



Physiological and Molecular Basis of Salt Stress Tolerance in Rice – A Review

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ABSTRACT

*The rice (*Oryza sativa* L.) plant is a glycophyte and soil salinity is a major constraint for its production. Crop growth, development and yield are adversely affected by high salt concentrations in soils. To engineer more salt-tolerant rice plants, it is crucial to untangle the key components of the plant salt tolerance mechanisms. The present article attempts to review the principal salt-tolerance mechanisms in rice plants. Several recent studies highlight the stress sensing and signalling components that regulate the plant salinity stress response. Various types of salt stress on plants, genetic diversity for salt tolerance, genes associated with several physiological mechanisms, together with Na⁺ transport and detoxification pathways on salinity tolerance are reviewed. In addition, the progress has been made towards engineering salt tolerance in different monocots, including marker assisted selection and gene stacking techniques are reviewed. The major problems and constraints to be addressed by researchers in future, in order to incorporate greater salinity tolerance into high yielding rice varieties through transgenic and breeding approaches are emphasized.*

Key words: Salt Tolerance, Gene-expression, Rice Breeding, Genetic diversity, Ion Transporter

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INTRODUCTION

Rice (*Oryza sativa* L.) is a cereal grain and moderately tolerant to abiotic stresses like salinity and drought. Over 800 million hectares (6 % of the world's total land area) of land throughout the world are salt-affected, either by sodicity (434 million ha) or salinity (397 million ha) conditions [1]. Most of this salinity and all sodicity is natural. However, a significant proportion of cultivated agricultural land has become saline due to the process of irrigation. Of the 1500 million ha of land farmed by dryland agriculture, 32 million (2%) are affected by secondary salinity. Of the current 230 million ha of irrigated land, 45 million ha are salt-affected (20%) [1]. Irrigated land is only 15% of total cultivated land, but as irrigated land has at least twice the productivity of rainfed land, it produces one third of the world's food. Increased salt tolerance of perennial species used for fodder production is a key component in reducing the spread of secondary salinity, while increased salt tolerance of crops will directly improve production in soils with primary salinity.

Increased salt tolerance requires new genetic sources of this tolerance and more efficient techniques for identifying salt tolerant germplasms. Powerful new molecular tools for manipulating genetic resources are presently available, but the applications of the new technologies are not yet fully utilized to introduce new genes for tolerance into current cultivars. This review summarizes the major adaptive mechanisms of salt tolerance at the physiological and molecular levels. The main mechanisms for salt tolerance are regulated by various candidate genes specific for ion transportation, osmo-protection and tissue tolerance by making plants grow consistently better in saline soil. Studies on gene expression in relation to physiological aspects are reviewed and suggestions are put forward to increase the value of these studies in enhancing plant performance on saline soil.

The article is organised into ten sections. The introduction is followed by a section on genetic diversity for salt tolerance in rice. The third section deals with rice genomics, followed by a discussion on salt

tolerance between other cereals and rice. The fifth section is about physiological mechanisms of salt tolerance and associated gene expressions. This is followed by a section on salt tolerance and transport processes of sodium and potassium ions. The seventh section deals with factors responsible for maintaining low sodium in cytoplasm, followed by a section on HKT's in salt tolerance. The ninth section discusses marker-assisted selection as a tool for salt tolerance breeding programme. The last section provides concluding remarks.

GENETIC DIVERSITY FOR SALT TOLERANCE

The extensive genetic diversity for salt tolerance that exists in rice distributed over numerous genera [2, 3 and 4]. The presence of excess salt is one of the most widespread soil toxicity problems in many rice growing areas. In particular, it accounts for 8.5 million hectares of land in India and the yield reduction is estimated at 30-50% [5]. The success of any breeding programme is dependent on the available genetic divergence in the crop. Rice germplasm is known to be a rich source of salt tolerant genes [6] for improving salinity tolerance in high yielding varieties. A narrow genetic base among released cultivars and the practice of using elite line x elite line crosses have been implicated in slowing the rate of genetic advance for yield [7]. Meanwhile, divergent parents in a cross have a greater scope of obtaining heterotic F1's and a broad spectrum of variability in the segregating generations in any varietal improvement programme [8]. Hence, analysis of genetic relationships among extant genotypes is an important component of crop improvement programme as it provides information about genetic diversity and helps to stratify breeding populations.

Variations in regard to salt tolerance among rice varieties were reported in crosses of blue bonnet, IR-8, Jhona-349 and Magnolia indicating that the salt tolerance may be possible within hybrid populations [9]. Like-wise, several other crops were also reported for their genetic diversity for salinity tolerance. A recent study on the exploration of genetic diversity for salt tolerance in *Miscanthus*, a woody rhizomatous C4 grass, has revealed several relatively salt tolerant genotypes that lowered Na⁺ concentrations and exhibited relatively high K⁺/Na⁺ ratios in the shoots under saline conditions indicating salt tolerant mechanisms which further can serve as starting material for breeding programmes [10]. Few studies, utilized image-based phenotyping to understand the genetic variation in chickpea for salt tolerance in around 245 diverse accessions and proposed seed number as a selection trait in breeding salt tolerant chickpea cultivars [11].

Modern biotechnology including marker-assisted selection and genetic engineering along with breeding techniques need to be increasingly used to introduce the specific combination of genes into elite crop cultivars. The approaches like trait selection, group of genes and the whole transcriptomic approaches aim at providing input for marker-assisted breeding or genetic engineering of new crop varieties [12]. Importantly, the introduction of genes and their effects need to be evaluated at field level to determine their role in salt tolerance and yield improvement.

RICE GENOMICS

Rice has the smallest genome of all common cereals [13]. Rice was the first cereal to be fully sequenced and both the indica and japonica genome sequences were published decades back [14 and 15], reporting that the indica genome is 420 Mb in size and contains between 32,000 and 50,000 genes and japonica variety is larger at 466 Mb and contains around 46,022 - 55,615 genes [16].

In India, rice is grown in 44 million hectares which is 28%, about 74 kg of milled rice per capita was consumed during 1999, providing about 30% of total calories/capita/day. The demand of rice is expected to increase to 114 million tons in 2030 - an increase of about 35%. This increase in production is to be achieved without major increase in arable land and with rapidly degrading ecosystem. Such a challenge requires deployment of all possible means of higher production, which to large extent involves development of better varieties tolerant to biotic and abiotic stress with efficient agricultural management [17].

It is necessary to understand survival strategy for rice growth under salt stress conditions and innovate means for rapid integration of the same in genetic improvement programmes. Advent of molecular biotechnology decades back has opened up a new possibility for genetic improvement in rice. Specific genetic traits are associated with genes could be mapped and introgressed leading to molecular breeding and genetic engineering which allowed movement of genes across the incompatibility barriers [18]. Few decades back, International Rice Genome Sequencing Program (IRGSP) was initiated to sequence the entire genome of rice, drawing upon the information already available on rice genetics generated by the Rice Genome Program (RGP) in Japan. The components of IRGSP have developed an integrated physical and genetic map as well as a comprehensive transcript map of rice [19 and 20]. Several private sectors including Monsanto and Syngenta have produced a draft sequence of the variety being investigated by

IRGSP and made available their data to IRGSP [21 and 15]. Further, an indica variety genome was sequenced in China [14]. It is estimated that the rice genome may contain 37,544 genes [22]. Functional analysis of these genes and their useful alleles would be a challenge for rice scientists in near future and requires global attention and initiatives.

Various nations have contributed in investigating the functional genomics of plants. Prominent among them are Plant Genomics Programs supported by the National Science Foundation USA, European Functional Genomics Programme, Australian Centre for Plant Functional Genomics, Rice Simulator Programme of Japan and Chinese Programme on Functional Genomics of rice. India, being a major rice producer and consumer, obviously needs to take a lead in the functional genomics of rice. The International Rice Research Institute (IRRI), Philippines, has helped to form an International Rice Functional Genomics Working Group, which has contributed towards the development of tools and resources for genome-wide understanding of gene functions viz., analysis of expression profiles, generation of mutants and tagged lines or target-specific silencing of genes, functional validation in transgenics, allele mining, comparative genomics and virtual plant concept [23 and 24].

National Consortium for Functional Genomics of Rice (NCFGR) was formed to develop programmes on the functional genomics of indica rice in selected areas and approaches are expected to unravel the function of most crucial group of genes from rice, i.e. signal transduction and transcription components. The analysis of the rice genome reveals that such genes might be represented by as much as 8000 genes. Their activity is responsible for perceiving environmental (abiotic and biotic) and intrinsic (developmental and hormonal) signals and prepare the plant to respond to adverse environment to survive and follow a set pattern of life-cycle. The programme, therefore, starts with analysis of expression and data mining to select useful genes which would be a target for functional validation.

SALT TOLERANCE IN OTHER CEREALS COMPARED TO RICE

The characterization of a salt-tolerant crop variety includes Na^+ exclusion, K^+/Na^+ discrimination, retention of ions in the leaf sheath, tissue tolerance, ion partitioning into different-aged leaves, osmotic adjustment, transpiration efficiency, early vigor and early flowering leading to a shorter growing season, the latter increasing water use efficiency [25]. The physiological mechanisms of several other cereal crops and associated salinity tolerant mechanisms [18] in comparison to rice are further discussed. For instance, halophytic wild relatives are sources of new genes for enhanced Na^+ exclusion [26] and with the possibility that these sources of Na^+ exclusion might continue to be effective even at high external NaCl concentrations. Similarly, of all the soil mineral stresses or chemical toxicities, acidity, associated Al^{3+} toxicity and salinity are the most important constraints to sorghum productivity in tropical environments [27]. An annual meet on Sorghum highlighted and reported few hybrids like ICSA 405 \times JJ 1041, ICSA 766 \times ICSV 96020, ICSA 276 \times S 35, etc., that were clustered into one group for their suitability in iso-climatic conditions of Gangavathi to tolerate salinity stress conditions in India [28].

Wheat (*Triticum aestivum*) is a moderately salt-tolerant crop [29 and 30]. In the field, where salinity rises to 100 mM NaCl (about 10 dS m^{-1}) rice (*Oryza sativa*) will die before maturity, while wheat will produce a reduced yield. Durum wheat (*Triticum turgidum* ssp. durum) is less salt tolerant than bread wheat, as are maize (*Zea mays*) and sorghum *Sorghum bicolor*. Even barley (*Hordeum vulgare*), the most-tolerant cereal, dies after extended periods at salt concentrations higher than 250 mM NaCl i.e., equivalent to 50% seawater [31 and 32]. Thus, there are crop varietal differences in the extent of accumulation of Na^+ and Cl^- in leaves [33 and 34], but the relationship between Na^+ or Cl^- accumulation and salt tolerance has not been fully established in barley to the same extent as in wheat and rice [34, 35].

Thinopyrum ponticum is a perennial grass is known for its salt tolerance as they grow on salt-affected lands. Several accessions survived at 750mM NaCl [35] and some also maintained reasonable growth at an ECe 13.9 dS m^{-1} [36]. Moreover, *Th. ponticum* introgressions into bread wheat appear to have resulted in improved salt tolerance [37]. Finally, to make use of amphiploids as a new salt-tolerant feed wheat, the most tolerant accessions of wild relatives should be used to make amphiploids with a range of modern high-yielding, locally-adapted varieties for the target environments (as recommended for *Th. bessarabicum* [38].

Thinopyrum bessarabicum survives prolonged exposure to 350 mM NaCl , although growth reductions are large even at 250 mM [39]. So, although *Th. bessarabicum* is much more salt tolerant than bread wheat, productivity is still greatly diminished when salinity reaches half of that in seawater [40].

Hordeum marinum (XX) is tolerant to salinity [41] and can be hybridized with wheat. They also have reported that *H. marinum* displays a capacity to control concentrations of Na^+ (and also Cl^-) in leaves and even when exposed to very high (namely 450 mM) concentrations of NaCl in the root-zone.

In *Aegilops cylindrica*, under hydroponics conditions at EC 15 dS m^{-1} , the best introgression line yielded 23% of the grain weight of its non-saline control, whereas the bread wheat did not produce grain. In the

field plots, irrigated to be moderately saline (EC of 5 to 10 dSm⁻¹), one introgression line yielded 20% higher than the bread wheat [42] and in soil with higher salinity (EC of 20 to 34 dSm⁻¹), the best line had a 36% reduction in grain yield compared with a 54% reduction for a commercial bread wheat [42].

SALT TOLERANCE PHYSIOLOGICAL MECHANISMS AND ASSOCIATED GENE-EXPRESSIONS

To cope with the effect of salt stress, plants have developed the ability to sense both changes in osmolytes and the ionic K⁺/Na⁺ component. These two metabolic entities are evident in plants under salt stress conditions. A high salt concentration in the soil creates hyperosmotic stress on roots. To date, a study was reported on the plant model organism, *Arabidopsis* (*Arabidopsis thaliana*) that *At*-histidine kinase receptor protein *HK1* has shown to complement the loss of the yeast osmosensor *Sln1* [43]. Plants exhibit many physiological responses to osmotic stress. However, recent research has shown that some of these responses are altered in *hk1* mutants, but others remain unaffected. Plant osmolytes are likely to be closely coupled with Ca²⁺ channels given that plants exhibit a rapid rise in cytosolic Ca²⁺ levels within seconds of exposure to NaCl [44]. Thus, there is immediate need of genetic identification of osmotic and Na⁺ sensors that would be instrumental in resolving these early sensory mechanisms.

Transcription factors in rice are vital in linking salt-sensory pathways to many tolerance responses. Genes of transcription factor (TF) family are differentially expressed in response to elevated external salinity [45], including basic leucine zipper (bZIP) [46], APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) [47], WRKY [48], MYB [49], basic helix-loop-helix (bHLH) [50] and NAC [51] families. These transcription factors, in turn, regulate the expression levels of various genes that may ultimately influence the level of salt tolerance of plants. Partially, the transcriptional regulation of these stress-response genes in plants is mediated by dynamic changes in hormone biosynthesis [52]. After stress induction an initial quiescence period is followed by a growth recovery phase, both of which correlate with changes in the levels of the plant hormones abscisic acid (ABA), jasmonate (JA), gibberellic acid (GA) and brassinosteroid (BR). In response to high salinity, most stress-induced transcriptional changes occur approximately 3 hours after application of salt stress [52]. ABA prevents lateral root elongation into surrounding media with high salt concentrations [53]. Even though several key components of the plant salt stress response network have been identified in recent years, there are significant knowledge gaps that need to be fulfilled. In addition to the hormones mentioned above, ethylene is shown to confer salt tolerance in soil grown *Arabidopsis* plants by improving the Na⁺/K⁺ ratio in shoots [54]. Excision of *ETHYLENE OVERPRODUCER1* (*ETO1*) resulted in elevated ethylene levels, which stimulates reactive oxygen species (ROS) production by the respiratory burst oxidase homologue F (RBOHF). The increased stele ROS accumulation results in reduced net Na⁺ influx in roots, decreased Na⁺ xylem loading and to root K⁺ retention, subsequently enhancing salinity tolerance [54].

SALT TOLERANCE AND TRANSPORT PROCESSES OF SODIUM (Na⁺) AND POTASSIUM (K⁺) IONS

Generally, plant membrane transporters play key roles in resistance mechanisms to biotic and abiotic stress, particularly K⁺ and Na⁺ transporters for resistance to salt stress [55]. Several transporters including calcium-permeable non-selective cation channels (NSCCs) [56], CYCLIC NUCLEOTIDE-GATED CHANNEL (CNGC) [57] and the GLUTAMATE-LIKE RECEPTOR (GLR) [58] families are permeable to Na⁺ and thus, entry point of Na⁺ into the cell. Furthermore, the rice (*Oryza sativa*) Na⁺ transporter *OshKT2;1* has shown to mediate Na⁺ influx into roots under K⁺-starvation [59].

To stabilize the toxic effects of Na⁺ accumulation, plants require K⁺ acquisition and distribution during Ion homeostasis [60]. Over-expression of the relatively Na⁺-impermeable rice K⁺ transporter *OshAK5* confers salinity tolerance [61]. Thus, inward-rectifying K⁺ channels and outward-rectifying K⁺ channels have been identified as mechanisms for long-term net K⁺ selective influx and K⁺ efflux in plant cells, respectively [62] and may also reduce Na⁺ toxicity.

ROLE OF NHX1 AND SOS1 IN MAINTAINING LOW Na⁺ IN CYTOPLASM

Two major factors that maintain low cytoplasmic Na⁺ concentrations in plant cells are the tonoplast-localized NHX1 and plasma membrane-localized SALT OVERLY SENSITIVE 1 (SOS1, also known as NHX7) Na⁺/H⁺ antiporters [63], whereas, most NHXs are essential for Na⁺ detoxification via sequestration of Na⁺ within the vacuole. Constitutive overexpression of *OsNHX1* and its orthologs in Rice and other plant species, such as tomato (*Solanum lycopersicum*) or *Arabidopsis*, led to increased plant salinity tolerance [64]. Recent studies have shown that the expression of *OsNHX1*, *OsNHX2*, *OsNHX3*, and *OsNHX5* is regulated differently in rice tissues and is increased by salt stress, hyperosmotic stress and ABA [64]. Over-expression of *AtNHX1* in tomato increases vacuolar K⁺ as well as K⁺ transport from root to shoot [65], a beneficial enhanced intracellular (K⁺)/(Na⁺) ratio that reduces Na⁺ stress. Interestingly, over-expression of the vacuolar type I H⁺-PPase AVP1 improves plant salt tolerance by mediating vacuolar

Na⁺ sequestration [66]. The potential of altering H⁺-PPase in crops was shown by *AtAVP1* over-expression in *Hordeum vulgare*, which conferred increased tolerance to salinity under polyhouse conditions but also improved grain yield in a field trial with saline soil [67].

HIGH-AFFINITY POTASSIUM TRANSPORTER (HKTs) AND SALT TOLERANCE

Detailed analyses have demonstrated a major role of *AtHKT1;1* and its rice ortholog *OsHKT1;5* in the removal of Na⁺ from the xylem sap into the xylem parenchyma cells, thereby protecting leaves from Na⁺ toxicity [16]. Targeted *AtHKT1;1* over-expression in the stele enhances salt tolerance. QTL analyses for Na⁺ resistance have suggested that similar xylem Na⁺-unloading mechanisms which are essential for salt tolerance in rice and wheat (*Triticum turgidum*). In both cases, major salt tolerance QTL map to regions that include *HKT1;5* orthologs, encoding a more Na⁺-selective class I HKT transporter. Recently, comparative analyses using salt-tolerant *indica* cultivars and a sensitive *japonica* cultivar have led to the hypothesis that *OsHKT1;4* restricts leaf sheath-to-blade Na⁺ transfer in rice plants under salinity stress [68]. The recent HKT marker-assisted introduction of a wheat *HKT1;5* from an ancestral wheat relative *Triticum monococcum* into commercial durum wheat (*Triticum turgidum* ssp. *durum* var. *Tamaroi*) has led to significant increases in grain yields in field trials on natural saline soil in Australia [18]. Together, these findings demonstrated that class I HKT transporters are an essential mechanism for monocot plants to protect photosynthetic organs from Na⁺ over-accumulation during salinity stress.

For the purpose of engineering more salt tolerant plants, further knowledge is needed about synergistic effects of certain combinations of tolerance traits. A correlation of elevated shoot Na⁺ and increased salt tolerance was reported in a study with a class II HKT transporter in barley. Enhanced Na⁺ root uptake and higher Na⁺ xylem sap concentrations were due to over-expression of *HvHKT2;1*. In contrast to rice and wheat, in barley higher rates of Na⁺ translocation to the shoot and elevated salt-inclusion might be an important tolerance strategy [69].

MARKER-ASSISTED SELECTION AS A TOOL FOR SALT TOLERANCE BREEDING PROGRAMME

Improving yield performance of crops is an ultimate aim of plant breeding programmes [55]. Beyond that, obtaining salt-tolerant crop using conventional breeding methods is a cumbersome process and takes a long time. Traditionally, crops are out-crossed to genetically diverse germplasms and then selected, based on their phenotype as evaluated in the field. This process later streamlined by using QTL analyses coupled with marker-assisted selection (MAS) [70]. MAS require linkage of a quantitative trait with a genetic marker that is polymorphic between parental lines. Salt-tolerant rice varieties such as Pokkali and Nona Bokra originated in coastal areas of India, and are the source of salt tolerance in most rice breeding programmes. Hence the essential basis for successful breeding with MAS is an in-depth knowledge of genetic traits and variability within the desired plant species. An example for successful process is *Saltol*, a favorable QTL identified in rice that is responsible for the bulk of genetic variation in ion uptake under saline conditions [71]. Similarly, several other genome regions have shown to play major roles in salt tolerance, molecular markers and next-generation sequencing that are likely to be crucial in guiding future breeding plans.

MAS possess the potential to contribute towards the development of more salt-tolerant crops. A major bottleneck is the selection of appropriate markers specific to salinity tolerance. Many scientists are attempting to improve rice salt tolerance by genetic manipulation of certain genes. However, some of these genes may not be sufficient to improve crop viability significantly in highly saline-prone area. Few reported to enhance stress tolerance by manipulating only one or a few main components of the regulatory gene network instead of engineering several such molecular mechanisms [45]. This approach is limited by independent segregation of genetic traits, which complicates breeding strategies. An emerging method to circumvent this issue, involves the use of trait landing pads, whereby engineered sequence - specific nucleases, such as zinc-finger nucleases to target multiple transgenes to the same locus [72]. While so far only zinc-finger nucleases have been used in such trait landing pads, it is expected that the rapidly emerging CRISPR/Cas method would further facilitate targeted insertion of promoters and genes of interest [73].

CONCLUDING REMARKS

Research, over the past few decades, helped to understand salt stress responses and tolerance mechanisms in rice. However, the regulation of gene expression and signalling cascades that regulate many Na⁺ transporters remain to be properly understood. Furthermore, it is important to determine the ion transport processes reviewed in order to enhance rice production in saline soil conditions. Molecular breeding tools together with conventional plant breeding methods would help develop rice crop cultivars with enhanced salt tolerance. In this context, specific genes controlling physiological mechanisms for

enhanced salinity tolerance could be combined to include Na⁺ transporters like *HKTs* and *NHXs*, ROS scavengers and other traits in salt homeostasis and influence the capability of the rice plant to produce high yield even under elevated salt stress conditions.

CONFLICT OF INTEREST

No Conflict Of Interest.

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