Plant salt-tolerance mechanisms

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Abstract

Crop performance is severely affected by high salt concentrations in soils. To engineer more salt-tolerant plants it is crucial to unravel the key components of the plant salt tolerance network. Here we review our understanding of the core salt-tolerance mechanisms in plants. Recent studies have shown that stress sensing and signaling components may play important roles in regulating the plant salinity stress response. We also review key Na+ transport and detoxification pathways and the impact of epigenetic chromatin modifications on salinity tolerance. In addition, we discuss the progress that has been made toward engineering salt tolerance in crops, including marker assisted selection and gene stacking techniques. We also identify key open questions that remain to be addressed in the future.

Keywords

Plant salinity tolerance; NaCl; abiotic stress; engineering of salt-tolerant plants; biotechnology

Soil salinization and its impact on plants

Soil salinization is a growing problem for agriculture worldwide. Salt accumulation in arable soils is mainly derived from irrigation water that contains trace amounts of sodium chloride (NaCl) and from seawater [1,2]. Increased soil salt concentrations decrease the ability of a plant to take up water and, once Na^+ and Cl^- are taken up in large amounts by roots, both Na^+ and Cl^- negatively affect growth by impairing metabolic processes and decreasing photosynthetic efficiency [1,3]. Thus plant salt stress can be subdivided into early-occurring

osmotic stress and slowly increasing ionic Na^+ stress [4,5] with additional Cl^- stress (reviewed in [6]). Plants enact mechanisms to mitigate osmotic stress by reducing water loss while maximizing water uptake. Furthermore, plants minimize the harmful effects of ionic Na^+ stress by exclusion of Na^+ from leaf tissues and by compartmentalization of Na^+, mainly into vacuoles [5,7]. Despite these tolerance mechanisms, salt stress decreases crop yields and is leading to continuing loss of arable land. Such losses are compounded by the additional challenge that agriculture needs to provide enough nutrition for a world population that is rapidly expanding (estimated to reach 9.6 billion by the year 2050) and which has a steadily increasing quality of life [8,9]. In this context, engineering crops to enhance salt-tolerance mechanisms is a promising approach to address these challenges. In this Review, we examine the key mechanisms that mediate plant salt tolerance and give an overview of recent literature on salinity stress sensing and signaling as well as regulation of gene expression as part of the salt stress response in plants. Furthermore, the understanding of the plant Na^+ transport network is updated and an evaluation of methods than can help with the engineering of salt- tolerant crops is made.

Sensory mechanisms of salt stress

To mount an effective response to cope with salt stress, plants have developed the ability to sense both the hyper-osmotic component and the ionic Na^+ component of the stress. These two sensory modalities are evident in that some responses to NaCl remain distinct from responses to purely osmotic stress. A high salt concentration in the soil solution produces hyperosmotic stress on roots. To date, the molecular identities of plant hyper-osmotic sensors and Na^+ sensors have remained elusive. The *Arabidopsis* (*Arabidopsis thaliana*) histidine kinase receptor protein HK1 has been shown to complement the loss of the yeast osmosensor Sln1 [10] and overexpression/loss-of-function lines exhibit drought and osmotic stress- associated phenotypes [11,12]. Plants exhibit many physiological responses to osmotic stress. However, recent research has shown that some of these responses are altered in *hk1* mutants, but others remain unaffected. Therefore, other proteins must still be perceiving the osmotic stress in the *hk1* mutant [13]. Plant hyper-osmotic sensors are likely to be closely coupled with Ca^{2+} channels given that plants exhibit a rapid rise in cytosolic Ca^{2+} levels within seconds of exposure to NaCl or mannitol [14]. This Ca^{2+} response originates within the roots [15] and occurs in several cell types [16,17]. This observation has led to speculation that hyper-osmotic stress may be sensed by a mechanically gated Ca^{2+} channel [18]. In support of a mechano-osmotic sensory modality, mutations affecting cuticle development interfere with many osmotic-induced responses, including downstream ABA production [19]. The cuticle provides structural support to the plasma membrane/cell wall and could alter the water diffusibility into the cell. Thus altering cuticle properties may affect the mechanical properties of water stress on the cell. Other second messengers are also induced by salt or hyper-osmotic stress and are linked to Ca^{2+} signaling, for example Reactive Oxygen Species (ROS) [20] (Figure 1), and *Arabidopsis* annexins have been reported to mediate both NaCl and ROS- induced Ca^{2+} responses [21,22]. Downstream of Ca^{2+}, kinases may become activated, including Calcium-dependent protein kinases (CPKs) [23,24] and calcineurin B-like proteins (CBLs) with CBL-interacting protein kinases (CIPKs) [25], which may transduce the hyper- osmotic signal to downstream protein activity

and gene transcription. Furthermore, transcription factors may be activated by Ca^{2+}/Calmodulin directly, including Calmodulin Binding Transcription Activators (CAMTAs) [26], GT-element-binding-like proteins (GTLs) [27], and MYBs [28]. Although the rapid Ca^{2+} increase is a hallmark response to osmotic stress, there may also exist Ca^{2+}-independent osmotic sensory mechanisms. Genetic identification of osmotic and Na^+ sensors is likely to be instrumental in resolving these early sensory mechanisms.

Gene regulation in roots in response to salt stress

Transcription factors are integral in linking salt-sensory pathways to many tolerance responses. Core sets of transcription factor (TF) family genes are differentially expressed in response to elevated external salinity [29], including basic leucine zipper (bZIP) [30], WRKY [31], APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) [32], MYB [33], basic helix–loop–helix (bHLH) [34] and NAC [35] families. These transcription factors, in turn, regulate the expression levels of various genes that may ultimately influence the level of salt tolerance of plants (Figure 1). To counteract the water potential decrease resulting from the osmotic component of enhanced salinity, genes relevant for inorganic ion uptake and osmolyte synthesis are up-regulated [36]. To some extent, transcriptional regulation of these stress-response genes in plants is mediated by dynamic changes in hormone biosynthesis [36,37] (Figure 1). After stress induction an initial quiescence period is followed by a growth recovery phase, both of which correlate with changes in the levels of the plant hormones abscisic acid (ABA), jasmonate (JA), gibberellic acid (GA) and brassinosteroid (BR). Mining of data from the At Gen Express consortium has revealed a secondary signaling network that controls plant growth after salt stress [36,38]. In response to high salinity, most stress-induced transcriptional changes occur approximately 3 hours after application of salt stress [36]. The expression of 5590 genes was reported to be salt-regulated in roots of *A. thaliana* seedlings [39] and fluorescence-activated cell sorting (FACS) has revealed that root cortex cells were the most transcriptionally active [36]. Furthermore, molecular analyses have revealed that the root endodermis is the pivotal cell layer in the context of lateral root development under salt stress conditions. ABA prevents lateral root elongation into surrounding media with high salt concentrations [40]. Also another recent study has shown that plants try to circumvent highly saline media by altering the direction of root growth [41]. This phenotype was defined as halotropism and is not induced by osmotic stress but by salt-triggered auxin responses [41]. Further exploring molecular mechanisms behind halotropism may improve our understanding of plant salinity tolerance strategies. Even though several key components of the plant salt stress response network have been identified in recent years, there are significant knowledge gaps that need to be filled. In addition to the above mentioned hormones, ethylene was recently shown to confer plant salt tolerance in soil grown *Arabidopsis* plants by improving the Na^+/K^+ ratio in shoots [42]. Knock-out of *ETHYLENE OVERPRODUCER1* (*ETO1*) resulted in elevated ethylene levels, which stimulated root stele reactive oxygen species (ROS) production by the respiratory burst oxidase homologue F (RBOHF). The increase in stele ROS accumulation led to reduced net Na^+ influx in roots, decreased Na^+ xylem loading and to root K^+ retention and subsequent enhanced salinity tolerance [42].

Network of Na+ and K+ transport processes

Several plant membrane transporters play key roles in resistance mechanisms to biotic and abiotic stress, particularly Na^+ and K^+ transporters for resistance to salt stress [8]. Multiple Na^+-influx pathways into roots exist. Na^+ may cross the plasma membrane via nutrient channels and transporters. Some channel and transporter mutants reduce Na^+ accumulation in plant cells, but only a few transporter mutants have been directly shown to impair Na^+ influx into roots. Calcium-permeable non-selective cation channels (NSCCs) [2,43], including the CYCLIC NUCLEOTIDE-GATED CHANNEL (CNGC) [44–46] and the GLUTAMATE-LIKE RECEPTOR (GLR) [47] families are permeable to Na^+ and, thus, represent a likely entry point of Na^+ into the cell (Figure 1). Furthermore, the rice (*Oryza sativa*) Na^+ transporter OsHKT2;1 has been shown to mediate Na^+ influx into roots under K^+-starvation [48]. In addition, AtCHX21, a cation/H^+ antiporter expressed in the root endodermis, is involved in Na^+ transport from endodermal cells to the stele [49].

Na^+ enters the xylem by efflux out of stellar cells and is subsequently transported to aerial plant tissues. Potential candidates for the control of xylem loading of Na^+ are the outward-rectifying K^+ channels KORC and NORC [50,51]. AtSKOR, an ortholog of KORC in *Arabidopsis*, is involved in xylem loading of K^+ [52]. Furthermore, class I HKT transporters have an important function in removing Na^+ from the xylem [53,54], which is discussed in more detail below.

Ion homeostasis during salinity stress requires the maintenance of stable K^+ acquisition and distribution [55] given that K^+ accumulation in plant cells balances the toxic effects of Na^+ accumulation. Overexpression of the relatively Na^+-impermeable rice K^+ transporter *OsHAK5* confers salinity tolerance on tobacco bright yellow 2 (BY2) cells [56]. Inward-rectifying K^+ channels and outward-rectifying K^+ channels have been identified as mechanisms for long-term net K^+ selective influx and K^+ efflux in plant cells, respectively [57], and may also reduce Na^+ toxicity.

Role of NHX and SOS in maintaining low Na+ in the cytoplasm

Two major factors that maintain low cytoplasmic Na^+ concentrations in plant cells are the tonoplast-localized NHX1 [58] and plasma membrane-localized SALT OVERLY SENSITIVE 1 (SOS1, also known as NHX7) [59,60] Na^+/H^+ antiporters (Figure 1). Whereas most NHXs are essential for Na^+ detoxification via sequestration of Na^+ within the vacuole, the SOS signaling pathway was reported to export Na^+ out of the cell. Constitutive overexpression of *AtNHX1* and its orthologs in *Arabidopsis* and other plant species, such as tomato (*Solanum lycopersicum*) or rice, led to increased plant salinity tolerance [61,62]. Recent studies have shown that the NHX-type proteins are also important for compartmentalization of K^+ into vacuoles and for cellular pH homeostasis [63]. Over-expression of *AtNHX1* in tomato increases vacuolar K^+ as well as K^+ transport from root to shoot [63–65], which is beneficial because enhanced intracellular $(K^+)/(Na^+)$ ratios reduce Na^+ stress. Moreover, tomato *LeNHX3* maps to a quantitative trait locus (QTL) related to leaf Na^+ accumulation [66]. Recent work demonstrates that vacuolar NHX antiporters play multiple roles in osmoregulation, cell growth, and plant development [63,65], whereas

endosomal NHX antiporters are crucial for cell growth and might be involved in vesicular trafficking, protein processing, and cargo delivery [65,67]. Studies suggest an involvement of endosomal transport proteins, including NHXs, in plant salt tolerance, by controling organelle pH and ion homeostasis [60,65,67]. NHX5 and NHX6 colocalize to Golgi and *trans*-Golgi network markers and *nhx5 nhx6* double knock-out plants were more sensitive to salinity [64]. Furthermore, loss of vacuolar H^+-ATPase (V-ATPase) function did not alter salinity tolerance in *Arabidopsis*. In contrast, reduction of V-ATPase activity in the *trans*-Golgi network/early endosome (TGN/EE) resulted in increased salt sensitivity [66]. Interestingly, over-expression of the vacuolar type I H^+-PPase AVP1 improves plant salt tolerance by mediating vacuolar Na^+ sequestration [68]. The potential of altering H^+-PPase in crops was shown by *AtAVP1* over-expression in barley (*Hordeum vulgare*), which conferred increased tolerance to salinity under greenhouse conditions but also improved shoot biomass and grain yield in a field trial with saline soil [69].

HKT as major player in plant salt tolerance and root-to-shoot Na^+ partitioning

The identification of the wheat (*Triticum aestivum*) *HKT1* (*TaHKT2;1*) gene whose product mediates Na^+/K^+ cation transport [70,71] has led to the identification and characterization of many *HKT* genes from different plant species [72]. Sequence and transport analyses have revealed at least two distinct subgroups of HKT transporters, class I and II, which in most cases mediate more Na^+-selective transport [73,74] and $Na^+–K^+$ co-transport [71], respectively. Disruption mutations in the sole *HKT* gene in *Arabidopsis*, *AtHKT1;1*, which encodes a class I transporter, cause Na^+ hypersensitivity of leaves coupled to Na^+ over-accumulation in leaves upon salinity stress, with a concomitant reduction in root [Na^+] [3,75,76]. Detailed analyses have further demonstrated a major role of AtHKT1;1, and its rice ortholog OsHKT1;5, in the removal of Na^+ from the xylem sap into the surrounding xylem parenchyma cells, thereby protecting leaves from Na^+ toxicity [53,54,76,77] (Figure 1). Targeted *AtHKT1;1* overexpression in the stele enhances salt tolerance [78]. *In vivo* electrophysiological analyses by patch clamping on root stelar cells from wild type and *Athkt1;1* mutant plants have provided evidence that AtHKT1;1 mediates passive Na^+ channel- like transport [79]. AtHKT1;1-mediated Na^+ removal from the xylem has also been suggested to stimulate indirect K^+ loading into xylem vessels via outward-rectifying K^+ channels, resulting in a high K^+/Na^+ ratio in leaves [53,54], which also counteracts Na^+ stress.

QTL analyses for Na^+ resistance have suggested that similar xylem Na^+-unloading mechanisms are essential for salt tolerance in rice and wheat (*Triticum turgidum*) [53,80]. In both cases, major salt tolerance QTL map to regions that include *HKT1;5* orthologs, encoding a more Na^+-selective class I HKT transporter [53,81]. Na^+ tolerance QTL analyses of wheat led to the identification of another strong salt tolerance QTL named *Nax1* [82]. The *Nax1* locus, which maps to the region of the *TaHKT1;4* gene that also encodes a class I HKT transporter, was found to contribute to Na^+ removal from xylem in the leaf sheath to protect leaf blades from Na^+ over-accumulation [83]. Recently, comparative analyses using salt- tolerant *indica* cultivars and a sensitive *japonica* cultivar have led to the hypothesis that

OsHKT1;4 restricts leaf sheath-to-blade Na^+ transfer in rice plants under salinity stress [84]. The recent HKT marker-assisted introduction of a wheat *HKT1;5* from an ancestral wheat relative *Triticum monococcum* into commercial durum wheat (*Triticum turgidum* ssp. *durum* var. Tamaroi) has led to significant increases in grain yields in field trials on natural saline soil in Australia [85]. Together, these findings demonstrate that xylem parenchyma-localized class I HKT transporters are an essential mechanism for plants to protect photosynthetic organs from Na^+ over-accumulation during salinity stress.

The maintenance of K^+ acquisition with the exclusion of Na^+ from leaves has been found to be highly correlated with plant salt tolerance [86]. This is consistent with studies of class I HKTs being a crucial factor in determining a high K^+/Na^+ ratio in plants [53,54]. However, interestingly and conversely, elemental profiling of shoot tissue and a genome-wide association study using more than 300 *Arabidopsis* accessions indicated that accessions with higher Na^+ accumulation in leaves tend to grow in soils that were potentially impacted by salt such as in coastal regions [87,88]. Elevated leaf Na^+ levels of those accessions were found to be due to a weak allele of *AtHKT1;1*, which causes a reduction of *AtHKT1;1* expression in roots [87,88]. It has been suggested that these naturally occurring weak *AtHKT1;1* alleles promote a certain level of leaf Na^+ accumulation but still avoid Na^+ toxicity. This mechanism may be important for osmotic adjustment during salinity stress [88]. The weak *AtHKT1;1* allele may only be beneficial in genetic backgrounds of more tolerant accessions, which may have alterations in other salt tolerance mechanisms as well (hypothetically for example enhanced vacuolar Na^+ sequestration in leaves). In order to engineer more salt tolerant plants, further knowledge is needed about synergistic effects of certain combinations of tolerance traits. A similar correlation of elevated shoot Na^+ and increased salt tolerance was reported in a study with a class II HKT transporter in barley. Enhanced Na^+ root uptake and higher Na^+ xylem sap concentrations were due to overexpression of *HvHKT2;1* [89]. In contrast to rice and wheat, in barley higher rates of Na^+ translocation to the shoot and elevated salt-inclusion might be an important tolerance strategy [89].

Regulatory mechanisms of *AtHKT1;1* expression have been recently identified. The plant hormone cytokinin negatively regulates the expression of *AtHKT1;1* in roots of *Arabidopsis* plants via the type-B response regulators ARR1 and ARR12, thus in response to salinity stress cytokinin levels decrease and *AtHKT1;1* expression increases [90]. Recently, a similar negative regulation of root *AtHKT1;1* expression through the transcription factor ABA-INSENSITIVE 4 (ABI4) has also been reported [91]. Loss-of-function mutations in the *ABI4* gene rendered soil-grown plants more salt tolerant, with lower Na^+ content levels in shoots correlating with increased *AtHKT1;1* expression. By contrast, *ABI4*-overexpression lines had lower *AtHKT1;1* expression and were salt hypersensitive [91]. Several *cis*-regulatory elements in the *AtHKT1;1* promoter region have been identified in suppressor screens of the *sos3* mutant. A transfer DNA insertion in a tandem repeat sequence, which lies more than 3.9 kb upstream of the *AtHKT1;1* ATG start codon led to a substantial reduction of *AtHKT1;1* expression in roots of *sos3*-suppressed plants [92]. This tandem repeat was suggested to be an enhancer element regulating *AtHKT1;1* expression. The transcription factors that control enhanced *AtHKT1;1* expression have yet to be determined.

A genetic screen for *soil salinity-sensitive* (*sss*) mutants led to the isolation of the *sss1-1* mutant, which showed strong Na^+ hypersensitivity in shoots [93]. The *SSS1* locus encodes the AtrbohF protein, an NADPH oxidase catalyzing ROS production [93]. Interestingly, the lack of AtrbohF in root pericycle and vascular parenchyma cells abolished salinity-induced ROS accumulation in the vasculature and caused Na^+ over-accumulation in shoots with increased Na^+ levels in xylem sap [93]. AtrbohF-mediated ROS production might enhance AtHKT1;1- mediated Na^+ unloading from the xylem sap and, thus, protect leaves from salinity stress; however, more research is required to test this model or other possible mechanisms. These results together provide support for the hypothesis that *AtHKT1;1* expression is controlled by a complex signaling network through various *cis-* and t*rans-*elements under salinity stress.

Chromatin modifications and epigenetics in salt tolerance

Chromatin modifications, referred to as epigenetic modifications, have been proposed to contribute to the adaptation potential of plants to different environmental stresses [92,94]. Several studies have shown that chromatin modification is involved in the resistance responses of plants to salt stress in the same generation as the stress occurs. In this context, hyperosmotic priming was reported for *Arabidopsis* plants that have been treated with a mild salt stress in seedling stage, followed by cultivation under control conditions [95]. In this Na^+ stress-free period, no visible differences between pre-treated plants and control plants were observed. Subsequently, after an additional salt stress application, the pre-treated group accumulated less Na^+ and thus was more tolerant. This phenotype was attributable to epigenetic histone modifications that mainly affected expression of transcription factors. Interestingly, induction of *HKT1* expression was stronger in pre-treated plants compared with controls, which might explain the altered Na^+ accumulation [95]. Another study reported that failure of cytosine methylation at a putative small RNA target site of the *AtHKT1;1* promoter subsequently led to lower gene expression, resulting in hypersensitivity to salt stress [92]. Furthermore, salinity affected the DNA methylation status of many promoters and coding regions of four transcription factors in soybean (*Glycine max*), indicating that chromatin modification of these genes could enhance plant salinity tolerance [96]. Moreover, methylation as well as expression of chromatin modifier genes varied between diverse rice genotypes and tissue organs under salt stress [97,98]. Hence, demethylation of these genes in rice roots might be an active epigenetic response [98]. Note that, to date, research on salinity stress has not unequivocally shown epigenetic inheritance of salt tolerance from one generation to the next. Another important factor is the ploidy status of a plant. Autopolyploidy has recently been shown to account for resistance to high salinity and results in more effective potassium accumulation in *Arabidopsis* [99].

Importance of osmolytes

The accumulation of organic osmolytes, such as proline, glycine betaine, sugar alcohols, polyamines and proteins from the late embryogenesis abundant (LEA) superfamily, plays a key role in maintaining the low intracellular osmotic potential of plants and in preventing the harmful effects of salinity stress [100,101] (Figure 1). The metabolic rearrangements and regulatory networks controlling osmolyte levels are therefore pivotal to understanding plant

salinity tolerance. Molecular analyses have shown that salt stress stimulates proline synthesis whereas its catabolism is enhanced during recovery [102,103]. During this recovery phase, proline may function as an essential signaling molecule and has been proposed to regulate cell proliferation, cell death and expression of stress-recovery genes [104]. In *Arabidopsis*, knockout of the *P5CS1* gene, which encodes a -1-pyrroline-5-carboxylate synthetase, impairs proline synthesis resulting in salt hypersensitivity [102]. For many years, it was presumed that proline plays a crucial role in osmotic adjustment; however, alternative suggestions are that it acts as a reactive oxygen scavenger, redox buffer, or molecular chaperone, stabilizing proteins and membrane structures under stress conditions [105,106]. Like proline, glycine betaine is an organic osmolyte synthesized by several plant families to balance the osmotic potential of intracellular ions under salinity. There is evidence that glycine betaine is a compatible solute involved in protecting major enzymes and membrane structures [107,108]. Although glycine betaine has been reported to play a vital role in maintaining the activities of ROS scavenging enzymes [109], there is no evidence showing whether or not glycine betaine has any direct ROS scavenging capability.

Additional regulators of salt-tolerant plants

Several studies have demonstrated that manipulation of stress-responsive genes can result in altered salt tolerance of plants. Engineering plants with a reduced sensitivity to salinity requires knowledge of key components of the stress response network. One recently identified example is the R2R3-MYB transcription factor AtMYB20, which down-regulates expression of type 2C serine/threonine protein phosphatases (PP2Cs) [33]. Given that PP2Cs are negative regulators of the ABA signaling pathways [110], reduction of their transcript levels may enhance plant salt tolerance. Both seedlings and adult plants of *AtMYB20*-overexpression lines were shown to be more salt tolerant than wild type, whereas plant lines with reduced *AtMYB20* expression were salt hypersensitive [33]. Another gene recently suggested to be involved in the plant salt stress response is the *UBIQUITIN-SPECIFIC PROTEASE16* (*UBP16*). UBP16-deficient plants accumulate higher levels of Na^+ in leaf tissue and are salt hypersensitive at the seedling and adult stages [111]. Furthermore, *UBP16* expression is NaCl-induced and the UBP16 stabilizes plasma membrane Na^+/H^+ antiporter activity and SERINE HYDROXYMETHYLTRANSFERASE1 (SHM1) by de-ubiquitylation and thereby prevents protein degradation by the 26S proteasome [111]. Taken together, these results suggest that UBP16 may be an important regulator of sodium transport processes.

Engineering of salt-tolerant plants

An example of gene manipulation for salt tolerance is the salt stress-induced bZIP transcription factor bZIP24, which induces expression of several stress-response genes in *Arabidopsis* [29,30]. RNA interference-mediated knock-down of *bZIP24* expression in *Arabidopsis* results in increased plant salt tolerance. This can to some extent be explained because *AtHKT1;1* is one of the targets down-regulated either directly or indirectly by bZIP24 [30]. Originally, bZIP24 was discovered in a comparison of transcript regulation in *Arabidopsis* and in the halotolerant species sweet alyssum (*Lobularia maritima*) [112]. This

exemplifies the use of halophilic model species and comparative genomics in uncovering novel salt-tolerance mechanisms.

Given that *AtHKT1;1* expression is crucial for determining leaf Na^+ levels and, hence, salt tolerance, the molecular mechanisms regulating *AtHKT1;1* expression and activity could be an important point of action for the engineering of Na^+-resistant crops [85]. However, besides the negative regulators already mentioned, little is known about the positive regulators of *AtHKT1;1*.

In addition to studies that have been performed using the model plant *Arabidopsis*, studies in crops have revealed insights into plant salinity tolerance mechanisms [113]. The rice transcription factor *SALT-RESPONSIVE ERF1* (*SERF1*) has recently been identified as an enhancer of the ROS-activated MAP KINASE cascade during salt stress [114]. *SERF1* expression is induced in roots by high salinity and *SERF1*-deficient rice plants have been shown to lack expression of salt stress-induced tolerance genes [114]. Furthermore, hydroponically grown three- to four-week-old *serf1* mutants are salt sensitive, whereas *SERF1*-overxpression lines exhibit enhanced tolerance. This is, at least partially, because the Na^+/K^+ ratios in the leaves of *serf1* were significantly increased compared with wild-type plants [114].

Marker-assisted selection is a promising breeding tool

Improving yield performance of staple crops is an ultimate objective of plant breeding programs [8,115]. Given that obtaining salt-tolerant crops using conventional breeding methods takes a long time, alternative approaches are being considered in parallel. Traditionally, crops were outcrossed to genetically diverse germplasms and were then selected based on their phenotype as evaluated in the field. This process is now streamlined using QTL analyses coupled with marker-assisted selection (MAS) [115,116]. MAS is an approach that requires the linkage of a quantitative trait with a genetic marker that is polymorphic between parental lines [115]. Hence the essential basis for successful breeding with MAS is an in-depth knowledge of genetic traits and variability within the desired plant species [115]. One example is *Saltol*, a favorable QTL identified in rice that is responsible for the bulk of genetic variation in ion uptake under saline conditions [115,117]. Given that other genome regions have been shown to play major roles in salt tolerance as well, molecular markers and next-generation sequencing are likely to be crucial in helping to guide future breeding plans [96].

MAS clearly has the potential of contributing to the development of more salt-tolerant crops. A major bottleneck remains the selection of appropriate markers (i.e. key players in the context of salinity tolerance). Many studies are attempting to improve plant salt tolerance by genetic manipulation of certain genes; however, some of these genes might not have sufficient impact to improve crop viability significantly in highly saline environments. It has been proposed that enhancing plant stress tolerance is practicable by manipulating only one or a few main components of the regulatory gene network instead of engineering several molecular mechanisms [29]. Besides conventional breeding methods, stacking of traits (also known as pyramiding) is a promising approach based on the introduction of several

beneficial genes to improve plant performance. However, this approach is limited by independent segregation of traits, which complicates breeding strategies. An emerging method to circumvent this issue involves the use of trait landing pads, whereby engineered sequence- specific nucleases, such as zinc-finger nucleases, are used to target multiple transgenes to the same locus [118]. While so far only Zinc-finger nucleases have been used in practice for generating trait landing pads, it is expected that the rapidly emerging CRISPR/Cas method will further facilitate targeted insertion of promoters and genes of interest [119 and references therein].

Concluding remarks and outlook

As described above, great leaps and bounds toward understanding plant salt stress responses and tolerance mechanisms have been achieved in the past 15 years. However, many challenges still lie ahead. For example, the regulation of gene expression and signaling cascades that regulate many Na^+ transporters remain to be elucidated. Furthermore, it remains to be determined which of the transport processes reviewed here could be combined to enhance plant performance. Ultimately, both molecular breeding and advanced biotechnology methods should help scientists to develop crops with enhanced salt tolerance. In this context, relevant genes for enhancing salinity tolerance that could be combined are Na^+ transporters, such as HKTs and NHXs, ROS scavengers and other traits that have been shown to play major roles in salt homeostasis and that positively influence the capability of the plant to deal with elevated salinity.

Acknowledgments

References

1. Flowers TJ, Yeo AR. Breeding for salinity resistance in crop plants: where next? Aust J Plant Physiol. 1995; 22:875–884.

2. Tester M, Davenport R. Na^+ tolerance and Na^+ transport in higher plants. Ann. Bot. 2003; 91:503–527. [PubMed: 12646496]

3. Mäser P, et al. Altered shoot/root Na^+ distribution and bifurcating salt sensitivity in *Arabidopsis* by genetic disruption of the Na^+ transporter AtHKT1. FEBS Lett. 2002; 531:157–161. [PubMed: 12417304]

4. Sahi C, et al. Beyond osmolytes and transporters: novel plant salt-stress tolerance-related genes from transcriptional profiling data. Physiol. Plant. 2006; 127:1–9.

5. Munns R, Tester M. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 2008; 59:651–681. [PubMed: 18444910]

6. Teakle NL, Tyerman SD. Mechanisms of Cl(−) transport contributing to salt tolerance. Plant Cell Environ. 2010; 33:566–589. [PubMed: 19895402]

7. Blumwald E. Sodium transport and salt tolerance in plants. Curr. Opin. Cell Biol. 2000; 12:431–434. [PubMed: 10873827]

8. Schroeder JI, et al. Using membrane transporters to improve crops for sustainable food production. Nature. 2013; 497:60–66. [PubMed: 23636397]

9. United Nations, Department of Economic and Social Affairs, Population Division. World Population Prospects: The 2012 Revision, Highlights and Advance Tables. 2013. http://esa.un.org/wpp/Documentation/publications.htm

10. Urao T, et al. A transmembrane hybrid-type histidine kinase in Arabidopsis functions as an osmosensor. Plant Cell. 1999; 11:1743–1754. [PubMed: 10488240]

11. Tran LS, et al. Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in *Arabidopsis* . Proc. Natl. Acad. Sci. USA. 2007; 104:20623–20638. [PubMed: 18077346]

12. Wohlbach DJ, et al. Analysis of the Arabidopsis histidine kinase ATHK1 reveals a connection between vegetative osmotic stress sensing and seed maturation. Plant Cell. 2008; 20:1101–1117. [PubMed: 18441212]

13. Kumar MN, et al. Role of the putative osmosensor Arabidopsis *histidine kinase1* in dehydration avoidance and low-water-potential response. Plant Physiol. 2013; 161:942–953. [PubMed: 23184230]

14. Knight H, et al. Calcium signalling in Arabidopsis thaliana responding to drought and salinity. Plant J. 1997; 12:1067–1078. [PubMed: 9418048]

15. Tracy FE, et al. NaCl-induced changes in cytosolic free Ca^{2+} in *Arabidopsis thaliana* are heterogeneous and modified by external ionic composition. Plant Cell Environ. 2008; 31:1063–1073. [PubMed: 18419736]

16. Kiegle E, et al. Cell-type-specific calcium responses to drought, salt and cold in the *Arabidopsis* root. Plant J. 2000; 23:267–278. [PubMed: 10929120]

17. Marti MC, et al. Cell- and stimulus type-specific intracellular free Ca^{2+} signals in Arabidopsis. Plant Physiol. 2013; 163:625–634. [PubMed: 24027243]

18. Kurusu T, et al. Plant mechanosensing and Ca^{2+} transport. Trends Plant Sci. 2013; 18:227–233. [PubMed: 23291244]

19. Wang ZY, et al. The plant cuticle is required for osmotic stress regulation of abscisic acid biosynthesis and osmotic stress tolerance in *Arabidopsis* . Plant Cell. 2011; 23:1971–1984. [PubMed: 21610183]

20. Jiang Z, et al. Relationship between $NaCl^-$ and H_2O_2-induced cytosolic Ca2+ increases in response to stress in *Arabidopsis* . PLoS One. 2013; 8:e76130. [PubMed: 24124535]

21. Laohavisit A, et al. *Arabidopsis* annexin1 mediates the radical-activated plasma membrane Ca^{2+}- and K^+-permeable conductance in root cells. Plant Cell. 2012; 24:1522–1533. [PubMed: 22523205]

22. Laohavisit A, et al. Salinity-induced calcium signaling and root adaptation in Arabidopsis require the calcium regulatory protein annexin1. Plant Physiol. 2013; 163:253–262. [PubMed: 23886625]

23. Harmon AC, et al. CDPKs - a kinase for every Ca^{2+} signal? Trends Plant Sci. 2000; 5:154–159. [PubMed: 10740296]

24. Boudsocq M, Sheen J. CDPKs in immune and stress signaling. Trends Plant Sci. 2013; 18:30–40. [PubMed: 22974587]

25. Weinl S, Kudla J. The CBL-CIPK Ca^{2+}-decoding signaling network: function and perspectives. New Phytol. 2008; 179:675–686. [PubMed: 18507772]

26. Pandey N, et al. CAMTA 1 regulates drought responses in *Arabidopsis thaliana* . BMC Genomics. 2013; 14:216. [PubMed: 23547968]

27. Weng H, et al. Poplar GTL1 is a Ca^{2+}/calmodulin-binding transcription factor that functions in plant water use efficiency and drought tolerance. PLoS One. 2012; 7:e32925. [PubMed: 22396800]

28. Yoo JH, et al. Direct interaction of a divergent CaM isoform and the transcription factor, MYB2, enhances salt tolerance in *Arabidopsis* . J Biol. Chem. 2005; 280:3697–3706. [PubMed: 15569682]

29. Golldack D, et al. Plant tolerance to drought and salinity: stress regulating transcription factors and their functional significance in the cellular transcriptional network. Plant Cell Rep. 2011; 30:1383–1391. [PubMed: 21476089]

30. Yang O, et al. The *Arabidopsis* basic leucine zipper transcription factor *AtbZIP24* regulates complex transcriptional networks involved in abiotic stress resistance. Gene. 2009; 436:45–55. [PubMed: 19248824]

31. Jiang Y, Deyholos MK. Functional characterization of Arabidopsis NaCl-inducible *WRKY25* and *WRKY33* transcription factors in abiotic stresses. Plant Mol. Biol. 2009; 69:91–105. [PubMed: 18839316]

32. Kasuga M, et al. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nat. Biotechnol. 1999; 17:287–291. [PubMed: 10096298]

33. Cui MH, et al. An Arabidopsis R2R3-MYB transcription factor, AtMYB20, negatively regulates type 2C serine/threonine protein phosphatases to enhance salt tolerance. FEBS Lett. 2013; 587:1773–1778. [PubMed: 23660402]

34. Jiang Y, et al. Functional characterization of the Arabidopsis *bHLH92* transcription factor in abiotic stress. Mol. Genet. Genomics. 2009; 282:503–516. [PubMed: 19760256]

35. Tran LS, et al. Isolation and functional analysis of *Arabidopsis* stress-inducible NAC transcription factors that bind to a drought-responsive cis-element in the early responsive to dehydration stress 1 promoter. Plant Cell. 2004; 16:2481–2498. [PubMed: 15319476]

36. Geng Y, et al. A spatio-temporal understanding of growth regulation during the salt stress response in *Arabidopsis* . Plant Cell. 2013; 25:2132–2154. [PubMed: 23898029]

37. Dinneny JR, et al. Cell identity mediates the response of *Arabidopsis* roots to abiotic stress. Science. 2008; 320:942–945. [PubMed: 18436742]

38. Kilian J, et al. The At Gen Express global stress expression data set: protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses. Plant J. 2007; 50:347–363. [PubMed: 17376166]

39. Chen WQ, et al. Expression profile matrix of Arabidopsis transcription factor genes suggests their putative functions in response to environmental stresses. Plant Cell. 2002; 14:559–574. [PubMed: 11910004]

40. Duan L, et al. Endodermal ABA signaling promotes lateral root quiescence during salt stress in *Arabidopsis* seedlings. Plant Cell. 2013; 25:324–341. [PubMed: 23341337]

41. Galvan-Ampudia CS, et al. Halotropism is a response of plant roots to avoid a saline environment. Curr. Biol. 2013; 23:2044–2050. [PubMed: 24094855]

42. Jiang C, et al. An Arabidopsis soil-salinity-tolerance mutation confers ethylene-mediated enhancement of sodium/potassium homeostasis. Plant Cell. 2013; 25:3535–3352. [PubMed: 24064768]

43. Tyerman SD, Skerrett IM. Root ion channels and salinity. Sci. Hort. 1999; 78:175–235.

44. Hua BG, et al. Plants do it differently. A new basis for potassium/sodium selectivity in the pore of an ion channel. Plant Physiol. 2003; 132:1353–1361. [PubMed: 12857817]

45. Gobert A, et al. *Arabidopsis thaliana* cyclic nucleotide gated channel 3 forms a non-selective ion transporter involved in germination and cation transport. J Exp. Bot. 2006; 57:791–800. [PubMed: 16449377]

46. Guo KM, et al. The cyclic nucleotide-gated channel, AtCNGC10, influences salt tolerance in *Arabidopsis* . Physiol. Plant. 2008; 134:499–507. [PubMed: 18823330]

47. Tapken D, Hollmann M. *Arabidopsis thaliana* glutamate receptor ion channel function demonstrated by ion pore transplantation. J. Mol. Biol. 2008; 383:36–48. [PubMed: 18625242]

48. Horie T, et al. Rice OsHKT2;1 transporter mediates large Na^+ influx component into K^+-starved roots for growth. EMBO J. 2007; 26:3003–3014. [PubMed: 17541409]

49. Hall D, et al. Functional analysis of CHX21: a putative sodium transporter in *Arabidopsis* . J. Exp. Bot. 2006; 57:1201–1210. [PubMed: 16513816]

50. de Boer AH, Wegner LH. Regulatory mechanisms of ion channels in xylem parenchyma cells. J. Exp. Bot. 1997; 48:441–449. Spec No, [PubMed: 21245223]

51. Wegner LH, de Boer AH. Properties of two outward-rectifying channels in root xylem parenchyma cells suggest a role in K^+ homeostasis and long-distance signaling. Plant. Physiol. 1997; 115:1707–1719. [PubMed: 12223889]

52. Gaymard F, et al. Identification and disruption of a plant shaker-like outward channel involved in K^+ release into the xylem sap. Cell. 1998; 94:647–655. [PubMed: 9741629]

53. Ren ZH, et al. A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nat. Genet. 2005; 37:1141–1146. [PubMed: 16155566]

54. Sunarpi, et al. Enhanced salt tolerance mediated by AtHKT1 transporter- induced Na unloading from xylem vessels to xylem parenchyma cells. Plant J. 2005; 44:928–938. [PubMed: 16359386]

55. Schroeder JI, et al. Perspectives on the physiology and structure of inward- rectifying K^+ channels in higher plants: biophysical implications for K^+ uptake. Annu. Rev. Biophys. Biomol. Struct. 1994; 23:441–471. [PubMed: 7919789]

56. Horie T, et al. Rice sodium-insensitive potassium transporter, OsHAK5, confers increased salt tolerance in tobacco BY2 cells. J. Biosci. Bioeng. 2011; 111:346–356. [PubMed: 21084222]

57. Schroeder JI, et al. Voltage dependence of K channels in guard-cell protoplasts. Proc Natl. Acad. Sci. USA. 1987; 84:4108–4112. [PubMed: 16593851]

58. Blumwald E, Poole RJ. Na/H Antiport in Isolated Tonoplast Vesicles from Storage Tissue of Beta vulgaris. Plant Physiol. 1985; 78:163–167. [PubMed: 16664191]

59. Qiu QS, et al. Regulation of SOS1, a plasma membrane Na^+/H^+ exchanger in *Arabidopsis thaliana* by SOS2 and SOS3. Proc. Natl. Acad. Sci. USA. 2002; 99:8436–8441. [PubMed: 12034882]

60. Yamaguchi T, et al. Sodium transport system in plant cells. Front. Plant Sci. 2013; 4:410. [PubMed: 24146669]

61. Apse MP, et al. Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in *Arabidopsis* . Science. 1999; 285:1256–1258. [PubMed: 10455050]

62. Zhang H-X, Blumwald E. Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. Nature Biotechnol. 2001; 19:765–768. [PubMed: 11479571]

63. Barragan V, et al. Ion exchangers NHX1 and NHX2 mediate active potassium uptake into vacuoles to regulate cell turgor and stomatal function in *Arabidopsis* . Plant Cell. 2012; 24:1127–1142. [PubMed: 22438021]

64. Leidi EO, et al. The AtNHX1 exchanger mediates potassium compartmentation in vacuoles of transgenic tomato. Plant J. 2010; 61:495–506. [PubMed: 19912566]

65. Bassil E, et al. The *Arabidopsis* intracellular Na^+/H^+ antiporters NHX5 and NHX6 are endosome associated and necessary for plant growth and development. Plant Cell. 2011; 23:224–239. [PubMed: 21278129]

66. Villalta I, et al. Genetic analysis of Na^+ and K^+ concentrations in leaf and stem as physiological components of salt tolerance in Tomato. Theor. Appl. Genet. 2008; 116:869–880. [PubMed: 18251001]

67. Krebs M, et al. Arabidopsis V-ATPase activity at the tonoplast is required for efficient nutrient storage but not for sodium accumulation. Proc. Natl. Acad. Sci. USA. 2010; 107:3251–3256. [PubMed: 20133698]

68. Undurraga SF, et al. Arabidopsis sodium dependent and independent phenotypes triggered by H^+-PPase up-regulation are SOS1 dependent. Plant Sci. 2012; 183:96–105. [PubMed: 22195582]

69. Schilling RK, et al. Expression of the *Arabidopsis* vacuolar H^+-pyrophosphatase gene (*AVP1*) improves the shoot biomass of transgenic barley and increases grain yield in a saline field. Plant Biotechnol J. 2013:1–9.

70. Schachtman DP, Schroeder JI. Structure and transport mechanism of a high-affinity potassium uptake transporter from higher plants. Nature. 1994; 370:655–658. [PubMed: 8065452]

71. Rubio F, et al. Sodium-driven potassium uptake by the plant potassium transporter HKT1 and mutations conferring salt tolerance. Science. 1995; 270:1660–1663. [PubMed: 7502075]

72. Horie T, et al. HKT transporter-mediated salinity resistance mechanisms in *Arabidopsis* and monocot crop plants. Trends Plant Sci. 2009; 14:660–668. [PubMed: 19783197]

73. Uozumi N, et al. The *Arabidopsis HKT1* gene homolog mediates inward Na^+ currents in *Xenopus laevis* oocytes and Na^+ uptake in *Saccharomyces cerevisiae* . Plant Physiol. 2000; 122:1249–1259. [PubMed: 10759522]

74. Mäser P, et al. Glycine residues in potassium channel-like selectivity filters determine potassium selectivity in four-loop-per-subunit HKT transporters from plants. Proc. Natl. Acad. Sci. USA. 2002; 99:6428–6433. [PubMed: 11959905]

75. Berthomieu P, et al. Functional analysis of *AtHKT1* in *Arabidopsis* shows that Na^+ recirculation by the phloem is crucial for salt tolerance. Embo J. 2003; 22:2004–2014. [PubMed: 12727868]

76. Horie T, et al. Calcium regulation of sodium hypersensitivities of *sos3* and *athkt1* mutants. Plant Cell Physiol. 2006; 47:622–633. [PubMed: 16540484]

77. Davenport RJ, et al. The Na^+ transporter AtHKT1;1 controls retrieval of Na^+ from the xylem in *Arabidopsis* . Plant Cell Environ. 2007; 30:497–507. [PubMed: 17324235]

78. Moller IS, et al. Shoot Na^+ exclusion and increased salinity tolerance engineered by cell type-specific alteration of Na^+ transport in *Arabidopsis* . Plant Cell. 2009; 21:2163–2178. [PubMed: 19584143]

79. Xue S, et al. AtHKT1;1 mediates nernstian sodium channel transport properties in *Arabidopsis* root stelar cells. PLoS One. 2011; 6:e24725. [PubMed: 21931830]

80. James RA, et al. Physiological characterization of two genes for Na^+ exclusion in durum wheat, *Nax1* and *Nax2*. Plant Physiol. 2006; 142:1537–1547. [PubMed: 17028150]

81. Byrt CS, et al. HKT1;5-like cation transporters linked to Na^+ exclusion loci in wheat, *Nax2* and *Kna1* . Plant Physiol. 2007; 143:1918–1928. [PubMed: 17322337]

82. Munns R, et al. Genetic control of sodium exclusion in durum wheat. Aust J Agr. Res. 2003; 54:627–635.

83. Huang S, et al. A sodium transporter (HKT7) is a candidate for *Nax1*, a gene for salt tolerance in durum wheat. Plant Physiol. 2006; 142:1718–1727. [PubMed: 17071645]

84. Cotsaftis O, et al. A two-staged model of Na^+ exclusion in rice explained by 3D modeling of HKT transporters and alternative splicing. PLoS One. 2012; 7:e39865. [PubMed: 22808069]

85. Munns R, et al. Wheat grain yield on saline soils is improved by an ancestral Na^+ transporter gene. Nature Biotech. 2012; 30:360–364.

86. Hauser F, Horie T. A conserved primary salt tolerance mechanism mediated by HKT transporters: a mechanism for sodium exclusion and maintenance of high K^+/Na^+ ratio in leaves during salinity stress. Plant Cell Environ. 2010; 33:552–565. [PubMed: 19895406]

87. Rus A, et al. Natural variants of At *HKT1* enhance Na^+ accumulation in two wild populations of *Arabidopsis* . PLoS Genet. 2006; 2:e210. [PubMed: 17140289]

88. Baxter I, et al. A coastal cline in sodium accumulation in *Arabidopsis thaliana* is driven by natural variation of the sodium transporter AtHKT1;1. PLoS Genet. 2010; 6:e1001193. [PubMed: 21085628]

89. Mian A, et al. Over-expression of an Na^+-and K^+-permeable HKT transporter in barley improves salt tolerance. Plant J. 2011; 68:468–479. [PubMed: 21749504]

90. Mason MG, et al. Type-B response regulators ARR1 and ARR12 regulate expression of *AtHKT1;1* and accumulation of sodium in *Arabidopsis* shoots. Plant J. 2010; 64:753–763. [PubMed: 21105923]

91. Shkolnik-Inbar D, et al. *ABI4* downregulates expression of the sodium transporter *HKT;1* in Arabidopsis roots and affects salt tolerance. Plant J. 2013; 73:993–1005. [PubMed: 23240817]

92. Baek D, et al. Regulated *AtHKT1* gene expression by a distal enhancer element and DNA methylation in the promoter plays an important role in salt tolerance. Plant Cell Physiol. 2011; 52:149–161. [PubMed: 21097475]

93. Jiang C, et al. ROS-mediated vascular homeostatic control of root-to-shoot soil Na delivery in *Arabidopsis* . EMBO J. 2012; 31:4359–4370. [PubMed: 23064146]

94. Zhu JK. Epigenome sequencing comes of age. Cell. 2008; 133:395–397. [PubMed: 18455978]

95. Sani E, et al. Hyperosmotic priming of *Arabidopsis* seedlings establishes a long-term somatic memory accompanied by specific changes of the epigenome. Genome Biol. 2013; 14:R59. [PubMed: 23767915]

96. Song Y, et al. The dynamic changes of DNA methylation and histone modifications of salt responsive transcription factor genes in soybean. PLoS One. 2012; 7:e41274. [PubMed: 22815985]

97. Karan R, et al. Salt stress induced variation in DNA methylation pattern and its influence on gene expression in contrasting rice genotypes. PLoS One. 2012; 7:e40203. [PubMed: 22761959]

98. Wang W, et al. DNA methylation changes detected by methylation-sensitive amplified polymorphism in two contrasting rice genotypes under salt stress. J. Genet. Genomics. 2011; 38:419–424. [PubMed: 21930101]

99. Chao DY, et al. Polyploids exhibit higher potassium uptake and salinity tolerance in *Arabidopsis* . Science. 2013; 341:658–659. [PubMed: 23887874]

100. Tarczynski MC, et al. Stress protection of transgenic tobacco by production of the osmolyte mannitol. Science. 1993; 259:508–510. [PubMed: 17734171]

101. Verslues PE, et al. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. Plant J. 2006; 45:523–539. [PubMed: 16441347]

102. Szekely G, et al. Duplicated *P5CS* genes of Arabidopsis play distinct roles in stress regulation and developmental control of proline biosynthesis. Plant J. 2008; 53:11–28. [PubMed: 17971042]

103. Sharma S, Verslues PE. Mechanisms independent of abscisic acid (ABA) or proline feedback have a predominant role in transcriptional regulation of proline metabolism during low water potential and stress recovery. Plant Cell Environ. 2010; 33:1838–1851. [PubMed: 20545884]

104. Szabados L, Savoure A. Proline: a multifunctional amino acid. Trends Plant Sci. 2010; 15:89–97. [PubMed: 20036181]

105. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 2007; 59:206–216.

106. Verbruggen N, Hermans C. Proline accumulation in plants: a review. Amino Acids. 2008; 35:753–759. [PubMed: 18379856]

107. Guinn EJ, et al. Quantifying why urea is a protein denaturant, whereas glycine betaine is a protein stabilizer. Proc. Natl. Acad. Sci. USA. 2011; 108:16932–16937. [PubMed: 21930943]

108. Raza SH, et al. Glycinebetaine-induced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. Environ. Exp. Bot. 2007; 60:368–376.

109. Chen TH, Murata N. Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. Plant Cell Environ. 2011; 34:1–20. [PubMed: 20946588]

110. Merlot S, et al. The ABI1 and ABI2 protein phosphatases 2C act in a negative feedback regulatory loop of the abscisic acid signalling pathway. Plant J. 2001; 25:295–303. [PubMed: 11208021]

111. Zhou H, et al. UBIQUITIN-SPECIFIC PROTEASE16 modulates salt tolerance in *Arabidopsis* by regulating Na^+/H^+ antiport activity and serine hydroxymethyltransferase stability. Plant Cell. 2012; 24:5106–5122. [PubMed: 23232097]

112. Popova OV, et al. Differential transcript regulation in *Arabidopsis thaliana* and the halotolerant *Lobularia maritima* indicates genes with potential function in plant salt adaptation. Gene. 2008; 423:142–148. [PubMed: 18703123]

113. Yamaguchi T, Blumwald E. Developing salt-tolerant crop plants: challenges and opportunities. Trends Plant Sci. 2005; 10:615–620. [PubMed: 16280254]

114. Schmidt R, et al. Salt-responsive ERF1 regulates reactive oxygen species-dependent signaling during the initial response to salt stress in rice. Plant Cell. 2013; 25:2115–2131. [PubMed: 23800963]

115. Ashraf M, Foolad MR. Crop breeding for salt tolerance in the era of molecular markers and marker-assisted selection. Plant Breeding. 2013; 132:10–20.

116. Collins NC, et al. Quantitative trait loci and crop performance under abiotic stress: where do we stand? Plant Physiol. 2008; 147:469–486. [PubMed: 18524878]

117. Gregorio GB, et al. Progress in breeding for salinity tolerance and associated abiotic stresses in rice. Field Crops Res. 2002; 76:91–101.

118. Ainley WM, et al. Trait stacking via targeted genome editing. Plant Biotechnol. J. 2013; 11:1126–1134. [PubMed: 23953646]

119. Belhaj K, et al. Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. Plant Methods. 2013; 9

Highlights

- Recent major advances have been made in identifying plant salt tolerance mechanisms

- Potential targets for improvement are uptake, sensing, signaling and detoxification

- Uptake and detoxification are mediated by membrane-bound transporters and channels

- Tolerance can be engineered by combining this knowledge with novel genetic techniques
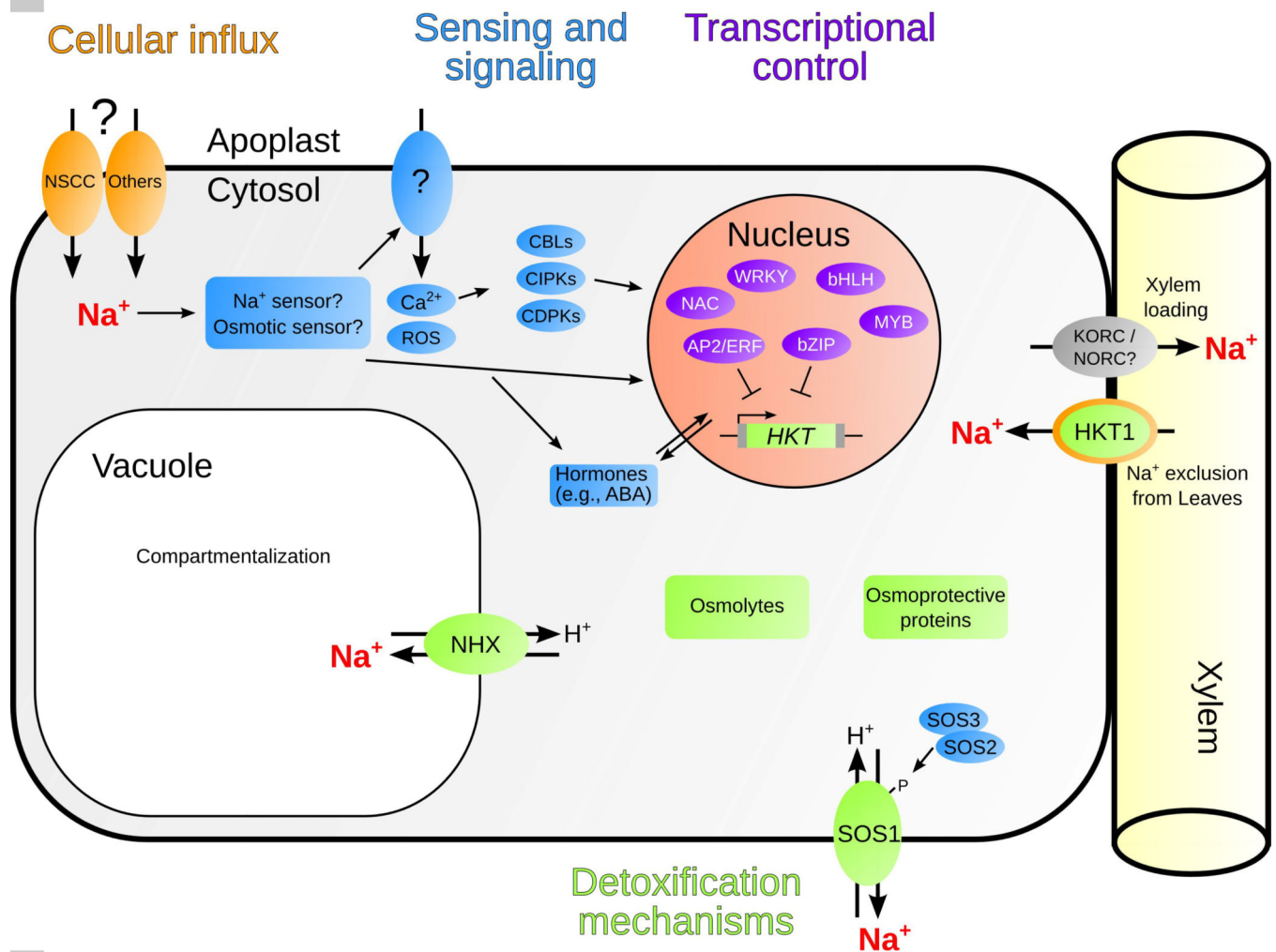
Figure 1.
Overview of cellular Na^+ transport mechanisms and important components of the salt stress response network in plant root cells. Na^+ (depicted in red) enters the cell via non- selective cation channels (NSCCs) and other, as yet largely unknown membrane transporters (cellular Na^+ influx mechanisms highlighted with orange). Inside the cell, Na^+ is sensed by an as yet unidentified sensory mechanism. At the next step, Ca^{2+}, ROS and hormone signaling cascades are activated. CBLs, CIPKs and CDPKs are part of the Ca^{2+} signaling pathway (sensing and signaling components highlighted with blue), which can alter the global transcriptional profile of the plant (transcription factor families in the nucleus depicted in purple; an AP2/ERF and a bZIP transcription factor that negatively regulate *HKT* gene expression are shown as an example). Ultimately these early signaling pathways result in expression and activation of cellular detoxification mechanisms, including HKT, NHX and the SOS Na^+ transport mechanisms as well as osmotic protection strategies (cellular detoxification mechanisms highlighted with light green). Furthermore, the Na^+ distribution in the plant is regulated in a tissue-specific manner by unloading of Na^+ from the xylem.