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Review article

A COMPREHENSIVE REVIEW ON "GENETIC COMPONENTS OF SALINITY TOLERANCE IN RICE (*ORYZA SATIVA* L.)"

M.Sudharani[1], P.Raghava Reddy[2], V.Jayalakshmi[3]

[1](Corresponding Author), Scientist (Plant Breeding) Seed Research and Technology Centre, Rajendranagar, Hyderabad-30. E mail:madugula.sudharani@yahoo.com

[2] Former Vice-Chancellor, ANGR Agril.University, Rajendranagar, Hyderabad-30.

[3]Senoir Scientist (Plant Breeding), Regional Agricultural Research Station, Nandyal.

Rice (*Oryza sativa L.*) is the most important food crop in the world, which accounts for more than 21% of the calorific needs of the world's population and up to 76% of the calorific intake of the population of South East Asia (Ma *et al.*, 2007 and Melissa *et al.,* 2009). Rice production employs one billion people and is essential for the economic development of rural areas in India, Bangladesh and Southeast Asia and provides rural employment and prosperity.

Though significant improvement in productivity has been achieved over the years, a series of biotic and abiotic stresses limits its productivity worldwide. Abiotic stresses alone contributes to 50 per cent of the total yield losses. Among abiotic stresses, salinity, drought and extreme temperatures are major barriers to limit rice crop production. High salt concentration in soil is the major constraint to rice production in Bangladesh and India (Mohammadi-Nejad *et al*., 2008). The loss of farmable land due to salinisation is directly in conflict with the needs of the world population. Therefore, increasing the yield of rice in poor soils and in less productive salinised lands is essential for feeding the world.

Nearly 20 per cent of the world`s cultivated area (800 M ha) and nearly half of the world's irrigated lands are affected by salinity (Zhu *et al*., 2001 and Maser *et al.*, 2002). The area is still increasing as a result of secondary salinization and land clearing (FAO, 2005 and Metterhichi and Zinck, 2003).Hence, through understanding of the effects and mechanism of salt tolerance would be very useful for further genetic improvement.

1. Effect of salinity on rice and importance of growth stage.

Gregorio (1997) emphasized that salinity symptoms were prominent on the first and second leaves and were visualized by leaf rolling, formation of new leaf, brownish and whitish leaf tip, drying of leaves and also reduction in root growth, stunted growth and stem thickness leading to complete cessation of growth and dying of seedlings. Extremely high salt stress conditions cause severe damage to plants, while moderate to low salt stress affects the plant growth rate along with most of the growth and yield parameters like low tillering, stunting, spikelet sterility, less florets panicle^{-1}, low 1000-grain weight and leaf scorching etc.

Rice is relatively tolerant during germination, becomes very sensitive during early seedling stage, gains tolerance during active tillering, but becomes sensitive during panicle initiation, anthesis and fertilization and finally relatively more tolerant at maturity (Makihara *et al.*, 1999 and Singh *et al.*, 2004). Studies have shown that a very poor correlation exists between tolerance at seedling stage with that during reproduction, suggesting that tolerance at these two stages is regulated by a different set of genes (Moradi *et al.*, 2003). The reproductive stage is crucial as it ultimately determines the grain yield. However, the importance of the seedling stage cannot be undermined as it affects crop establishment. Salinity reduces the growth of plant through osmotic effects, reduces the ability of plants to take up water and this causes reduction in growth. There may be salt specific effects. If excessive amount of salt enters the plant, the concentration of salt will eventually rise to a toxic level in older transpiring leaves causing premature senescence and reduces the photosynthetic leaf area of a plant to a level that cannot sustain growth (Munns, 2002).

Alam *et al.* (2004) attributed the possible reasons for decrease in the shoot and root growth in salinized plants as reduction of photosynthesis, which in turn limits the supply of carbohydrates needed for growth and reduction of turgor in expanding tissues resulting from lowered water potential in root growth medium.

2. Different kinds of tolerance mechanisms to salt stress in plants (Glycophytes)

Under salt stress conditions, the crop plants either try to avoid the stress, which is indeed not an actual tolerance mechanism or employ the following mechanisms to overcome the salt damage in a sequential adaptations.

a. Seedling Vigour: Rice cultivars differ substantially in their growth rate with the most vigorous lines being the traditional varieties. Naturally occurring salt resistant varieties invariably belong to these traditional tall varieties. The high vigour of land races may enable them to tolerate growth reduction. Vigorous growth also has a dilution effect. The Na^+ uptake of the salt tolerant land race 'Pokkali' is not less than the salt sensitive dwarf IR-28 but the low Na^+ concentration in Pokkali is attributed to the diluting effect of its rapid vegetative growth (Yeo and Flowers, 1984 and Bohra and Doerffling, 1993).

b. Initial entry of salts from roots: Plant roots experience the salt stress when Na^+ and Cl^- along with other cations are present in the soils in varying concentration (1 to 150 mM for glycophytes and more for halophytes). The toxic ions sneak into the plant along with the water stream which moves from soil to the vascular system of the root by different pathways like symplastic and apoplastic. Na^+ and K^+ are mediated by different transporters which is clearly demonstrated by Garciadebleas *et al.* (2003). Substantial genetic variability in the rate of sodium uptake by rice roots is present signifying a sizeable potential for genetic improvement.

c. Intra-cellular Compartmentation: Number of mechanisms are reported to affect the salt tolerance in plants based on cell level tolerance.

i. Ion Homeostasis Pathway: Ion homeostasis in cell is taken care of by the ion pumps like antiporters, symporters and carrier proteins on membranes (plasma membrane or tonoplast membrane). Salt Overly Sensitive (*SOS*) regulatory pathway is one good example of ion homeostasis. This pathway is activated after the receptor perceives the salt stress to alter protein activity and gene transcription by signaling intermediate compounds (Liu and Zhu, 1998; Ishitani *et al.*, 2000; Halfter *et al.*, 2000; Qui *et al.*, 2002; Quintero *et al.*, 2002 and Guo *et al.* 2004). Addition of salt induces the Na^+/H^+ antiporter activity but it increases more in salt tolerant than salt sensitive species (Staal *et al.*, 1991). Na^+ which enters leaf cells is pumped into vacuole before it reaches to toxic level for enzymatic activities. This pumping activity is controlled by valuolar Na^+/H^+ antiporters (Blumwald *et al.*, 2000). This mechanism has been emphasized by certain experiments where over-expression of vacuolar transporter (*NHX1)* has increased the salinity tolerance of *Arabidopsis* (Apse *et al.*, 1999), tomato (Zhang and Blumwald, 2001), *Brassica napus* (Zhang *et al.*, 2001) and rice (Fukuda *et al.*, 2004). This increase uptake of Na^+ to short vacuoles could facilitate enhanced storage of Na^+ and ultimately conferring greater tolerance by reducing Na^+ in cytosol.

ii. Synthesis of Osmoprotectants: Organic solutes such as sugar, alcohol proline and ammonium compounds are produced in response of osmotic stress (Johnson *et al.*, 1968). These are localized in cytoplasm and the inorganic ions such as Na^+ and Cl^- are preferentially sequestered into vacuole, thus leads to the turgor maintenance for the cell under osmotic stress (Flowers *et al.,* 1977 and Bohnert *et al.*, 1995). Trehlose, a non-reducing sugar, possess a unique feature of reversible water storage capacity to protect biological molecules from desiccation damages. Garg *et al.* (2002) demonstrated the expression of trehlose biosynthesis in conferring the tolerance to multiple abiotic stress. The increase in trehlose levels in transgenic rice lines of Pusa Basmati-1 using either tissue specific or stress dependent promoter, resulted into the higher capacity for photosynthesis and concomitant decrease in the extent of photo-oxidative damage during salt, drought and low temperature stresses.

iii. Signal Pathway – Transcription factors: The promoter regions *viz.,* dehydration-responsive elements (*DRE*s) and ABA responsive elements (*ABRE*s) which are involved in the plant responses respond to the osmotic effect. The transcription factor *DREB1A* specifically interacts with *DRE* and induces the expression of stress tolerance genes. Constitutive over-expression of source of the genes encoding for these proteins can induce the constitutive expression of many genes resulting into increased tolerance but it was associated with reduced growth even under unstressed condition. Hence, the stress inducible promoters (*rd29A*) are preferred to have normal plants showing enhanced stress tolerance (Shinozaki and Yamaguchi-Shinozaki, 2000).

iv. Stress activated protein pathway: Plants produces many kinds of stress responsive proteins induced by various kinds of stresses like heat, cold, salt or drought etc. Major one of them are *LEA*, dehydrins etc. (Baker *et al.*, 1988; Dure, 1992; Moons *et al.*, 1995 and Bray, 1997). These are reported to play an important protective role during desiccation/salt stress in rice plants.

d. Plant level transport of salt and its compartmentation: Plant level compartmentation is the most important mechanism conferring the salt tolerance.

i. Compartmentation in older leaves, leaf sheath and culm: Leaf to leaf gradients of sodium content are very prominent in rice. This gradient develops rapidly on exposure to salinity (Yeo and Flowers, 1983). There is progressive filling up of the leaves but the younger leaves are protected especially in tolerant varieties. The gradient of compartmentation is more pronounced in tolerant than susceptible cultivars. Compartmentation has also been observed within leaf. At moderate salinity, the Na^+ content in the leaf sheath may be 2 to 3 times higher than in the blade.

ii. More selective uptake into reproductive organ and flag leaf: Salt tolerant cultivars maintain substantially lower salt concentration in the panicle and with concentration being lowest in grains compared to husks and rachis. Flag leaf health is also critical for higher yield under salt stress. Tolerant lines tend to maintain lower concentration of salt in the flag leaf. Selection for healthier flag leaf at flowering could be used to screen for salinity tolerance at reproductive stage.

3 Effect of salinity on seed germination and seedling growth

i. Germination (%): The osmotic effect due to salinity was the main inhibitory factor that reduced germination as indicated by Akbar and Ponnamperuma (1982). Dubey (1984) ascribed the reduction in seed germination and seedling vigour under salinity stress to the adverse influence on the activity of key enzymes like α amylase, Rnase and protease in the endosperm and consequent depletion of food reserves in embryo.

Dubey and Sharma (1989) reported delayed differentiation of root and shoot and reduction in seedling vigour index with increase in salt concentration. The shoot growth was found to be more inhibited than root growth. Similarly, Roy *et al.* (1992) observed that salt stress tended to reduce the root and shoot growth of germinating seeds. The reduction was more prominent in salt sensitive variety, Ratna than resistant cultivar, CSR-4. Peris and Rana Singhe (1993) reported that, irrespective of the varietal tolerance, the dry weight of the seedlings was reduced in the saline treatment. Salt sensitive entry, IR-5931-110-1 exhibited greater (65% over control) reduction in total dry weight than salt tolerant variety Nonabokra (33% reduction over control). Mandal and Pramanik (1995) studied the response of 35 cultivars to different levels of salt stress (8, 12 and 16 dS m^{-1}) and inferred that *japonica* and *javonica* cultivars are more tolerant at higher salinity level than *indica* cultivars. Ashwani Pareek *et al.* (1998) observed delayed germination, reduction in germination per cent, reduced growth of primary root and shoot axis and arrest of root branching at higher salinity levels. Severe effects of salinity on germination and seedling growth were reported by Chakrabarty and Chattopadhyay (2000). Similarly, Abeysiriwardena and De (2004) also reported the reduction in germination per cent with increasing salinity and observed that tolerant cultivars recorded higher germination per cent than the sensitive cultivars. The gradual decrease in root length with the increase in salinity as observed might be due to more inhibitory effect of NaCl salt to root growth compared to that of shoot growth (Rahman *et al.* 2001). Subasinghe *et al.* (2007) studied several traditional, old improved, new improved and hybrid rice cultivars at different levels of salinity and categorized Pokkali, AA 354, Nona Bokra and AT- 401 as salt tolerant, where Pokkali recorded the highest germination under the highest salinity level. Reduction in emergence and seedling growth both in hybrids and parents of rice with increasing salinity levels was reported by Maiti *et al*. (2009) and Hasamuzzaman *et al.* (2009).

Shereen *et al.* (2005) and Haq *et al.* (2009) screened seven rice cultivars at 100 mM of salt concentration and reported that with increase in salinity, a significant reduction was observed in shoot dry weight, shoot fresh weight and number of tillers $plant^{-1}$ after 42 days of salt stress. Similarly, Hakim *et al.* (2010) studied the response of twelve rice varieties against six salinity levels (0, 4, 8, 12, 16 and 20 dS m^{-1}) at germination and early seedling stages and found that salinity decreased the final germination per cent and led to reduction in shoot and root length and dry weight in all varieties. Further they noticed that magnitude of reduction increased with increasing salinity stress.

ii. Dry weight: Zeng and Shannon (2000) observed significant reduction in root dry weight of rice genotypes at 1.9 and 3.4 dS m^{-1} of salinity. While Ali *et al.* (2004a) emphasized the importance of root shoot ratio in screening the rice germplasm against salinity as the lines with higher root shoot ratio recorded lower visual score of 4-5. Growth reduction immediately after the application of 12.5 dS m^{-1} of EC was observed by Alam *et al.* (2004) in rice, but no significant variation was seen at lower levels (8.5 and 4.5 dS m^{-1}). They observed severe effects on leaf area, shoot and root fresh weight besides effect on all plant parts.

Sharma and Brar (2005) stated that seedling stage was observed to be the most sensitive to salinity. They stated that chlorophyll and protein content decreased while soluble sugars and catalase activity increased and attributed the reduction in dry matter at higher salinity to the toxic effect of added salts and physiological scarcity of water or altered carbon and nitrogen metabolism. While Islam *et al.* (2007) reported that root growth was less effected than shoot growth due to salinity based on dry weight basis. Rahmanzadeh *et al.* (2008) evaluated four rice cultivars both in pots and under *in vitro* conditions at various levels of salinity and found Tichung-65 as most sensitive cultivar, based on reduction in seedling dry weight, wet weight, shoot length and root length. The researchers Awala *et al*. (2010) screened 54 genotypes of *Oryza glaberrima*, NERICA (21) and *O. sativa* (41) and grown in pots by irrigating with NaCl (80 mM) solution. They observed that relative root biomass was significantly lower in *Oryza glaberrima* than others.

iii. Root and shoot length: With increase of salinity, reduction of root length, shoot length, dry weight of root and dry weight of shoot was observed by Roy *et al.* (2002) and further reported that the rice cultivar Annada was least effected by induced salinity, while Govinda Raju and Balakrishnan (2002) stated that effect of salinity was more in susceptible varieties than resistant varieties.

Most of the seedling parameters *viz.,* germination, root length, shoot length, vigour index and dry matter accumulation were reduced by NaCl solution (Djanaguiraman *et al.*, 2003). Similarly, Jamil and Rha (2007) also screened transgenic lines of rice at seedling stage and observed reduction of shoot and root length in all the lines and found T-99 and T-112 as tolerant than T-115 and T-121. Similar findings were reported by Anatai *et al.* (2009).

iv. Na^+ /K^+ ratio: Selection within varieties or lines with low Na^+ transport has been made in rice (Yeo *et al.*, 1990). The Na^+/K^+ ratio shows relative decrease in K^+ content compared to Na^+ content in the genotypes along the salinity gradient (Mass and Poss, 1989) and higher salinity disturbs Na^+/K^+ ratio in the plant which impairs the protein metabolism of the plants. Gill (1990) studied the differential Na^+ accumulation in different plant parts of cultivar Jaya and wild rice when subjected to salt stress and the salt injury in Jaya to greater translocation of Na^+ ion to the shoot system. Generally, the salt tolerant varieties of rice maintain low concentration of Na^+ in their leaves than those of salt sensitive lines, when exposed to salt stress (Lutts and Guerrir, 1995). Therefore, Na^+/K^+ in the leaves of crop plants can be used as an important indicator of salinity tolerance and breeding for low ion accumulation could be a simple way to improve salt tolerance.

Lee *et al.* (2003) observed significantly lower reduction of all growth parameters of tolerant *indica* varieties than *japonica* varieties. They further observed that tolerant *indica* cultivars were good Na^+ excluders with high K^+ absorption and maintained a low Na^+/ K^+ ratio in shoot, and indicated that tolerance level of *indica* was higher than that of *japonica*. They also observed that the cultivar with low Na^+/ K^+ ratio was highly tolerant and the susceptible one had high Na^+/ K^+ ratio.

Ten advanced rice lines (NR-2, NR-3, NR-4, NR-5, Basmati-385 x NIAB-IRRI-9, Basmati-320, DM-51-24, NIAB-IRRI-9 x DM25, Jhona-349 and Pokkali) were screened by Ali *et al.* (2004a) and found that tolerant lines had higher root and shoot ratio thus provided a clue about salt tolerance parameter of a genotype. However, Suriya–Arunroj *et al.* (2005) screened and categorized rice cultures in three groups based on response to different levels of salinity. The tolerant group consisted of Pokkali, FL- 496 and FL-530, moderately tolerant group had FL-358, FL-367, FL-411 and KDMC-105 and RD- 6 as susceptible. While Javed *et al.* (2006) stated that selectivity of K^+ over Na^+ appeared to be an important salt tolerance indicator at the seedling stage.

Walia *et al*. (2007) also revealed similar reports in their study on both *indica* (IR 6373 and IR-29) and *japonica* (Agami and M-103) varieties of rice and found that tolerant genotypes maintained much lower shoot Na^+ than sensitive genotypes under salinity stress Similarly, Sexcion *et al.* (2009) observed the morpho-physiological traits associated with salinity tolerance in rice cultivars and classified Pokkali, Cheriviroppu, FL-478 and IR-651 as salt tolerant due to consistent expression of high vigour, low SES, high shoot root biomass, lower shoot Na^+ accumulation and lower shoot Na^+ / K^+ ratio compared to sensitive genotypes.

Haq *et al*. (2009) reported significant variation in leaf Na^+ under salt stress but not in control. The tolerant variety (CO-34) accumulated lower Na^+ (14.9 mol m^{-3}) while susceptible variety (Monoberekan) accumulated 52.9 mol m^{-3} in the leaf sap. They further reported larger reduction in K^+/Na^+ ratio under salt stress compared to control. They revealed that the key feature of plant salt tolerance was the ability of plant cells to maintain optimum K^+/Na^+ ratio in the control.

v. Effect of salinity on grain yield: Ali *et al.* (2004b) observed significant reduction of yield in many rice genotypes at a salinity level of 8.5 dS m^{-1} besides the reduction of many yield contributing parameters *viz.,* chlorophyll content, productive tillers $plant^{-1}$, panicle length and fertility percentage.

Uddin *et al.* (2007) stated that salinity reduced the number of effective tillers $plant^{-1}$, number of grains $panicle^{-1}$, 100-grain weight and yield $plant^{-1}$. Hosamuzzaman *et al.* (2009) reported that 1000-grain weight and grain yield decreased with increase in levels of salinity. Similarly, Mohammadi-Nejad *et al.* (2010) found that salinity stress caused reduction in overall vigour especially in the number of filled grain $panicle^{-1}$ and yield $plant^{-1}$.

4 Effect of salinity on yield contributing parameters

i. Plant height: Govindaraju and Balakrishnan (2002) indicated that plant height, number of productive tillers $hill^{-1}$, 1000-grain weight, grain yield, straw yield, chlorophyll content and photosynthetic ability decreased with increase of salinity. Natarajan *et al.* (2005) observed that high yielding and tolerant genotype IR 60494-2B-18-3-2-3 significantly recorded superior number of productive tillers $hill^{-1}$ and number of filled grains $panicle^{-1}$. Similar observation was reported by Krishna Murthy *et al.* (1987) and Makihara *et al.* (1999) for these parameters under saline conditions.

ii. Days to 50 per cent flowering: Salinity delayed flowering, reduced the productive tillers $plant^{-1}$, fertile florets $panicle^{-1}$, seed set (weight $grain^{-1}$), 1000-seed weight and overall grain yield (Khatun *et al.,* 1995). Further it was noted that tolerant genotypes had lesser reduction in floret fertility than sensitive genotypes.

iii. Tiller production: Tiller production gradually decreased with increased levels of salinity. In case of variety BR11, more than 30 per cent reduction of effective tillers was observed at 150 mM NaCl treatment compared to control (Zeng and Shannon, 2000). Similarly, it was observed that number of productive tillers $hill^{-1}$ decreased with increase in salinity levels (Sajjad (1984b), Heeman *et al*. (1998) and Hasamuzzaman *et al.* (2009).

iv. Panicle length (cm): Reduction in panicle length was observed with increase of salinity level by Sajjad (1984b), Heenan *et al.* (1988) and Khatun *et al.* (1995) and Hasamuzzaman *et al.* (2009).

vi. Number of filled spikelets $panicle^{-1}$: Filled spikelets $panicle^{-1}$ also decreased significantly with increase in level of salinity. The lowest filled grains $panicle^{-1}$ was observed at 150 mM NaCl level (Hasamuzzaman *et al.* (2009).

vii. Panicle weight (g): Reduction in panicle weight under salinity was reported by Sajjad (1984b), Heenan *et al.* (1988) and Khatun *et al*. (1995).

viii. Harvest Index: Low harvest index of susceptible low yielding genotypes was recorded by Singh (1984b) and Natarajan *et al*. (2004). While Zeng and Shannon (2000) reported that harvest index significantly decreased when salinity was 3.40 dS m^{-1} and higher.

ix. Spikelet fertility(%): Drastic decrease in spikelet fertility observed with increase in salinity level. Maximum reduction of spikelet fertility observed between 90 and 120 mM Nacl treatment (Hasamuzzaman *et al.* (2009), Khatun *et al*. (1995), Sajjad, (1984b) and Heenan *et al.* (1988).

Sabouri *et al*. (2008) evaluated 45 Iranian land races, 25 improved cultivars and 5 exotic cultivars at 1, 2, 4 and 8 dS m^{-1} of salt stress and classified the genotypes into 3 groups. The cultivars Tarom-mohalli, Gharib, Shahpasand mazandaran and Ahlami-Tarom had low Na^+/K^+ ratio, high root and shoot dry weight, high biomass, high root length and high shoot length and were tolerant. While, Khazan, Speedroud, IR-28 and IR-29 were most sensitive.

Screening of rice genotypes at two levels of salinity (4 and 8 dS m^{-1}) was done by Rao *et al.* (2008) and found that vegetative events were less affected by salinity stress than reproductive stage, higher floret fertility contributed to higher seed set and grain yield in tolerant genotypes while higher spikelet sterility led to poor seed set and lower grain yield in sensitive genotypes. They further observed that reduction of 1000-grain weight at an EC of 4 dS m^{-1} and concluded that reproductive stage is more useful to identity tolerant rice genotypes.

x. SES based on visual salt injury: Ali *et al.* (2004b) screened different rice genotypes under salinity condition (12 dS m^{-1}) and the entries IR-552182, IR-59418, IR-65195, IR-71657, NR-1 and IR-9 were graded as tolerant by scoring visually.

Identification of plant genotypes with tolerance to salt and incorporation of desirable traits into economically useful crop plants, may reduce the effects of salinity on productivity. Developing crop plants tolerant to salinity has the potential of making an important contribution to food production in many countries. Great efforts is therefore, being diverted towards the development of salt tolerant crop genotypes through use of plant breeding strategies.

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