



SALINITY STRESS IN RICE : AN OVERVIEW

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Abstract

Molecular and physiological traits imparting resistance/tolerance and susceptibility to a particular biotic and abiotic stress are important from crop production point of view. There are a number of biotic (*viz.*, insect-pests and diseases) and abiotic stresses (*viz.*, drought, water logging, salinity/alkalinity, low temperatures etc.), which affect crop, among abiotic stresses, high salinity stress is the most severe environmental stress, which impairs crop production on at least 20% of irrigated land worldwide. In response to high salinity stress, various genes get upregulated, the products of which are involved either directly or indirectly in plant protection. Some of the genes encoding osmolytes, ion channels, receptors, components of calcium signaling and some other regulatory signaling factors or enzymes are able to confer salinity-tolerant phenotypes, when transferred to sensitive plants. Overall, the susceptibility or tolerance to high salinity stress in plants is a coordinated action of multiple stress responsive genes. Molecular markers would provide reliable information about genetic diversity of crops. Since these molecular markers are numerous in number and thus, will have better coverage of whole genome especially for the traits associated with salinity tolerance. Salt stress effectively decreases the availability of water in the soil to plants not only this but, it also poses other effects such as salt ions impair enzyme function, inhibit protein synthesis, affect the structure and permeability of cell membranes, inhibit photosynthesis and lead to the production of toxic reactive oxygen species. By doing functional genomics analysis, we would be able to have better understanding of salinity tolerance mechanism *i.e.*, transcription factors involved in regulation pathway of different mechanisms of the plants under saline conditions detailed understanding of genome structure and functioning will lead to great developments. For instance, combining techniques permitting modifications at the nucleotide level with the outcome of genomics will probably have a tremendous impact on breeders' activities in a medium-term perspective. The objective of this review is to analyze the available literature and estimate yield losses for rice crop grown in salt-affected soils around the world. In particular, rice (*Oryza sativa*) studied as examples of staple food crops. Today, rice stands as a forerunner amongst the cereals in terms of details known on its genetics.

Key words : Saline, rice, screening, biotechnology, genome.

Introduction

Modern definition of biotechnology represents it as a mechanism/ practice providing powerful and useful tools, in a continuum of technical evolution that contributes or could contribute to the improvement of crop production, food quality and safety, while preserving the environment. This field also addresses the complex regulatory framework surrounding modern biological sciences, as well as tools in the pipeline, and intellectual property aspects related to the technology. Biotechnology represents the most promising way to increase salt tolerance in plants. Salt tolerant plants developed through genetic engineering are already a reality and it is expected that continuing progress in existing areas of investigation, together with

anticipated advances in biotechnology itself, will lead to further significant advances with commercial release of salt tolerant varieties expected within next decade. Salinity is one of the most important limiting factor that causes substantial crop yield losses throughout the globe among other abiotic stresses (drought, water logging temperature etc.) and its menace is increasing day by day. However, the appropriation of such stress tolerance is a question of agronomy and natural resource management rather than biotechnology itself. Salt tolerant perennials with high water use efficiency will have a particular role in contributing to rehabilitation of saline land and addressing the underlying problem of salinity. Recent research on the physiology of salt tolerance has demonstrated that the overall trait is determined by several sub-traits, any of which can in turn be determined by several genes.

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Sustainable productivity is the highest priority for developing nations like India for their ever growing populations. In contrast to the expanding need for food fuel and other commodities, many countries are already confronted with loss of farmland, diminishing source of water and expanding biotic and abiotic stress.

Role of Biotechnology

Biotechnological tools have greatly contributed to the production and supply of improved quality seed and planting material to farmers worldwide. Among other uses, biotechnology is employed to:

- speed-up the multiplication process for vegetatively propagated crops,
- detect diseases transmitted by seed or planting material,
- eradicate diseases transmitted by planting material,
- Protect seed with biological control agents, and test varietal identity and purity.

So biotechnology represents the most promising alternative to utilize such salt affected land for production of salt resistant crops that can survive well under such environment. There are many different aspects as to how agriculture can be benefitted from biotechnological techniques. The appropriate development of biotechnological tool to ensure long-term sustainability is a question of agronomy and natural resources management rather than biotechnology itself requiring complementary land management to address the cause and spread of such salt affected land. Salt tolerant plants with high water use when managed appropriately, will be a particular tool in contributing to rehabilitation of plants under saline conditions.

Adverse effect of salinity stress

The following adverse effects are observed in response to high salinity stress.

1. High salinity interferes with plant growth and development and can also lead to physiological drought conditions and ion toxicity (Zhu, 2002). Therefore, high salinity and drought stresses affect mostly all aspects of plant physiology and metabolism and cause both hyperionic and hyperosmotic stresses, which lead to plant demise.
2. The basic physiology of high salinity stress and drought stress overlaps with each other as a high salt deposition in soil leads to a deposition of a low water potential zone in the soil. This makes it increasingly difficult for the plant to acquire water as well as nutrients.

3. Salinity causes ion-specific stresses resulting in an altered K^+/Na^+ ratio. The external Na^+ can negatively impact intracellular K^+ influx.
4. Salinity leads to a buildup of Na^+ and Cl^- concentrations in the cytosol, which can be ultimately detrimental to the cell. The Na^+ can dissipate the membrane potential and therefore facilitates the uptake of Cl^- down the gradient.
5. Higher concentrations of sodium ions (above 100 mM) are toxic to cell metabolism and can inhibit the activity of many essential enzymes, cell division and expansion, membrane disorganization and osmotic imbalance, which finally can lead to growth inhibition.
6. Higher concentrations of sodium ions can also lead to a reduction in photosynthesis and the production of reactive oxygen species.
7. Potassium ions are one of the essential elements required for growth. Alterations in K^+ ions (because of the impact of high salinity stress) can disturb the osmotic balance, the function of stomata, and the function of some enzymes.
8. High salinity can also injure cells in transpiring leaves, which leads to growth inhibition. This salt-specific or ion-excess effect of salinity causes a toxic effect of salt inside the plant. The salt can concentrate in the old leaves and the leaves die, which is crucial for the survival of a plant (see Munns *et al.*, 2006).
9. Shoji *et al.* (2006) demonstrated that high salinity affects cortical microtubule organization and helical growth in *Arabidopsis*.

Salinity and its effect on rice crop

Generally, all soils contain a mixture of soluble salt, some of which are essential for growth. The saline soil usually consists of chloride and sulphates of sodium, calcium and magnesium. Electrical conductivity in the saturation extract of such soils is greater than 4.0 mScm^{-1} and exchangeable sodium percentage (ESP) is less than 15. The response of various plants to such saline environment differs in nature *i.e.*, glycophytes are plant that tolerate low concentration of salt and usually referred as salt susceptible whereas halophytes are the tolerant plants that can tolerate relatively higher concentration of salts. Physiologically, high salt concentration affect the plant growth and development in three ways; firstly by decreasing the osmotic potential of soil and producing a water stress in plants, secondly causing ion toxicity, since Na^+ is not readily sequestered into vacuoles as in halophytes resulting into altered Na^+/K^+ ratio and thirdly by suppressing the supply of one or more mineral nutrient

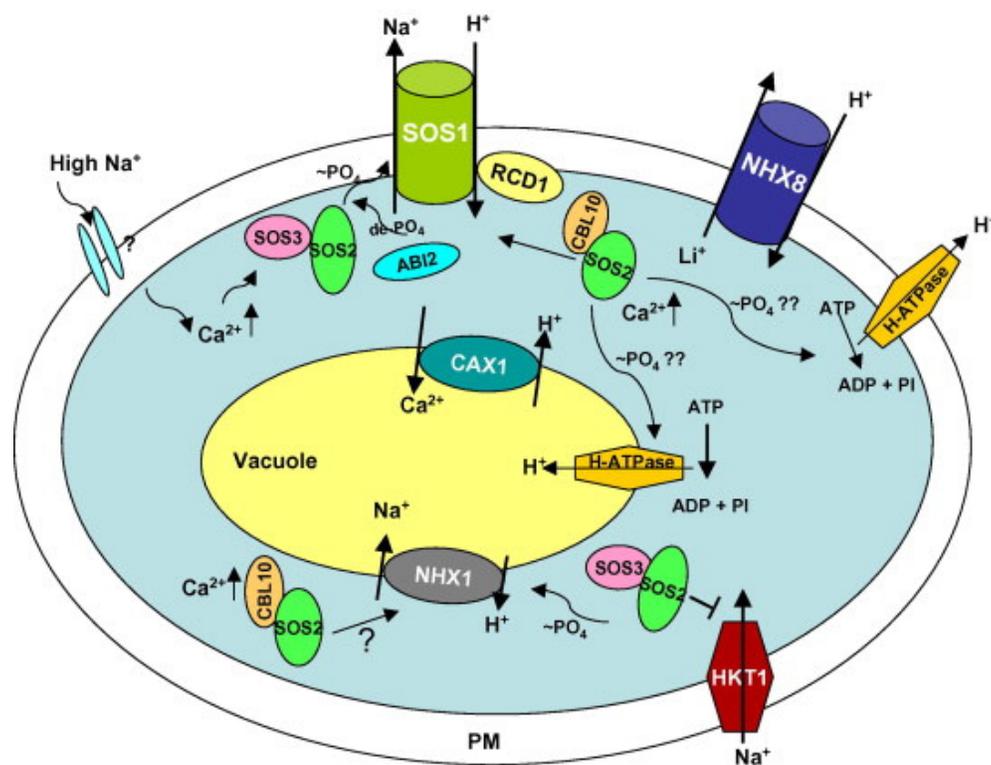


Fig. 1 : Regulation of ion homeostasis by various ion pumps. The salinity stress signal is perceived by a receptor or salt sensor present at the plasma membrane of the cell. This signal is responsible for activating various ion pumps present at plasma and vacuolar membranes. This signal also activates the SOS pathway, the components of which help in regulating some of these pumps. The various pumps/channels are the K⁺ inward-rectifying channel (KIRC), histidine kinase transporter (HKT), nonspecific cation channels (NSCC), K⁺ outward-rectifying channel (KORC), Na⁺/H⁺ antiporters (SOS1), vacuolar Na⁺/H⁺ exchanger (NHX) and H⁺/Ca²⁺ antiporter (CAX1). Na⁺ extrusion from plant cells is powered by the electrochemical gradient generated by H⁺-ATPases, which permits the Na⁺/H⁺ antiporters to couple the passive movement of H⁺ inside along the electrochemical gradient and extrusion of Na⁺ out of the cytosol. The stress signal sensed by SOS3 activates SOS2, which activates SOS1.

or mineral deficiency. The consequences of all these can ultimately lead to plant death as result of growth arrest and molecular damage (Mc Cure and Hanson, 1990).

Rice responses to salinity stress

In general, plants that are more salt-tolerant tend to grow more slowly at low salinity levels than less salt-tolerant plants. Broadly adaptable crop plants can produce good yields, where strong temporal changes of soil salinity occur in the soil. But, such plants, which tolerate a wide range of salinities, may perform less efficiently at any salinity than less adaptable plants at their optimal salinity. Salt-tolerant plants tend to produce less than sensitive plants because of: (1) greater allocation of organic in the roots of tolerant plants at the expense of leaf area; (2) decreased use of solar radiation; and (3) low transpiration rate. Adaptation to soil salinity is, in part, a reduction in the capacity for photosynthetic carbon assimilation consequent to the necessity of minimizing evapotranspiration. The efficiency of photosynthetic

pigments and nitrate reductase may decrease (Lal, 1996). The effects of soil salinity may interact with those related to climate and other edaphic factors in determining plant growth and development. Reduced availability of water in the soil is concomitant to higher evaporative demand in the leaves. The accumulation of compatible low molecular-weight compounds for osmotic adjustment may be in part related to their alternative function of protection from photo-oxidative damage. On the other hand, the diversion of nitrogen to form organic osmolytes is likely to increase the effect of salinity-dependent nutrient deficiencies and further decrease photosynthesis and growth (Ball and Sobrado, 1999). At low fertility levels, plants may appear more tolerant to salinity than at high fertility levels, if salinity is not the limiting factor of yield (Maas and Hoffman, 1977). On the other hand, nutrient deficiencies induced by salinity and sodicity can further reduce crop yields due to low soil fertility (Bernstein, 1964 & 1974).

The most important cereal crop in the world is rice, yielding one-third of the total carbohydrate source. Three billion people consider rice as their staple food, accounting for 50–80% of their daily calorie intake. Rice is a salt-sensitive monocot (Maas and Hoffman, 1997; Francois and Mass, 1994). In particular, seedlings and the reproductive stages are very sensitive to salinity, while germination is relatively tolerant (Lutts *et al.*, 1996; Zheng and Shannon, 2000; Zheng *et al.*, 2001). Understanding the mechanisms by which plants perceive salt stress signals and convey them to the cellular machinery to activate adaptive responses, is important for the development of classical breeding programs as well as for biotechnological methods to improve the salt tolerance of crops. Salt tolerance depends on a complex signaling network, involving multiple signal transduction pathways, allowing plants to respond rapidly and properly to salt stress (Zhu, 2001). These include reactive oxygen species, nitric oxide, calcium, protein kinases, including calcium-dependent protein kinase (CDPK), sucrose non-fermenting-related kinases (SnRKs) and mitogen-activated protein kinases (MAPKs), and various lipid second messengers. Rice cultivars vary in their ability to tolerate salt stress; with both salt-tolerant and salt-sensitive lines being available (Zeng *et al.*, 2004 and Zeng, 2005) the response of rice to salinity varies with growth stage. In the most commonly cultivated rice cultivars, young seedlings were very sensitive to salinity (Lutts *et al.*, 1995; Zeng and Shannon, 2000b). Tillering and booting phases are two physiologically important growth stages contributing to good plant population stand as well as yield (Alamgir and Ali, 2006). Rice is considered moderately tolerant to exchangeable Na. The tolerance to sodicity of rice may lead to soil sodication if irrigation is carried out with sodic water. Therefore, in order to maintain long-term soil productivity, rice may be excluded from crop rotations if alkaline water is used for irrigation (Eynard *et al.*, 2005). Zeng and Shannon (2000b) showed that shoot dry weights of main culms were not significantly reduced by salinity until EC was 6.1 dS/m or higher. Asch and Wopereis (2001) studied the effect of field-grown irrigated rice cultivars to varying levels of floodwater salinity and concluded that floodwater EC levels >2 dS/m may lead to losses up to 1 t/ha per unit. Other effects of salinity include nutrient uptake and metabolism, ion accumulation and photosynthesis. Salt stress in the soil generally involves osmotic stress and ion injury. High salt environments can break the ion homeostasis of plant cells, destroy the ionic balance, and affect the distributions of K^+ and Na^+ in the cells. It is necessary to re-establish the ion homeostasis in cells for plant living under salt-stress.

Plant survival and growth in saline environments is a result of adaptive processes such as ion transport and compartmentation, compatible solutes synthesis and accumulation. Many of these compatible solutes are N-containing compounds, such as amino acids and betaines; hence the nitrogen metabolism is of central importance for salt tolerance. It is well known that salt stress has different effects on old and young tissues. Thus, the understanding of comparative effects of salt stress on old and young tissues may be important for salt tolerance research. Although, plant biologists have extensively reported the difference between old and young tissues, many questions remain. Salt stress in soil generally involves osmotic stress and ion-induced injury, and Na^+ is the main toxic ion in salinized soil. Low Na^+ and high K^+ in the cytoplasm are essential for the maintenance of a number of enzymatic processes. Transmembrane K^+ movements in plants are mediated by several types of channels, including the AKT family and by transporters that belong to two families, KcsA-TRK (HKT) and KUP/HAK/KT (HAK). The extent of tolerance by plants to Na^+ stress depends on ion compartmentalization and exclusion. In higher plants, the salt overly sensitive protein 1 (SOS1) functions in Na^+ exclusion from root epidermal cells into the rhizosphere, which also plays a role in retrieving Na^+ from shoots to roots. SOS3–SOS2 (CIPK24–CBL4) protein kinase pathway mediates regulation of the expression and activities of Na^+ transporters such as SOS1 and Na^+/H^+ exchanger (NHX) that mediates Na^+ compartmentalization into vacuoles. In rice plants, a high affinity K^+ transporter (HKT) family, OsHKT1; 5, mediates Na^+ exclusion from shoots via Na^+ .

Biotechnological approach

In general terms, biotechnology is referred as genetic manipulation involving transfer of genes across species boundaries or cloning of animals. However, in broad sense, it also involves conventional breeding in which genome of one individual is combined with other using pollen as vector. With the advent of recent techniques like genome mapping, gene sequencing, marker assisted breeding etc, new opportunity for understanding the salt stress resistance mechanisms in plants has arisen. Following are certain approaches which are usually adopted for genetic manipulation in plants:

a) Genetic mapping and marker assisted breeding : The direct selection of superior salt-tolerant genotypes under field conditions is hindered by the significant influence that environmental factors have on the response of plants to salinity (Richards, 1996). There is also evidence supporting the notion that salt tolerance is a complex trait involving the functions of many genes

(Fooland *et al.*, 1999 and Flowers, 2004). Genetic mapping work to identify abiotic stress tolerance genes has been limited relative to work on gene discovery and transformation. Genomic regions or quantitative trait loci (QTL) has been identified for salt tolerance in the *Citrus grandis* × *Poncirus trifoliata* F1 hybrid and its back cross to *Citrus grandis* (Tozlu *et al.*, 1999a) and for salt tolerance under waterlogged conditions in the *Eucalyptus camaldulensis* × *E. grandis* hybrid (Dale *et al.*, 1999). Moreover, QTLs associated with salt tolerance at the germination stage in barley (Mano and Takeda, 1997), tomato (Fooland *et al.*, 1999) and Arabidopsis (Gregario *et al.*, 2002) were different from those QTLs associated with salt tolerance at the early stage of growth; the plants selected by their ability to germinate at high salinity did not display similar tolerance during vegetative growth.

The development of molecular biology techniques has enabled the development of DNA markers that can be used to identify QTLs. The use of QTLs has improved the efficiency of selection, in particular, for these traits controlled by several genes. The development of high density DNA maps that incorporate microsatellite markers, RFLP (Restriction fragment-length polymorphisms) and AFLP (Amplified fragment-length polymorphisms) and advances in marker assisted selection techniques will facilitate pyramiding traits of interest to attain substantial improvement in crop salt tolerance.

b) Gene discovery, sequencing and micro array analysis : Most genes involved in salt stress mechanism discovered so far, have been identified by traditional molecular biology techniques such as differential hybridization, subtractive hybridization etc. and by genetic data analysis of homologous gene from model organism. Moreover, protein crystallography has enabled detailed investigation of the structure and function of protein transcribed for salt tolerant genes.

c) Plant transformation : Once genes involved in stress tolerance have been identified, they may be employed to modify stress tolerance in crop plants either as tagged genes or transfer genes across species boundaries through plant transformation. Techniques for transferring foreign genes into plants include electroporation, PEG mediated gene transfer, microinjection, particle or micro projectile bombardment and Agrobacterium-mediated gene transfer (Holmberg and Bulow, 1998). These techniques are now available for several crops (Bartels and Nelson, 1994). The salt tolerance of the plants in the field needs to be evaluated, particularly evaluated as a function of yield. Although,

there have been many successes in developing stress-tolerant transgenic in model plants *i.e.*, tobacco, arabidopsis or rice (Grover, 2003), there is urgent need to test these success in other crops.

Conclusion

An increase in rice production on saline soil should meet the demand of the world's current population growth rate. A less costly means to achieve this aim of increase rice productivity even under salinity stress, wherever possible. Many rice-producing countries, even in the developed countries, still show a big gap between the present and potential yield. Hence, efforts should be focused on identifying the causes of this gap, especially in the production factors and the various features of crop management. An adequate understanding of physiological aspects of rice productivity under saline condition will facilitate the above efforts and provide relevant measures for the improvement of rice production at the field level. At present, many national rice research programmes are well organized and developed. Upstream research deserves more attention from funding donors and scientists in order to increase the rice productivity and production in adverse saline soil, with more emphasis being placed on rainfed and marginal ecologies to support the world's current population growth. As financial and human resources are limited in many countries, regional/inter-regional and international collaboration and cooperation in rice research would offer practical solutions to meet the world's increased food demands. Increasing the yield of rice crop in normal soil as well as in salinized lands is an absolute requirement for feeding the world. The development and use of new variety that can tolerate the high levels of salinity in the soils would be a practical contribution towards addressing the problem. The approaches that identify specific genes are up or down regulated either through the analysis of RNA or proteins might provide a scientific focus for transformation. Transgenic technology will undoubtedly continue to aid the search for the cellular mechanism that underlines tolerance. In our opinion, there is urgent need on research work aiming at isolation of new genes designing of vectors, transformation and evaluation of progenies. If it could happen, it will be an asset for the appropriate utilization of salt affected soil for agriculture productivity solving the global food crisis.

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