

Salinity tolerance in *Oryza sativa* L. and its wild relatives

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ABSTRACT: Cultivated rice (*Oryza sativa* L.) is salt sensitive, and its growth, physiology and yield are negatively affected by moderate salinity. Rice employs three major physiological mechanisms to minimize salt-induced damage i.e., maintenance of Na^+/K^+ homeostasis, osmotic adjustment, and reactive oxygen species (ROS) detoxification. *Oryza coarctata* is the only halophyte member in the genus *Oryza*. It can survive and grow abundantly at high salinity level up to 40 dS/m in the coastal regions of many South Asian countries. The superior salt tolerance of *O. coarctata* is associated with several unique morphological and physiological features including salt-secreting hairs on the leaf surface, Kranz-like leaf anatomy and activity of C_4 -related enzymes, synthesis of pinitol as a unique organic osmoprotectant, compartmentalization of Na^+ in roots, efficient restriction of Na^+ xylem loading in roots and transport to shoots, and the ability to use Na^+ as cheap osmoticum. Interestingly, *O. coarctata* possesses highly efficient vacuolar Na^+ sequestration resulting in tissue tolerance which is an uncommon mechanism in cultivated rice. Some non-halophytic wild rice species that display higher level of salt tolerance than the salt-tolerant cultivated rice varieties also possess the tissue tolerance mechanism. This review provides an account on some physiological mechanisms of salt tolerance in cultivated rice, halophytic rice, and some wild rice relatives to explore new traits and candidate genes for future genetic improvement of rice.

KEYWORDS: ion homeostasis, halophytic rice, salt stress, sodium exclusion, tissue tolerance, wild rice

INTRODUCTION

Soil salinity is one of the major threats for agricultural productivity and sustainability in more than 100 countries across all continents. Salt-affected areas in Asia were estimated at 194.7 million ha accounting for 55% of the world's saline soils [1]. In Thailand, the total area of salt-affected soils is 2.302 million ha, of which 1.904 million ha are inland saline soils and the rest are coastal [2]. The majority of inland saline soils are distributed in the northeast of Thailand at approximately 1.841 million ha accounting for 18% of agricultural land [3]. Salinity levels of soils are classified into slightly, moderately, highly, and severely affected based on electrical conductivity of saturated soil extract (EC_e) at 2–4, 4–8, 8–16, and > 16 dS/m, respectively [4]. Most glycophytic and crop plants are sensitive to salinity and suffer severe growth reduction even in the slightly salt-affected soil. Among cereal crops, rice is highly sensitive to salinity compared with other cereal crops [5]. In the northeast of Thailand, only slightly and moderately affected saline soils can be used for rice cultivation [2], although with very low survival rates and yields. A study involving a total of 51 farmers' fields in the northeast of Thailand from 2005–2007 revealed an average of 20% decrease in yield of RD6 (an elite glutinous rice) in slightly saline soils, having EC_e between 3–5 dS/m, compared to those in non-saline soils [6]. In a heavy saline field having the maximum soil EC_e of 6.50 dS/m during grain filling

stage, yield of 'KDML105' rice was reduced by 58% compared with that in the nearby non-saline plot, but no yield reduction was recorded in the slightly saline field having the maximum soil EC_e of 4.83 dS/m [7].

High concentration of sodium chloride in saline soils causes osmotic stress to plant roots and accumulation of sodium ions in plant tissues leads to ion toxicity stress. Soils with an EC_e value of 4 dS/m, equivalent to approximately 40 mM NaCl, generate an osmotic pressure of 0.2 MPa which causes an osmotic stress that hinders root water uptake of sensitive plants and leads to a rapid growth reduction [5]. Subsequently, ion toxicity is induced by a marked increase in Na^+ and Cl^- in leaf tissues. Excess Na^+ accumulation disrupts ionic balance, reduces cytosolic K^+ uptake causing erratic metabolic processes and overproduction of reactive oxygen species (ROS), which consequently hampers plant growth and development [8]. To counteract the osmotic stress effect, plants exploit an adaptive mechanism, 'osmotic adjustment', by synthesizing and accumulating organic solutes in the cytoplasm to increase their hyperosmotic tolerance against salt-induced water loss from the cells [9]. Compartmentation of high concentration of inorganic ions like Na^+ and Cl^- in the vacuoles also contributes to osmotic adjustment particularly in halophytes [10]. The ability of tissues to function while containing a high internal Na^+ and Cl^- concentrations is known as 'tissue tolerance', a common strategy of salt tolerance for halophytes [9]. To prevent ion toxicity, many ion channels and trans-

porters function to exclude toxic Na^+ from tissues, but increase uptake of essential K^+ , thereby maintain 'ion homeostasis' [11].

This review provides some accounts on three major physiological mechanisms for salt tolerance, namely ion homeostasis, osmotic adjustment and ROS scavenging, in cultivated rice, followed by the mechanisms of tolerance in halophytic and non-halophytic wild rice relatives, and some prospects for opportunities in utilizing wild rice as gene donors in future rice breeding.

MECHANISMS OF SALT TOLERANCE IN *Oryza sativa* L.

Maintenance of ion homeostasis

The ability of plants to maintain low Na^+ and high K^+ , hence low Na^+/K^+ ratio particularly in the young active leaves, is the most important strategy to resist adverse effects of Na^+ toxicity. Low Na^+/K^+ is by far the most reliable physiological indicator for salt tolerance and highly correlated with less growth reduction and greater survival [12]. Sodium ions enter the root stele by symplastic, transcellular, and apoplastic route. In rice, it was reported that a high proportion of Na^+ enters the stele by the bypass flow of water through Casparian strips in both the exodermis and endodermis [13]. A study using radiotracer fluxes of $^{24}\text{Na}^+$, and the fluorescent apoplastic tracer PTS (trisodium 8-hydroxyppyrene-1,3,6-trisulfonic acid) to estimate the transpirational bypass flow revealed that under salt stress the % bypass flow and Na^+ flux from root to shoot were considerably lower in the salt-tolerant 'Pokkali' than the salt-sensitive 'IR29' rice variety [14].

Sodium ions that enter the root xylem vessels either by symplastic or apoplastic pathways will be unidirectionally transported to shoots in the transpirational stream, therefore the mechanisms that reduce Na^+ loading into or facilitate Na^+ removal from the root xylem vessels are crucial for hampering Na^+ arrival to the shoot. In addition to Na^+ influx into rice roots by apoplastic bypass flow, Na^+ is also taken up via ion channels/transporters localized in plasma membrane of root epidermal/cortical cells and transported to the stele via the symplast and transcellular pathways. The Na^+ transporters in rice roots responsible for Na^+ uptake included nonselective cation channels (NSCC) and K^+ selective channels [11]. In addition, a member of high-affinity K^+ transporter (HKT) family transporter, *OsHKT2;1*, was found to mediate large Na^+ influx into rice roots in the condition of low K^+ [15]. To prevent the buildup of Na^+ in root cells to a toxic level, the plasma membrane localized Na^+/H^+ antiporter (*OsSOS1*) in the epidermal and cortical cells mediates Na^+ exclusion from root cells into apoplastic space and back to the soils [16]. It was estimated that

roots must exclude 97–98% of Na^+ , allowing only 2–3% to be transported to shoots to prevent the buildup of Na^+ to lethal concentrations [17]. The action of *OsSOS1* transporter in parenchyma cells in the root stele, on the other hand, also contributes to the flow of Na^+ into xylem vessels.

In rice, a plasma membrane-localized Na^+ transporter, *OsHKT1;5*, plays the most significant role in reabsorption of Na^+ from root xylem vessels to adjacent parenchyma cells in the stele [5]. Under salt stress, the salt tolerant rice variety 'Nona Bokra' was reported to have more efficient functions of *HKT1;5* than the salt sensitive 'Koshihikari' associated with much lower Na^+ accumulation in the shoots of 'Nona Bokra'. The greater Na^+ transport activity of 'Nona Bokra' *HKT1;5* protein was related to four amino acid substitutions resulting in greater Na^+ selectivity [18]. The *OsHKT1;5* gene is located in *Saltol*, the major salt tolerant quantitative trait locus (QTL) originally mapped on chromosome 1 of 'Pokkali' [19] and has been transferred to many salt sensitive elite rice cultivars to enhance salinity tolerance. During reproductive stage another plasma membrane-localized HKT protein, *OsHKT1;4* played important role in sodium exclusion by reabsorption of Na^+ from xylem vessel in the leaf sheath, leaf blade and the peduncle to exclude Na^+ from leaf blades and developing panicles [20]. In addition, the gene *OsHKT1;1* mainly expressed in the phloem of leaf blade is responsible for Na^+ recirculation from leaf back to the root [21].

In both root and shoot cells of rice, the salt overly sensitive (SOS) signaling pathway and the function of *OsSOS1* is considered the major mechanism of Na^+ exclusion from cytosol to the cell exterior. The SOS signaling pathway is ubiquitous in higher plants and was the first signaling pathway of gene regulation under abiotic stress to be fully characterized based on *Arabidopsis* [22] and was demonstrated to be conserved in rice [16]. When rice cells are exposed to salt stress, excessive Na^+ is detected by plasma membrane sensors which activate calcium channels resulting in increased fluxes of Ca^{2+} into the cytosol. The elevated Ca^{2+} binds and activates the calcium-binding protein *OsSOS3* (*OsCBL4*, calcineurin-like 4) which then forms complex with *OsSOS2* (*OsCIPK24*, CBL-interacting protein kinase 24). The *SOS3-SOS2* complex in turn phosphorylates and stimulates *OsSOS1* to actively pump Na^+ out to the apoplast in exchange for H^+ [16, 21]. The higher expression of *OsSOS1* gene in the roots of more tolerant weedy rice, *JYGY-1* and *JYFN-4*, under salt stress was associated with lower Na^+/K^+ and greater salt tolerance in comparison to the salt-sensitive 'Nipponbare' [23]. Earlier and sustained expression of *OsSOS3* in 'Pokkali' indicated the faster and stronger sensing of Na^+ resulting in lower Na^+/K^+ in 'Pokkali' than 'ND019' [24].

In addition to the SOS system, cytosolic Na^+ is

also controlled by Na^+ sequestration into vacuoles in exchange for H^+ via the actions of Na^+/H^+ exchanger (NHX) protein family. The active transport of Na^+ into the vacuoles is driven by electrochemical gradients generated by two proton pumps i.e. vacuolar H^+ -pyrophosphatase (V-PPase) and vacuolar ATPase (V-ATPase) [25]. In rice, *OsNHX1* was localized in the vacuolar membrane of shoot and root cells, and the *OsNHX1* gene was up-regulated by salt stress. Other members of rice NHX including *OsNHX2*, *OsNHX3* and *OsNHX5* were identified and found to have similar roles as *OsNHX1* but were expressed in different tissues in rice plants [23]. The functions of *OsNHX1* not only keep cytosolic Na^+ concentrations below toxic levels but also maintain cell turgor because high vacuolar Na^+ acts as an osmoticum hence lowers the cell osmotic potential [10]. The salt tolerant 'Pokkali' was found to have much higher expression of *OsNHX1*, *OsNHX2*, *OsNHX3* and *OsNHX5* genes than other sensitive genotypes when subjected to 150 mM NaCl for up to 48 h [25]. The vacuolar Na^+ sequestration is considered to be the key salt tolerance trait of both monocot and dicot halophytes enabling them to thrive in saline soils with high leaf Na^+ concentration [26].

In order to maintain low Na^+/K^+ or high K^+/Na^+ under salt stress, plants also need to have an efficient K^+ uptake system. Several genes encoding K^+ uptake transporters and channels have been identified in rice. *OsKATI*, predominantly localized at the internodes, was demonstrated to confer salt tolerance of rice at the cellular level by increasing cellular K^+ content under salt stress [27]. The K^+ transporter genes *OsHAK1*, localized at root epidermis and stele, root-shoot junctions, stems, leaves, and panicle axes, are induced by salt stress mediating K^+ uptake and transport to maintain a high K^+/Na^+ ratio under salt stress [28]. *OsHAK5*, abundantly expressed in root epidermis and stele, vascular tissues, and mesophyll cells, plays important roles in K^+ uptake and root-to-shoot transport, particularly under K^+ deficient external medium [29]. Elevation of Na^+ uptake under salt stress results in membrane depolarization in root cells, which activates outward-rectifying K^+ selective channels (GORK) resulting in a massive efflux of K^+ , therefore K^+ retention in salt-stressed roots is positively correlated with salt tolerance [30]. Both *OsAKT1* (a gene for K^+ uptake channel) and *OsGORK* were up-regulated by salt stress in roots, but tolerant rice genotype was able to retain root K^+ more effectively than the salt sensitive one due to the smaller upregulation in *OsGORK* and higher up-regulation of *OsAKT1* [31]. Sodium uptake, exclusion, and transport within the rice plant is depicted in Fig. 1.

Osmotic adjustment

Low water potential (Ψ) of saline soils retards root water uptake, therefore, in order to maintain water uptake and cell turgor plants need to adjust its cellular

Ψ to the level below that of the saline soil via osmotic adjustment (OA). OA is achieved by accumulating osmotically active solutes inside the cells to reduce the cell's Ψ [32]. Two types of solutes contribute to OA, small organic molecules and inorganic ions. Organic molecules are also referred to as compatible solutes because they can accumulate at high concentrations in the cytosol without affecting cellular metabolism and enzyme activity [17]. Organic compatible solutes are collectively referred to as osmoprotectants due to their multiple protective roles including detoxifying ROS, maintaining membrane integrity, stabilizing enzymes, hormonal functions, and inducing expression of stress-responsive genes [33]. Inorganic ions that contribute to OA under salt stress are composed predominantly of K^+ in cytosol and Na^+ and Cl^- in the vacuoles [17]. Although OA using inorganic ions cost less energy than synthesizing organic solutes, most glycophytes and crop plants including cultivated rice predominantly employ organic solutes for OA because these plants cannot tolerate high concentrations of Na^+ and Cl^- [34]. Sucrose, proline, glycinebetaine, mannitol, sorbitol, polyamine, amino acids and trehalose have been implicated as compatible solutes in several plant species including rice (Fig. 1).

Proline is the predominant compatible solute that accumulates in response to salinity in many plants. The level of proline in plants is tightly controlled by the key biosynthetic enzyme pyrroline-5-carboxylate synthetase (P5CS1) and the catabolic enzyme proline dehydrogenase (PDH1) [35]. In response to salinity, salt tolerant rice genotypes were reported to accumulate higher proline content than salt sensitive ones [36]. Higher proline was related to better OA under salt stress in 'CSSL8-94', an improved rice line introgressed with drought tolerance QTL on chromosome 8, compared to its salt sensitive parent 'KDML105' [37]. Effects of proline accumulation in rice were complex and varied with genotypes, developmental stage, as well as strength, method, and duration of salt treatments. Therefore, there is still a debate as to whether proline accumulation is a symptom of damage, a stress response, or an adaptive mechanism [38]. Nevertheless, proline was indicated to also function in scavenging ROS, stabilizing protein structure, buffering cellular redox potential, activating genes, and serving as a nitrogen source during stress recovery [39].

Trehalose, a non-reducing disaccharide of glucose, plays important protective roles in plants under abiotic stress as an osmolyte, osmoprotectant, ROS scavenger, membrane stabilizer, a regulator of sugar/starch metabolism, and an initiator of signal transduction cascade [40]. The biosynthesis of trehalose in plants involves the generation of trehalose-6-phosphate (T6P) from glucose-6-phosphate and UDP-glucose by trehalose-6-phosphate synthase (TPS), and

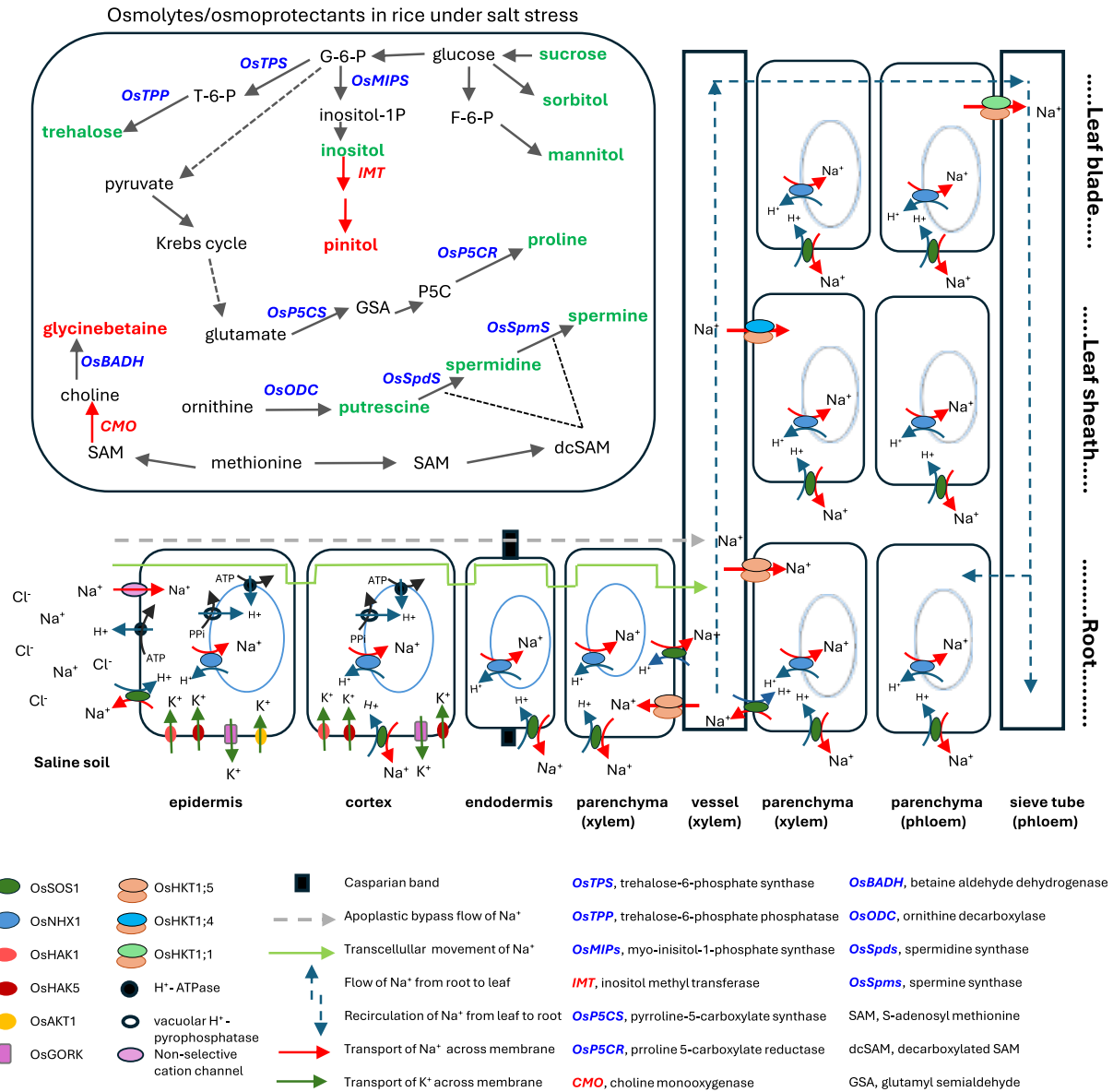


Fig. 1 Uptake and transport of Na⁺ and salt induced osmoprotectants in rice (*O. sativa*). Organic osmolytes in green are known to be synthesized in cultivated rice while glycinebetaine is not commonly synthesized in rice. Pinitol is only synthesized in halophytic rice (*O. coarctata*). The enzymes choline monooxygenase (CMO) and inositol methyl transferase (IMT) are not functional in cultivated rice. For clarity, vacuolar ATPase and vacuolar H⁺-pyrophosphatase are omitted from vacuolar membranes of most cells. Similarly, four transporters for K⁺ are illustrated in only epidermal and cortical cells.

the subsequent dephosphorylation of T6P to trehalose by trehalose-6-phosphate phosphatase (TPP) [41]. Characterization of transgenic rice overexpressing *OsTPS8* and the loss-of-function *Ostps8* mutants confirmed that active *OsTPS8* conferred enhanced salinity tolerance by enhancing synthesis of various sugar osmolytes (trehalose, sucrose, maltose, and fructose), and controlling thickening of suberin in Casparian bands in the roots through abscisic acid (ABA) signaling [42]. Exogenous application of trehalose

alleviated salt stress damage in rice by maintaining Na⁺/K⁺ balance, preserving chlorophyll, activating activity of ROS scavenging enzymes, reducing H₂O₂ and malondialdehyde [43, 44].

Glycinebetaine (GB), a quaternary ammonium compound, plays critical roles in osmoregulation in halophytes and some crop plants under abiotic stress. In plants, GB is synthesized from choline through betaine aldehyde by two-step reactions through the activity of choline monooxygenase (CMO) followed by

betaine aldehyde dehydrogenase (BADH) [45]. Rice does not normally accumulate GB because it lacks a functional gene of CMO, but rice supplied with exogenous betaine aldehyde could accumulate GB via the activity of BADH and developed significant tolerance to salt stress [46]. Transgenic rice overexpressing CMO gene accumulated GB and was superior in salt tolerance than the wild type [47].

ROS scavenging

ROS plays dual roles in plant responses to stress. At low concentrations, ROS acts as a second messenger, signaling molecules, mediating induction of large number of stress responsive genes in response to initial stage of stress. On the other hand, under long term stress, ROS becomes toxic due to an imbalance of the rates of ROS generation and ROS scavenging creating a state of oxidative stress [48]. Under salinity stress, all four forms of ROS, namely singlet oxygen (1O_2), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\cdot OH$), are excessively produced, which actively oxidize nucleic acids, proteins, lipids, membrane, and chlorophyll leading to cellular damage, metabolic disorder, premature senescence, and cell death [49]. Plants are equipped with antioxidant defense systems to detoxify excess ROS. The antioxidant system that reduces and detoxifies ROS is composed of several enzymes including catalase (CAT), superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and non-enzymatic antioxidants such as ascorbic acid, glutathione, flavonoids, isoprenoids, carotenoids, tocopherol, proline, and gamma-aminobutyric acid [50, 51]. These antioxidants maintain a non-toxic level of ROS and permit transient accumulation of ROS to act as signaling molecules [50]. Salt tolerant rice genotypes were reported to suffer less damage from ROS under salt stress compared with the salt sensitive ones as reflected by lower ROS (H_2O_2 and O_2^-), lower electrolyte leakage (EL), and malondialdehyde (MDA). These lower salt-induced damages in salt tolerant rice were accompanied by higher activity of antioxidant enzymes and higher content of non-enzymatic antioxidants [52–54]. Overexpression of genes encoding antioxidant enzymes conferred salt tolerance to transgenic rice, accompanied by improved physiological traits such as lower MDA, lower EL, and higher net photosynthesis rate compared with the wild type plants [55]. Signaling roles of ROS was also proposed to involve an interaction with different hormones (abscisic acid, ethylene, salicylic acid, jasmonic acid, auxin, and gibberellic acid), which consequently regulate expression of stress-responsive genes in various metabolic pathways contributing to adaptation to salt stress [48].

SALINITY TOLERANCE MECHANISMS OF WILD SPECIES OF THE GENUS *Oryza*

The halophytic wild rice *O. coarctata* Roxb

Plants in the genus *Oryza* are glycophytes and generally salt sensitive. *O. coarctata* Roxb., formerly known as *Porteresia coarctata* (Roxb.) Tateoka, is the only halophytic wild species, growing in vast areas in mangrove swamps along the coastal regions of India, Bangladesh, and Pakistan [56]. It is highly salt tolerant and can survive salinity up to 40 dS/m [57]. *O. coarctata* is an allotetraploid having chromosome number $2n = 4x = 48$, and genome size of 665 Mb [56]. This halophytic wild rice has many morphological and anatomical features which are distinct from cultivated rice and other wild species in the genus. It has an extensive underground rhizome and shallow root system which provides for good anchorage and efficient nutrient absorption [58]. Unlike cultivated rice, leaves of *O. coarctata* are thick, succulent, and waxy with the adaxial (upper) surface displaying prominent alternate ridges and furrows [58]. The furrow walls are lined with numerous unicellular fingerlike micro-hairs ('salt hairs'), which secrete salt crystals to the leaf surface under high salinity, another distinctive feature not found in cultivated rice [56]. Numerous salt-secreting hairs are also present on the abaxial (lower) leaf surface. These hairs are peg-like and rupture after secreting salts [59]. The unique feature of salt hairs in *O. coarctata* plays critical roles in both salt exclusion and prevention of water loss through transpiration during high daytime temperature in the mangrove environment [60]. Another striking feature of *O. coarctata*, not found in other species of *Oryza*, is the presence of Kranz-like anatomy including enlarged bundle sheath cells containing large centrifugally oriented chloroplasts with no grana, higher leaf vein density and higher ratio between bundle sheath and mesophyll cell area compared with 'IR64' rice. These anatomical features indicated that *O. coarctata* is evolving toward C_4 mode of photosynthesis in order to cope with adverse environmental conditions [61]. In association with Kranz-like anatomy, *O. coarctata* displayed higher net photosynthesis rate and higher expression of genes encoding C_4 -related enzymes (NADP malic enzyme and pyruvate phosphate dikinase) compared with 'Pokkali' [62]. The morphological, anatomical, and physiological features of cultivated rice and *O. coarctata* are summarized in Table 1.

Growth and physiological responses under salinity of *O. coarctata* displayed typical characteristics of halophytes such as growth stimulation by salinity. Compared with 'Pokkali', a salt-tolerant variety of *O. sativa*, shoot growth (length) of *O. coarctata* was profoundly stimulated under salinity being longest in the 400 mM NaCl (≈ 40 dS/m) treatment compared with the control while shoot growth of 'Pokkali' was severely in-

Table 1 Comparison of characters related to salt tolerance between *O. sativa* L. and *O. coarctata* Roxb.

	Cultivated rice <i>O. sativa</i>	Halophytic wild rice <i>O. coarctata</i>
Genome type	AA, diploid	KKLL, allotetraploid
Plant habit	Annual herb	Perennial herb
Underground system	Normal fibrous root system	Extensive system of rhizomes from which shallow roots arise
Level of salt tolerance	Severe growth reduction at 8–16 dS/m	Tolerate up to 40 dS/m
Leaf morphology	Thinner, non-succulent, and non-waxy Smooth surface	Thick, succulent, and waxy Presence of prominent ridges and furrows
Salt secretion	No salt-secreting hairs No salt secretion from leaf	Presence of numerous salt hairs on both leaf surface Salts are secreted through ‘salt hairs’
Leaf anatomy	Kranz-like anatomy not evident	Kranz-like anatomy; higher vein density; enlarged bundle sheath cells with thick cell wall and numerous mitochondria and small chloroplast (without grana).
Mode of photosynthesis	C ₃ photosynthesis Lower expression of genes encoding C ₄ -related enzymes	Initial stage toward an evolution of C ₄ -photosynthesis Higher expression of genes for C ₄ -related enzymes (<i>NADPME</i> , <i>PEPC</i> , and <i>PPDK</i>)
Osmoprotectant	No pinitol was detected under control and salt stress	Pinitol accumulates under salt stress and serves as the major osmoprotectant
Ion homeostasis	At the initial stage of salt exposure <i>OsHKT1;5</i> is enhanced to restrict flow of Na ⁺ to shoots but fails to maintain this ability in long term stress; Less efficient <i>OsNHX1</i>	At the initial stage of salt exposure, it allows more Na ⁺ to enter the shoot for osmotic adjustment; with long term stress <i>OcHKT1;5</i> is efficiently enhanced to restrict Na ⁺ entering the shoots; Highly efficient <i>OcNHX1</i>

Adapted from References [56] to [66].

hibited at 200 mM NaCl. In addition, *O. coarctata* displayed stable biomass, relative water content, and photosystem II efficiency together with more efficient Na⁺ exclusion while these features were severely retarded in ‘Pokkali’ under high salinity [59]. Salt tolerant varieties/lines of rice (‘Pokkali’, ‘Nona Bokra’ and ‘FL478’) could survive 240 mM NaCl treatment at seedling stage for only 12–14 days while *O. coarctata* remained undamaged and grew up to reproductive stage [63].

In addition to the ability to secrete salts by ‘salt hairs’, *O. coarctata* displays several salt tolerance mechanisms differing from cultivated rice. During the early stage (first 24 h) after salt application, *O. coarctata* transported more Na⁺ to the shoot, compared with the cultivated rice. It was inferred that *O. coarctata* uses Na⁺ for OA while the cultivated rice invests more energy and carbon sources to synthesize organic solutes for such purpose [17, 64]. The effective and rapid OA of *O. coarctata* was associated with the ability to maintain leaf water status and higher stomatal conductance leading to higher photosynthetic efficiency and less cellular damage [62]. With long term salt stress, *O. coarctata* maintained low concentrations of shoot Na⁺ by increasing expression of *OcHKT1;5* to reduce loading of Na⁺ into root xylem vessel, and that of *OcNHX1* to sequester Na⁺ into vacuoles of root cells

[64]. Moreover, *HKT1;5* of *O. coarctata* (*OcHKT1;5*) showed higher Na⁺ transport capacity than ‘Nipponbare’ *OsHKT1;5* based on an electrophysiological measurement of *Xenopus* oocyte expressing rice *HKT1;5* protein. Homology modeling revealed that the more efficient Na⁺ transport of *OcHKT1;5*, compared to that of ‘Nipponbare’ *OsHKT1;5* was attributed to four amino acid changes at positions 239, 207, 214 and 363 [65]. The most significant tolerance mechanism of *O. coarctata* was associated with greatly enhanced expression of *OsNHX1* in both root and leaf cells [62, 64]. The highly efficient sequestration of Na⁺ into vacuoles allows the cells of *O. coarctata* to continue metabolic functions even at high concentrations of total leaf Na⁺ content, thus, it possesses the ‘tissue tolerance’ mechanism [64].

In addition to Na⁺ exclusion and tissue tolerance, *O. coarctata* was known to synthesize pinitol, a unique osmoprotectant absent in cultivated rice [66]. Pinitol is a methylated derivative of inositol, a sugar alcohol implicated to play vital roles in osmotic adjustment. Inositol is synthesized by conversion of glucose-1-phosphate to inositol-1-phosphate by *L-myo*-inositol-1-phosphate synthase (MIPS), the rate-limiting enzyme of the pathway, followed by dephosphorylation of inositol-1-phosphate to inositol, which is then methylated to pinitol by inositol methyl transferase (IMT)

[59]. A 37 amino acid stretch between Trp-174 and Ser-201 in *O. coarctata* MIPS enzyme possesses some unique amino acid sequences that are different from that of cultivated rice. This 'salt-tolerance' determinant domain allows MIPS enzyme of *O. coarctata* to remain active in the presence of high salt concentrations while MIPS in cultivated rice become aggregated and non-functional [59]. Both gene transcripts and proteins of MIPS and IMT were up-regulated in *O. coarctata* under salt stress resulting in 10-fold increase in pinitol concentration in leaves compared to the level before stress [66]. The transgenic salt sensitive 'IR64' rice overexpressing MIPS, IMT or both genes from *O. coarctata* exhibited significantly higher salt tolerance without growth reduction up to 200 mM NaCl with concomitant strong accumulation of inositol and pinitol [67]. This is indicative of possible manipulation of inositol metabolism for enhancing salt tolerance in rice.

Non-halophytic wild relatives of *O. sativa*

Wild rice species, being weedy in nature, possess numerous genes controlling a variety of mechanisms adapted to multiple stresses, therefore are considered valuable genetic resources for breeding for stress tolerance including salt stress [68]. Among 22 wild *Oryza* species tested for salt tolerance based on visual injury scores and survival rate evaluated after 10-day exposure to 150 mM NaCl, *O. coarctata*, *O. alata* and *O. latifolia* were identified as highly tolerant (HT), while *O. minuta*, *O. grandiglumis*, *O. eichingeri* and *O. rhizomatis* were tolerant (T; same level as the tolerant checks 'Pokkali', 'Nona Bokra' and 'FL478'). Notably, all HT and T wild rice displayed significantly higher leaf Na⁺ content and higher Na⁺/K⁺ than the tolerant checks but showed lower % chlorophyll reduction and lower lipid peroxidation indicating the presence of 'tissue tolerance' ability in these wild species [63]. A comparison of physiological responses of six cultivars of cultivated rice ('Pokkali', IR1, Nipponbare, H-86, Pusa Masmati, and IR29) with four wild species (*O. alata*, *O. barthii*, *O. australensis*, and *O. punctata*) revealed that the wild species collectively showed higher salt tolerance with lower growth reduction, better chlorophyll retention, higher shoot Na⁺ and K⁺ content, but lower Na⁺ and higher K⁺ in the xylem sap. This indicated that wild species, particularly the tolerant species *O. alata*, *O. barthii* and *O. australensis*, showed more efficient control over xylem Na⁺ loading, employed Na⁺ as cheap osmoticum for osmotic adjustment, and exhibited tissue tolerance mechanism [69]. Similarly, higher salt tolerance in *O. alata* and *O. latifolia* compared with 'Pokkali', was associated with tissue tolerance via vacuolar Na⁺ sequestration as indicated by higher leaf mesophyll Na⁺ content, up to 30 times higher expression of *NHX1* gene, and better retention of K⁺ in leaf cells [26].

Among wild species *O. rufipogon* Griff. is the pro-

genitor of *O. sativa*, grows naturally and possesses various characteristics resistant to biotic and abiotic stress, therefore it provides valuable gene pools for genetic improvement of rice including the salt tolerance traits [70]. Using introgression lines derived from rice cultivar 'NJ16' and *O. rufipogon*, nine QTLs associated with salt tolerance were discovered and located on chromosomes 1, 3, 4, 5, 6, 8 and 10. One of the introgression lines ('DJ15') was crossed with salt sensitive rice cultivar 'Koshihiraki' resulting in some recombinant inbred lines that showed superior salt tolerance in the field conditions [71]. Additionally, evaluation of a population of introgression lines derived from a cross between salt sensitive rice cultivar '93-11' and *O. rufipogon* resulted in localization of 10 QTLs related to salt tolerance, and one of the introgression lines '9L136' showed increased survival rate as well as lower MDA and higher activity of ROS scavenging enzymes, compared with '93-11' recurrent parent [72]. A comparison of physiological responses between *O. rufipogon* and salt sensitive rice 'Vealona Nano' subjected to 80 mM NaCl for 7 days revealed that *O. rufipogon* had better control of Na⁺ transport to shoots showing 2.5 times lower leaf Na⁺ content and Na⁺/K⁺ ratio, subsequently led to lower growth reduction and better maintenance of photosynthetic apparatus. Roots of *O. rufipogon* contained higher Na⁺ content and were more succulent suggesting an efficient vacuolar Na⁺ sequestration [73].

EXPLOITATION OF WILD RICE FOR IMPROVEMENT OF SALT TOLERANCE IN CULTIVATED RICE

Several hundred QTLs related to salt tolerance in rice have been mapped using populations derived from crosses between salt-sensitive cultivars and salt-tolerant landrace varieties, for example 'Pokkali' [74] and 'Nona Bokra' [75], among others. Most of these QTLs were linked to Na⁺ and K⁺ content, Na⁺/K⁺, and growth performance at seedling stage. Despite numerous QTLs, only *Saltol* QTL, responsible for K⁺/Na⁺ homeostasis, have been successfully transferred from a limited number of donors to some popular elite varieties through marker-aided backcross breeding (MABB) in many countries to create new salt-tolerant cultivars [76]. In Thailand, the improved lines of 'KDML105' introgressed with *Saltol* showed greater tolerance and higher yield under salt stress at 10–12 dS/m [77]. Subsequently, one elite line was released to farmers as cultivar 'RD73' by the Thailand Rice Department in 2017 [78]. Improvement of 'RD6' (a popular glutinous rice) for salt tolerance was achieved by an introgression with *Saltol* QTL (for salt tolerance) and with *qBI* (1, 2, 11 and 12) for blast resistance producing a BC₄F₄ line which exhibited superior salt tolerance, blast resistance, reduced neck blast, and better agronomic performance than the original 'RD6' in the field conditions [79]. However,

most improved lines using *Saltol* QTL could not thrive in highly saline conditions, and it was suggested that this strategy of rice breeding has probably reached a plateau and cannot deliver any further improvement in salt tolerance [80].

With global warming becoming intensified, it was estimated that approximately 4 million ha of global agriculture land is abandoned annually due to excessive salts [81]. In order to develop superior salt-tolerant rice, which can cope with stronger levels of salinity in wider range of environments at various stages of growth, breeders should encompass several attributes of tolerance mechanisms. It was suggested that future breeding strategies place more emphasis on identification and introgression of candidate genes/QTLs from wild rice species for traits that are not available in cultivated rice [80]. Improved rice varieties which can tolerate highly saline soils from seedling to reproductive stage will be much needed in the future. Therefore, future salt tolerant varieties should be superior not only in Na⁺ exclusion and K⁺/Na⁺ homeostasis but also tissue tolerance, osmotic adjustment, and other attributes. Candidate genes/QTLs responsible for different mechanisms, particularly those related to tissue tolerance, need to be identified from salt-tolerant wild rice species and pyramided into elite cultivars for enhanced salt tolerance [76, 80]. Rice varieties that can effectively balance both Na⁺ exclusion and tissue tolerance may have dual advantage of using less energy for Na⁺ exclusion and lower cost of organic osmolyte production as Na⁺ can act as osmoticum, thus more resources will be partitioned to growth under salt stress [82]. The most critical bottleneck restricting gene transfer from distantly related wild relatives like *O. coarctata* to cultivated rice is the interspecific crossability barrier. It was suggested that embryo rescue, chromosome doubling, or the use of bridging species could be used to facilitate gene transfer [80]. Newly identified genes from wild rice are also useful for functional genomic studies for more in-depth analysis of salt tolerance mechanisms and for improvement through biotechnology approaches including transgenic technology and gene editing [63]. Therefore, opportunities are widely open to explore novel traits/genes/QTLs from a wide variety of indigenous rice and wild rice to be exploited for breeding superior salt-tolerant rice to overcome the challenge of limited parental resources.

CONCLUSION

Salinity is one of the major abiotic stresses that severely hampers commercial rice production. Salt tolerance of rice depends on three major physiological mechanisms, i.e., Na⁺/K⁺ homeostasis, osmotic adjustment, and ROS scavenging. Most popular elite rice varieties are salt-sensitive, and their genetic improvement via marker-assisted breeding currently depends on an in-

troggression of QTLs controlling Na⁺/K⁺ homeostasis from a limited number of salt-tolerant parents. Some wild rice species are more tolerant to salinity than cultivated rice due to their ability to control those mechanisms more efficiently, together with the presence of mechanisms not common or not present in cultivated rice such as tissue tolerance, salt secretion, unique anatomical features and C₄-like photosynthesis. Future research direction should focus on employing advanced tools including genomics, transcriptomics, proteomics, metabolomics, phenomics and physiomics for more in-depth understanding of salt tolerance mechanisms in wild rice and uncovering novel candidate genes for use in genetic improvement for rice salt tolerance.

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