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Genomics, Physiology, and Molecular Breeding Approaches for Improving Salt Tolerance

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Abstract

Salt stress reduces land and water productivity and contributes to poverty and food insecurity. Increased salinization caused by human practices and climate change is progressively reducing agriculture productivity despite escalating calls for more food. Plant responses to salt stress are well understood, involving numerous critical processes that are each controlled by multiple genes. Knowledge of the critical mechanisms controlling salt uptake and exclusion from functioning tissues, signaling of salt stress, and the arsenal of protective metabolites is advancing. However, little progress has been made in developing salt-tolerant varieties of crop species using standard (but slow) breeding approaches. The genetic diversity available within cultivated crops and their wild relatives provides rich sources for trait and gene discovery that has yet to be sufficiently utilized. Transforming this knowledge into modern approaches using genomics and molecular tools for precision breeding will accelerate the development of tolerant cultivars and help sustain food production.

Contents

INTRODUCTION.....	406
MECHANISMS ASSOCIATED WITH PLANT RESPONSES	
TO SALT STRESS.....	407
Membrane Na ⁺ Transport Systems Functioning in Avoidance of Na ⁺ Toxicity....	407
Channels That Mediate Invasive Na ⁺ Influx into Roots During Salt Stress.....	414
Channels and Transporters That Affect K ⁺ Homeostasis and Salt Tolerance.....	415
Cl ⁻ Transport and Homeostasis During Salt Stress.....	416
GENETIC DIVERSITY IN SALT TOLERANCE IN MAJOR CROPS.....	417
PROGRESS IN BREEDING SALT-TOLERANT CEREAL CROPS.....	418
Progress in Developing Salt-Tolerant Rice.....	418
Progress in Developing Salt-Tolerant Wheat	423
Progress in Developing Salt-Tolerant Barley.....	424
Exploiting Wild Relatives of Crop Plants	425
PROSPECTS FOR PRECISION BREEDING	
OF SALT-TOLERANT CROPS.....	425

INTRODUCTION

More than 6% of the world's total land area is affected by excess salts, and a considerable proportion of cultivated land is becoming saline because of human acts of land clearing, excessive irrigation, and other factors related to climate change, including salt intrusion into coastal zones resulting from sea-level rise and an increased number of storms (132). Excessive salt in soils has harmful effects on plant growth and productivity that can substantially reduce food production, particularly for major crops such as rice and wheat (101, 129). The adverse effects of salt stress in reducing plant growth and productivity have two main causes: (a) Osmotic stress reduces water uptake by roots and causes internal dehydration, with effects similar to those caused by drought, and (b) direct accumulation of salts leads to ion toxicity that disturbs metabolic processes, particularly in photosynthetic cells. Injury occurs when salts loaded in transpiring tissues surpass the ability of plants to extrude them from the cytoplasm, which largely relies on the mechanisms of Na⁺ extrusion from roots, Na⁺ unloading from the xylem, and Na⁺ sequestration in vacuoles (68, 101).

The majority of agricultural products, including major cereals, are produced from glycophytes, which are highly sensitive to salt stress. By contrast, halophytes benefit from high-salt conditions and can grow in soil or a growth medium that has substantial amounts of salts. Halophytes can be tolerant to salt concentrations as high as those of seawater (or even higher) owing to several different systems, including efficient sequestration of cytosolic Na⁺ to vacuoles and salt bladders, accumulation of compatible solutes, greater cytosolic K⁺ retention, efficient control of xylem ion loading and unloading, succulence, and salt-secreting glands (41, 125, 126). Because of their stable performance even with seawater irrigation, some halophytic species, such as quinoa (*Chenopodium quinoa*), that produce highly nutritious seeds are thought to be ideal for saline agriculture (125). Although part of the salt-tolerance mechanisms in halophytes depends on the distinct structure of tissues or organs, many other aspects are comparable to those of glycophytes. Therefore, learning about those mechanisms in halophytes is of great importance in order to develop salt-tolerant glycophytic crops (125).

Salt-tolerant cultivars of glycophytes have several advantages for food production and the environment. They generally require less leaching to remove salts from topsoil; can increase and sustain productivity on saline soils, especially in coastal zones; help expand agricultural lands to less favorable areas; and can be used to reclaim saline and sodic soils (68, 99). Despite the considerable progress in our understanding of plant responses to salt stress, breeding of salt-tolerant crops and cultivars has been slow, with only limited progress in crops such as rice and wheat (54, 99, 129). This review covers progress in research on the metabolic and molecular processes involved in salt-stress responses in plants, including salt uptake, transport, and accumulation in plant tissues and how these processes are regulated. We attempt to highlight gateways that evolved in plants to control salt accumulation in plant tissue and reduce its impacts on growth and productivity. We also provide examples of how conventional methods and modern genomic tools are being used to develop salt-tolerant crops. The wealth of knowledge of genes and metabolic networks associated with the critical processes required to maintain ion homeostasis in active plant tissues will help fast-track breeding of tolerant varieties.

MECHANISMS ASSOCIATED WITH PLANT RESPONSES TO SALT STRESS

Over the past few decades, substantial knowledge has accumulated about the responses and mechanisms associated with salt tolerance in plants. Moreover, our understanding of the pathways and genes involved in the physiological and biochemical processes affected by salt overaccumulation in the rhizosphere and plant tissues has also improved. The pathways associated with salt uptake by roots, regulation of its movement through roots to shoots, and accumulation in active tissues and cells have been investigated in depth. Furthermore, the roles of metabolites involved in signaling, osmoregulation, and protection of cellular metabolism have been documented. Here, we provide a brief summary of these essential traits and their genetic control mechanisms. Future breeding programs could rely on genetic variation in these mechanisms for use in crop improvement by combining genes and functional alleles that regulate salt accumulation in active plant tissues.

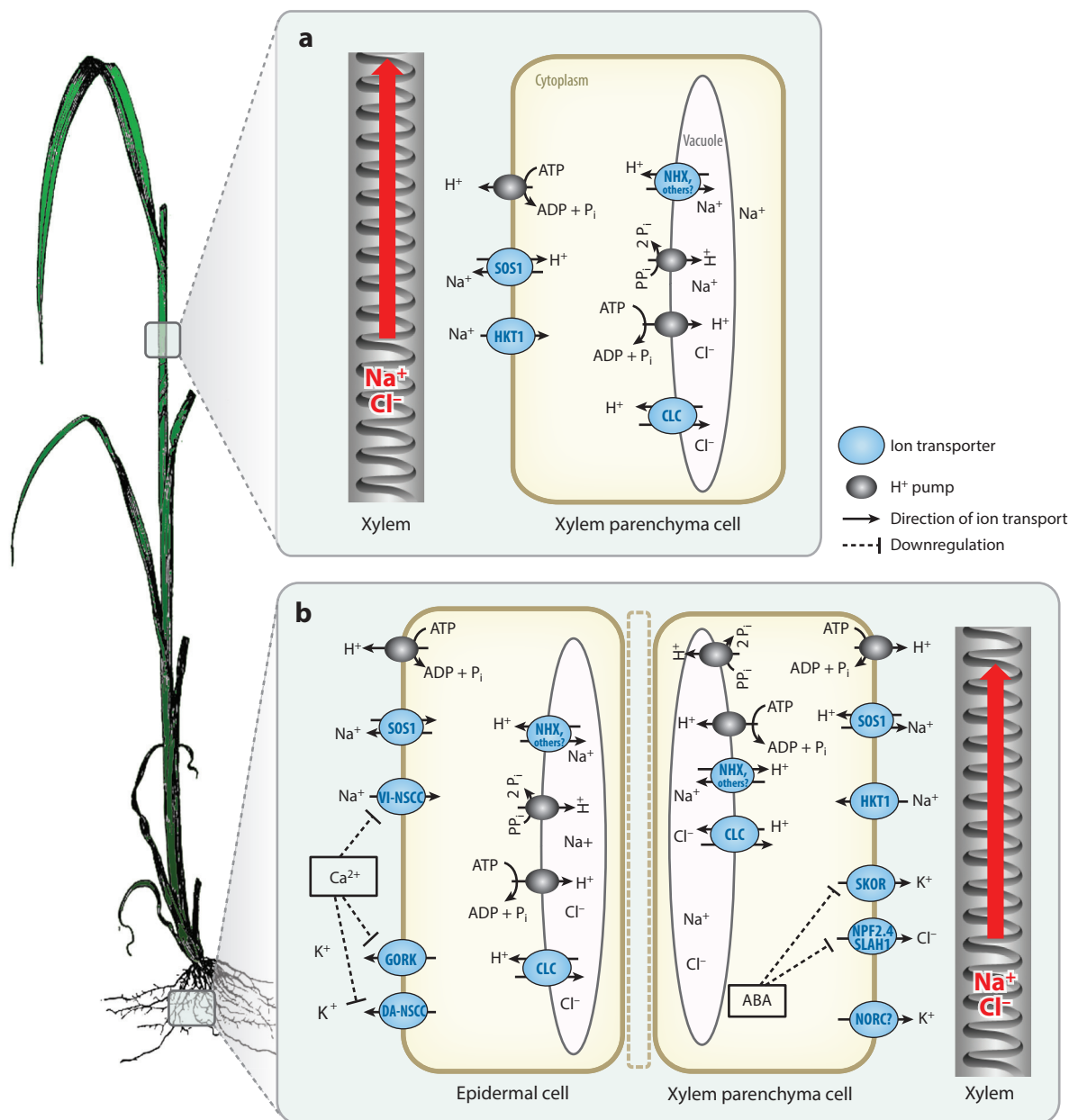
Membrane Na^+ Transport Systems Functioning in Avoidance of Na^+ Toxicity

Na^+ is a major toxic element that can accumulate to high levels in salt-affected soils. During salt stress, glycophytes generally prefer to maintain low Na^+ in shoots, and mechanisms of shoot Na^+ exclusion are crucial for the survival of these plants in salt-affected environments (31, 57, 98, 101). Therefore, features of Na^+ transport that correlate with survival in saline environments and the proteins mediating such processes have been targets of intensive study. Three essential Na^+ transport systems have been extensively studied: Na^+ efflux from roots to the rhizosphere, Na^+ sequestration in vacuoles, and Na^+ loading and unloading at the xylem, all of which contribute to lowering shoot Na^+ .

Na^+ efflux from roots to the rhizosphere. Na^+ exclusion from the cytosol under salt stress requires thermodynamically active transport. In plants, this energy-consuming Na^+ transport process is coupled with the activity of H^+ -ATPases that establish a H^+ electrochemical potential gradient across the plasma membrane as the driving force of Na^+ efflux (14). This process, including its molecular aspects, has been well characterized since the discovery of the salt overly sensitive (SOS) signaling pathway and the key plasma membrane Na^+/H^+ antiporter SOS1 (56, 174) (Figure 1). Detailed characterization of the *atsos1* mutant provided evidence that SOS1-dependent ion transport in the root tip and xylem parenchyma cells is indispensable for salt tolerance in

Arabidopsis (130). Assessment of the influence of histone modification on salt tolerance using various chemicals revealed that the class-I histone deacetylase regulates several salt-stress-related genes, including *SOS1* in *Arabidopsis* (119). These results suggested that *SOS1* expression is negatively regulated by histone deacetylation under salt stress and that inhibition of the histone-deacetylase-mediated process increases salt tolerance in *Arabidopsis* through the predominant contribution of *SOS1*-dependent Na^+ exclusion (119).

SOS1 orthologs were identified in rice (*Oryza sativa*), bread wheat (*Triticum aestivum*), durum wheat (*Triticum turgidum* ssp. *durum*), and tomato (*Solanum lycopersicum*) as *OsSOS1*, *TaSOS1*,



TdSOS1, and *SISOS1*, respectively, and were found to function in Na⁺ efflux when expressed in a yeast mutant strain (40, 85, 104, 163). *SISOS1*-silenced tomato plants exhibited salt-hypersensitive phenotypes, and the Na⁺/H⁺ exchange activity in their plasma membrane vesicles was largely reduced (104). Furthermore, expression of *OsSOS1* and a truncated *TdSOS1* in the *atsos1-1* mutant complemented the salt hypersensitivity of the mutant (39, 85). These results suggest that the SOS1-mediated system for salt tolerance is also preserved in these crops. Indeed, electrophysiological analyses using the noninvasive microelectrode ion flux estimation (MIFE) technique on the net Na⁺ flux in roots of bread and durum wheat cultivars demonstrated that the active Na⁺ efflux system energized by the P-type H⁺-ATPase is present in the epidermis of wheat roots and that such activity was highest in a salt-tolerant cultivar under salt stress (29). The use of amiloride, an inhibitor of Na⁺/H⁺ exchange activity, in MIFE experiments on a salt-tolerant wheat cultivar and *Arabidopsis* plants, including the *sos1* mutant, further indicated that the Na⁺ efflux system in wheat roots is similar to the one mediated by *AtSOS1* in *Arabidopsis* (29). More recently, Gao et al. (47) performed a comparative analysis of the products of four *SOS1* genes from ornamental *Chrysanthemum* species. *SOS1* genes from three salt-tolerant relatives encode SOS1 transporters that exhibit superior Na⁺ exclusion when expressed in a yeast mutant and in a salt-sensitive commercial variety (47). These results suggest that the SOS1-dependent Na⁺ exclusion system is widely conserved among plant species and has a large impact on the nature of salt tolerance in plant species.

Na⁺ sequestration in vacuoles. The active sequestration of Na⁺ in vacuoles by tonoplast-localized Na⁺/H⁺ antiporters maintains low Na⁺ in the cytosol under salt stress (14). The finding that *Arabidopsis thaliana* Na⁺/H⁺ EXCHANGER 1 (*AtNHX1*) overexpression increases salt tolerance in transgenic plants indicates that vacuolar Na⁺ sequestration is carried out by tonoplast Na⁺/H⁺ exchangers such as *AtNHX1* in *Arabidopsis* (2, 14) (**Figure 1**). Overexpression of *NHX1* transporters in several plant species, including rice, wheat, and maize, enhanced their salt tolerance, further supporting the notion that *NHX* transporters mediate a major part of the Na⁺/H⁺ exchange activity in the tonoplast (165).

In addition, biochemical analyses of vacuoles from leaves of the *Arabidopsis nhx1* mutant and reconstituted liposomes that encompass *AtNHX1* purified from *AtNHX1*-expressing yeast cells

Microelectrode ion flux estimation (MIFE):

an electrophysiological method that uses ion-selective microelectrodes, by which net membrane transport processes across the membranes of living organisms can be studied

Figure 1

Schematic summary of mechanisms of ion transport and homeostasis in plants during salt stress. (a) Partial ion homeostasis mechanisms for toxic elements, Na⁺, and Cl[−] in the leaf sheath. Some class-I HKTs mediate Na⁺ unloading from the xylem to prevent Na⁺ transfer into young leaf blades (60, 99, 101). SOS1, by contrast, has been proposed to mediate Na⁺ loading into the xylem (130). Tonoplast-localized *NHX*s mediate Na⁺ sequestration into vacuoles (2, 14). The possible involvement of other transporters in vacuole Na⁺ sequestration has also been suggested (7). Cl[−] sequestration into the vacuole may be mediated by a member of the *CLC* family (155–157). (b) Partial ion homeostasis mechanisms for toxic (Na⁺ and Cl[−]) and beneficial (K⁺ and Ca²⁺) elements in the root. An increase in the concentration of extracellular Ca²⁺ ameliorates damage resulting from ion toxicity by inhibiting NSCC-dependent toxic Na⁺ influx into roots and K⁺ efflux mediated by K⁺-permeable channels (32, 128). In roots, GORK corresponds to the K⁺ out channel that mediates stress-induced K⁺ efflux (32, 34). In the xylem parenchyma cell, SKOR is the K⁺ out channel that mediates K⁺ efflux for K⁺ loading into the xylem (50, 151). K⁺ loading also occurs via the NORC (57, 60). ABA is involved in direct or indirect downregulation of K⁺ and Cl[−] loading into the xylem (78, 109, 151). The functions of HKT1 and SOS1 in the plasma membrane and of *NHX* and *CLC* in the tonoplast are the same as in panel a. Abbreviations: ABA, abscisic acid; *CLC*, chloride channel; DA-NSCC, depolarization-activated nonselective cation channel; GORK, guard cell outward-rectifying K⁺ channel; HKT, high-affinity K⁺ transporter; *NHX*, Na⁺/H⁺ exchanger; NORC, nonselective outward-rectifying channel; NPT, nitrate transporter 1/peptide transporter; NSCC, nonselective cation channel; P_i, inorganic phosphate; PP_i, inorganic pyrophosphate; SKOR, stelar K⁺ outward rectifier; SLAH, slow-type anion channel-associated homolog; SOS, salt overly sensitive; VI-NSCC, voltage-insensitive nonselective cation channel.

demonstrated that this mutant exhibits robust K^+/H^+ exchange activity along with Na^+/H^+ exchange activity, implying that AtNHX1 is a dominant Na^+ , K^+/H^+ antiporter (3, 150). Out of six AtNHXs, AtNHX1 and AtNHX2 are the predominant paralogs that are functionally redundant and expressed throughout the tissues in *Arabidopsis* (7, 9, 116). Two independent studies using *nhx1 nhx2* double mutants revealed that mutating both of these *NHX* genes leads to strong phenotypes, predominantly for K^+ transport and homeostasis (7, 9). Bassil et al. (9) reported severe defects in growth and development of double mutants in both vegetative and reproductive stages. They further demonstrated that the lack of NHX1 and NHX2 transporters brought about more acidification and approximately 70% reductions in K^+ concentration in the vacuoles of the double mutants. Barragán et al. (7) subsequently demonstrated that *nhx1 nhx2* double mutants, the tonoplast vesicles of which exhibited significant attenuation in both Na^+/H^+ and K^+/H^+ exchange activity, displayed hypersensitivity to external K^+ and osmotic stress caused by impairments in osmoregulation and turgor generation, which the authors attributed to a reduced ability to retain K^+ in the vacuole. Interestingly, the *nhx1 nhx2* double mutants did not exhibit increased salt sensitivity in these studies. Instead, the addition of 30-mM Na^+ partially rescued the growth defect of double mutants, whereas the addition of 30-mM K^+ exacerbated the growth (9). Furthermore, *AtNHX1* overexpression in tomato improved K^+ but not Na^+ compartmentalization in vacuoles, which increased salt tolerance (77). The results described above indicate that further investigation is needed to clarify the role of NHX1 and NHX2 proteins in Na^+ sequestration in vacuoles during salt stress (7). In addition to NHXs, other transporters could mediate Na^+/H^+ exchange in the tonoplast (**Figure 1**).

In *Arabidopsis*, NHX1–6 can be divided into two subgroups: NHX1–4, which are located in the vacuole, and NHX5 and NHX6, which belong to a clade of endosomal antiporters (106). Studies of the latter subgroup in *Arabidopsis* provided evidence that AtNHX5 and AtNHX6 are functionally redundant and localize to both the Golgi apparatus and *trans*-Golgi network as well as the prevacuolar compartment (8, 113). Phenotypic evaluation of *nhx5 nhx6* double mutants indicated that dysfunctional mutations in both *NHX* genes cause defects in vacuolar trafficking that severely reduce plant growth and development and render the plants Na^+ hypersensitive (8). Reguera et al. (113) recently demonstrated that endomembrane pH homeostasis mediated by NHX5 and NHX6 transporters is crucial for the association of vacuolar sorting receptors and their cargo proteins in *Arabidopsis*. The authors hypothesized that ion transport mediated by AtNHX5 and AtNHX6 might be crucial for maintaining the process of protein trafficking from the Golgi apparatus and *trans*-Golgi network to the vacuoles and that missorting of vacuolar transporters, such as the Na^+/H^+ exchangers that are required for Na^+ sequestration, could account for the Na^+ hypersensitivity of the double mutants. Interestingly, two independent studies of endosomal NHX proteins (tomato LeNHX2 in tomato and wheat TaNHX2 in alfalfa plants) revealed that overexpression of these proteins leads to better K^+ homeostasis, including enhanced K^+ absorption during salt stress when there is a sufficient K^+ supply, thereby conferring salt tolerance on the transgenic plants (65, 164, 172). Altogether, these results suggest that the principal physiological role of the NHX family might be the regulation of cellular pH and K^+ homeostasis.

More recently, by fine mapping a quantitative trait locus (QTL) for salt tolerance in soybean, Guan et al. (55) isolated *Glycine max* salt tolerance–associated gene on chromosome 3 (*GmSALT3*), the product of which localizes to the endoplasmic reticulum. This gene encodes a membrane transporter that belongs to the cation/ H^+ exchanger family and is strongly expressed in the root xylem and phloem of the salt-tolerant cultivar during salt stress (55). A defect in *GmSALT3* function was associated with salt hypersensitivity and Na^+ overaccumulation in leaves and stems in soybean, but the underlying *GmSALT3*-mediated molecular mechanism has not been elucidated.

Apart from the more complex recent view of the molecular identity of Na^+/H^+ antiporters that function in vacuolar Na^+ sequestration, evidence for the importance of Na^+ sequestration in the salt tolerance of crop plants is accumulating. A study of salt-tolerant and salt-sensitive bread wheat cultivars suggested that superior Na^+ sequestration in roots is an important factor for salt tolerance, along with better SOS1-like root Na^+ efflux activity (29). Moreover, Wu et al. (160) found that higher Na^+ sequestration activity preferentially occurs in cells of the mature root zone in salt-tolerant wheat cultivars. Evidence also came from studies of the salt-tolerant S111-9 variety of soybean, a somatic hybrid descended from a salt-tolerant wild soybean variety and a salt-sensitive cultivar (58, 167). He et al. (58) found that S111-9 plants exhibit greater NAD(P)H-dependent cyclic electron flow activity and greater ATP accumulation in leaves in the presence of 150-mM NaCl, which in turn enhance the vacuolar Na^+ sequestration driven by the increased vacuolar H^+ -ATPase activity and, therefore, increase salt tolerance.

Na^+ loading and unloading at the xylem. To minimize the transfer and accumulation of Na^+ in shoots and, eventually, in leaves during salt stress, the control of net Na^+ loading into the xylem is as important as Na^+/H^+ exchanger-mediated Na^+ exclusion from roots and sequestration in root vacuoles. Na^+ unloading (retrieval) from the xylem is an ideal mechanism to achieve low Na^+ concentrations in the xylem sap under salt stress. Several independent research groups have shown that this process indeed occurs in several plant species, including *Arabidopsis*, rice, and wheat, as an essential salt-tolerance mechanism mediated by some high-affinity K^+ transporter (HKT) proteins (57, 60, 101) (**Figure 1**). The first HKT protein was identified as a high-affinity K^+ uptake system in plants that also transports Na^+ (124), but HKT proteins were later found to belong to two subfamilies: those that can transport K^+ and Na^+ (118, 124) and the later-isolated group-1 HKT transporters, which were determined to be more Na^+ -selective transporters (149). Thus, HKT transporters can be classified into at least two subgroups. The first group, the HKT1s, mediate preferred Na^+ -selective transport and are involved in reducing Na^+ accumulation in leaves by mediating xylem Na^+ unloading (86, 114, 134). The second group, the HKT2s, can mediate Na^+ - K^+ cotransport, and at typical high Na^+ concentrations (>10 mM), they also mediate Na^+ transport (60). The group-1 transporter AtHKT1;1, a product of the single *HKT* gene in *Arabidopsis*, functions in Na^+ unloading from the xylem and confers salt tolerance (30, 95, 134).

A QTL analysis of salt tolerance in rice revealed that OsHKT1;5 [shoot K^+ content 1 (SKC1)] has a physiological function similar to that of AtHKT1;1, reducing Na^+ and increasing K^+ levels in shoots during salt stress (114, 134). Further evidence came from studies based on salt-tolerance QTLs in wheat. Two strong loci named Na^+ EXCLUSION 1 (*Nax1*) and *Nax2*, which restrict Na^+ accumulation in shoots under salt stress, were identified using a durum wheat genotype that retains genetic elements introgressed from the ancestral wheat species *Triticum monococcum*. Both loci control Na^+ unloading from the xylem in roots and thus the rate of Na^+ transfer from roots to shoots (72). *Nax1* also governs net Na^+ loading into the xylem in leaf sheaths, which keeps Na^+ accumulation in leaf blades low (72). Fine-mapping analyses have led to identification of the causal genes: *TmHKT1;4* for *Nax1* (63) and *TmHKT1;5* for *Nax2* (16). In fact, the introgression of *TmHKT1;5-A* into a commercial salt-sensitive durum wheat cultivar indeed improved Na^+ exclusion from leaves, which brought about approximately 25% increases in wheat yield in salt-affected fields (100; for reviews with additional details, see 60, 98, 101). As for the *Nax1* locus, two *HKT1;4* genes, *TmHKT1;4-A1* and *TmHKT1;4-A2*, were located in the candidate region (63). Recent analyses by Tounsi et al. (146) revealed that the level of *TmHKT1;4-A2* transcripts is more robust in roots, and *TmHKT1;4-A2* expressed in *Xenopus laevis* oocytes showed superior Na^+ transport activity, with threefold-lower affinity and threefold-higher maximal inward conductance for Na^+ compared with those of *TmHKT1;4-A1*. These results support Huang et al.'s (63) hypothesis

that *TmHKT1;4-A2* is the dominant factor for *Nax1*-dependent Na^+ exclusion. Furthermore, a study by Byrt et al. (17) strongly suggested that a major salt-tolerance locus of bread wheat, *K⁺/Na⁺ DISCRIMINATION 1* (*Kna1*)—which has long been sought as the locus controlling the high cytosolic K^+/Na^+ ratio in leaves during salt stress (52)—is the *TaHKT1;5-D* gene, based on their characterization of transgenic bread wheat plants that show reduced *TaHKT1;5-D* expression following RNA interference (RNAi)-mediated gene silencing.

Cotsaftis et al. (28) suggested that, in rice, the *OsHKT1;4* gene mediates Na^+ exclusion in leaf sheaths; this exclusion is more efficient in salt-tolerant *indica* genotypes than in a salt-sensitive *japonica* cultivar because of the more efficient production of the full-length *OsHKT1;4* mRNA in the alternative splicing process. RNAi-triggered reductions of *OsHKT1;4* expression in a *japonica* rice cultivar revealed that *OsHKT1;4* contributes to xylem Na^+ unloading not only in leaf sheaths but also in stems, particularly when rice plants are at their reproductive stage (136). These results suggest that the *HKT1;4* gene in rice can also function in the control of net Na^+ loading into the xylem in upper tissues to prevent excessive Na^+ transfer into leaf blades under salt stress.

Studies have also recently highlighted the impact of *HKT1;1*-mediated Na^+ transport on the mechanism of salt tolerance in rice plants. Jabnourne et al. (70) reported that *OsHKT1;1* is a unique Na^+ -selective transporter in rice that shows voltage-dependent inward-rectifying Na^+ transport, with a K_m of 76-mM Na^+ in *Xenopus* oocytes. The *osbkt1;1* null mutant is hypersensitive to Na^+ , exhibiting significant increases in Na^+ content in both xylem and phloem sap and leaf blades under salt stress (154). Based on the strong *OsHKT1;1* expression detected in the vicinity of the xylem and phloem in leaves, the authors suggested that *OsHKT1;1* may contribute to both Na^+ unloading from the xylem and Na^+ loading into the phloem in leaves for Na^+ recirculation, and phloem-mediated Na^+ recirculation from shoots to roots or from younger leaves to older leaves is another possible strategy to control the level of Na^+ in young leaves, where salt-sensitive vital photosynthetic activity is high. Berthomieu et al. (12) have indeed proposed that *AtHKT1;1*-mediated Na^+ recirculation occurs in *Arabidopsis*. A subsequent study also found a lower concentration of Na^+ in the phloem in *AtHKT1;1* knockout mutants suffering salt stress in addition to the evidence for Na^+ unloading mediated by *AtHKT1;1* (134), although this phloem phenotype may be due to an indirect effect. The involvement of *AtHKT1;1* in Na^+ recirculation needs further investigation (30).

By contrast, Takagi et al. (137) isolated the *hitomebore salt tolerant 1* (*hst1*) rice mutant, which shows increased salt tolerance, from an ethyl methanesulfonate (EMS)-treated mutant population. They found that the gene encodes a type-B response regulator, *Oryza sativa* RESPONSE REGULATOR 22 (*OsRR22*), that could function in cytokinin signaling. They also found that the expression levels of 21 genes differed significantly between the *hst1* mutant and wild-type plants, including *OsHKT1;1*, which was upregulated in the mutant. Intriguingly, Mason et al. (87) showed that *AtHKT1;1* expression is controlled by cytokinin through the type-B response regulators *Arabidopsis* RESPONSE REGULATOR 1 (*ARR1*) and *ARR12* in *Arabidopsis* roots. These independent results suggest that a cytokinin-dependent signaling pathway is involved in *HKT1*-mediated Na^+ exclusion in plants (87, 137). In addition, because *OsHKT1;1* expression was demonstrated to be under the transcriptional control of a MYB-type transcription factor, *OsMYBc* (154), *OsRR22* and *OsMYBc* could function in the same signaling pathway that is important for rice salt tolerance. Together, these reports indicate that *OsHKT1;1* is one of the key factors in *OsHKT1*-mediated Na^+ exclusion mechanisms in rice and could work coordinately with *OsHKT1;4* and *OsHKT1;5* during salt stress.

Reactive oxygen species signaling and essential Na^+ transport systems for plant salt tolerance. Reactive oxygen species (ROS) are important signaling molecules that regulate multiple biological processes, although excessive ROS accumulation causes oxidative stress (10, 147).

Hydrogen peroxide (H_2O_2) plays an important role in the regulation of K^+/Na^+ homeostasis during salt stress and in resistance to stress in callus cells of salt-tolerant *Populus euphratica* (133, 171). Furthermore, among NADPH oxidase genes that catalyze ROS production, *respiratory burst oxidase homolog C* (*RbohC*), but neither *RbohD* nor *RbohF*, was involved in establishing the stability of *SOS1* mRNA in salt-stressed *Arabidopsis* plants, suggesting ROS-mediated regulation of salt tolerance via *SOS1* (23). On the other hand, dissection of the *soil salinity sensitive 1-1* (*sss1-1*) mutant of *Arabidopsis*, which shows strong shoot hypersensitivity to Na^+ , has revealed that *AtrbohF* encoded in the *sss1* locus mediates vascular ROS accumulation in response to salt stress, which is crucial for reducing Na^+ loading into the xylem sap and thus Na^+ transfer to shoots (74). Compared with *Arabidopsis atrbohD* and *atrbohF* single mutants and wild-type plants under salt stress, *atrbohD atrbohF* double mutants exhibited a significant increase in Na^+ sensitivity and decreased K^+/Na^+ ratios at the whole-plant level, which can be partially rescued by supplemental external H_2O_2 (81). Using a mapping population of *Arabidopsis*, Roy et al. (117) identified the *Arabidopsis thaliana* *CBL-INTERACTING PROTEIN KINASE 16* (*AtCIPK16*) gene, which encodes a protein kinase that is crucial for Na^+ exclusion from shoots. Whether this trait is functionally linked to ROS-mediated control of shoot Na^+ accumulation is unknown.

Zhu et al. (177) recently reported that salt-tolerant barley (*Hordeum vulgare* L.) cultivars display a distinct feature in controlling the net Na^+ loading into the xylem under salt stress, such that xylem Na^+ content initially increases up to certain concentrations (approximately 5 mM or less, depending on the cultivar) but then starts declining after reaching the peak. By contrast, salt-sensitive barley cultivars display constant increases in the xylem Na^+ concentration. Their electrophysiological MIFE analyses of ion fluxes in the steles of wild barley, salt-tolerant barley cultivars, and salt-sensitive barley cultivars suggested that abscisic acid stimulates Na^+ loading into the xylem, with the highest degree of Na^+ loading occurring in a salt-sensitive cultivar. Their results also suggested that apoplastic H_2O_2 production increases Ca^{2+} influx into root stele cells, which could in turn affect H_2O_2 production by NADPH oxidase in stele cells. Because the *AtSOS1* gene is robustly expressed in xylem parenchyma cells and epidermal cells of the root tip region in *Arabidopsis*, *AtSOS1* can also mediate Na^+ loading into the xylem in addition to Na^+ efflux out of the cells at the root surface (130) (**Figure 1**). These findings suggest that *SOS1*-mediated Na^+ loading into the xylem and *HKT1*-mediated Na^+ unloading from the xylem could influence each other and, therefore, the salt tolerance of the plant. In fact, a study in durum wheat investigating near-isogenic lines of *Nax1* and *Nax2* indicated that both systems might interact (175). MIFE experiments indicated that the presence of introgressed *Nax1* and *Nax2* loci in a durum wheat cultivar leads to a significant reduction in the Na^+/H^+ exchanger-dependent Na^+ efflux activity in the root epidermis but an increase in Na^+ retrieval from the xylem in leaf blades and sheaths (175). Interestingly, the presence of *Nax1* and *Nax2* genes (corresponding to *TmHKT1;4-A2* and *TmHKT1;5-A*, as described above) also downregulated the expression of the *SOS1*-like gene such that the salt-sensitive background cultivar showed the highest expression of *SOS1*; by contrast, however, the *SOS1* expression level in the *Nax1 Nax2* near-isogenic line is less than one-fourth that of the salt-sensitive cultivar. These results imply that the functions of the *SOS1* and *HKT1* transporters somehow may be co-regulated and that an unknown molecular mechanism controls the balance of Na^+ efflux and loading/unloading of the xylem.

A study of the xerohalophyte *Zygophyllum xanthoxylum* suggested a more complex view of the correlation among essential systems for Na^+ and K^+ homeostasis (170). In response to salt stress, *ZxNHX*-silenced plants showed significant reductions in transcript levels of *ZxSOS1* and *ZxHKT1;1* (both of which control net xylem Na^+ loading) as well as *Zygophyllum xanthoxylum* K^+ transporter 1 (*ZxAKT1*) (predicted to mediate K^+ uptake into roots) and *Zygophyllum xanthoxylum* stelar K^+ outward rectifier (*ZxSKOR*) (which mediates K^+ efflux and loads K^+ into the xylem)

Two-electrode voltage clamp (TEVC):

an electrophysiological method by which ion flow across the membranes of large cells such as *Xenopus laevis* oocytes can be measured as an electric current

compared with wild-type plants. Further study of the coordinated interaction of essential transport systems for Na^+ and K^+ by feedback regulation during salt stress will be important in order to elucidate the overall mechanism of plant salt tolerance.

Channels That Mediate Invasive Na^+ Influx into Roots During Salt Stress

Na^+ exclusion from shoots (and, ultimately, from leaves) is a preferable strategy for many glycophytic plant species to acquire resistance to high-salt environments (57, 101). In addition to the functions of the above-mentioned essential Na^+ transport systems, the rate of unidirectional Na^+ influx into roots has a large impact on Na^+ accumulation in shoots under conditions of high soil salinity. The electrophysiological properties of Na^+ currents in the root epidermis and cortex cells and features of Na^+ influx into intact roots suggest that, in principle, toxic Na^+ influx is passively mediated by voltage-independent (or weakly voltage-dependent) nonselective cation channels (NSCCs) in plants (1, 35, 148) (**Figure 1**).

NSCCs are constitutively active, without strict dependence on membrane voltage or specific ligands. They show low selectivity among monovalent cations, with typical K^+/Na^+ selectivity ratios between 0.3 and 3 (1, 35, 151). NSCCs can be divided into three subgroups based on their voltage dependence (if any)—voltage-insensitive NSCCs (VI-NSCCs), depolarization-activated NSCCs (DA-NSCCs), and hyperpolarization-activated NSCCs (HA-NSCCs) (35)—and thus may reflect more than one channel type. Multiple observations have indicated that, of these three groups, VI-NSCCs appear to mediate a large portion of toxic Na^+ influx into roots (33, 35, 36, 83). The physiological functions of VI-NSCCs have not been established; however, nonselective transport of cations—including not only monovalent cations but also divalent ones, such as Ca^{2+} and Mg^{2+} —without dependence on the membrane voltage status can be beneficial in terms of plant nutrition. Almost all of the VI-NSCCs characterized so far are permeable to K^+ , implying that they contribute to K^+ acquisition as well as uptake of other important monovalent cations (35). Additionally, many NSCCs are permeable to Ca^{2+} , which led to the assumption that such NSCCs are relevant to Ca^{2+} nutrition and signaling in addition to Na^+ uptake during salt stress. Demidchik et al. (33) indeed found that VI-NSCC-mediated Ca^{2+} transport, which exhibits $P_{\text{Ca}}/P_{\text{Na}}$ and $P_{\text{Ca}}/P_{\text{K}}$ ratios of 0.19 and 0.14, respectively, contributes to Ca^{2+} acquisition and can be involved in the growth of *Arabidopsis* roots. However, such a flexible nutritional uptake pathway mediated by VI-NSCCs seems to be the primary entry for Na^+ into roots once the Na^+ level in the soil environment rises. Importantly, Ca^{2+} exhibits a strong blocking effect on VI-NSCC-mediated Na^+ currents and Na^+ influx into intact roots (38, 148). This phenomenon can at least partially account for the ameliorative effect of increasing external Ca^{2+} on plant salt tolerance (35, 111) (**Figure 1**).

Despite extensive research, however, the molecular identities of NSCCs have not been elucidated. The two most important candidate families are the cyclic nucleotide gated channels (CNGCs) and ionotropic glutamate receptors (GLRs) (32). CNGCs may contribute to the uptake of monovalent cations in roots; however, no direct evidence of CNGC-mediated toxic Na^+ influx during salt stress has been provided (35). Evidence has recently accumulated that some GLRs can mediate Ca^{2+} currents in pollen tubes and heterologously expressed cells (90, 138, 152). In particular, two-electrode voltage clamp (TEVC) experiments using *Xenopus* oocytes that expressed GLR1.4 from *Arabidopsis* showed permeability for monovalent cations (including Na^+) as well as robust amino acid-dependent Ca^{2+} transport (138).

More recent studies have identified two novel channels that mediate Ca^{2+} currents in response to osmotic stress in *Arabidopsis*: REDUCED HYPEROSMOLALITY-INDUCED [Ca^{2+}]_i INCREASE 1 (OSCA1) and CALCIUM PERMEABLE STRESS-GATED CATION CHANNEL 1 (CSC1) (62, 169). TEVC experiments using *Xenopus* oocytes that expressed AtCSC1

demonstrated that the transport activity of AtCSC1 is triggered by hyperosmotic shocks resulting from increases in the concentration of both mannitol and NaCl, and that AtCSC1 mediates the transport of cations such as Ca^{2+} , Na^+ , and K^+ in a nonrectifying manner with no strict voltage dependence (62). However, the interaction between the activity of GLRs and novel channels and the feature of toxic Na^+ influx into roots in plants during salt stress has not been investigated.

More recent and surprising results were reported by Byrt et al. (18). The authors discovered that the *Arabidopsis thaliana* PLASMA MEMBRANE INTRINSIC PROTEIN 2;1 (AtPIP2;1), a well-known aquaporin water channel, exhibits nonselective cation conductance in addition to the water channel activity when expressed in *X. laevis* oocytes. Interestingly, the solo expression of AtPIP2;1, but neither the solo expression of AtPIP2;2 nor the coexpression of AtPIP2;1 with AtPIP1;2, elicited Na^+ currents in *X. laevis* oocytes. Moreover, AtPIP2;1-mediated ionic conductance was inhibited by either the presence of extracellular Ca^{2+} with an IC_{50} of 0.32 mM or a low external pH with an IC_{50} of pH 6.8. Whether the feature of nonselective cation transport by AtPIP2;1 can also be observed in *Arabidopsis* plants is an important subject for future research.

Channels and Transporters That Affect K^+ Homeostasis and Salt Tolerance

In terms of the mechanism of plant salt tolerance, K^+ is just as important as Na^+ , because maintaining a high K^+/Na^+ ratio in leaves is highly correlated with the salt tolerance of glycophytes (57). K^+ efflux systems have been of major interest because of their deep relevance for K^+ retention capacity during salt stress (32, 128).

K^+ efflux from roots occurs in response to salt stress (34, 128) (**Figure 1**). Shabala & Cuin (127) found a strong correlation between K^+ retention in salt-stressed roots and salt tolerance in several plant species, including barley and wheat, highlighting the importance of cytosolic K^+ homeostasis, which has a large impact on cytosolic K^+/Na^+ ratios. Because maintaining high cytosolic K^+/Na^+ ratios in photosynthetically active mesophyll cells in leaf blades during salt stress is of vital importance, large-scale validations of K^+ retention in the leaf mesophyll and salt tolerance have been conducted using barley and wheat varieties with different levels of salt sensitivity (161, 162). In barley, varieties that can retain a higher K^+ content in leaves exhibited better K^+ retention ability (i.e., lower K^+ efflux activity) under salt stress (162). A similar assessment of wheat varieties indicated that the lower activity of salt-induced K^+ efflux from leaf mesophyll cells strongly correlates with overall salt tolerance, particularly in bread wheat compared with the more sensitive durum wheat (161). These results strongly suggest that a superior K^+ retention ability in leaf mesophyll cells can greatly contribute to maintaining high cytosolic K^+/Na^+ ratios and thus increase salt tolerance in these plants.

In terms of the mechanism of salt-induced K^+ efflux, studies have suggested that *Arabidopsis* has two K^+ -permeable channels: (a) a slowly activating, time-dependent component mediated by the K^+ efflux channel and (b) an instantaneously activating component mediated by DA-NCSSs (32, 34, 128) (**Figure 1**). External Na^+ inhibits the K^+ efflux channel, but the effect on DA-NSCCs is not significant, suggesting that DA-NSCCs make a large contribution to K^+ efflux in response to salt stress (32, 34, 128). In fact, an increase in the concentration of external Ca^{2+} prevents K^+ loss by blocking those two K^+ efflux components, which indicates that the ameliorative effects that adding Ca^{2+} has on plant salt tolerance result from the blocking of K^+ loss in addition to the inhibition of toxic Na^+ influx (32, 35, 128) (**Figure 1**). Wu et al. (161) detected the involvement of both K^+ efflux components during salt-induced K^+ efflux from wheat mesophyll cells (similar to what occurs in *Arabidopsis*), implying that NSCCs make a larger contribution to the K^+ efflux.

In rice, two members of a K^+ transporter gene family, *Oryza sativa* high-affinity K^+ (*OsHAK1*) and *OsHAK5*, are upregulated by salt stress and associated with salt tolerance, mediating K^+ uptake

and thereby maintaining high K^+/Na^+ ratios during salt stress (20, 166). In barley, genes encoding major K^+ -transporting systems, including HAK proteins, are not upregulated in response to salt stress in leaf mesophyll cells and roots, which suggests that the K^+ uptake system makes a relatively small contribution to salt tolerance compared with the K^+ efflux system in barley (15, 127). Interestingly, radio tracer efflux analysis using ^{42}K indicated that the extent of K^+ efflux is not correlated with the salt tolerance of rice during long-term salt stress (27). These results suggest that rice plants might retain distinctive tolerance mechanisms in terms of K^+ homeostasis during salt stress.

Cl⁻ Transport and Homeostasis During Salt Stress

Mechanisms of Na^+ homeostasis in response to salt stress have been extensively studied over the past few decades, as reviewed above. Cl^- is an essential micronutrient for plant growth and is involved in stabilizing the membrane potential as a counteranion and regulating turgor and pH in the cell (140). Apart from its beneficial roles in plant nutrition, Cl^- can be toxic in the cytosol when plants are grown in saline soils, where Cl^- is predominant and can accumulate in excess (101, 140). However, mechanisms of Cl^- transport and detoxification during salt stress have not been studied as much as those for Na^+ . Cl^- exclusion from shoots correlates with the salt tolerance of crop and woody plants, such as soybean, grapevine, citrus, and lotus (51, 140). A study of the impact of Na^+ and Cl^- on four barley cultivars revealed that some genotypes show a reduced photosynthetic capacity after the application of large amounts of Cl^- (139). Similarly to Na^+ detoxification, the key systems of Cl^- transport are net xylem loading, intracellular sequestration, and extrusion from roots (140). A recent comparative transcriptomic analysis of root pericycle and cortical cells from salt-stressed *Arabidopsis* plants identified a novel gene that regulates Cl^- loading from xylem parenchyma cells into xylem vessels and, thus, Cl^- accumulation in shoots (78) (**Figure 1**). This gene encodes NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER 2.4 (NPF2.4), which localizes in the plasma membrane of *Arabidopsis* cells and shows passive Cl^- selective efflux activity when expressed in *Xenopus* oocytes (78). A study by Qiu et al. (109) suggested that an additional Cl^- transporter that localizes in the plasma membrane of root stele cells, *Arabidopsis thaliana* SLOW-TYPE ANION CHANNEL–ASSOCIATED HOMOLOG 1 (AtSLAH1), contributes to controlling long-distance root-to-shoot Cl^- transport (**Figure 1**). These results suggest that the net Cl^- loading into the xylem could be controlled by multiple systems.

An analysis using *Xenopus* oocytes showed that the CATION-CHLORIDE COTRANSPORTER 1 (CCC) protein from *Arabidopsis* mediates transport of K^+ , Na^+ , and Cl^- through its function as a $Na^+:K^+:Cl^-$ cotransporter (24). Two independent transfer-DNA insertions in the *AtCCC1* gene caused dwarf organ formation and significantly higher Cl^- accumulation in shoots following the application of large amounts of Cl^- salts, which suggested that AtCCC1 plays essential roles in development and Cl^- homeostasis during salt stress (24). In particular, the results indicated that AtCCC1-mediated retrieval of Cl^- (shoot Cl^- exclusion) occurs in the plasma membrane of root stele cells (24). Recent investigation of CCC1 proteins from *Arabidopsis* and grapevine, however, revealed that both CCC1 transporters localize at the Golgi apparatus and *trans*-Golgi network in plant cells and that CCC1 is most likely not directly involved in long-distance Cl^- transport for shoot Cl^- exclusion during salt stress (59). More recently, Chen et al. (22) identified a single amino acid substitution in OsCCC1 that causes a loss of function and leads to reductions in the sizes of root and shoot cells. Dissection of *osccc1* mutant and wild-type plants, including measurements of root cell sap osmolality and ion concentration, suggested that OsCCC1-dependent ion homeostasis, in particular for Cl^- and K^+ , is necessary to maintain

cellular osmotic potential and cell elongation (22). Immunostaining of OsCCC1 in rice tissues indicated that OsCCC1 localizes to the plasma membrane (22), but determining whether this localization applies across all plants will require further investigation.

Membrane proteins that belong to the chloride channel (CLC) family have been a target of research on Cl^- transport and its involvement in the mechanisms of plant salt tolerance. Recent studies of tonoplast-localized GmCLC1 in soybean revealed that Cl^- transport mediated by GmCLC1 is dependent on cytoplasmic pH (implying that this transporter functions as a Cl^-/H^+ antiporter) and that overexpression of *GmCLC1* throughout the entire *Arabidopsis* plant and in the hairy roots of soybean plants resulted in significantly lower Cl^- accumulation in shoots during salt stress (156, 159) (**Figure 1**). In addition, constitutive overexpression of *CsCLC-c* from trifoliate orange (*Poncirus trifoliata*) in the *Arabidopsis atlc-c* mutant and of *ZmCLC-d* from maize in the *Arabidopsis atlc-d* mutant rendered the mutants more salt tolerant, with decreased Cl^- accumulation in the whole plant in comparison with nontransformants and wild-type plants (155, 157). These studies suggest that CLC-type transporters play a beneficial role in Cl^- exclusion during salt stress, presumably through Cl^- sequestration in vacuoles.

GENETIC DIVERSITY IN SALT TOLERANCE IN MAJOR CROPS

Crop species vary considerably in their tolerance of salt stress. Cereal crops (such as barley, triticale, and wheat) and other crops (such as cotton, canola, and sugar beet) are relatively more salt tolerant, whereas corn, rice, and beans are considered salt sensitive (82). Cultivated species and wild relatives, including halophytes, vary significantly in their salt tolerance (26, 44, 88, 92). However, efforts to exploit this natural variation have led only to modest improvements in a few crops because of several factors, including the complexity of salt tolerance (as reviewed above), its interactions with other agronomic traits that are important for yield and quality, and the limited understanding of the physiology and genetics of tolerance at sensitive stages.

In most crops, tolerance is developmentally regulated, several traits are involved in tolerance at each sensitive stage, and several to numerous genes are involved in each trait (44, 68, 101, 102). For example, rice is relatively tolerant during germination, tillering, grain filling, and maturity but sensitive during the seedling stage, panicle initiation, flowering, and pollination. Some barley genotypes are extremely tolerant during germination (84) but sensitive at the seedling and early vegetative stages, then become more tolerant during grain filling and maturity. For some crops, such as rice (97) and tomato (44), tolerance at the seedling stage does not correlate with tolerance during reproduction or other stages, and combining tolerance traits at all sensitive stages is necessary to develop resilient varieties. Knowledge of the specific processes that are hindered or negatively affected by salt stress at critical stages will facilitate targeting of specific genes or alleles to improve tolerance.

When developing salt-tolerant crops and varieties, crop quality is often as important as productivity under salt stress. Plants grown in saline soils accumulate higher concentrations of certain osmotically active metabolites (57, 101). For example, the total soluble solids (including sugar content) increased in crops such as melon, tomato, and sugar beet when they were grown in saline soils (93). Tomato varieties developed from the salt-tolerant relative *Lycopersicon cheesmanii* and cultivated tomato have a higher salt tolerance and accumulate more soluble solids than cultivated tomato (121). In cereals, salt stress negatively affects several aspects of grain quality. In rice, for example, salinity reduces grain filling, head rice recovery, and amylose content and increases chalkiness (112). Similarly, the grain quality of triticale (120) and wheat (45) is negatively affected by soil salinity. Little is known about how salt stress affects the quality of these economically important crops, and even less is known about genetic control and diversity in associated traits, even

though quality is as essential as yield in terms of the acceptance and adoption of new cultivars. Salt accumulation in plant tissue reduces photosynthetic carbon assimilation (96), reducing the assimilate supply to developing grains, which in turn reduces grain filling and head rice recovery. Panda et al. (105) reported an increase in transcript levels of ethylene receptors and signaling components under salt stress, together with a decrease in the transcripts of certain cell cycle regulators and the activity of enzymes associated with grain filling. However, determining whether these changes are direct consequences of salt accumulation in plant cells will require further studies.

Reliable, high-throughput phenotyping for salt tolerance in crops is necessary to determine whether there is a genetic basis for selection for overall tolerance, for dissecting specific traits associated with tolerance, and for subsequent breeding. Such protocols should carefully consider several aspects, including the stage of development when soil salinity is high, the most sensitive development stages, and weather conditions, especially temperature and relative humidity at the sites targeted by specific breeding programs, which directly affect the rate and amount of salt accumulation in plant tissue. Screening in one environment is often not a reliable way to identify suitable genotypes for a different environment. Several approaches have been developed that rely either on field hot spots or on confined environments that simulate conditions of high salinity (54) or high salinity and alkalinity (135) using hydroponics or other artificial media. Participatory evaluation of breeding lines in farmers' fields also facilitates selection and formal release of salt-tolerant varieties that incorporate local adaptation and farmers' preferences. This approach has been successfully adopted in rice (66). Nondestructive imaging techniques are being developed for high-throughput phenotyping of traits associated with salt tolerance, as has been done in rice (19) and *Arabidopsis* (6). However, these techniques are more suitable for the discovery of genes that underlie plant development and stress responses when combined with single-nucleotide polymorphism (SNP)-based genotyping tools that rely on whole genome sequences. Determining the reliability of these techniques for breeding varieties that are tolerant to salt stress in farmers' fields will require additional studies.

PROGRESS IN BREEDING SALT-TOLERANT CEREAL CROPS

The complexity of plant responses to salt stress and the apparent effects on the growth, yield, and quality of economic products substantially slowed the breeding of salt-tolerant cultivars using standard methods that rely solely on phenotypic selection. However, the existence of sufficient genetic diversity provides opportunities to develop varieties with better tolerance than existing donors when functional alleles of the genes underlying major tolerance traits are identified and combined in high-yielding backgrounds (44, 68). The intrinsic genetic diversity observed in cultivated pools of several crop species is being exploited to breed salt-tolerant varieties, and modest progress has been made in developing commercial salt-tolerant cultivars in a few crops, including rice and wheat (68, 102, 129) (**Figure 2, Table 1**). Progress in understanding the physiology and genetics of the traits underpinning tolerance is also being made in other crops, such as tomato (44) and soybean (55), paving the way for further improvements. Here, we provide examples from three important cereal crops—rice, wheat, and barley—in which the crop's responses to salt stress are relatively well understood and progress has been made in breeding salt-tolerant cultivars (particularly in rice and wheat).

Progress in Developing Salt-Tolerant Rice

Rice is grown in a wide range of ecological environments, from dry uplands to flooded soils in irrigated and rain-fed lowlands, and across a wide geographic distribution, from 50°N in northern



Figure 2

Photographs of salt-tolerant rice varieties, which are helping farmers to enhance and sustain food production in salt-affected fields. (a) The salt-tolerant CSR36 variety (*left*) and salt-sensitive local Ganga Kaveri variety (*right*) at the vegetative stage in salt-affected soil in Uttar Pradesh, India. (b) The salt-tolerant BRRI Dhan 10 variety (*right*) and salt-sensitive BRRI Dhan 28 variety (*left*) at the reproductive stage in a saline field in coastal Bangladesh. Abbreviation: BRRI, Bangladesh Rice Research Institute. Photographs courtesy of the International Rice Research Institute.

China to 35°S in South Australia and Argentina. This wide adaptation has resulted in immense genetic variability, making rice one of the most widely grown cereal crops in extreme geographic and ecological conditions. This has also made rice a good model for genetic studies to improve adaptation to adverse weather and soil conditions.

Genetic variation in responses to salt stress in rice. Rice is generally salt sensitive, with low thresholds of 1.9–3.0 decisiemens per meter (dS/m) (19–30 mM), above which growth and yield are affected (53, 82). In spite of this high sensitivity, rice is grown on a range of salt-affected soils, including saline soils in coastal tropical zones (where salinity is mostly dynamic and seasonal) and saline and alkaline/sodic soils inland (where salt stress persists throughout the season) (69). This adaptation to saline ecosystems results from rice’s ability to grow well on flooded soils, in which free salts are diluted and leached from the mostly shallow rooting zone.

The Genetic Resources Center at the International Rice Research Institute (<http://irri.org/about-us/our-organization/genetic-resources-center>) hosts more than 127,000 rice accessions collected worldwide, including approximately 4,000 wild relatives, providing a rich source of diversity. This is reflected in the considerable genetic variability in responses to salt stress that

Table 1 Examples of salt-tolerant rice varieties released recently in Bangladesh, India, and the Philippines for salt-affected areas

Name of variety	Designation of breeding line	Year of release	Country of release	Parentage	Donor	Tolerance
BRR1 Dhan 47	IR63307-4B-4-3	2007	Bangladesh	IR51511-B-B-34-B/TCCP 266-2-49-B-B-3	TCCP 266-2-49-B-B-3 (Pokkali somaclonal variant)	12-14 dS/m
CR Dhan 402	CR2095-181-1	2009	India	Mashuri/Ormundakan	Mashuri	5-8 dS/m
CR Dhan 403	CR2096-71-2	2009	India	Mashuri/Chakrakanda	Mashuri	5-8 dS/m
NDRK 5088	IR262-43-8-1	2009	India	M40-431-24-114/Jaya	Jaya	6-11 dS/m
BINA Dhan 8	IR66946-3R-149-1-1	2010	Bangladesh	IR29/Pokkali B	Pokkali B	12-14 dS/m
BRR1 Dhan 53	BR5778-156-1-3-HR14	2010	Bangladesh	BR10 (BR51-46-5)/BR23/BR847-76-1-1	BR23	8 dS/m
BRR1 Dhan 54	BR5999-82-3-2-HR1	2010	Bangladesh	BR1185-2B-16-1/BR548-128-1-1-3	BR1185-2B-16-1	8 dS/m
BRR1 Dhan 55	IR73678-6-9-B (AS996)	2011	Bangladesh	IR73382-121/IR64	IR73382-121 (IR64/Lua Ma)	8-10 dS/M
CSR 43	CSR-89IR-8	2011	India	KDML105/IR4630-22-5-1-3/IR20925-33-3-1-28	IR4630-22-5-1-3	7 dS/m
NSIC Rc290 (Salinas 6)	PR28377-AC97-54	2011	Philippines	PSB Rc50/PSB Rc10	PSB Rc50 (derived from IR5657-33-2/IR4630-22-2-5-1-3)	Moderate
NSIC Rc294 (Salinas 8)	PR28378-AC96-36	2011	Philippines	PSB Rc50/IR64	PSB Rc50	Moderate
NSIC Rc296 (Salinas 9)	IR71896-3R-8-3-1	2011	Philippines	IR55182-3B-14-3-2/IR65195-3B-13-2-3	IR55182-3B-14-3-2 [derived from IR9884-54-3-1E-P1 (PSB Rc48)/Cheriviruppu]	Moderate
BINA Dhan 10	IR64197-3B-14-2	2012	Bangladesh	IR42598-B-B-B-12/Nona Bokra	Nona Bokra	12 dS/m
CR Dhan 405 (Luna Sankhi)	IR72046-B-R-3-3-1	2012	India	IR31142-14-1-1-3-2/IR71350	IR31142-14-1-1-3-2 (G. Heenati/IR4563-52-1-3-6/IR13292-5-3)	5-8 dS/m
CR Dhan 406 (Luna Barial)	Jaya/Lumishree	2012	India	Jaya/Lumishree	Jaya	5-8 dS/m

(Continued)

Table 1 (Continued)

Name of variety	Designation of breeding line	Year of release	Country of release	Parentage	Donor	Tolerance
BRRI Dhan 61	BR7105-4R-2	2013	Bangladesh	IR64419-3B-4-3/BR29	IR64419-3B-4-3	12-14 dS/m
NSIC Rc324 (Salinas 10)	PR31607-2-B-B-B	2013	Philippines	IR65185-3B-8-32/Asominori	IR65185-3B-8-32	Moderate
NSIC Rc326 (Salinas 11)	IR84084-B-B-1-1	2013	Philippines	IR66946-3R-176-1-1/IR64680-81-2-2-1-3	IR66946-3R-176-1-1 (derived from Pokkali)	Moderate
NSIC Rc328 (Salinas 12)	IR62700-2B-9-2-3	2013	Philippines	IR8192-200-3-3-1-1/BG367-4/Suakoko 8	Suakoko 8	Moderate
NSIC Rc330 (Salinas 13)	PR37435-30-1	2013	Philippines	PSB Rc90/PR292264-AC10/IR64-1-14/IR70030-7-2-2-1-2	PSB Rc90	Moderate
NSIC Rc332 (Salinas 14)	PR38566-Wagwag V9-3-2-15-2	2013	Philippines	Wagwag V9-3	Induced somoclonal Wagwag variant	Moderate
NSIC Rc334 (Salinas 15)	IR83410-6-B-4-1-1-2	2013	Philippines	PSB Rc88/IR64680-81-2-2-1-3	PSB Rc88 (IR4630-22-2-5-1-3 from Pokkali)	Moderate
NSIC Rc336 (Salinas 16)	IR84095-AJY3-8-SD01-B	2013	Philippines	IR68144-2B-2-2-3-1/IR66946-3R-78-1-1/IR77080-B-4-2-2	IR66946-3R-78-1-1 (derived from Pokkali)	Moderate
NSIC Rc338 (Salinas 17)	PR30665-1B-1-B-B-Cg	2013	Philippines	PR39455-1-B/Pokkali	Pokkali	Moderate
NSIC Rc340 (Salinas 18)	IR84096-AY4-2-SD04-B	2013	Philippines	IR72593-B-2-3-3/IR72875-94-3-3-2/IR66946-3R-156-1-1	IR66946-3R-156-1-1 (derived from Pokkali)	Moderate
NSIC Rc390 (Salinas 19)	IR83140-B-28-B	2014	Philippines	IR06G103/IR06G108	Multiple cross	Moderate
NSIC Rc392 (Salinas 20)	IR84675-58-4-1-B-B	2014	Philippines	IR84674/IR82855	Multiple cross	Moderate

Abbreviations: BINA, Bangladesh Institute of Nuclear Agriculture; BRRI, Bangladesh Rice Research Institute; CR, Central Rice Research Institute, India; CSR, Central Soil Salinity Research Institute, India; dS/m, decisiemens per meter; NDRK, Narayani D.R. Karigowda Institute of Technology, India; NSIC, National Seed Industry Council, Philippines; PSB, Philippine Seed Board.

has been reported based on screening of thousands of accessions (43, 107, 110). Platten et al. (107) evaluated a set of 550 accessions chosen to represent collections from salt-affected areas worldwide across the cultivated rice species *O. sativa* and *Oryza glaberrima*. They identified approximately 103 accessions that were moderate to highly salt tolerant, including 12 salt-tolerant *O. glaberrima* accessions. These tolerant genotypes were extremely diverse, and most of them originated from the *indica* subspecies, with a significant number from the aromatic subgroup and a few that originated from the *aus* and tropical *japonica* subspecies (107). Rahman et al. (110) evaluated a set of 111 rice accessions assembled from Bangladesh, India, Sri Lanka, and the Philippines, most of which were collected from salt-affected areas, and observed a similar abundance of tolerance within the *indica* subspecies. They further identified eight landraces being grown by farmers in the coastal saline areas of southern Bangladesh that have a high salt tolerance, similar to that of the Pokkali and Nona Bokra landraces, the two most salt-tolerant donors traditionally used in rice breeding. However, these new landraces vary in some of the traits known to be associated with tolerance, are genetically distinct, and carry distinct alleles at the *Saltol* locus (54, 141). This richness of genetic diversity, even within cultivated rice gene pool, provides resources to identify novel genes and alleles for salt tolerance, which can then be combined to develop resilient varieties using modern breeding and genomics tools (68, 168).

Traits associated with salinity tolerance in rice. Most studies and breeding efforts have emphasized enhancing salt tolerance during the early seedling stage to overcome the high salinity often encountered during crop establishment at the start of the rainy season (69). However, given the increasing interest and pressure to expand dry-season rice using irrigation and the increasing incidence of drought during the monsoon season, especially in inlands, introducing salt tolerance during both the seedling and reproductive stages has become essential (68, 61). Considerable progress has been made in revealing the mechanisms of tolerance during the seedling stage, and several key traits have been identified, with salt exclusion being the dominant mechanism (68, 96, 107, 168). Other important mechanisms include compartmenting of Na^+ in roots and older leaves, higher tissue tolerance, control of ROS, and regulation of stomatal function to control transpiration and passive salt uptake. Vigorous growth and early maturity reduce the salt load in active plant tissues. Tolerant genotypes also exclude salt from flag leaves and developing panicles during sensitive reproductive stages and produce fertile pollen (94, 96, 97). The details of these mechanisms of salt tolerance in rice have been previously reviewed (68, 102, 168).

The numerous salt-tolerance traits identified and characterized in tolerant accessions are not equally expressed; one or a few traits dominate in any given accession, sometimes at a specific developmental stage. An approach that combines adaptive traits through molecular breeding by pyramiding superior functional alleles has been proposed and is expected to considerably improve salt tolerance in rice (68, 107). As reflected above, our understanding of the genes associated with regulation of salt uptake and accumulation has advanced significantly in recent years, providing opportunities to combine tolerance traits in new breeding materials using molecular and genomic tools (142, 143).

Breeding salt-tolerant rice varieties. Although salt tolerance is complex and developmentally regulated in rice, reasonable progress has been made recently in developing salt-tolerant varieties through conventional breeding methods. This success results from several factors, including the availability of tolerant landraces as donors, phenotyping protocols that allow the evaluation of large populations in a relatively short time, progress in understanding the genetics and physiology of tolerance, and the approaches used for systematic evaluation and selection of elite breeding lines in naturally salt-affected fields with the participation of farmers (54, 66, 67). Several salt-tolerant

rice varieties were recently released and commercialized in several countries in Asia, including Bangladesh, India, and the Philippines (**Table 1**). These varieties have benefited rice production in salt-affected areas (**Figure 2**) through increased grain yield (by 0.5 to more than 2 tons per hectare in farmers' fields) (67, 131), stable production, and the ability to bring new and previously abandoned lands into production (67). However, the development of these varieties spanned 10–15 years of rigorous evaluation of many breeding lines, incurring high costs in the process, which makes alternative approaches (such as marker-assisted breeding and other genome-based methods) more attractive (142, 143). Understanding the physiological and genetic bases of tolerance and the genes conferring tolerance in these varieties will help to guide precision breeding and accelerate future varietal development.

Molecular breeding to accelerate varietal development. Numerous studies have identified QTLs associated with salt tolerance in rice, some of which have major effects (68, 102, 141, 144). An example is the locus on chromosome 1 that contains the major QTLs *Saltol* (derived from the Pokkali landrace) and *SKC1* (derived from the Nona Bokra landrace). *SKC1* is associated with shoot K^+ concentration and was later identified as *OsHKTI;5* (114), located within the *Saltol* region (141). The *Saltol* locus is involved in Na^+/K^+ homeostasis under salt stress (102, 107, 141); it was recently fine mapped, and markers were developed and used to transfer the locus into several popular varieties through marker-assisted breeding (13, 80, 141, 142). Global gene expression profiling at the *Saltol* locus using FL478 (a salt-tolerant derivative of Pokkali) and IR29 (a salt-sensitive variety) revealed that numerous genes that lie mainly within the *Saltol* locus are associated with salt tolerance in addition to *OsHKTI;5* and that these genes are upregulated in the shoot of FL478 under salt stress, including genes related to ion transport (153). Moreover, expression analyses of transcription factors within the *Saltol* locus identified 14 transcription factors, 8 of which were strongly correlated with salinity tolerance (103). These studies suggest that a cluster of genes might be associated with salinity tolerance within the *Saltol* region. Several other QTLs have been identified with moderate to large effects on several other chromosomes, and DNA markers are being validated that can then be pyramided in popular varieties and elite lines (A.M. Ismail, unpublished data).

The fundamental knowledge of salt-response mechanisms in plants (**Figure 1**) forms the basis for the development of effective approaches for improving salt tolerance in crops such as rice (68). Each of the numerous physiological mechanisms associated with salt tolerance involves several genes, as reviewed above. Studies are necessary to identify novel QTLs and genes and identify the strongest alleles for marker development and use in breeding. SNPs have become the marker system of choice for genetic mapping, diversity, and association analysis and for use in marker-assisted breeding because of their polymorphic abundance and the availability of rapid, high-throughput platforms for genotyping (46). For example, the Illumina GoldenGate 384-plex SNP genotyping assay has been successfully used to carry out genome-wide diversity studies and to assess allelic diversity at major loci such as *Saltol*, in addition to its use in marker-assisted breeding (21, 143). In-depth studies of allelic diversity at key loci for traits underpinning salt tolerance will determine whether new accessions have better and novel alleles. Overall, these approaches can enable marker-guided precision breeding based on multiple genes. The availability of SNP platforms has made it possible to track several genes associated with salt tolerance while selecting for traits of agronomic importance, such as yield and quality.

Progress in Developing Salt-Tolerant Wheat

Wheat is moderately tolerant of salt stress and is known to be more sensitive than barley but more tolerant than rice, maize, and sorghum (82). Genetic variation in salt tolerance in wheat has

Marker-assisted breeding: the use of molecular markers to reconstitute the desired genetic makeup of a variety while introducing one or more important traits during variety development in order to improve the efficiency of selection

Genome editing: the precise modification of the nucleotide sequence of an organism's genome or cell by inserting, deleting, or replacing DNA at a specific site, usually through the use of engineered nucleases

been reported (99, 122); however, breeding of salt-tolerant varieties has been slow, despite the importance of wheat as a food crop and the considerable losses inflicted by salt stress. Conventional breeding has led to modest progress in the development of salt-tolerant wheat varieties in several countries, including India, Pakistan, Australia, and Egypt. A few salt-tolerant varieties adapted to local conditions have been commercialized, some of them with reasonably high grain yield and quality, such as the S-24 variety released in Pakistan (99, 129).

Salt-tolerant wheat is efficient in excluding Na^+ from shoots, especially from young tissue and leaves. This exclusion mechanism reduces the Na^+ load and maintains a greater and more favorable K^+/Na^+ ratio in plant tissue, with consequent reduction in leaf death, sustained growth, and higher grain yield under salt stress. Bread wheat generally has better control over Na^+ transport to shoots and higher selectivity for K^+ over Na^+ in shoots than durum wheat does, leading to a higher K^+/Na^+ ratio in its shoot (26, 37, 99). This exclusion trait is associated with the *Kna1* locus in bread wheat (37), and the underlying gene has been identified as *TaHKT1;5-D*, which resides on the plasma membrane of root xylem parenchyma cells (**Figure 1**) and acts by reducing the Na^+ load in the xylem in order to minimize its translocation to the shoot (17). Na^+ exclusion from durum wheat leaves was attributed to two loci, *Nax1* and *Nax2*, both of which originated from the related species *T. monococcum* (63, 72), as discussed above. Both of these genes are most likely *HKT* genes, which function by removing Na^+ from roots and the lower parts of leaves, leading to lower concentrations in functioning leaf blades (71, 99). *Nax1* maps to chromosome 2A and was identified as *TmHKT1;4-A2*, whereas *Nax2* maps to chromosome 5A and was identified as *TmHKT1;5-A* (16, 63, 79). Introgression of *Nax2* into durum wheat cultivars considerably reduced the Na^+ concentration in leaves and substantially increased the grain yield in saline soils (by up to 25%) (100), suggesting the effectiveness of this approach for breeding of salt-tolerant varieties. In both wheat and rice, the mechanisms by which distinct salt-tolerance QTLs containing the *HKT* genes enhance salt tolerance—whether by expression levels, altered Na^+ transport activity of the *HKT* protein (114), altered cellular targeting, or other mechanisms—remain incompletely understood, and this deserves further attention because it could be relevant to pyramiding strategies.

Using molecular markers to combine the alleles underpinning *Nax1*, *Nax2*, and *Kna1* into high-yielding cultivars will improve the salt tolerance of bread wheat and durum wheat. Moreover, these QTLs could potentially be used to improve the salt tolerance of other cereal crops through transformation or genome editing, provided that they will not negatively affect grain yield or quality. Further improvement of salt tolerance in these important food crops will be critical to maintaining production gains in lands that are progressively deteriorating, bringing new lands into production, and keeping pace with increasing food demands. More screening and diversity studies are needed within the cultivated and wild relatives of both bread and durum wheat to identify additional loci and genes associated with salt exclusion and other important traits (summarized in **Figure 1**), which can then be combined in new genotypes using molecular breeding tools.

Progress in Developing Salt-Tolerant Barley

Among glycophytes, barley is the most salt-tolerant crop (82), and it can potentially be considered a model for improving the salt tolerance of other important food crops. Cultivated and wild relatives of barley show considerable genetic variability in salt tolerance (73, 176), which provides opportunities for crop improvement to develop varieties suitable for salt-affected areas. However, despite this genetic variation, little progress has been made in developing salt-tolerant barley varieties, presumably because of barley's inherent tolerance of salt stress compared with other cereals. Like other species, barley exhibits several traits associated with salt tolerance, the most important of which are salt exclusion, high leaf K^+ concentration, high stomatal density, and osmotic

adjustment through accumulation of inorganic ions in the shoot (176, 177). Overexpression of HvHKT2;1, a transporter permeable to both Na⁺ and K⁺, enhanced Na⁺ uptake and translocation to the shoot under salt stress and increased the salt tolerance of barley (89). By contrast, Zhu et al. (177) recently reported that a low Na⁺ concentration and high K⁺/Na⁺ are associated with salt tolerance in barley under mild stress. These contradictory responses are probably genotype specific, and concrete evidence can be established using a larger set of diverse genotypes. Several large-effect QTLs associated with salt tolerance in barley have been identified (123, 173) and can be further targeted to develop effective breeding approaches using molecular markers.

Type-I H⁺-PPase:

a proton-translocating energy pump localized in the tonoplast, coupling hydrolysis of pyrophosphate to metal cation sequestration in vacuoles

Exploiting Wild Relatives of Crop Plants

Interspecific hybridization is playing an increasingly important role in improving the performance of crop plants, especially for tolerance of abiotic stresses (5, 25). Wild relatives of several crops are being used as sources of tolerance of both biotic and abiotic factors and to enhance productivity, although doing so often requires special techniques, such as embryo rescue. A good example is the identification of sources of salt tolerance and candidate genes involved in wild rice germplasm (5, 91, 92). The salt tolerance of the halophytic wild rice relative *Oryza coarctata* has been studied for several decades in order to identify ways that it can be used to improve the salt tolerance of cultivated rice (42, 48). Colmer et al. (25) have reviewed the prospects for improving salt tolerance in wheat using wild relatives. Wild tomato relatives such as *L. cheesmanii* have provided sources of salt tolerance for the cultivated tomato *Lycopersicon esculentum* (44, 115). Genome sequences and dense genetic and physical maps of some wild relatives of a few crops are becoming available (145, 158), providing substantial genetic information that can help accelerate gene discovery and use to improve the salt tolerance of domesticated crops.

PROSPECTS FOR PRECISION BREEDING OF SALT-TOLERANT CROPS

The development of salt-tolerant varieties can be accelerated through the use of DNA markers to assist in selection while transferring tolerance-associated alleles to sensitive cultivars, or through transformation to introduce genes that increase salt tolerance. Traditionally, standard breeding has been the strategy of choice for developing salt-tolerant cultivars in major crops, as discussed above for rice and wheat (**Figure 2, Table 1**). However, developing a variety with this breeding pipeline requires considerable time and resources, because several generations of selection are necessary to produce a phenotype that combines tolerance with the desirable yield and quality.

Knowledge of the genomics, physiology and molecular biology of plant responses to salt stress has advanced considerably in recent years (**Figure 1**), and the use of this knowledge and related tools will hasten the breeding of salt-tolerant varieties. The genomes of several economically important species have been sequenced, and large amounts of genetic marker data as well as multiple genotyping platforms are now available (143). Marker-assisted breeding is being used to conveniently transfer major loci into popular varieties and elite lines (4, 141, 142). Moreover, approaches for using “big data” generated by next-generation sequencing and high-throughput phenotyping are being developed to accelerate the mapping of genes for adaptive and yield traits through genome-wide association studies (11, 64). Next-generation sequencing is facilitating genotyping by sequencing and its effective application in breeding and genomic studies (108). Transgenic approaches have been used for several decades, and numerous transgenic plants with variable levels of salt tolerance have been developed across several species (75). A typical example is the overexpression of type-I H⁺-PPase genes in several plant species [including *Arabidopsis*,

tobacco (*Nicotiana tabacum*), cotton (*Gossypium hirsutum*), alfalfa (*Medicago sativa*), and maize (*Zea mays*)], which enhances salt and drought tolerance (summarized in 49). The underlying mechanisms of these phenotypes are relevant but incompletely understood and are being investigated. Further studies are needed to determine whether this approach can be effective for the development of commercial salt-tolerant varieties. Technologies involving targeted genome editing using sequence-specific nucleases have great potential for crop improvement (76). Such technologies are particularly useful when favorable alleles associated with specific traits of interest are known, as is the case for salt tolerance (**Figure 1**). Overall, this recent progress in genomics, physiology, and molecular biology is expected to transform breeding pipelines by accelerating the precise development and selection of appropriate progenies and by shortening and reducing the number of breeding cycles necessary to develop salt-tolerant cultivars.

SUMMARY POINTS

1. Na^+ is one of the major toxic elements hindering the growth and productivity of glyco-phytic plants. Three major membrane transport systems have been elucidated as essential mechanisms to avoid Na^+ toxicity: Na^+ efflux from roots to the rhizosphere, Na^+ sequestration into vacuoles, and Na^+ exclusion from leaf blades.
2. K^+ and Ca^{2+} are beneficial elements for plants to cope with salt stress. The salt-stress-induced loss of K^+ is mediated by K^+ efflux-mediating channels, which is a crucial factor for salt tolerance in plants. Ca^{2+} is known to block both salt-induced K^+ loss and invasive Na^+ influx through the nonselective cation channels, the molecular identities of which have not been determined, in the plasma membrane of the root.
3. Along with Na^+ , Cl^- can be another major toxic element in salt-affected soils. In fact, mechanisms of Cl^- transport and detoxification during salt stress have been much less of a focus than those of Na^+ thus far. However, studies are beginning to elucidate the functions of some essential Cl^- transporters that mediate Cl^- homeostasis under salt stress.
4. Genetic variation in salt tolerance is present within and among plant species and their wild relatives, providing opportunities to improve the salt tolerance of food and industrial crops in order to enhance and sustain productivity in salt-affected areas.
5. Substantial progress has been made in unraveling the bases of salt tolerance in several crops and model plant species, yet little progress has been made in developing salt-tolerant crops owing to several factors, including the complexity of plant responses to salt stress; the apparent effects on the growth, yield, and quality of economic products; and a reliance on conventional phenotyping.
6. Recent advances in genetics, physiology, genomics, and phenomics are providing opportunities for precision breeding to select appropriate progenies and to shorten breeding cycles and variety development pipelines.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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