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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

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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 that 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 *hkl* mutants, but others remain unaffected. Therefore, other proteins must still be perceiving the osmotic stress in the *hkl* mutant [13]. Plant hyper-osmotic sensors are likely to be closely coupled with Ca²⁺ channels given that plants exhibit a rapid rise in cytosolic Ca²⁺ levels within seconds of exposure to NaCl or mannitol [14]. This Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ signaling, for example Reactive Oxygen Species (ROS) [20] (Figure 1), and *Arabidopsis* annexins have been reported to mediate both NaCl and ROS-induced Ca²⁺ responses [21,22]. Downstream of Ca²⁺, 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]. Overexpression 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 controlling 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 *trans*-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 δ -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*-overexpression 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.

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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

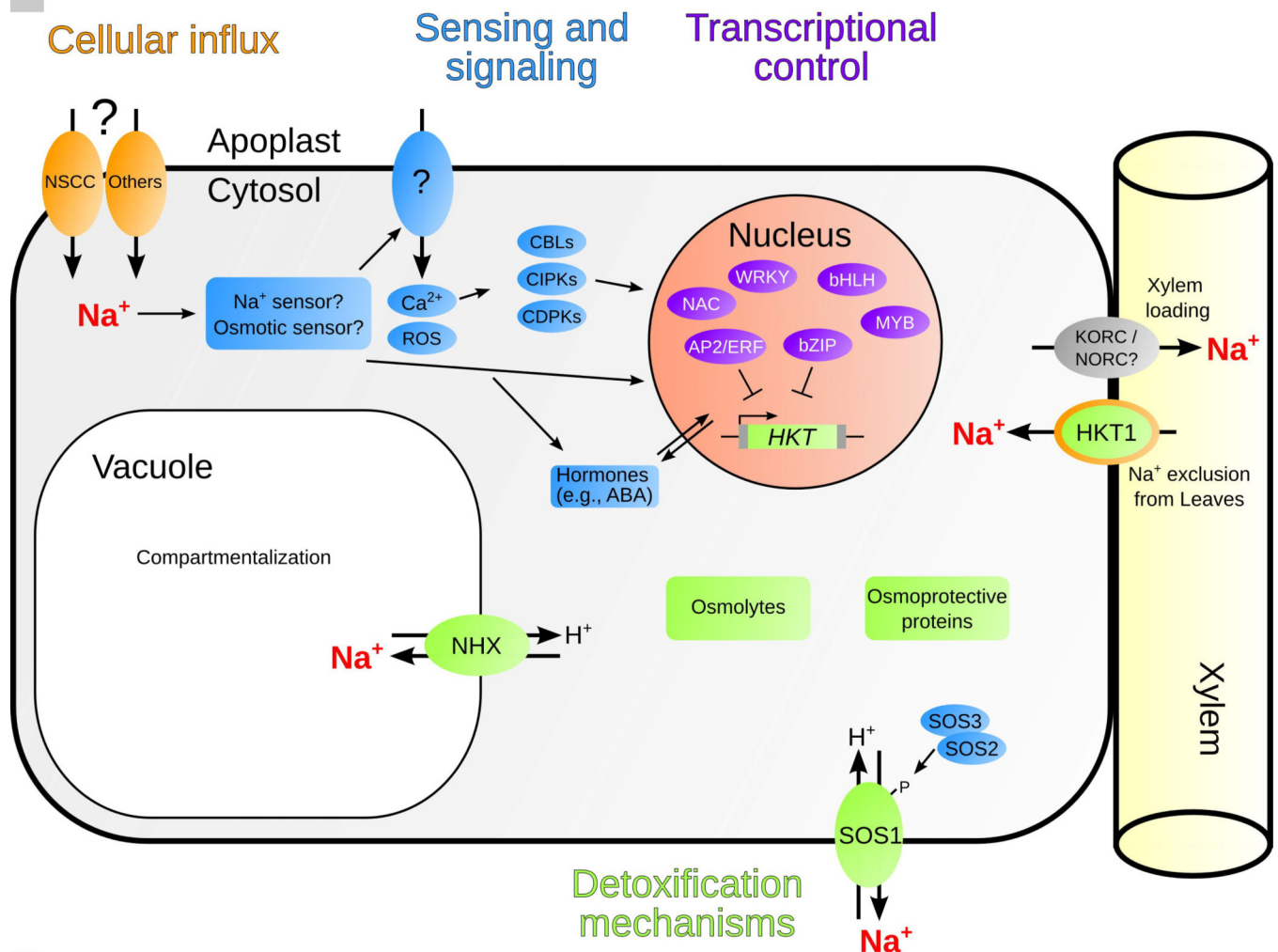


Figure 1.

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, Ca²⁺, ROS and hormone signaling cascades are activated. CBLs, CIPKs and CDPKs are part of the Ca²⁺ 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.