Review Article

Rice breeding for salt tolerance

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Human exploitation of earth's resources is leading to new problems day by day and hence newer methods are devised to overcome the same. One such case is breeding for abiotic stress, utilizing even non arable land, to feed the ever increasing population. A major stress leading to yield loss can be due to inland and coastal salinity. Rice is the most important cereal crop which feeds humans on earth. This review tries to analyse the efforts of breeders in minimising the effects of salt stress on the yield of rice crop.

Abiotic stress and biotic stress breeding are essential ways to combat yield reduction. Scientists around the world are putting in their best efforts to produce varieties and hybrids with improved heterosis under stress affected environments. The article aims to review the efforts put in by breeders to solve salinity associated problems.

Importance of Rice

Rice, most loved cereal of Asia, feeds the majority of the world's population. More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's people and about two-thirds of the world's poor live (Khush and Virk, 2000). Green revolution helped to solve the world's demand for food, but is not enough to meet the 21st century's exploding population. Improved rice varieties and hybrids developed by institutes throughout the world including IRRI have helped to improve the quality and quantity of rice production.

Land resource

Cultivable area under rice needs to be increased to improve the production demands. Due to over usage of land for cultivation by dumping in of inorganic fertilizers, poor crop rotation, use of underground water etc., the fertility of arable lands are undergoing a decline. Reclamation of land for acidity, salinity, alkalinity, hardpan, nutrients etc., needs to be undertaken periodically. Ahmed *et al*. (2001) state that, to cope up with the ever increasing demand for rice it should be met with quantum jump in production in fixed cultivable area. This is a daunting task, in view of plateauing trend observed in yield potential of high yielding varieties and decreasing and declining natural resource base.

Salt stress

About 6.5% (831 million ha) of the world's total area (12.78 billion ha) is affected by salt in soils (FAO). Area under salt stress is on the increase due to many factors including

climate change, rise in sea levels, excessive irrigation without proper drainage in inlands, underlying rocks rich in harmful salts *etc.,* Vast areas of land are not utilised due to salinity and alkalinity problems.

According to Reynolds *et al.* (2001), soil salinity (accumulation of salts in the surface zone) has a number of causes, which differ in different geological and climatic regions. The causes can be natural, be due to clearing of native vegetation ('dryland salinity'), or due to irrigation. Salinity is often accompanied by other soil properties, such as sodicity, alkalinity, or boron toxicity, which exert their own specific effects on plant growth. Water logging often accompanies salinity. Crop species show a spectrum of responses to salt, although all have their growth and, eventually, their yield reduced by salt. Salt effects are the combined result of the complex interaction among different morphological, physiological and bio-chemical processes.

Saline soils are classified based on electrical conductivity (EC) of the soil solution which detects osmotic problems and exchangeable sodium percentage (ESP) indicative of a physical dispersion problem. Salt affected soils are classified as non-saline/non-sodic soils (ESP ≤15%; EC ≤ 4 mmhos cm-1), saline soil (ESP ≤ 15%; EC > 4 mmhos cm-1), sodic soil (ESP > 15%; EC ≤ 4 mmhos cm-1) and as saline sodic soil (ESP > 15%;   EC > 4 mmhos cm-1). The pH of saline soils is generally less than 8.5, of saline sodic soils about 8.5 and of sodic soils more than 8.5 (USDA, 1954).

Sensitivity of Rice to salt stress

Salinity, a serious problem affecting one third of all the irrigated land in the world (Mass and Hoffman, 1977), impairs normal growth and limits the realization of yield potential of modern cultivars. Salinity is one of the major obstacles to increase production in rice growing areas worldwide. Rice is rated as an especially salt-sensitive crop (Shannon *et al.*, 1998). According to the classification of salt tolerance to salinity, the rice crop is within the sensitive division from 0 dsm-1 to 8 dsm-1 (Mass, 1986). There are two essential parameters sufficient for expressing salt tolerance. Threshold means the maximum allowable salinity without yield reduction and slope means the percent of yield reduction per unit increase in salinity beyond the threshold. The threshold and slope of rice (*Oryza sativa*) are 3.0 dsm-1 and 12% per dsm-1 of saturated soil extract (EC_e), respectively (Mass and Hoffman, 1977). Relative salt tolerance of rice at 50 % yield and at 50 % emergence are 3.6 dsm-1 and 18 dsm-1 of EC_e, respectively (Wahhab, 1961).

Rice is differentially affected by salinity at different stages. The effects of salinity on the growth of rice were found to be related to the stage of plant development, salt concentration, type of salt, duration of exposure to salt, soil pH, water regime, temperature, humidity and solar radiation (Akbar, 1986). Rice crop is relatively tolerant to salinity and alkalinity during germination stage. The germination though affected by salinity can be appreciably overcome in the later stages (Akbar and Yabuno, 1974).

Janaguiraman *et al*. (2003) have reported that, tolerant genotypes of rice record a higher germination percentage, root length, shoot length, vigour index and amylase and dehydrogenase activity with less accumulation of anthocyanin in their roots. Deepa Sankar *et al*. (2006) have ranked salt tolerant rice lines based on germination and seedling growth under salt stress conditions. Under the high concentration of 1.0 M $MgCl_2$: NaCl : $CaCl_2$: Na_2SO_4 (1:4:5:10) and at 1.6 % NaCl , the genotype CSR 23 and CSR 10 performed well for germination per cent, CO 43 and Nona Bokra performed well for total biomass to utilized seed ratio and CSSRI 60 for vigour index based on LD_{50} values.

Breeding for salt stress

In recent years the development of salt-resistant varieties has been proposed as a means of expanding agriculture into the regions affected by salinity (Epstein, 1980).Breeding rice varieties with in-built salt tolerance is realized as the most promising, less resource consuming, economically viable and socially acceptable approach. Salt tolerance is a multigenic trait that allows plants to grow and maintain economic yield in the presence of non-physiologically high and relatively constant levels of salt, in particular NaCl (Hurkman, 1992).

Rice productivity in salt-affected areas is very low, <1.5 t/ha, but can reasonably be raised by at least 2 t/ha (Ponnamperuma 1994), providing food for more than 10 million of the poorest people living off these lands.

There are only six rice varieties released for salt tolerance in the country so far through Central Variety Release Committee (CVRC) as reported by Singh *et al*. (2004). There are many rice varieties released for combating salt related problems related to particular state of India. There are a number of salt tolerant rice varieties available for different levels of soil stresses *viz*., CSR 10 for very high stress; CSR 13, CSR 27, Narendra Usar 2 and Narendra Usar 3 for moderate to high stress and a basmati CSR 30 rice for moderate stress and they are being utilized for land reclamation programmes in Uttar Pradesh.

Major progress has been made in breeding salt tolerant high yielding rice varieties for various inland saline, coastal saline and alkaline soils of fragile ecosystems (Mishra *et al*., 2003). Of 32 salt tolerant rice varieties developed by Central Soil Salinity Research Institute (CSSRI), Karnal, CSR 10 was the first dwarf high yielding salt tolerant early maturing rice variety released. The recent adapted varieties in coastal saline soils are SR 26 B, CSR 1, CSR 2, CSR 3, CSR 27, CSR 13, Panvel 1, Panvel 2, Panvel 3, Pokkali (in Kerala only), Vytilla 1 and Vytilla 2. CSR 22, CSR 23, CSR 26, CSR 27 and CSR 30 have been identified to suit sodic soils.

Salt tolerant rice varieties like Pokkali, Vytilla 1, Vytilla 2, Vytilla 3, Vytilla 4 and Vytilla 5 have been developed by the Kerala Agricultural University to solve the coastal salinity problems of the state. SR 26 B from Orissa, Kalarata, Bhurarata, Panvel 1, Panvel 2, Panvel 3 from Maharashtra and BTS 24 from Andaman and Nicobar Islands has been developed to combat salinity problems (IRRI).

In Tamil Nadu the coastal area is about 0.68 million ha. Out of which problem

soils constitute 30 % and saline soils constitute 21.7% of the total problem soils indicating vast area under alkaline and saline-alkali soils (Jauhar Ali *et al.,* 1999). High yielding varieties *viz*., Savitri, Ponni, Swarnadhan, Mansarovar, Salivahan and Pavizham were found suitable for shallow water salinity condition. Varieties like BR 10, Patnai 23, SR 26 B, PVR 1, PY 1, CSR 1, CSR 4, Co 43, AD 85002, IET 8113, TRY 1 and TRY 2 are generally found suitable for saline areas of Tamil Nadu.

The hybrid CORH 2 developed by three line breeding system (A/B/R) has been found suitable for solving inland salinity problems. With the availability of many types of TGMS lines and no restriction of restorer it is relatively easier to develop desirable new two-line hybrids than three line rice hybrids (Thiyagarajan *et al*., 1999). TGMS can combine with other cultivars without the need for specific restorers (Yuan, 1990) and hence can be utilized for developing high yielding saline tolerant hybrids.

In vitro screening for salt stress
Conventional approaches are not only cumbersome but also time consuming. Plant cell and tissue culture techniques offer a potent tool in developing salt tolerant lines (Stavarek and Rains, 1984).

Reddy and Vaidyanath (1985) studied the response of salt stress on embryo derived calli of rice to identify cellular phenotypes associated with the stress. They reported that callus growth decreased markedly with increasing NaCl concentration in the medium and proline content was enhanced several fold in the salt stressed calli. Regeneration

frequency in the salt selected lines was enhanced when compared to unselected lines.

Chauhan *et al.* (1997) suggested that the ability of the cells to maintain higher concentrations of K^+ and lower levels of Na^+ and Cl^- coupled with the maintenance of higher concentration of sterols and polyamines contribute to the salt tolerance in rice callus.

Pushpam and Sree Rangaswamy (2000) confirmed that there was a decline in callus growth and score at higher concentration of NaCl compared to low levels. Decline in growth of callus in the NaCl environment was due to diversion of some quantum of energy for growth and metabolism. They also observed that the unstressed calli grew faster and recorded highest growth and weight as compared to stressed calli.

In vitro screening for salt stress in different national rice hybrids was performed by Shanmuganathan (2001). It led to the identification of three hybrids CORH 2, DRRH 1 and PSD 1 as salt tolerant and hybrids KRRH 1 and CNRH 3 as moderately salt tolerant.

Sangita Basu *et al.* (2002) reported that the presence or retention of K^+ in rice callus was a key factor for salt tolerance as it was found to be positively correlated with growth while proline was probably the last metabolic device that rice calluses opted for when exposed to salt stress.

Deepa Sankar *et al.* (2009) analysed five temperature sensitive genic male sterile lines(TGMS), eight salt tolerant testers and 40

hybrids obtained by crossing them in line x tester design for slat tolerance under *in vitro* condition. Hybrid GD 98029/CSSRI 13 ranked first followed by hybrids GD 98028/CO 43, GD 98028/CSR 23, GD 98029/CSR 10, GD 98029/Nona Bokra and the parent Nona Bokra in MS media supplemented with 2,4-D 2 mg l^{-1}, kinetin 0.25 g l^{-1} and casein hydrolysate 1 g l^{-1} along with NaCl at 1.6 per cent concentration exhibiting their potential for salt tolerance.

Field screening for salt stress

Screening of rice genotypes based on traits like Spikelet sterility, Na$^+$: K$^+$ ratio is being followed by breeders in salt affected soils. A high percentage of spikelet sterility relates to a low level of salt tolerance in rice. Low Na$^+$/K$^+$ ratio of ion uptake is positively correlated with a high level of salt tolerance and can be taken into consideration as a desired characteristic while screening rice lines (Mishra *et al.*, 1997).

Karim and Haque (1986) reported that plant height, root length, number of tillers per plant, straw yield and dry weight of root was affected by salinity during vegetative stage. In a study performed by Ansari *et al.* (2003) cultivars Ganga White, Nona Bokra, IR 6 and Shua-92 had better seedling survival, whereas IR 28 and Basmati had the lowest seedling survival. These cultivars had a more or less similar ranking in straw or grain weight. Zeng *et al*. (2000) reported that the reduction in spikelet number per panicle was a major cause of yield loss under salinity. Ansari *et al.* (2003) have reported that sodium uptake increased and potassium decreased with increasing salinity.

Genetics of salt tolerance in rice

Combining ability for various economic traits *viz.,* days to 50 per cent flowering, plant height, productive tillers per plant, panicle length, grains per panicle, spikelet fertility, 100 grain weight, single plant yield, Na$^+$: K$^+$ ratio and salinity tolerance in rice under saline conditions has been performed by various scientists which helps breeders to design their breeding programme for improving the trait. Kalaiyarasi *et al.* (2002) reported the preponderance of non additive gene action in terms of yield components from a study based on two line inter-subspecific rice hybrids. Deepa Sankar *et al.* (2008) have reported that the traits days to 50 per cent flowering, productive tillers per plant, panicle exsertion, panicle length, panicle weight, filled grains per panicle, spikelet fertility, 100 grain weight, Na$^+$: K$^+$ ratio, harvest index and grain yield per plant are controlled by dominance gene action and plant height was controlled by additive gene action.

Heterosis in rice under saline/ alkaline condition

The phenomenon of heterosis in rice was first reported by Jones in 1926. Successful development and extensive adoption of hybrid rice technology in China prompted augumentation of hybrid rice breeding in India since 1980 (Siddiq, 1987). Several workers have reported the occurrence of heterosis for various yield and yield components in rice. However reports on heterosis in rice under salinity are very limited.

In a study undertaken at CSSRI, significant heterosis over the mid-parent and better parent was observed for almost all the characters studied (Mishra *et al.*, 2003). Out of the 15 F_1's, crosses Pokkali/IR 28 (79.87), CSR 10/IR 28 (67.18), CSR 13/IR 28 (54.58) and CSR 1/IR 28 (48.56) indicated a positive and significant heterotic response over the mid-parent and cross Pokkali/IR 28 (49.31) over the better parent in alkali soil, whereas CSR 13/ IR 28 (35.17) and CSR 10/CSR 13 (26.72) over the mid-parent and only one cross (CSR 10/CSR 13, 24.53) over the better parent in saline soil exhibited better heterotic effects.

Deepa Sankar *et al.* (2008) have reported the presence of heterosis for grain yield per plant in normal and salt affected environments. The TGMS based rice hybrid GD 98179/CSSRI 60 recorded the highest standard heterosis per cent for the trait grain yield per plant in all the environments followed by GD 98168/Vytilla 3, GD 98029/CSSRI 60, GD 98029/CSR 23 and GD 98028/ Nona Bokra. In salt affected environments the hybrids GD 98028/CSR 23 and GD 98021/CO 43 recorded high heterosis for both $Na^+ : K^+$ ratio and grain yield per plant.

Stability of genotypes in salt affected environments

Varietal adaptation to environmental fluctuations is important for the stabilization of crop production both over region and years. Stability refers to the suitability of a variety for general cultivation over a wide range of environments. The Genotype x Environment structure is an important aspect of both plant breeding programmes and the introduction of new crop cultivars (Freeman, 1985). Rice varieties suited to normal non-

stress soil condition rarely or mostly may not perform under high salt stress conditions. Thus, there is urgent need to evaluate salt tolerant stable rice varieties, which could withstand the varying salt stress condition during crop growth period (Mishra *et al.,* 2000).

Rogbell (1995) studied stability in rice for salt tolerance involving 12 parents and their 35 hybrids. The analysis showed that G x E interaction was significant for all the characters except filled grains per ear and 100 grain weight. The parents IR 64 and IR 54717-C10-113-1-2-2-2 and hybrid combinations namely CAN 4121 x IR 54717-C10-43-1-2-2-2, CAN 4206 x IR 4630-22-2-5-13 and IR 64 x IR 54717-C10-113-1-2-2-2 were stable over environments for grain yield per plant.

Seventeen elite rice genotypes including CSR 10 (tolerant) and Jaya (high yielding) were evaluated at five different locations ranging from non-stress to highly deteriorated sodic soils (pH >10.2) in two successive years (Kharif 1995 and 1996) by Mishra *et al.* (2000). The mean performance of the genotypes in different environments differed significantly for yield and yield components. The linear component of variation (b_i) was found to be non-significant for yield and component traits, indicating that the genotypes do not respond to the stress linearly and behave differently under different set of environment depending upon their level of tolerance. The non-linear component of variation (S_{di}^2) was found to be significant for yield and other traits. Test of deviation from the unit regression (bi = 1) indicated for all the traits a number of genotypes significantly differ in their

regression coefficient value from unity, thus suggesting the differential behaviors of genotypes under different environments. Salt tolerant genotype CSR-88IR-12 appeared to be most stable and highly adaptable over environments.

Stability analysis for salt stress in different national rice hybrids was performed through Eberhart and Russell and AMMI models by Shanmuganathan (2001). The analysis showed that the G x E interaction was significant for all the characters studied. Hybrids ADTRH 1, KRRH 1 and PHB 71 for Kharif season and hybrid PHB 71 for Rabi season were observed as stable performers. CORH 2 and PHB 71 were adjudged as stable and adaptable for cultivation in both Kharif and Rabi seasons of Tamil Nadu. KRRH 1 was the stable hybrid for the trait per day productivity regardless of season indirectly indicating the remunerative nature of this hybrid among farming community.

Mishra *et al*. (2003) have identified stable salt tolerant genotypes for yield and its components on the basis of stability parameters under multiple stress environments. The most stable and adaptable genotypes identified are CSR 10, CSR 11, CSR 13, CSR 27, CSR 1, Pokkali, CSR 18 and CSR 21for grain yield , filled grains per panicle, low spikelet fertility(%), Na/K ratio and K absorption.

Deepa Sankar *et al.* (2008) have evaluated five TGMS lines, eight salt tolerant testers and 40 hybrids under normal and salt affected environments. AMMI analysis revealed that among parents CO 43 and among hybrids GD 98179/CSSRI 60 to be the best adapted genotype or winner in the mega-environment.

Marker assisted selection for salt tolerance

Marker assisted selection can help in locating salt tolerance genes which can be introduced into high yielding varieties by conventional breeding or by genetic engineering. Mapping Quantitative trait loci for salt tolerance in rice have been reported by many scientists.

Zhong-Hai Ren *et al.* (2005) have reported that, a rice quantitative trait locus, *SKC1* for salt tolerance which encodes a sodium transporter. Physiological analysis suggested that *SKC1* is involved in regulating K^+/Na^+ homeostasis under salt stress, providing a potential tool for improving salt tolerance in crops. International Rice Research Institute (IRRI) is working on a project that aims to identify donors of superior salt tolerance mechanisms, and develop and use DNA markers to combine underlying QTLs/genes into varieties and breeding lines adapted to target areas. A major QTL, designated Saltol was recently fine-mapped and a marker-assisted backcrossing system is being developed for its incorporation into popular varieties by scientists working in IRRI.

Transgenic rice for salt tolerance

Breeders around the world are trying to create transgenic plants for solving varied problems like improving yield, stress tolerance, increase in shelf life etc., Transgenic plants which can improve salt tolerance in rice have been attempted.

Hoshida *et al.* (2000) have reported on enhanced tolerance to salt stress in transgenic

rice that over expresses chloroplast glutamine synthetase gene. Salt tolerance of transgenic rice with *mtlD* gene and *gutD* gene were analysed by Huizhong Wang *et al.* 2000. Analysis of sugar alcohol showed that transgenic rice plants could produce and accumulate mannitol and sorbitol. The salt tolerance of transgenic plants was much higher than that of their controls. Fengyun Zhao *et al.* (2006) have reported on the expression of yeast Na^+/H^+ antiporter *SOD2* in transgenic rice. These transgenic plants accumulated more K^+, Ca^{2+}, Mg^{2+} and less Na^+ in their shoots compared with those of non-transformed controls. Xujun Chen and Zejian Guo (2008) have reported that tobacco OPBP1 enhances salt tolerance and disease resistance of transgenic rice.

There is an International concern on increasing the cultivable area under salinity. Rice is a major crop consumed by most of the human population around the world and its demand is always increasing. Improving the yield of rice under salt affected areas is an immediate requirement. The services rendered by breeders towards solving the problem are appreciable.

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