaptation to osmotic stress

Our understanding of plant stress-inducible signaling has been greatly facilitated by research on TFs that regulate and control subsets of stress-responsive gene expression.

Thus, WRKY proteins regulate diverse plant processes ranging from development to various biotic and abiotic stresses as well as hormone-mediated pathways (Ramamoorthy et al. 2008). Involvement of WRKY factors in plant salt adaptation were shown for WRKY25 and WRKY33 that increased salt tolerance and ABA sensitivity independent of the SOS-pathway when over-expressed in *A. thaliana* (Jiang and Deyholos 2009). In *A. thaliana*, *wrky63* knock out mutants showed decreased sensitivity to ABA and drought (Ren et al. 2010). In these plants, the stomatal closure and the expression of the AREB1/ABF2 TF were affected indicating involvement of WRKY factors in the ABA-dependent pathway of drought and salt adaptation (Ren et al. 2010). Potential of WRKY-type TFs to confer increased salt tolerance by transgenic expression is further supported by the different salt-induced regulation of a WRKY protein in salt-sensitive rice and a halophytic rice-relative model species (Diédhiou et al. 2009a, b). Interestingly, *A. thaliana* WRKY25 and WRKY33 are not only responsive to osmotic stresses but they are also regulated by oxidative stress (Miller et al. 2008). In addition, down-stream regulated target genes of WRKY33 include transcripts with function in ROS detoxification as peroxidases and glutathione-S-transferases (Jiang and Deyholos 2009) suggesting function of WRKY factors as key regulators in both osmotic and oxidative stress adaptation. Alternatively, it is tempting to hypothesize involvement of WRKY factors in the osmotic stress signaling via control of the intracellular stress-induced ROS levels (Fig. 2).

Interestingly, Zat proteins (TFIIIA-type Cys2/His2 zinc finger proteins) have been suggested to control and regulate WRKY functions (Miller et al. 2008). Thus, in soybean overexpression of GmWRKY54 conferred increased salt and drought tolerance and regulation of the GmWRKY54 by Zat10/STZ was hypothesized (Zhou et al. 2008). In addition, in rice stomatal closure is regulated by the Cys2/His2 zinc finger protein DST (drought and salt tolerance) via ABA-independent targeting of genes that are involved in ROS homeostasis (Huang et al. 2009). These findings further support involvement of zinc finger proteins and probably WRKY TFs in osmotic adaptation via ROS signaling (Fig. 2). Interestingly, although both drought and salt stress might result in intracellular accumulation of toxic amounts of ROS, hydrogen peroxide ( $H_2O_2$ ) and nitric oxide (NO) also function as signaling molecules in ABA-mediated stomatal responses (Miller et al. 2010; Wilkinson and Davies 2010). Mutation of a cellulose synthase-like protein induced accumulation of ROS, changed sensitivity to salt stress and to water deficit, and regulation of plant osmotic stress tolerance via control of intracellular stress-induced ROS levels has been suggested (Zhu et al. 2010a).

### Stress adaptation and multi-transcriptional regulation: AP2/ERF, MYB, and bHLH TFs

Next to TFs with possible upstream position in the hierarchical network of stress adaptation as the bZIP factors described above, integrative stress-adaptive functional roles of regulatory proteins from other diverse groups have been reported. These factors might be either integrated in the main pathways of environmental adaptation, likely under control of the key regulatory TFs, or they might have functions in regulating sub-networks of adaptation to drought and salt stress and in linking these stress adaptations to other stresses, developmental and hormonal responses. Thus, dual roles in both biotic and abiotic stress responses have been demonstrated for AP2/ERF proteins as soybean GmERF3 and the ABA-responsive RAP2.6 from *A. thaliana* (Zhang et al. 2009; Zhu et al. 2010b). Overexpression of Arabidopsis light and drought responsive RAP2.4 led to defects in multiple developmental processes regulated by light and ethylene as well as drought tolerance (Lin et al. 2008). Complementary to these observations, overexpression of AP2/ERF GmERF3 in tobacco induced the expression of PR genes and of osmotin accompanied by enhanced accumulation of free proline and soluble carbohydrates (Zhang et al. 2009). Members of the DREB/CBF subfamily of the AP2/ERF TFs have been recognized for a decade for their roles in stress tolerance via ABA-dependent and -independent pathways and for their regulation of a stress-response sub-transcriptome with more than hundred target genes inclusive regulatory factors as ZAT12 and RAP2.1 (Shinozaki and Yamaguchi-Shinozaki 2000). However, constitutive overexpression of the DREB/CBF pathway led to serious developmental defects of transgenic plants although accompanied by increased tolerance to drought, salt, and cold (Kasuga et al. 1999). These data clearly demonstrate complexity of the stress adaptive network that requires major control points of the multiple transcriptional sub-regulons as well as cooperative and integrative function of the different stress sub-clusters to prevent impairing side effects. Nevertheless, members of the AP2/ERF TF family are integrated as a hub in signaling interconnections of complex biotic and abiotic environmental cues. Supporting the undeniable key function of AP2/ERF in terms of drought and salt tolerance the picture of integrative function of these factors in plant developmental processes as well as biotic and/or abiotic stress signaling in an interconnecting and linking way is, however, only emerging.

As another example for multi-functional regulations, the R2R3-MYB TF AtMYB41 is transcriptionally induced in response to ABA, drought, salinity, and cold (Lippold et al. 2009). In addition, the factor influences cell expansion and cuticle deposition suggesting a linking function in abiotic



stress response and cell wall modifications (Cominelli et al. 2008). Interaction and competition of complex signaling pathways in fine-tuning cellular responses is further illustrated by the *A. thaliana* basic-helix-loop helix TF bHLH92. The factor regulates only the expression of a subset of salt- and drought-responsive genes (Jiang et al. 2009). However, different peroxidases are down-stream targets of the factor and bHLH92 might have a function in the control of ROS-mediated signaling thus linking salt and drought adaptation to ROS signaling (Fig. 2). Here, more detailed work will be necessary to elucidate the precise integration of the diverse TFs in the cellular network of stress adaptation and to understand their potential in genetic engineering of improved stress tolerance, probably via targeted engineering of defined subsets of stress adaptive mechanisms or sub-pathways of signaling to customize specific features of stress adaptation.

### NAC-triggered gene expression and miRNA

NAC type proteins are not only involved in diverse processes as developmental programs, defense, and biotic stress responses (Olsen et al. 2005) but they also have a key function in abiotic stress tolerance inclusive drought and salinity. Thus, in rice *ONAC5* and *ONAC6* are transcriptionally induced by ABA, drought, and salt stress (Rabbani et al. 2003; Takasaki et al. 2010). *ONAC5* and *ONAC6* transcriptionally activate stress-inducible genes as *OsLEA3* by direct binding to the promoter and they interact in vitro suggesting functional dimerization of these TFs (Takasaki et al. 2010). Interestingly, overexpression of the Arabidopsis factors ANAC019, ANAC055, and ANAC072 caused increased drought tolerance of transgenic plants but they only changed transcription of a limited number of non-particularly salt- and drought-responsive genes (Tran et al. 2004). These important results strongly suggest interaction or co-regulation of NAC factors with other regulatory pathways or subsets of stress-inducible molecular mechanisms for achieving the significant increased stress tolerance that was observed (Tran et al. 2004). Improved drought and salt tolerance could also be achieved by transgenic overexpression of diverse NAC factors in species ranging from *A. thaliana* and rice to chickpea, wheat, and tomato (Peng et al. 2009; Yokotani et al. 2009; Xia et al. 2010; Yang et al. 2011). Interestingly, in tomato two NAC TFs were salt-inducible in a salt-tolerant cultivar but showed different expression in salt-sensitive tomato plants (Yang et al. 2011). These data indicate that differences in plant salt tolerance might be due to different and specific transcriptional activation of NAC-dependent regulatory pathways.

As important examples for conferring increased stress tolerance under field conditions, in rice transgenic overexpression of *SNAC1* enhanced salt and drought tolerance and *OsNAC10* improved drought tolerance and grain yield (Hu et al. 2006; Jeong et al. 2010). *OsNAC10*-regulated target genes mainly included protein kinases and TFs of AP2, WRKY, LRR, NAC, and Zn-finger types as well as the stress-responsive genes cytochrome P450 and the potassium transporter *HAK5* (Jeong et al. 2010). These results support the view that NAC type TFs might be part of the general frameworks of drought and salt adaptation by connecting or regulating subsets of linear adaptive pathways but the NAC factors themselves are likely to be controlled by global regulatory factors of the network of stress adaptive transcription and metabolism. Thus, important evidence for cooperative regulation of stress responses by members of different TF families was provided by the study of Tran et al. (2007) that showed interaction and co-function of the drought, salt, and ABA inducible zinc finger protein ZFHD1 and a NAC factor.

As it was recognized recently, members of the CCAAT-HAP TF family also have a potential key function in conferring stress tolerance to crops. Transgenic maize plants with increased expression of the CCAAT-HAP-type factor *ZmNF-YB2* showed improved drought tolerance under field conditions (Nelson et al. 2007). This effect was achieved by mechanisms independent of ABA and DREB/CBF pathways supporting the hypothesis of concerted action of different TF families within subsets of regulatory modules in the cellular stress-response network.

Interestingly, members of the NAC TF family are potential regulatory targets of the small RNA (miRNA) posttranscriptional silencing machinery (Rhoades et al. 2002; Guo et al. 2005). As an example, recently a NAC domain containing TF was identified as a target of miR164 in switchgrass (Matts et al. 2010). Thus, regulation of NAC TFs by miRNA-mediated cleavage of mRNAs together with data showing differential regulation of NAC factors in response to drought and salt stress indicate that these TFs might participate in the regulation of environmental adaptation through miRNA pathways. Next to NAC proteins, TFs e.g. of SCL, MYB, and TCP types were identified as targets of drought and salt inducible miRNAs as miR159, miR168, miR171, and miR396 (Liu et al. 2008). Accordingly, it might be hypothesized that the cellular networks of drought- and salt-stress tolerance are regulated by miRNA-mediated targeting of convergent and divergent adaptive pathways under control of different stress-specific TFs. Accordingly, relevance of modification of drought and salt stress-specific signaling pathways via the miRNA machinery in a biotechnological context might be a powerful approach for genetic engineering of improved tolerance but remains to be discovered.

## Epigenetics: what is next in terms of biotechnological application?

Next to transcriptional regulations of abiotic stress responses, epigenetic processes are becoming a new and current chapter in plant environmental adaptation. Efficiency of gene expression is highly influenced by chromatin structure that might be modulated epigenetically by processes as DNA methylation and posttranslational modifications of histones. The histone-mediated structure of nucleosomes in the chromatin might be posttranslationally modified at the *N*-terminal tails of the core histone complexes (H2A, H2B, H3, H4) and thus influence nucleosome density, binding efficiency of TFs, and transcriptional activity (Chinnusamy and Zhu 2009; Kim et al. 2010). In addition to methylations of histones, also acetylations and phosphorylations as well as other posttranslational modifications of histones as ubiquitination, biotinylation, and sumoylation might have a modulating impact on the regulation of stress-specific gene expression (Chinnusamy et al. 2008).

Meanwhile, it is accepted knowledge that phenotypes within one species may transmit different epigenetic information based on covalent modifications of DNA or histones (Fazzari and Grealley 2004). Thus, plant populations from stress exposed habitats may carry inherited memories of stress adaptation and transfer this epigenetically to next generations. As an example, the desert shrub *Zygophyllum dumosum* was posttranslationally methylated at histone H3 under wet but less under dry growth conditions indicating posttranslational regulation of gene expression activity (Granot et al. 2009). As it was also reported recently, natural populations of mangroves were DNA hypomethylated when grown under saline conditions in contrast to populations from non-saline sites (Lira-Medeiros et al. 2010). Based on these results, it seems obvious to think on simulation of inherited memories of stress adaptation in biotechnological applications to confer increased drought and salt tolerance to naturally sensitive species. However, in contrast to the detailed knowledge on influences of epigenetic mechanisms on developmental processes, information on epigenetic regulation of abiotic stress resistance is still rare.

As a few examples, salinity-induced phosphorylation of histone H3 and acetylation of histone H4 in *A. thaliana* and tobacco have been reported (Sokol et al. 2007). In addition, altered acetylation as well as trimethylation of histone H3 under drought stress in drought-responsive genes of *A. thaliana* have been observed (Kim et al. 2008). In rice, expression of cytosine DNA methyltransferases was modified by salt stress indicating functional importance of epigenetic modulation of genome activity also in monocot species (Sharma et al. 2009).

Detailed knowledge on the specific mechanisms that underlay epigenetic regulation under environmental exposure is, however, only slowly emerging. Thus, trans-generational modifications of stress adaptations as salt stress include altered genomic DNA methylation as well as function of Dicer-like proteins suggesting involvement of small RNA pathways in epigenetic regulations (Boyko et al. 2010). Interestingly, in barley expression of Polycomb proteins with function in histone methylation was influenced by abscisic acid (ABA) suggesting involvement of ABA-mediated pathways in epigenetic modifications (Kapazoglou et al. 2010).

Thus, according to the current knowledge, an application of epigenetic processes to improve the stress-regulating function of TFs will be a challenging and novel biotechnological approach for the engineering of plant tolerance to drought and salinity, however, many detailed information are still missing. Particularly, despite the importance of elucidating epigenetic mechanisms in model plants, it will be obligatory to extend investigations to systematic and comprehensive comparisons of stress relevant epigenetics in sensitive- and naturally tolerant species. Linking epigenetic processes to the key regulatory components of the general stress adaptive frameworks will be essential to further support the feasibility of epigenetics in the customized engineering of stress adaptation.

## Conclusion and perspectives

Cellular effects of environmental stresses as drought and salinity are not only imbalances of ionic and osmotic homeostasis but also impaired photosynthesis, cellular energy depletion, and redox imbalances. Regulatory systems inclusive TFs that link sensing and signaling of the environmental conditions and the cellular adaptive responses are emerging but are not well understood yet. As a next step, it will be important to identify master regulators and master pathways of stress adaptation in naturally stress-tolerant species as well as integration of the diverse regulatory factors in the network of intracellular stress adaptation pathways (Fig. 2). Within this hierarchical network, cellular stress responses might be fine tuned by interaction and competition of TFs that regulate sub-clusters of the stress transcriptome. Here, systematic and comprehensive data on the timing of all stress responsive TFs upon stress will be indispensable for detailed hierarchical linking of all regulatory factors. In addition, more detailed understanding of shared and competing transcriptional regulation as well as modulated intramolecular interactions of different factors and epigenetic processes will be essential for targeted and efficient genetic engineering of improved drought and salt tolerance in plants.

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