

Salinity in rice production



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Rice (*Oryza sativa*) is the most important crop in the world after wheat, with more than 90% currently grown in Asia. Rice is the grain that has shaped the cultures, diets and economies of billions of Asians. For them, rice is more than food; rice is life. About 120, 000 varieties are grown across the world in an extensive range of climatic soil and water condition. It is grown on an area of 149. 151 million hectares (ha) yielding 550. 193 million tons of paddy with a yield of 3689 kg ha⁻¹ (Alam et al., 2001). In Asia, China is the major rice producing country followed by India, Indonesia and Bangladesh. However, yield per hectare is highest 6. 1 tons in Japan, followed by 5. 1 tons ha⁻¹ in China. Rice breeders have used genetic variability to produce cultivars that have high yield potential and that resist disease and insect damage and that tolerate cold, drought, and even floods. But apart from some sporadic work in Sri Lanka and India, little has been done until recently to identify any breed/cultivars adaptable to adverse soil conditions such as salinity. Salinity is a major threat to crop productivity in the southern and south-western part of Bangladesh, where it is developed due to frequent flood by sea water of the Bay of Bengal and on the other hand introduction of irrigation with saline waters. In Bangladesh, there are approximately 2. 85 million ha of coastal soils (Ponnamperuma, 1977) which occur in the southern parts of the Ganges tidal floodplain, in the young Meghna estuarine floodplain and in tidal areas of the Chittagong coastal plain and offshore islands (Brammer, 1978). About one million ha of land of these coastal and offshore areas are affected by varying degrees of salinity. These coastal saline soils are distributed unevenly in 64 thanas of 13 coastal districts covering 8 agroecological zones (AEZ) of the country. The majority of the saline land (0. 65 million ha) exists in the districts of Satkhira, Khulna,

Bagerhat, Barguna, Patuakhali, Pirojpur and Bhola on the western coast and a smaller portion (0.18 million ha) in the districts of Chittagong, Cox's Bazar, Noakhali, Lakshmipur, Feni and Chandpur. According to the report of Soil Resource Development Institute (SRDI) of Bangladesh, about 0.203 million ha of land is very slightly (2-4 dSm⁻¹), 0.492 million ha is slightly (4-8 dSm⁻¹), 0.461 million ha is moderately (8-12 dSm⁻¹) and 0.490 million ha is strongly (> 12 dSm⁻¹) salt affected soils in southwestern part of the coastal area of Bangladesh. Large fluctuations in salinity levels over time are also observed at almost all sites in these regions. The common trend is an increase in salinity with time, from November-December to March-April, until the onset of the monsoon rains. The electrical conductivity (EC) of the soils and water are lowest in July-August and highest in March-April at all sites. Soil salinity, at any time, is maximum in the surface layers (0-15 cm), the salinity gradient being vertically downwards. The salinity in subsoil is usually much lower than that in the top soil. The underground water within 1-2 meters below the soil surface at all locations is moderately to strongly saline in the dry season. The compositions of the soluble salts in these saline soils can indicate possible management strategies for crop production. Sodium has been found to be the dominant cation, and Cl⁻ the dominant anion species. Next in importance are Mg²⁺ and SO₄²⁻. Hence the salts are of the sodium-magnesium and chloride-sulphate types. A very important aspect of the soluble salt composition of the underground water is the large excess of magnesium relative to calcium. Thus proper measures to maintain ionic balance may be needed for good plant growth even under low salinity conditions. There is a general lacking of suitable salt tolerant modern variety (MV) of rice to suited different AEZ in the coastal areas of Bangladesh. The

scarcity of good quality irrigation water is a major problem in these areas. The surface water resources are insufficient and irrigated agriculture is largely dependent on ground water resource. The use of such water for irrigation without proper management may render the irrigated soils as salt affected and consequently crop production may be hindered.

For centuries, farmers have salt-tolerant cultivars on the saline soils of India, Burma, Thailand, Indonesia, the Philippines and Vietnam. But, because of lodging and susceptibility to disease and insect damage, yields are about 1 ton ha⁻¹. Recognition of the potential of saline lands for rice production in the densely populated countries of south and southeast Asia prompted the inclusion of salt tolerance as a component of the programme of the International Rice Research Institute (IRRI). Of the adverse soil conditions, salinity received most attention, because of its widespread occurrence in current and potential rice lands. Salt tolerance studies are usually conducted in growth chambers and greenhouse, with plants raised in plastic trays or in small pots. The salt tolerance of any crop is usually expressed as decrease in yield associated with a given level of soil salinity as compared with yield under non-saline conditions. The primary salinity factors influencing plant growth are the kind and concentration of salt present in the soil solution. Salt concentration in soil is usually determined by measuring EC of a soil saturation extracts (EC_e) obtained from the active root zone. Recently, simple, rapid and reliable instruments such as salinity sensors and four electrode probes, have been developed for measurement of electrical conductivity of soil water (EC_{sw}).

Rice is the most suited crop for saline soils because it can tolerate standing water, which is necessary for reclamation of saline soils. Soils are considered saline if they contain soluble salts in quantities sufficient to interfere with the growth of most crop species. Thus, the criterion for distinguishing saline from non-saline soils is arbitrary (Marschner, 1995). According to the definition, a saline soil has an electrical conductivity (EC) greater than 4 milli mhos cm^{-1} or 4 micro Siemens cm^{-1} or deci Siemens m^{-1} and an exchangeable sodium percentage (ESP) and pH of less than 15 and 8.5, respectively. The saline soils with an $\text{ESP} > 15$ and $\text{pH} > 8.5$ are termed as saline-alkaline or saline-sodic soils. However, many different units have been used for salinity level expression. These are molarity (M), milli molarity (mM) (based on molecular weight of the salt); milli mhos cm^{-1} (mmhos cm^{-1}); micro Siemens cm^{-1} ($\mu\text{S cm}^{-1}$), deci Siemens m^{-1} (dS m^{-1}) (based on electrical conductivity) and % salt (based on percent concentration of the salt). Among these, mM, dS m^{-1} and % salt concentrations are most commonly used. Approximately 58.5 mgL^{-1} NaCl = 1mM solution of NaCl and 640 mgL^{-1} NaCl is equivalent to 1 mmhos cm^{-1} (= 1 dS m^{-1}) EC (Shannon et al., 1998). Therefore, 1 dS m^{-1} salinity is equivalent to about 11 mM salt solution.

The present population of Bangladesh is about 140 million and rice is the principal food item of its population. The alarming growth of population and loss of arable land due to urbanization are main causes of concern for finding ways and means for augmenting food production particularly rice. The possibility of increasing food production by increasing land area is quite out of question in Bangladesh. The only feasible alternative is to increase the cultivable land areas by bringing salt affected soils under cultivation with

high yielding salt tolerant rice cultivars. The lack of an effective evaluation method for salt tolerance in the screening of genotypes is one of the reasons for the limited success in conventional salt tolerant breeding. Two yield parameters, tiller number per plant and spikelet number per panicle, have proved most sensitive to salinity and are highly significantly correlated to final seed yield in rice cultivar under salt stress (Zeng and Shannon, 2000).

Salinity in soil or water is one of the major stresses, can severely limit crop production (Shannon, 1998). The deleterious effects of salinity on plant growth are associated with (i) low osmotic potential of soil solution (water stress), (ii) nutritional imbalance, (iii) specific ion effect, or (iv) a combination of these factors (Ashraf, 1994a; Marschner, 1995). All these cause adverse pleiotropic effects on plant growth and development at physiological and biochemical levels (Munns, 2002) and at molecular level (Mansour, 2000). It is often not possible to assess the relative contribution of these major constraints to growth inhibition at high substrate salinity, as many factors are involved. These include ion concentration, duration of exposure, plant species, cultivar and root stock (excluder and includer), stage of plant development, plant organ and environmental conditions. So, to cope with the above constraints, salt stressed plants mainly adopt three mechanisms for salt tolerance such as (i) osmotic adjustment, (ii) salt inclusion/ exclusion and (iii) ion discrimination (Volkmar et al., 1998).

Plant growth was seriously affected due to salinity which reduced turgor in expanding tissues and osmoregulation (Steponkus, 1984). Alam et al. (2001) stated that the critical EC level of salinity for seedling growth was about 5 dSm⁻¹. They observed that dry matter, seedling height, root length and

emergence of new roots of rice decreased significantly at an electrical conductivity value of 5-6 dSm⁻¹ and during the early seedling stage, more higher salinity caused rolling and withering of leaves, browning of leaf tips and ultimately death of seedlings. They speculated that both osmotic imbalance and Cl⁻ was responsible for suppress of the growth. These authors maintained that the shoot growth was more suppressed than that of root and salt injury was more severe at high temperature (35oC) and low humidity (64%) due to increased transpiration and uptake of water and salt by rice plants. At the reproductive stage, salinity depressed grain yield much more than that at the vegetative growth stage (Alam et al., 2001). These authors maintained that at critical salinity levels straw yield was normal but produced little or no grain. The decrease in grain yield was found proportional to the salt concentration and the duration of the saline treatment. When the plants were continuously exposed to saline media, salinity affected the panicle initiation, spikelet formation, fertilization of florets and germination of pollen grains hence caused an increase in number of sterile florets. The greatest injurious effect was on the panicle. Salinity severely reduced the panicle length, number of primary branches per panicle, number of spikelet per panicle, seed setting percentage and panicle weight and reduced the grain yield. The weight of 1000 grains was also reduced. Salt injury resulted in the production of small grains in grain length, width and thickness. Most rice cultivars were severely injured in submerged soil cultures at EC of 8-10 dSm⁻¹ at 25o C; sensitive ones were hurt even at 2 dSm⁻¹. At comparable EC's injury was less in sea water than in solutions of common salt, in neutral and alkaline soils than in acid soils, at 20oC than at 35oC and in 2-week old seedling than in 1-week old seedlings. Since rice

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plant is susceptible to salinity at transplanting and gains tolerance with age, they advised that aged seedlings (6 weeks old) be planted in saline fields.

Salinity affected rice during pollination, decreased seed setting and grain yield (Maloo, 1993). Finck (1977) suggested that deficiency of K and Ca elements might play a significant role in plant growth depression in many saline soils. Girdhar (1988) observed that salinity delayed germination, but did not affect the final germination up to the EC of 8 dSm⁻¹ by evaluating the performance of rice under saline water irrigation. In normal conditions, the Na⁺ concentration in the cytoplasm of plant cells was low in comparison to the K⁺ content, frequently 10⁻² versus 10⁻¹ and even in conditions of toxicity, most of the cellular Na⁺ content was confined into the vacuole (Apse et al., 1999).

Abdullah et al. (2001) performed an experiment on the effect of salinity stress (50 mM) on floral characteristics, yield components, and biochemical and physiological attributes of the sensitive rice variety IR-28. The results showed significant decrease in panicle weight, panicle length, primary branches per panicle, filled and unfilled grain, total grains and grain weight per panicle, 1000-grain weight and total grain weight per hill. They further observed significant reduction in both chlorophyll a and chlorophyll b content in different parts of the rice leaves at saline condition. In another experiment, Abdullah et al. (2002) studied the effect of salinity on photosynthate translocation in panicle branches and developing spikelets, carbohydrate content of different vegetative parts and suggested that reduction in grain number and grain weight in salinized panicles was not merely due to reduction in pollen viability and higher accumulation of Na⁺

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and less K^+ in different floral parts but also due to higher accumulation of photosynthates (sugar) in primary and secondary panicle branches, panicle main stalk and panicle stem coupled with reduced activity of starch synthetase in developing grains.

Gypsum ($CaSO_4 \cdot 2H_2O$) is widely used for ameliorating saline/sodic soils due to its tendency of replacing its Ca^{2+} with exchangeable Na^+ on the soil complex. In addition, gypsum application to saline/sodic soils improve yield of paddy and forage grasses in arid and semi arid regions due to the effects of Ca^{2+} on plant composition such as decrease in the concentration of Na and improve plant-tissue concentrations of P, K, Zn, Cu, Mg and K: Na ratio (Rengel, 1992). The addition of supplemental Ca to the root environment was a means of enhancing plant tolerance to salt stress (Epstein, 1998). This might favour the increase of Na^+ inside the cells, change enzyme activity resulting in cell metabolical alterations; disturbance in K^+ uptake and partitioning in the cells, and throughout the plant that might even affect stomatal opening, thereby, impairing the ability of the plant to grow. This author assumed that the addition of Ca^{2+} to the root environment of salt stressed plants would maintain or enhance the selective absorption of K^+ at high Na^+ concentrations and prevent the deleterious effects of the excess of Na^+ . Another role attributed to supplemental Ca^{2+} addition was its help in osmotic adjustment and growth via the enhancement of compatible organic solutes accumulation (Girija et al., 2002). Under salt stress conditions there was a decrease in the Ca/Na ratio in the root environment which affected membrane properties, due to displacement of membrane-associated Ca^{2+}

by Na⁺, leading to a disruption of membrane integrity and selectivity (Cramer et al., 1985; Kinraide, 1998).

Aslam et al. (1993) observed significant reduction in shoot and root fresh weights by different types of salinity such as NaCl alone, NaCl + CaCl₂, Na₂CO₃ alone and a salts mixture. On the plant growth, NaCl alone was found to be the most toxic, Na₂CO₃ alone was the least harmful, and NaCl + CaCl₂ and the salts mixture were intermediate. They found similar results in both solution culture experiment and the experiments conducted in salinized soils. They considered the better root growth under high salinity condition as the capacity of the tolerant genotypes to combat the adverse effect of salinity. Aslam et al. (2001) investigated the effect of supplemental Ca on rice growth and yield in solution and soil cultures, and in naturally salt affected field. In solution culture, Ca was applied at 5, 10, 20, 40, 80 and 160 µg/mL with 80 mM NaCl and without NaCl and in soil culture 0, 50, 100 and 200 kg Ca ha⁻¹ was applied to artificially prepared salinity (EC 9 dSm⁻¹). Three cultivars, differing in salt tolerance, were used, namely K8-282 (salt tolerant), BG 402-4 (moderately tolerant) and IR-28 (salt sensitive). Application of Ca at 20-40 µg/mL improved tillering capacity, shoot and root length, shoot and root weights in solution culture in the presence of NaCl. Shoot Na⁺ and Cl⁻ decreased, whereas K⁺ concentration and K⁺/Na⁺ ratio increased because of Ca supply to saline medium. Grain and straw yields, plant height and panicle length were significantly higher in saline compared to saline sodic soil. Application of 200 kg Ca ha⁻¹ proved statistically superior to the control in respect of panicle length, numbers of tillers, grain and straw yields under both saline and saline sodic soil as well as in naturally salt-

affected field. Seed setting was improved in all cultivars because of external Ca supply to saline and saline sodic soils. Aslam et al. (2003) stated that an increase in potassium and K^+/Na^+ ratio was an indication of salt tolerance due to the application of additional Ca in both salt tolerant and susceptible rice cultivars under saline environment. These authors maintained that salt affected soils showed an improvement in the paddy yield of both salt tolerant and salt sensitive rice cultivars due to Ca application as gypsum at the rate of 25% of gypsum requirement of soil.

Franco et al. (1999) studied the effect of supplemental $CaCl_2$ on growth and osmoregulation in NaCl stressed cowpea seedlings. They found that salinity inhibited the length of root and shoot of cowpea but the inhibitory effect could be ameliorated by the addition of Ca^{2+} . The concentration of organic osmoregulators (proline, soluble carbohydrates, soluble amino-nitrogen, and soluble proteins) increased in root tips of seedlings grown in salt-stressed condition with supplemental Ca. They indicated that Ca^{2+} could have a protective effect in root tips, which is of fundamental importance for the maintenance of root elongation in NaCl stressed cowpea seedlings.

Considerable improvements in salinity tolerance have been made in crop species in recent times through conventional selection and breeding techniques (Shannon, 1998; Ashraf, 1994a; 2002). Most of the selection procedures have been based on differences in agronomic characters, which represent the combined genetic and environmental effects on plant growth and include the integration of the physiological mechanisms conferring salinity tolerance. Typical agronomic selection parameters for salinity

tolerance are yield, biomass, plant survivality, plant height, leaf area, leaf injury, relative growth rate and relative growth reduction.

Many scientists have suggested that selection is more convenient and practicable if the plant species possesses distinctive indicators of salt tolerance at the whole plant, tissue or cellular level (Ashraf, 2002; Epstein and Rains, 1987; Jacoby, 1999; Munns, 2002). Physiological criteria are able to supply more objective information than agronomic parameters or visual assessment while screening for component traits of complex characters (Yeo, 1994). There are no well-defined plant indicators for salinity tolerance that could practically be used by plant breeders for improvement of salinity tolerance in a number of important agricultural crops. This is partly due to the fact that the mechanism of salt tolerance is so complex that variation occurs not only amongst species but, in many cases, also among cultivars within a single species (Ashraf, 1994a; 2002). During the course of plant growth, the form and functions of various organs undergo significant change and the ability of the plant to react to salinity stress depend on those genes that are expressed at the stage of development during which the stress is imposed (Epstein and Rains, 1987). The mechanism of salinity tolerance becomes even more complicated when the response of a plant also varies with the concentration of saline medium and the environmental conditions in which the plant is grown.

Osmotic adjustment in plants subjected to salt stress can occur by the accumulation of high concentration of either inorganic ions or low molecular weight organic solutes. Although both of these play a crucial role in higher plants grown under saline conditions, their relative contribution varies

among species, among cultivars and even between different compartments within the same plant (Ashraf, 1994a). The compatible osmolytes generally found in higher plants are of low molecular weight sugars, organic acids, amino acids, proteins and quaternary ammonium compounds.

According to Cram (1976), of the various organic osmotica, sugars contribute up to 50% of the total osmotic potential in glycophytes subject to saline conditions. The accumulation of soluble carbohydrates in plants has been widely reported as response to salinity or drought, despite a significant decrease in net CO₂ assimilation rate (Popp and Smirnov, 1995; Murakeozy et al., 2003). Ashraf and Tufail (1995) determined the total soluble sugars content in five sunflower accessions differing in salt tolerance. They found that the salt tolerant lines had generally greater soluble sugars than the salt sensitive ones. Ashraf and Harris (2004) suggested that considerable variations in the accumulation of soluble sugars in response to salt stress were evident at both inter-specific and/or intra-specific levels and even among lines of which all were salt tolerant.

Several salt-induced proteins have been identified in plant species and have been classified into two distinct groups such as (i) salt stress proteins, which accumulate only due to salt stress and (ii) stress associated proteins, which also accumulate in response to heat, cold, drought, water-logging and high and low mineral nutrients (Pareek et al., 1997; Ali et al., 1999; Mansour, 2000). Proteins that accumulate in plants grown under saline conditions may provide a storage form of nitrogen that is neutralized when stress is over and may play a role in osmotic adjustment (Singh et al., 1987). A higher content of soluble proteins has been observed in salt tolerant than in salt sensitive

cultivars of barley, sunflower (Ashraf and Tufail, 1995) and rice (Lutts et al., 1996; Pareek et al., 1997). Pareek et al. (1997) also suggested that stress proteins could be used as important molecular markers for improvement of salt tolerance using genetic engineering techniques.

Amino acids have been reported to have accumulated in higher plants under salinity stress (Ashraf, 1994b; Mansour, 2000). The important amino acids are alanine, arginine, glycine, serine, leucine and valine, together with the imino acid – proline and the non-protein amino acids- citrulline and ornithine (Mansour, 2000). Lutts et al. (1996) found that proline did not take part in osmotic adjustment in salt stressed rice and its accumulation seemed to be a symptom of injury rather than an indicator of salt tolerance. On the contrary, Garcia et al. (1997) reported that exogenously applied proline exacerbated the deleterious effects of salt on rice. The salt tolerant rice cultivars Nona Bokra and IR 4630 accumulated less proline in their leaves than the salt sensitive Kong Pao and IR 31785 (Lutts et al., 1996). These contrasting reports on the role of proline in salt tolerance and its use as selection criterion for salt tolerance in rice has been questioned.

Regulation of ion transport is one of the important factors responsible for salt tolerance of plants. Membrane proteins play a significant role in selective distribution of ions within the plant or cell (Ashraf and Harris, 2004).

According to Du-Pont (1992) the membrane proteins involved in cation selectivity and redistribution of Na^+ and K^+ . These proteins are: (a) primary H^+ -ATPases which generate the H^+ electrochemical gradient that drives ion transport, (b) Na^+/H^+ antiports in the plasma membrane for pumping excess Na^+ out of the cell, (c) Na^+/H^+ antiports in the tonoplast for

extruding Na^+ into the vacuole and (d) cation channels with high selectivity for K^+ over Na^+ . It is well established that Na^+ moves passively through a general cation channel from the saline growth medium into the cytoplasm of plant cells (Marschner, 1995; Jacoby, 1999; Mansour et al., 2003) and the active transport of Na^+ through Na^+/H^+ antiports in plant cells is also evident (Shi et al., 2003). Salt tolerance in plants is generally associated with low uptake and accumulation of Na^+ , which is mediated through the control of influx and/ or by active efflux from the cytoplasm to the vacuoles and also back to the growth medium (Jacoby, 1999). Energy-dependent transport of Na^+ and Cl^- into the apoplast and vacuole can occur along the H^+ electrochemical potential gradients generated across the plasma membrane and tonoplast (Hasegawa et al., 2000). The tonoplast H^+ pumps (H^+ -ATPase and H^+ -pyrophosphatase) also play a significant role in the transport of H^+ into the vacuole and generation of proton (H^+) which operates the Na^+/H^+ antiporters (Mansour et al., 2003; Blumwald, 2000).

In the past few decades, plant breeders in Bangladesh have achieved little success in developing some salinity tolerant crops specially rice through conventional breeding techniques, with relatively little/no direct input from physiologists or biochemists. Mutation breeding, a modern technique for creating variability has also played a vital role for generating new valuable cultivars of rice. Incorporation of mutation programme for achieving a desired character(s) in a variety can thus reduce the time required to breed an improved variety with the conventional hybridization method. Several cultivars derived from direct utilization of induced mutants have shown that traits such as short straw, earliness and resistance to certain diseases, can

be introduced in otherwise well-adapted varieties without significantly altering their original attributes. Study on the response of rice to salinity stress may be helpful in breeding salt tolerant cultivars by identifying physico-chemical potential of salinity tolerance such as accumulation of toxic Na^+ and Cl^- in the older parts of the plant, higher photosynthetic efficiency of the leaves, escaping ability to uptake Na^+ and Cl^- . The direct use of mutation is a valuable approach especially when the improvement of one or two easily identifiable characters is desired in an otherwise well-developed variety. In order to develop practicable strategies for selecting salt tolerant rice mutants/lines/genotypes adaptable in coastal belt of Bangladesh notably during boro season, detailed information needs to be gathered on the changes in physiological and biochemical aspects due to salt stress are attributable against detrimental effects of salt stress.

In addition to the development of salt tolerant cultivars, better understanding of nutritional disorders in the context of plant nutrient uptake and physiological as well as biochemical mechanisms of salt tolerance in rice plants may suggest some strategies for plant breeders and growers for developing salinity tolerant varieties and management practices for cultivation in saline areas. There have been few such studies available in the country or elsewhere on rice for tolerance mechanism created especially in mutant genotypes. This research programme has been, therefore, planned with the aim of finding out the bio-chemical causes and possible soil amelioration programme in cultivating the rice mutants in the coastal saline soils of Bangladesh.

Keeping the above ideas, in mind, the present work has therefore, been designed and planned with the following objectives:

- investigating the effect of salinity on some bio-chemical aspects and growth of different rice genotypes at different growth stages,
- – finding out the mechanism of salinity tolerance of selected rice genotypes; and
- – suggest possible reclamation programme for better growth of rice under saline condition.