

## MINIREVIEW

## Transcriptional regulation of salinity stress: role and spatio-temporal expressions of ion-transporter gene promoters

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### Abstract

Ion transporters play an important role in ion homeostasis and control ion flow from its intrusion to exclusion in the entire plant system. Abiotic stress tolerance in plants depends immensely on the activity of these transporters. The transporter proteins are transcriptionally regulated by *cis*-elements present in their upstream region for specific activity. The presence of different *cis*-elements facilitates cross-talk between different signal transduction pathways. Depending on the *cis*-elements, a specific stress signalling pathway is activated, eliciting a physiological change towards maintaining ion homeostasis to alleviate stress. Beta-glucuronidase localization studies using various promoter regions indicated their expression specificity in organs/tissues. This review gives an overview about promoter activity of different transporters and its involvement under salinity stress.

*Additional key words:* abiotic stress tolerance, ion homeostasis, Na<sup>+</sup>/H<sup>+</sup> antiporter, signal transduction pathways.

### Introduction

Plant faces different abiotic stresses such as salinity, drought, and temperature extremes. The abiotic stresses cause adverse effects on growth, development, and productivity (a loss of yield more than 50 %) and lead to plant death in case of severity (Vinocur and Altman 2005). The productivity of over one-third of the arable land in the world is affected by soil salinity (Epstein 1972).

Salinity imparts both ionic stress and osmotic stress and causes adverse impact on plant growth by disturbing the ionic equilibrium and eliciting sodium toxicity. Plants follow different mechanisms to minimize the damage from Na<sup>+</sup> excess by minimizing an initial influx, loading into xylem, intercellular compartmentalization and secretion of salt from the leaf surface (Tester and Davenport 2003). Plants respond and adapt to these conditions by regulating a wide array of genes. Several genes and transcription factors (TFs) have been isolated

and well characterized from different plants. The over-expression of these genes through genetic engineering revealed stress tolerant phenotypes in several crop plants by tissue specific, temporal, and optimal expression. A plant genome comprises many different TFs, *e.g.*, 1 510 - 1 581 (20 % of all proteins) in *Arabidopsis* (Iida *et al.* 2005) and 1 611 in rice (Xiong *et al.* 2005). However, the genome of the yeast *Saccharomyces cerevisiae* contains only 12 % of TFs (Mewes *et al.* 1997). The TFs play a crucial role in initiation of gene transcription by recruiting RNA polymerase II to a respective gene promoter for the expression of a specific gene. Several stress inducible promoters have been identified and characterized, however, because of their weak expressions, they are not being used in developing genetically engineered crop plants. Promoters have different *cis*-elements whereby different TFs can bind at a different time according to a specific requirement of particular protein

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*Abbreviations:* ABA - abscisic acid ; ABRE - ABA responsive elements; GUS - β-glucuronidase; HKT- high affinity potassium transporter; MYB - myeloblastosis; MYC - myelocytomatosis; NHX - sodium proton exchanger; TFs - transcription factors.

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(Fig. 1). Promoters used in plant biotechnology are generally of three types - constitutive (active continuously in most or all tissues), spatio-temporal (tissue specific or stage-specific activity), and inducible (regulated by the application of an external chemical or physical signal; Potenza *et al.* 2004).

During stress, different functional proteins are expressed for imparting tolerance. Ion transporters are

one of the important class of membrane proteins, which are strongly involved in ion homeostasis (Shi and Zhu 2002). The ion transporters help in ion movement across the tonoplast and plasma membrane (Blumwald 2000). This review focuses on how ion transporters are regulated at different times, in different tissues, and by different ions for efficient ion homeostasis.

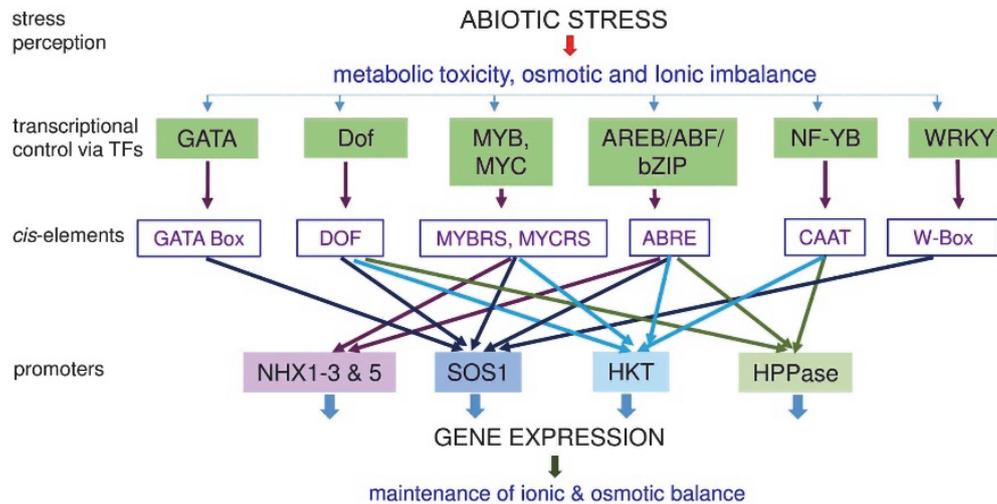


Fig. 1. A schematic representation of important *cis*-elements of different transporter promoters involved in stress tolerance mechanism. At the time of stress, different transcription factors like GATA, Dof, MYB, MYC, AREB, and WRKY regulate stress tolerance by binding to specific sequences in the promoter region and so activating different stress-responsive genes. The *cis*-elements are found common in different promoter sequences and are involved in specific expressions of genes at the time of stress for conferring stress tolerance. For all abbreviations, see a list on the first page.

### NHX1 Na<sup>+</sup>/H<sup>+</sup> antiporter

A novel antiporter NHX1 localized at a late endosome/pre-vacuolar compartment was identified from yeast cells and shows ion sequestration in a pH-dependent manner (Nass *et al.* 1997, Nass and Rao 1998). Later, the functional mechanism of the *NHX1* gene was deciphered from *Arabidopsis* and several other plant species (Apse *et al.* 1999, Gaxiola *et al.* 1999, Zhang and Blumwald 2001). The *NHX* homologs have also been identified in rice (*OsNHX1* to *OsNHX5*) and their expressions were found higher under salinity, osmotic stress, and abscisic acid (ABA) treatment (Fukuda *et al.* 2011). For understanding the actual function of gene, a detailed study of its regulon is prerequisite. A very few reports are available on the differential transcriptional regulation of the *NHX* genes. The analysis of *NHX* promoters revealed that their expressions are very tissue specific. Shi and Zhu (2002) observed that the *Arabidopsis AtNHX1* promoter expresses the  $\beta$ -glucuronidase (*GUS*) gene in all tissues except root tips. A strong *GUS* expression was observed in guard cells and root hair cells. The root hair cells have larger vacuoles as compared to root tips suggesting that *AtNHX1* play role in compartmentalizing

Na<sup>+</sup> in these cells. An *AtNHX1* promoter activity is substantially increased by NaCl, KCl, or ABA applications demonstrating that salt and ABA regulations of *AtNHX1* expression occur at the transcriptional level (Shi and Zhu 2002). Another *NHX1* promoter *BvNHX1* from *Beta vulgaris*, a salt tolerant plant, shows *GUS* expression in all vegetative tissues, especially in hydathodes and trichomes (Adler *et al.* 2010). In contrast to the *AtNHX1* promoter, the *BvNHX1* promoter does not show a higher expression in guard cells. However, root hairs show a higher *GUS* expression in root tips and elongation zones. Sepals and anthers show *GUS* expression, whereas no expression is in petals and carpels (Adler *et al.* 2010). The *BvNHX1* promoter induces a higher *GUS* activity after a long-term exposure to a mild stress as compared to a short exposure to a severe salt stress. Activity of *GUS* is induced by osmotic stress (Na<sub>2</sub>SO<sub>4</sub> or mannitol) and by ionic stress (KCl), but non-significantly by ABA. The *OsNHX1* and *OsNHX5* promoters were observed in steles, emerging parts of lateral roots, vascular bundles, water pores, and basal parts of seedlings (Fukuda *et al.* 2011). The *OsNHX1*

promoter was found in guard cells and trichomes, whereas an *OsNHX5* promoter-GUS complex was observed in root tips and pollen grains.

The presence of *NHX1* homologs in *Arabidopsis* (Yokoi *et al.* 2002) and rice (Fukuda *et al.* 2011) has developed an interest about their regulation, and interestingly, each promoter shows a unique expression. A detailed gene expression analysis revealed differences and similarities in the regulation of *NHX*-type antiporter gene expression between rice and *Arabidopsis*. The

### SOS1 Na<sup>+</sup>/H<sup>+</sup> antiporter

Another very important antiporter gene, *SOS1*, which plays a unique role in transport of Na<sup>+</sup> ions through the plasma membrane, was identified in *Arabidopsis* (Shi *et al.* 2000). Expression of the *SOS1* gene was regulated by *SOS2/SOS3* pathway. The first *SOS1* promoter was identified and characterized from *Arabidopsis*, it shows expression in epidermal cells at root tips and in parenchyma cells at the xylem/symplast boundary of roots, stems, and leaves (Shi *et al.* 2002). The active expression of *AtSOS1* in vascular system also highlights its role in Na<sup>+</sup> movement throughout the plant. Similarly, *Salicornia brachiata SOS1* overexpressing tobacco lines show Na<sup>+</sup> loading in xylem (Yadav *et al.* 2012). A *Salicornia brachiata SOS1* promoter isolated by Goyal *et al.* (2013) shows upregulation of GUS expression in stems and leaves by salt stress but not by ABA and cold stresses. *In silico* analysis of *SOS1* promoters from *Thellungiella parvula* and *T. salsuginea* halophytes and *Arabidopsis* shows conserved motifs between *TsSOS1* and *TpSOS1*, whereas no homology was observed between *AtSOS1* and *TpSOS1* or *TsSOS1* promoters (Oh *et al.* 2010).

Feki *et al.* (2014) isolated two *SOS1* promoters, named *PrSOS1-AB* and *PrSOS1-D*, from the *SOS1* alleles of wheat, which are localized on genome A and/or B, and genome D, respectively. The *PrSOS1-AB* is almost similar to

*AtNHX1* and *AtNHX2* promoters does not have an ABRE element, however, they contain MBC/MYB a transcription factor binding sequence, meaning that they are activated indirectly through ABA-dependent pathway, whereas *OsNHX1-3* possesses ACGT-containing ABA-responsive elements, but they are absent in *OsNHX5* (Fukuda *et al.* 2011). However, *OsNHX5* is regulated by ABA-dependent pathway through MYC/MYB TFs. This reflects unique complex signalling of the promoters in gene expression.

*PrSOS1-D* except five additional nucleotide sequences. Expressions of *PrSOS1-AB* and *PrSOS1-D* are similar at an early stage; however, after 20 d, the *PrSOS1-AB* line shows GUS expression in leaves and roots, and after 30 d, only in leaves. Abiotic stress treatments like NaCl, ABA, and mannitol induce a stronger GUS expression in the *PrSOS1-AB* line. Similarly to the *AtSOS1*, the *PrSOS1-AB-GUS* lines show a strong expression in root tips under NaCl, ABA, and mannitol, whereas it is not observed in the *PrSOS1-D* line. Both these promoters show no expression at the flowering stage. The *SOS2* promoter isolated from *Brassica juncea* induces a strong GUS activity under salinity, desiccation, and ABA treatments in both shoots and roots (Kaur *et al.* 2015).

Motif search analysis revealed that *SOS* promoters identified in different plants seem to follow ABA-dependent pathway and contain ABRE elements (Fig. 1). In addition, MYB and MYC TF binding sites are also present, which suggest that genes expressed *via* these TFs under stress conditions are ABA-mediated (Kaur *et al.* 2015). The presence of DOF, GT-1, GARE, TCA, and Aux RR-core suggest that *SOS* transcription is also facilitated by different hormones under stress and non-stress conditions (Goyal *et al.* 2013, Kaur *et al.* 2015). In contrast to the *NHX1* promoter, the *SOS1* promoter is more active in ion exclusion from roots (Feki *et al.* 2014).

### V-ATPase and V-PPase

Proton transport to the vacuole uses two different types of pumps, V-ATPase and V-PPase. The V-ATPase possesses two domains V<sub>1</sub> and V<sub>o</sub> comprising eight and six different subunits, respectively. The six c-subunits reported in the V<sub>o</sub> domain largely play an important role in H<sup>+</sup>-transport (Gao *et al.* 2011). The subunit *c* has been reported in many plants, and its role in NaCl transport was also confirmed. The V-ATPase shows different activities in different organs, *e.g.*, in *Mesembryanthemum crystallinum*, and its activity is induced by salinity stress (Low *et al.* 1996). In *Tamarix hispida*, similar V-ATPase activities have been observed in roots, stems, and leaves

(Gao *et al.* 2010). Expression of the *V-ATPase c*-subunit was observed in shoots and mildly in roots after NaCl treatment in *Pennisetum glaucum* (Tyagi *et al.* 2005). The *P. glaucum V-ATPase c* shows a localized *GUS* expression in trichomes, developing anthers, bottom parts of stamens, gynoeciums, and petals. The localization of *V-ATPase c* expression in the trichomes might be important in salt secretion (Tyagi *et al.* 2005). It has been observed that *SOS4* mutants of *P. glaucum* have hairless roots (Shi and Zhu 2002), so plausibly in this case, *PgVATPase c* is working together with *SOS4* in salt secretion. The promoter sequence of *VATPase* gene

revealed stress specific *cis*-elements like ABRE and DRE showing its involvement in ABA-dependent manner (Tyagi *et al.* 2005, Gao *et al.* 2010).

The V-H<sup>+</sup>-PPase comprises a single subunit and plays an important role in abiotic stress tolerance by providing energy to Na<sup>+</sup> transporters. The V-PPases are classified in two types; type-I AVP1 and type-II AVP2/AVPL1 (Drozdowicz *et al.* 2000). The type-I V-PPase works in a K<sup>+</sup> dependent manner, whereas type-II does not need K<sup>+</sup>. The type-I V-PPase has been characterized in detail in *Arabidopsis* (Gaxiola *et al.* 2001, 2002). Its over-expression enhances salinity tolerance *via* increased Na<sup>+</sup> uptake into the vacuole (Gaxiola *et al.* 2002). The promoter region of the *V-PPase* genes has been isolated from different plants to understand the specific regulation under different stresses. Sun *et al.* 2010 isolated a *TsVPI* promoter from a halophyte, *Thellungiella halophila*, a close relative of *Arabidopsis* and compared its activity with the *AVP1* promoter. Both the *AVP1* promoter and the *TSVPI* promoter show similar *GUS* expressions under controlled conditions, however, the expression was found higher in *TSVPI-GUS* plants after salt treatment. The

*TSVPI-GUS* transgenic lines show *GUS* expression in all tissues except seeds. During salt treatment of *TSVPI-GUS* transgenic plants, *GUS* activity is induced in both shoots and roots, especially in root tips. Both *AVP1* and *TsVPI* show almost 75 % sequence similarity, however, there exists a motif variation among them. The *TsVPI* promoter contains important *cis*-elements like light responsive elements, a motif named HD-Zip1 associated with palisade-mesophyll cells, an anaerobic induction responding element ARE, and drought responsive elements MBS, HSE, and ABRE. The type-II AVP2/AVPL1 works independent to K<sup>+</sup>, and does not express in vacuole because K<sup>+</sup> independent proton translocating activity is not observed in vacuole (Rea and Poole 1985). In contrast to expression of type-I *AVP1* in the vacuole, the *Arabidopsis* type-II *AVP2/AVPL1* shows *GUS* expression in the Golgi apparatus of trichomes and filaments (Mitsuda *et al.* 2001). Recently, a type-II H<sup>+</sup>-PPase gene (*ZmGAPP*) promoter was isolated and shows expression after dehydration, cold, and salt stresses (Hou *et al.* 2016).

### **HKT promoter**

The promoter HKT is one of the important classes of antiporters and is involved in Na<sup>+</sup> transport or Na<sup>+</sup>-K<sup>+</sup> symport in plants. On the basis of biophysical transport properties and amino acid composition in the first p-loop, the HKT transporters are classified into two subgroups; class I and class II (Maser *et al.* 2002, Platten *et al.* 2006). Members of class I HKTs (SGGG-type) consist of a serine residue instead of a signature glycine residue in the first p-loop (PA), whereas class II HKTs (GGGG-type) consist of all four glycine residues at the filter positions in the PA, PB, PC, and PD. Class I type HKTs mediate Na<sup>+</sup> transport. Class II HKTs transporters help in sequestering both Na<sup>+</sup> and K<sup>+</sup>, which depends on the external concentration of K<sup>+</sup> (Maser *et al.* 2002). It helps in Na<sup>+</sup> uptake when K<sup>+</sup> is limiting in external medium (Almeida *et al.* 2013). Albeit the *HKT* gene stands in a very important position in the class of ion transporter genes, less information is known about the role of its promoter structure and transcriptional regulation.

The *Arabidopsis AtHKT1* promoter consists of two tandem repeats of promoter 680 bp (R1 and R2) at a distal region located approximately 3.9 kb upstream of ATG start codon. The R1 and R2 repeats are separated by 34 nucleotides and act as either a repressor or an enhancer of *AtHKT1* expression (Baek *et al.* 2011). The *AtHKT1* promoter of about 250 nt consists target for the small RNA, which is highly methylated. This region is differently methylated in leaves (95.6 %) and roots (84.9 %) (Baek *et al.* 2011). The expression pattern of *HKT1*;1 and *HKT1*;2 genes in two near isogenic lines (NIL) of tomato (157-14 and 157-17) were found to be

complex because of the difference in their promoter sequences, as line 157-14 contains TATA box which is absent in line 157-17, which resulted in higher expression during salinity in the latter (Asins *et al.* 2013). High salinity has a less negative effect on root dry and fresh masses and shoot dry mass in 157-14 (a homo-zygote for the *cheesmaniae* alleles at the same genomic region containing *HKT1*;1 and *HKT1*;2) than 157-17 (a homozygote for the *lycopersicum* alleles at the genomic region containing *HKT1*;1 and *HKT1*;2) (Asins *et al.* 2013), due to presence of TATA-box. The promoter sequence of *HKT1*;1 was found more polymorphic than *HKT1*;2 in NILs 157-14 and NIL 157-17 (Table 1 Suppl.). The variation could lead to a different accumulation of Na<sup>+</sup> in roots and shoots. The concept of loading extra Na<sup>+</sup> in vascular tissue and reducing loading in the leaf mesophyll tissue was confirmed by the expression of a rice *OsHKT1*;1 promoter. The *oshkt1*;1 mutant plants showed less Na<sup>+</sup> in phloem sap and more in shoots, thus they are hypersensitive to salinity (Wang *et al.* 2015). The *OsHKT1*;1 expression is found mainly in xylem, phloem, and leaf blades because of its strong promoter activity. The *OsHKT1*;1 promoter sequence helps in isolation of the MYBc protein in Y1H assay since it has a specific binding site at -76 to -68 position (Wang *et al.* 2015). Like other promoters (*NHX1*, *SOS1*, and *H<sup>+</sup>PPase*), the *HKT* promoter of rice is also ABA-dependent. The presence of particular element and its frequency decides the expression of relevant genes at a specific time of requirement. Wild and bread wheat *HKT* promoters show complexity and variability in expressions

of the *cis*-elements, which can be correlated with their function. *Triticum monococcum* has a jasmonic acid response element, which is not present in *T. aestivum* (Babgohari *et al.* 2014). Jasmonic acid is involved in plant adaptation to biotic stresses and to some level in salt and osmotic responses. Similarly, core elements, TATA boxes, and CCAAT elements are frequent in *TmHKT1;5* and result in its higher expression as compared to

*TaHKT1;5*. Further, the frequency and position of TATA boxes suggest that a higher number of TATA boxes at position -30 are more effective during salinity stress as compared to TATA boxes present across the rest of the promoter region. Thus, a better response to environmental signals by wild wheat as compared to bread wheat can be owed to frequent and variable expressions of regulatory elements.

## Conclusions and perspectives

Regulatory sequences play responsible roles for controlling gene expressions in living systems. Therefore, it becomes critical to understand transcription control of promoters and their *cis*-elements. The stress-inducible promoters need to be tested over many generations for their sustainable expression before being employed in crop improvement *via* biotechnological applications. The constitutive expression of a protein at a stage and conditions when the protein is not desired causes in vain energy loss. The unique and versatile role of transporters in ion homeostasis draws attention towards the study of genes responsible for their spatio-temporal expression. The present review highlights that NHX promoters are active in different parts of plants except root tips probably because the root tips contain too small vacuoles for ion sequestration. The *SOS1* gene is involved in loading Na<sup>+</sup> into xylem, thereby reducing tissue Na<sup>+</sup> concentration. The antiporter promoters follow ABA-dependent

pathway as they have ABRE as the major *cis*-element. The upregulation of ion transporter genes might be controlled by basic leucine zipper transcription factors and ABRE-binding proteins (AREBs)/ABRE-binding factors (ABFs). The versatility of promoters for overexpressing abiotic stress responsive genes needs to be studied for the expression of gene of interest because most of the literature is based on reporter gene expression. Since the stress-inducible promoters have a low expression potential (Reynolds 1999), therefore it is essential to test its transcriptional efficacy for the magnitude of expression of downstream genes of the desirable function. The transgenic plants overexpressing these promoters should be tested in the field to conclude whether these promoters are strong enough to express the stress tolerant genes and can be used further in developing abiotic stress tolerant crop plants.

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