International Journal of Agricultural Technology 2014 Vol. 10(1):265-275
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Screening for Salt Tolerant Rice *(Oryza Sativa* L.) Genotypes at Early Seedling Stage

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N.A. Chachar, S.D. Chachar, Q.I. Chachar, M.I. Keerio, A. Shereen, and M.H. Chachar (2014). Screening for salt tolerant rice *(Oryza sativa* L.) genotypes at early seedling stage. International Journal of Agriculture Technology 10(1):265-275.

The environmental stresses such as salinity (soil or water) are serious obstacles for field crops especially in the arid and semi-arid tracts of the world. In order to investigate impact of salinity stress on rice seed germination, early seedling growth and ion accumulations, were conducted at Plant Physiology Division of Nuclear Institute of Agriculture (NIA), Tandojam, during 2012-13. Seeds of six rice genotypes were collected from Nuclear Institute of Agriculture (NIA) Tandojam. These seeds were grown in on plastic bowls at different salinity levels along with control in culture solution. The pH of culture solution was maintained at 5.0. Treatments i.e., distilled water (control), 40mM, 60mM, 80mM and 100mM NaCl. As it is manifest from present data that genotypes, treatments and their interaction were highly significant for germination (%), root/shoot length (cm) , root/shoot fresh and dry weight (mg) and solute accumulation in plants (inorganic solute Na^+ and K^+). Among the genotypes tested IR 66946-3 R-178-1-1 and IR 84675-58-4-1-B-B are the tolerant genotypes had the potential to perform better under saline conditions at early seedling stage, whereas Sarshar was moderate tolerant under saline conditions. Moreover the genotypes i.e. IR 84675-7-3-2-B-B and IR 84675-25-7-3-B-B are the moderate sensitive under salinity, subsequently, the genotype HHZ5-SAL10-DT2-DT1 showed sensitive one under saline environment. Further it is concluded that the rice genotypes adjust their osmotic potential by accumulating solutes (K^+ over Na^+), and their better performance might be related to the selective uptake of K^+ over Na^+.

Keywords: rice, salinity, early seedling

Introduction

Abiotic stress is the main factor that is negatively affecting crop growth and productivity worldwide (Chao *et al.*, 2007). Crop plants usually exposed to abundance of natural biotic and abiotic stresses, which limit their growth and productivity. Salinity is one of the major abiotic constraints on crop production

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and food security and adversely impact the social-economic fabric in many developing countries, affecting about 95 million hectares worldwide (Ghassemi *et al*., 2010). The saline area is three times larger than land used for agriculture (Binzel and Reuveni, 1994) According to an estimate, 1/3 of irrigated land has been affected by salinity and saline area is increasing each year. In Pakistan alone, 40,000 hectares of arable land is lost annually due to salinity (Ahmad *et al.*, 2006; Ashraf *et al.,* 2008; Mehmood *et al.*, 2009).

Salt stress affects many physiological aspects of plant growth which grow in the salt affected soils tend to show differences in physiological and biochemical activities from those grown on non-salt affected soils (Lutts *et al.*, 1995). Jamil *et al.* (2007) reported that salinity delayed germination and decreased seedling growth. Several physiological pathways, i.e., photosynthesis, respiration, nitrogen fixation and carbohydrate metabolism have been observed to be affected by high salinity (Chen *et al.*, 2008). Under salinity, plant has to face both osmotic and ionic stresses which ultimately cause reduction in growth (Munns and Tester, 2008). In the presence of high salt concentration in the medium, osmotic potential is negative enough to cause water to diffuse out of tissue. It has been reported that stress environment affects membrane selective efficiency in germinating seed (Lodhi *et al.,* 2009), which ultimately results in excess absorption of toxic ion.

Rice *(Oryza sativa* L*)* is one of the most important crops in the world and is the primary stable food for over two billion people. It is the second most important crop of the world after wheat with more than 90% currently grown in Asia (Anonymous, 1992). Rice is highly valuable cash crop that earns substantial foreign exchange for the country. . It is the second most important crop of the world after wheat with more than 90% currently grown in Asia (Anonymous, 1992). Rice is highly valuable cash crop that earns substantial foreign exchange for the country.

It is now well known that some plant species can tolerate high salinity (Schachtman and Munns, 1992). This crop is regarded as a salt sensitive especially at young seedling stage, where varying degree of mortality occurs at 50mM NaCl in solution culture and in most salt sensitive varieties 50% of the population may die within ten days of salinization at the age of 14 days (Flowers and Yeo, 1981). Reduction in rice yield because of salinity is 40-60% (Aslam *et al.*, 1993). The paddy yield is much low in Pakistan as compared to other rice producing countries. Rice is usually recommended as first choice during reclamation of salt-affected soils because of its special ability to grow in standing water as it helps in rapid leaching of salt-affected soils. There is enough scope for increasing yield from the salt affected soils by ensuring successful cultivation of rice cultivars capable of good yields is a pre-requisite. However, a significant difference for paddy yield under saline conditions has been reported among rice cultivars (Yeo and Flowers, 1984; Muhammad and Aslam, 1998).

Today, its production is not good because of increasing numbers of biotic and abiotic stresses such as various diseases and shortage of water and salinity, respectively (Oerke *et al.,* 1994; Ferrero *et al.,* 2001; Ferrero *et al.,* 2002). With the rapid growth in population consuming rice and the deteriorating soil and water quality around the globe, there is an urgent need to understand the response of this important crop towards these environmental abuses. With the ultimate goal to raise rice plant with better suitability towards changing environmental inputs, intensive efforts are on worldwide employing physiological, biochemical and molecular tools to perform this task.

Materials and methods

The present investigations were conducted at Plant Physiology Division of Nuclear Institute of Agriculture, Tandojam as collaborated Research between SAU and NIA, Tandojam during 2012-13. Seeds of six rice genotypes HHZ5-SAL10-DT2-DT1, IR 66946-3 R-178-1-1 (International Salt Tolerant Check), Sarshar (High Yielder Check), IR 84675-7-3-2-B-B, IR 84675-25-7-3-B-B and IR 84675-58-4-1-B-B were collected from Nuclear Institute of Agriculture (NIA) Tandojam. Approximately 400 healthy seeds of each rice genotypes were selected and surface sterilized with 3% sodium hypochlorite for 15 minutes and washed thoroughly with distilled water to avoid any fungal infection during germination. These seeds were grown in on plastic bowls at different salinity levels along with control in culture solution (Yoshida *et al.*, 1976), presented in table-2. The pH of culture solution was maintained at 5.0. Treatments i.e., distilled water (control), 40mM, 60mM, 80mM and 100mM NaCl. The bowls were kept in controlled incubator (Luminine Cube II, ANALIS Model L M-500) having 28-30 ^{O}C for 15 days after culturing.

Result and discussions

Seed germination (%)

It is well documented that the presence of soluble salts in the growing medium are the major factor to reduce germination and growth in rice. In the present work, there was significant decrease in seed germination in all rice genotypes. The lowest germination was recorded at highest salinity level (100 mM NaCl). The reduction in seed germination was also reported earlier (Khan, 2009; Jamil *et al.,* 2007 and Singh *et al.,* 2000). This decrease in germination with increasing salinity might be due to low osmotic potential of the growing media, resulting in less imbibition of water by seeds. Promila *et al.* (2000), also reported delay or reduce germination and inhibition of seedling growth during imbibition phase under salinity.

At high salinity treatment (100mM NaCl), the genotypes IR 66946-3 R-178-1-1 and IR 84675-58-4-1-B-B also remain on top with maximum seed germination percentage (60.0 each). On the other hand, minimum germination (45.0) at high salinity treatment (100mM NaCl), was recorded in HHZ5-SAL10DT2-DT1 (figure: 1).

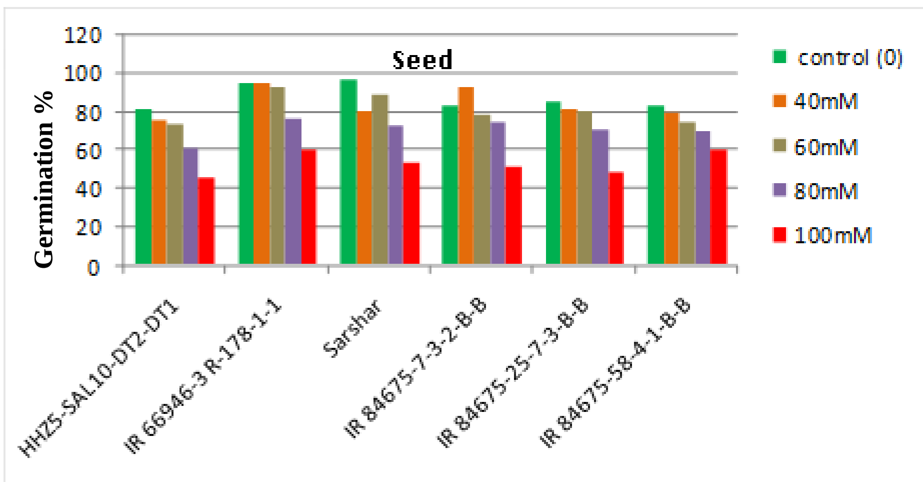

Fig. 1. Effect of different salinity levels on seed germination of rice genotypes

Genotypic differences were also found in the present study, indicating that the rice genotypes respond varyingly under saline environment. Rice genotypes IR 66946-3 R-178-1-1 and Sarshar showed resistant to salinity, having maximum seed germination (83.9 and 78.2%) under the various concentrations of NaCl.

Salinity also reduces vegetative growth of rice (Deivanai *et al.*, 2011). There was decrease in shoot length in all the rice genotypes with the increase of NaCl levels. At high salinity treatment (100mM NaCl), maximum shoot length was observed in genotype IR 66946-3 R-178-1-1 (i.e. 9.16cm), followed by IR 84675-58-4-1-B-B (8.56cm), while the genotypes Sarshar and IR 84675-58-4--B-B perform better (7.2, 8.5cm). On the other hand, minimum shoot length values were observed in HHZ5-SAL10-DT2-DT1 and IR 84675-25-7-3-B-B i.e. 3.0 and 5.95cm, respectively (Figure: 2).

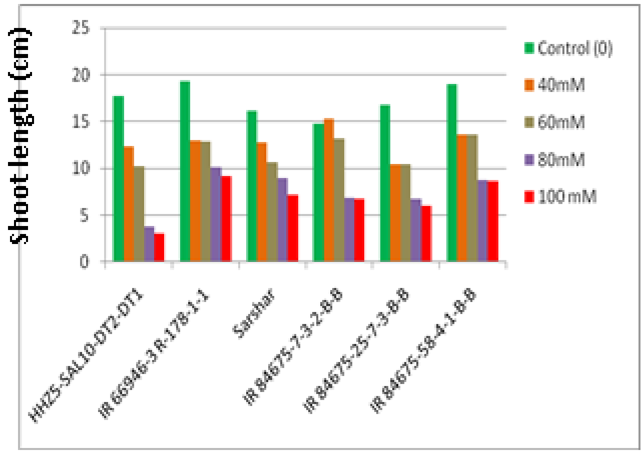

Fig. 2. Effect of different salinity levels on shoot length (cm) of rice genotypes

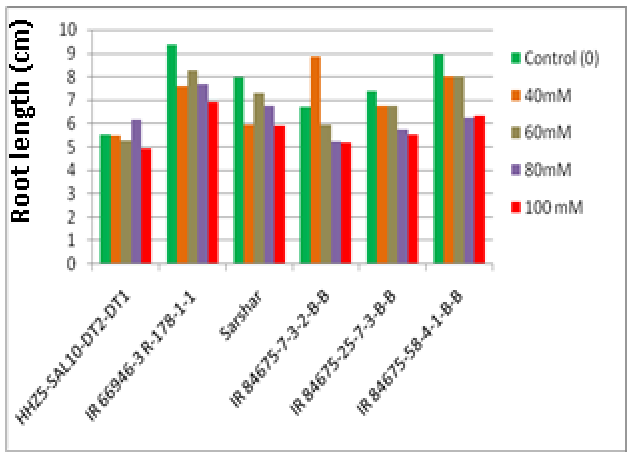

Fig. 3. Effect of different salinity levels on root length (cm) of rice genotypes

In the present study rice genotypes were tested under different NaCl levels, showed significant decline in shoot and root growth. Maximum shoot length was recorded in IR 84675-58-4-1-B-B followed by IR 66946-3 R-178-1-1. Similarly maximum root length was recorded in IR 66946-3 R-178-1-1 and IR 84675-58-4-1-B-B, while the minimum root length was recorded in HHZ5-SAL10-DT2-DT1 as shown in figure: 3. Decreasing trend in root growth was also reported by Hakim *et al.* (2011) under salinity. This decreasing trend in rice genotypes was also reported by Jamil *et al.* (2007), who reported that decreased plant growth at high salinity treatments, while the effect of NaCl up to 40 mM was almost low on shoot and root growth of rice genotypes, differing in salt tolerance, tested under hydroponics conditions.

Shoot fresh weight

Similar trends were found in shoot fresh weights, with increasing salinity levels in all rice genotypes. Maximum shoot fresh weights was observed in IR 66946-3 R-178-1-1(Figure: 4), whereas the minimum shoot fresh weight values were observed in HHZ5-SAL10-DT2-DT1 and IR 84675-25-7-3-B-B. The results are in agreement with those of Amirjani (2010) who reported that (NaCl) significantly reduced root/shoot fresh weight.

At high salinity treatments (100 mM NaCl), there was comparatively higher reduction in plant biomass with increasing salinity of the growing media. Here again Genotypes IR 66946-3 R-178-1-1 and IR 84675-58-4-1-B-B showed maximum shoot fresh weight (234 and 179 mg/10), followed by IR 84675-25-7-3-B-B (154mg/10), Sarshar (158 mg/10), and HHZ5-SAL10-DT2-DT1 (138 mg/10 shoots). While minimum shoot fresh weight (119mg /10 shoots) was observed in genotype IR 84675-7-3-2-B-B respectively (Figure: 4).

Root fresh weight

There was decreasing trend found in root fresh weight with the increase in salinity treatments in all rice genotypes. The decrease was more in 100 mM (NaCl) salinity treatment as compared to control. Mean values for root fresh weigh in five treatments were recorded as 230, 197, 146, 122 and 111 mg/10 roots under control, 40, 60, 80 and 100 mM (NaCl), respectively.

Root fresh weight at the highest salt treatments was observed as maximum in genotype IR 66946-3 R-178-1-1 (i.e. 184mg/10 roots), followed by Sarshar (111), IR 84675-7-3-2-B-B (105), IR 84675-58-4-1-B-B (138 and IR 84675-25-7-3-B-B (99mg/10 roots). While, the genotype HHZ5-SAL10-DT2-DT1 showed minimum (29 mg/10 shoots) values for root fresh weight at that concentration (figure: 5). This decreasing trend in rice genotypes was also reported by Jamil *et al.* (2007).

Shoot dry weight

The effect of salt (NaCl) stress on dry matter yield was recorded in terms of shoot dry weight (SDW). The two genotypes i.e. IR 66946-3 R-178-1-1 and IR 84675-58-4-1-B-B both got maximum shoot dry weight as (56 and 32 mg/10 shoots), at 100mM (NaCl). The other genotypes were also showing better SDW, was Sarshar (27), and IR 84675-25-7-3-B-B (29 mg/10 shoots) respectively. Minimum values for SDW (23 and 20mg/10 shoots) were recorded in genotype HHZ5-SAL10-DT2-DT1 and IR 84675-7-3-2-B-B, each respectively (Figure 6).

Root dry weight

At high salt treatments 100 mM NaCl, was bit changed, the genotype IR 66946-3 R-178-1-1 (17 mg/10 roots) with maximum root dry weight, followed by IR 84675-58-4-1-B-B (14), Sarshar (12), IR 84675-25-7-3-B-B (10), and IR 84675-7-3-2-B-B (9 mg/10), while minimum root dry weight under high salinity treatment was recorded in genotype HHZ5-SAL10-DT2-DT1 i.e. (3 mg/10 roots) presented in figure-7. The decreasing trend in root and shoot dry weight was also reported by other researchers (Amirjani, 2010; Sahfi *et al.*, 2006), who found that salinity had a significant effect on root and shoot dry weight.

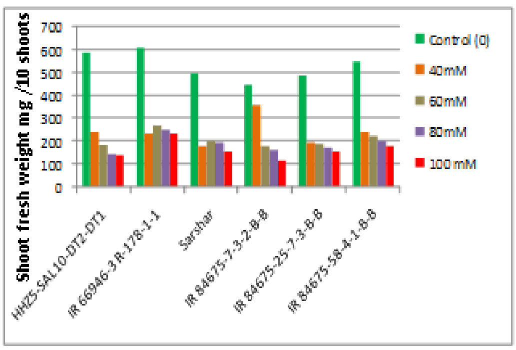

Fig. 4. Effect of different salinity levels on shoot fresh weight (mg/10 shoots) of rice genotypes

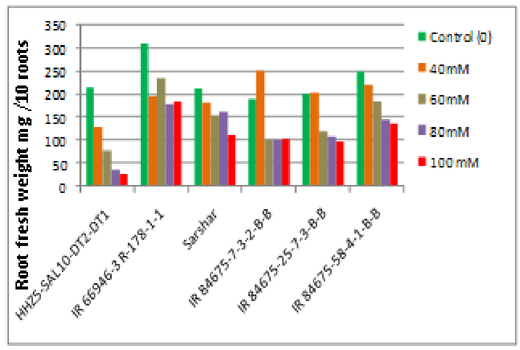

Fig. 5. Effect of different salinity levels on root fresh weight (mg/10 roots) of rice genotypes

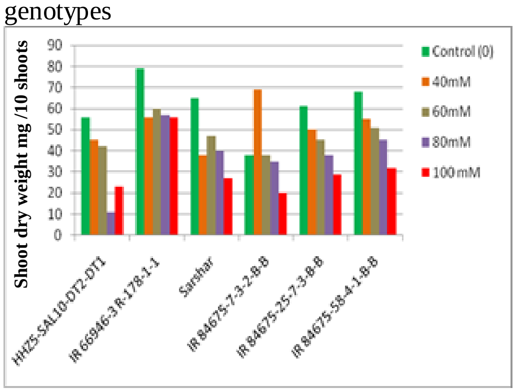

Fig. 6. Effect of different salinity levels on shoot dry weight (mg/10 shoots) of rice genotypes

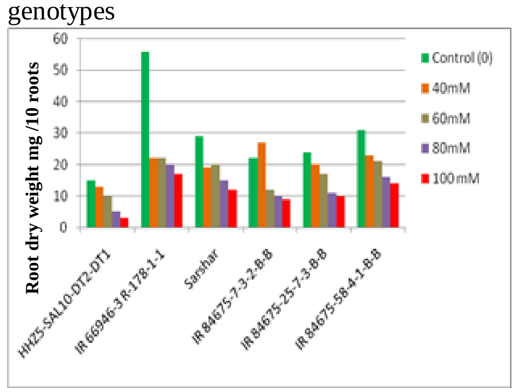

Fig. 7. Effect of different salinity levels on root dry weight (mg/10 roots) of rice genotypes

Potassium and sodium (K^+/Na^+) ratio

The result for potassium and sodium (K^+/Na^+) ratio of different rice genotypes, as affected by NaCl salinity is presented in figure -8. There was decrease in K^+/Na^+ ratio with the increasing in salinity treatment in all rice genotypes. The decrease was more in 100 mM NaCl as compared to control treatment. Mean value for K^+/Na^+ ratio in five treatments was observed as 1.86, 0.98, 0.76, 0.68, and 0.47% in control, 40, 60, 80 and 100 mM NaCl respectively.

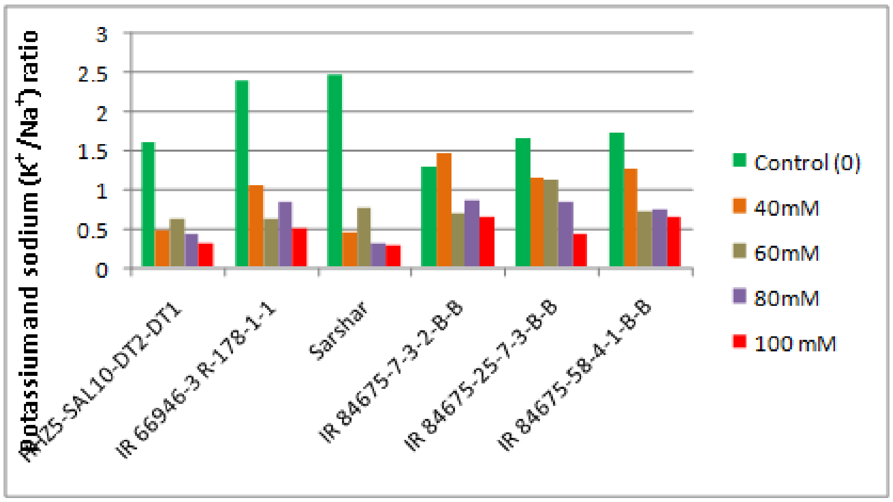

Fig. 8. Effect of different salinity levels on Potassium and sodium (K^+/Na^+) ratio of rice genotypes

At highest salinity level, maximum K^+/Na^+ ratio was observed in genotypes IR 84675-7-3-2-B-B and IR 84675-58-4-1-B-B (0.65 in each), followed by IR 66946-3 R-178-1-1 (0.51), IR 84675-25-7-3-B-B (0.44) and HHZ5-SAL10-DT2-DT1 (0.30). The genotype Sarshar showed minimum values for K^+/Na^+ ratio (0.29).

A substantial body of information in literature indicates that the plant may not exhibit the same response / function under saline conditions as it does under non-saline condition. Numerous studies have shown that the K concentration in plant tissue is reduced as Na salinity or the Na^+/Ca^{++} ratio in the root media is increased (Lutts & Guerrier, 1995; Grattan & Grieve, 1999; Shin & Lee, 1999).

The maintenance of adequate levels of K^+ is essential for plant survival in saline habitats. It contributes to reducing the osmotic potential in root cells and facilitates solute transport process to sustain the overall water balance of the plant. Because plasma membrane of the root cortical cells have a high affinity for K^+ over Na^+ even though the degree of selectivity can vary quite drastically among species. Sodium transport from the environment into the cytoplasm of the plant cell is a passive process. It depends on the electrochemical potential gradient of Na^+ and the presence of Na- permeable channels in the plasma membrane, which allow Na^+ permeation. Under saline conditions salinity affects sterols and phospholipids composition of plasma membrane thereby inducing structural changes in bilayer lipid membrane. This causes depolarization of plasma membrane which affect regulation and selectivity of these channels making it more permeable to ions. Regulation and selectivity of such channel seems to be responsible for Na exclusion in many salt tolerant plants (Jacoby, 1999). This differential selectivity of plasma membrane may be a contributing factor in sensitivity/tolerance of these genotypes.

Conclusion

As it was manifested from present data that genotypes, treatments and their interaction were highly significant for germination (%), root/shoot length (cm) , root/shoot fresh and dry weight (mg) and solute accumulation in plants (inorganic solute Na^+ and K^+). Among the genotypes tested IR 66946-3 R-178-1-1 and IR 84675-58-4-1-B-B are the tolerant genotypes had the potential to perform better under saline conditions at early seedling stage, whereas Sarshar was moderate tolerant under saline conditions. Moreover the genotypes i.e. IR 84675-7-3-2-B-B and IR 84675-25-7-3-B-B are the moderate sensitive under salinity, subsequently, the genotype HHZ5-SAL10-DT2-DT1 showed sensitive one under saline environment. Further it is concluded that the rice genotypes adjust their osmotic potential by accumulating solutes (K^+ over Na^+), and their better performance might be related to the selective uptake of K^+ over Na^+.

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(Received 25 November 2013; accepted 12 January 2014)