***Pak. J. Bot*., 44: 7-13, Special Issue March 2012.**

EFFECT OF SALINITY ON PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF DIFFERENT VARIETIES OF RICE

MUHAMMAD JAMIL[1*], SAMINA BASHIR[1], SOMA ANWAR[1], SARA BIBI[1], ASIA BANGASH[1], FARMAN ULLAH[1] AND EUI SHIK RHA[2]

[1]Deparment of Biotechnology and Genetic Engineering, Kohat University of Science and Technology (KUST), Kohat 26000, Pakistan
[2]Department of Well–being, College of Agriculture & Life Sciences, Sunchon National University, Suncheon 540-742, Republic of Korea
Corresponding author: E-mails: m.jamil@kohat.edu.pk, euishik@sunchon.ac.kr

Abstract

Seeds of three different varieties (Shaheen Basmati, Basmati-385 and NIAB-IR 9) of rice were exposed to increasing concentrations (0, 50, 100 and 150mM) of NaCl to investigate the effect of salinity on seed germination and seedling growth, ion content, photosynthetic pigments and total protein content . There was a regular decrease in seed germination and seedling growth raised in Petri dishes for ten days with increasing salt concentration. Highest germination was observed in NIAB-IR 9 and Shaheen Basmati as compared to Basmati-385. However, Basmati-385 showed better performance at seedling growth than NIAB-IR 9 and Shaheen Basmati. Plants grown in sand culture under salt stress rapidly accumulated sodium (Na) ion in salt-stressed tissues while potassium (K) and calcium (Ca) ions were quickly decreased. Basmati-385 accumulated excessive Na and decreases more K as compared to other varieties. However, leakage of Ca was more pronounced in Shaheen Basmati than others. Chlorophyll (Chl a), Chlorophyll b (Chl b) and total carotenoid content in salt stressed plants were significantly decreased depending on NaCl concentration. Salt stress affected Chl a more than Chl b. Chl a was more disrupted by salinity in Basmati-385 than others. A marked reduction in the protein content of the rice plants under stress was observed with increasing salt concentrations. The effect was more prominent in Shaheen Basmati as compared to Basmati-385 and NIAB-IR 9. It was concluded on the basis of physiological and biochemical characteristics that Shaheen Basmati is more sensitive to salt stress as compared to Basmati-385 and NIAB-IR 9.

Introduction

Rice (*Oryza sativa*) is a model monocot system (Sinclair & Sheehy, 1999), the world's most important agricultural species and a crop grown under extensive irrigation regimes (Yeo & Flowers, 1983; Sahi *et al*., 2006). In Asia, more than two billion people are getting 60-70 percent of their energy requirement from rice and its derived products (Thiyagarajan & Selvaraju, 2001). In Pakistan, rice is the premium food grain crop and is grown on an area of 2.2 million hectare for domestic consumption and export abroad with foreign exchange return of 33 million US $ (Zia *et al*., 1998; Bashir *et al*., 2010).

Abiotic stress is a major factor around the world in limiting plant growth and productivity (Osakabe *et al*., 2011 & Jamil *et al.*, 2010). Exposure of plants to a stressful environment during various developmental stages appears to induce various physiological and developmental changes (Islam *et al*., 2008). Soil salinity, particularly due to NaCl, can be considered as the single most widespread soil toxicity problem that global rice production faces at present (Hong *et al*., 2007). Some toxic effects of salt stress include decreased germination and seedling growth (Zeng & Shannon, 2000; Ashraf, 2010), and suppressed leaf expansion which ultimately reduces photosynthetic area and dry matter production (Mansour & Salama, 2004). K+ in plant tissues evidently decreases when plants are exposed to salt stress, especially rice genotypes (Basu *et al*., 2002). Translocation of salt into roots and to shoots is a outcome of the transpirational flux required to maintain the water status of the plant and unregulated transpiration may cause toxic levels of ion accumulation in the shoot (Yeo, 1998). Lower transpiration rate, coupled with reduced ion uptake by the roots, or reduced xylem loading, may cause poor supply via the xylem. So it is possible that an inadequate supply of ions to the expanding region may restrict cell division and/or expansion when plants are grown at high levels of NaCl (Berstein *et al*., 1995).

Many reports show salt induced reduction in photosynthetic pigments in many plant species such as rice (Cha-um *et al*., 2007). Plants also show the high chlorophyll degradation symptom, chlorosis, as a common morphological and physiological characteristic in response to salt stress (Harinasut *et al*., 2000). Chlorophyll content of salt stressed rice can be described as a function of the leaf sodium content (Yeo & Flowers, 1983). The response of plants to excess NaCl is complex and involves changes in their morphology, physiology and metabolism (Hilal *et al*., 1998). Keeping in mind all these observations, experiments were designed to study the effect of NaCl stress on germination percentage, seedling growth, ion content (Na, K and Ca), protein content and photosynthetic pigments i.e. Chl a, Chl b and carotenoids

Materials and Methods

Seeds of Shaheen Basmati, Basmati-385 and NIAB-IR 9 were obtained from NARC Islamabad. Seeds were hand sorted to eliminate broken, small & infected seeds. Seeds were surface sterilized in 5 percent (*v/v*) sodium hypochlorite solution for 10 minutes then rinsed repeatedly with distilled water & air dried at an ambient temperature of 25ºC in the laboratory.

Germination and seedling growth: Twenty seeds of each variety were placed on 2 layers of filter paper lined with plastic Petri dish containing 20mL NaCl treatments of (control), 50mM, 100mM and 150mM solutions. Seeds were allowed to germinate in incubator at 27±2°C under dark condition. The Petri dishes were arranged with three replications for each salt treatment. The seed germination was investigated after every 12 hrs for 4 days. A seed was considered to have germinated when ≥ 2mL radical had emerged. Emerged seedlings in the 4 treatments were sampled according to their developmental stages and then placed under white fluorescent light (16h light period /day) at 25°C and 60 percent relative humidity, in a growth chamber. Ten seedlings from each 3 replicates were randomly selected at 15^{th} day after experiment for the measurement of growth parameters. Lengths of shoot and root were measured with a scale. For fresh weight (FW), the shoots and roots were excised and weighed separately on electric balance in grams. For dry weight (DW), the excised shoots and roots were dried in incubator at 80°C for 48 hours and then weighed in grams.

Sand culture experiment: Two week old seedlings of each variety (that were grown in Petri dishes in distilled water) were transplanted separately into plastic pot of 12cm diameter and with a small hole in the bottom. The pots were filled with 700g washed and dried sand. All the pots were irrigated for 2 weeks with Hoagland nutrient solution. Nutrient solution was refreshed on weekly intervals and pH (5.8) was adjusted after every 3 days. Two weeks after transplanting, salt stress of 0 (control), 50mM, 100mM and 150mM NaCl concentrations was imposed for 4 days in full strength Hoagland solution. The plants were treated under different stress levels in different pots with 3 replicates. The plants were harvested 18 days after the start of the experiment. Fresh and dried samples of plant leafs were assessed for different biochemical tests.

Elemental analysis: Well ground leaf samples (weighed 25mg) were digested by a mixture of concentrated sulphuric acid and hydrogen peroxide mixed in 2:1 (v/v) ratio. After digestion, the volume of the each sample was made up to 20 ml with distilled deionized water. All cations (Na^+, K^+, and Ca^{2+}) were estimated with a flame emission spectrophotometer. This method was adopted, with little modification, from Awan & Salim (1997).

Photosynthetic pigment concentrations: Dried samples of leaves were analyzed for photosynthetic pigment contents. These pigments were extracted with 96 percent methanol as described by Lichtentaler & Wellburn (1985). The supernatant was separated and the absorbances were read on UV-visible spectrophotometer. The absorbance spectra of Chl a, Chl b and total carotenoids (C_{x+c}) of the extracts were measured at 666, 653 and 470 nm respectively. Total amount of pigments was determined with equations recommended by Lichtentaler & Wellburn (1985).

Determination of protein content: Protein content was determined using the micro-Kjeldahl technique. Dried plant material weighed 115mg was taken and transferred to digestion flask. 1.0g of digestion mixture (copper sulphate, iron sulphate and potassium sulphate) was added and followed by addition of 10ml of concentrated H_2SO_4. The solution was heated until the solution became clear. After cooling, it was diluted upto 25ml with distilled water. The digest was transferred to the distillation assembly and 10ml of 50% NaOH solution was added to it. The distillation was completed in 6 minutes with the change of color of boric acid to yellow due to the formation of ammonium borate indicating the trapping of nitrogen in the form of ammonium hydroxide. The boric acid having the trapped ammonia was titrated with 0.1N H_2SO_4. Titration was done by methyl red indicator for which about 40ml of boric acid was taken in a volumetric flask and methyl red indicator was added, a pink colored was appeared. During titration, the color of boric acid having ammonia changed again into pink. The protein was calculated by using the formula:

$$\text{Protein} = \frac{(\text{Titrant volume for sample} - \text{Titrant volume for blank}) \times 1.4007 \times 1}{\text{Dry weight of sample}} \times 6.25$$

where: 6.25 = Protein factor, 1.4007 = Nitrogen factor

Statistical analysis: Analysis of variance was performed by using MS-Excel (Add-In) Statistical software. Mean values of all parameters including germination and early seedling growth, ion content, photosynthetic pigments, and total protein content were compared by LSD using Tukey's *t* (Li, 1964).

Results

Seed germination: The different treatments showed different germination pattern in control and seeds stressed with NaCl. Increasing salt levels had detrimental effects on germination percentage. Decrease in germination was recorded with increase in salinity from 0 to 150mM. Overall high percentage was observed in Shaheen Basmati as compared to other varieties (Fig. 1). Application of salt stress significantly delayed the time to achieve 50% germination (Fig. 2). Under optimal conditions the rice seeds in control and 50mM treatment started to germinate after 1 day's imbibitions. At second day, the seeds started germination in all the treatments (Fig. 2).

Seedling growth: Rice seedlings growth was significantly ($p \le 0.05$) influenced by the levels of salt it contained. The growth of rice seedling was evaluated by root and shoot- lengths, fresh and dry weights. The maximum rice seedling growth occurred at 50mM salt concentration after control. The data showed that the length and weight of roots and shoots were reduced with the increasing salt concentrations (Figs. 3-8). These results indicated that NaCl inhibited the growth of rice seedling and led to a decrease in biomass. Salt-stress caused decreases in root-lengths more than shoot length (Fig. 3). Reduction in root and shoot length was more pronounced in Shaheen Basmati while Basmati-385 was least affected (Figs 3 & 4). Rice seedlings grown at the low levels of NaCl (0mM) reached relatively higher fresh and dry weights and did not imply toxicity symptoms, however, the plantlet fresh and dry weight was significantly reduced at higher levels of salinity (150mM) indicating the symptoms of salt toxicity as growth depression (Figs. 4 & 5). Basmati-385 and NIAB-IR 9 were better in response in term of fresh and dry weights at higher levels of salinity than Shaheen Basmati (Figs. 4 & 5). The concentration of NaCl that significantly reduced weight was 150mM in comparison to the control (Figs. 3-8). Shaheen Basmati was more affected than other 2 varieties in term of seedling growth (Figs. 3-8).

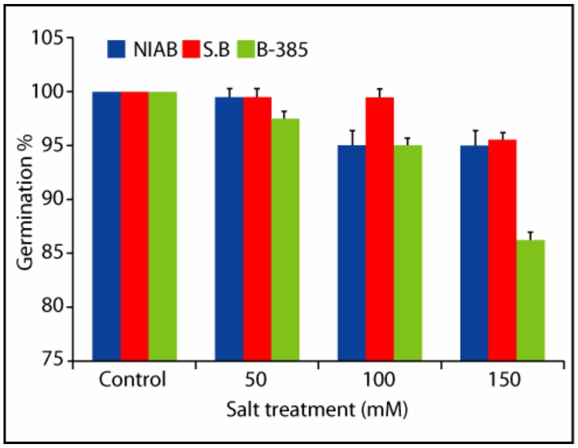

Fig. 1. Effect of different concentration of salt on germination percentage of NIAB-IR 9, S.B (Shaheen Basmati) and Basmati-385. Each value is expressed as mean + S.D. (n=5) statistically significant at $p<0.05$.

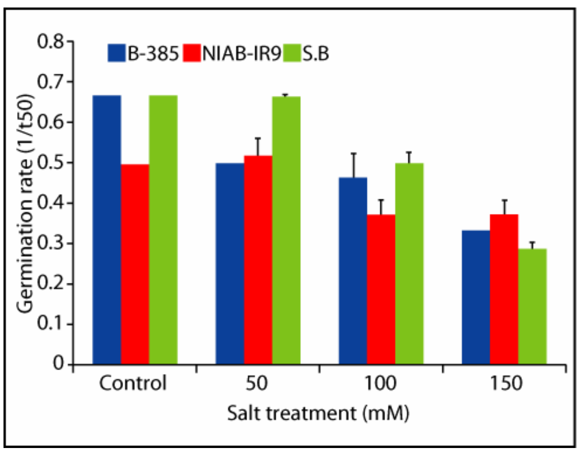

Fig. 2. Effect of different concentration of salt on germination rate of NIAB-IR 9, S.B (Shaheen Basmati) and Basmati-385. Each value is expressed as mean + S.D. (n=5) statistically significant at $p<0.05$.

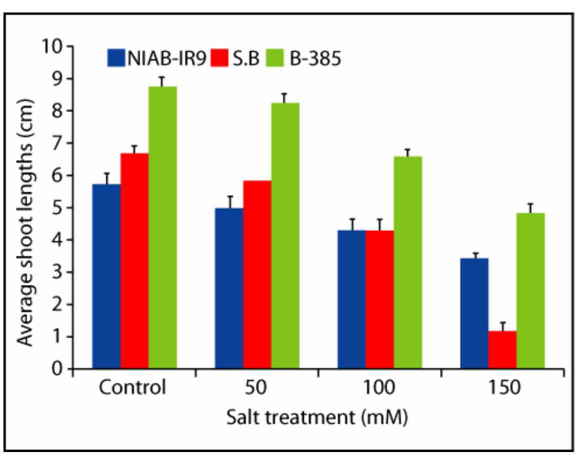

Fig. 3. Effect of different concentration of salt on root length of NIAB-IR 9, S.B (Shaheen Basmati) and Basmati-385. Each value is expressed as mean + S.D. (n=5) statistically significant at $p<0.05$.

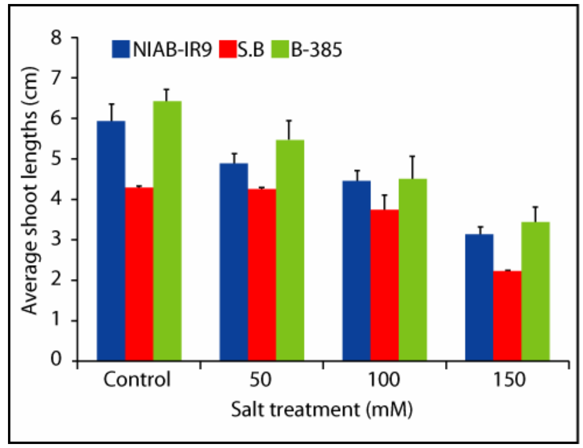

Fig. 4. Effect of different concentration of salt on shoot length of NIAB-IR 9, S.B (Shaheen Basmati) and Basmati-385. Each value is expressed as mean + S.D. (n=5) statistically significant at $p<0.05$.

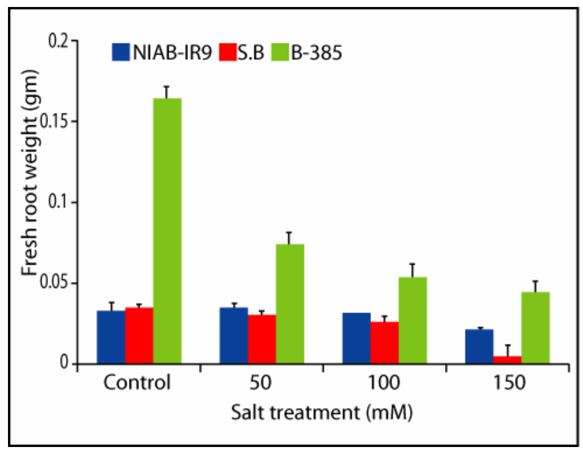

Fig. 5. Effect of different concentration of salt on fresh root weight of NIAB-IR 9, S.B (Shaheen Basmati) and Basmati-385. Each value is expressed as mean + S.D. (n=5) statistically significant at $p<0.05$.

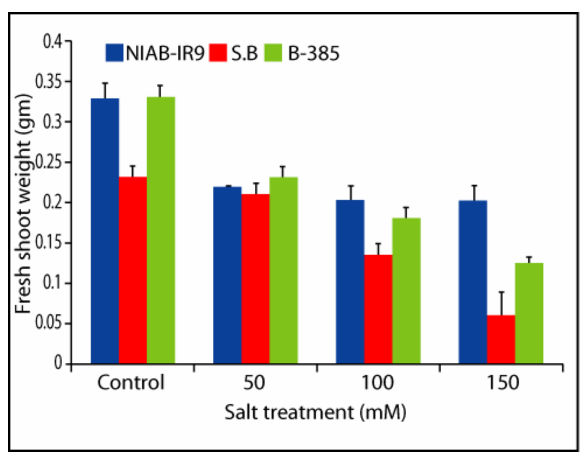

Fig. 6. Effect of different concentration of salt on fresh shoot weight of NIAB-IR 9, S.B (Shaheen Basmati) and Basmati-385. Each value is expressed as mean + S.D. (n=5) statistically significant at $p<0.05$.

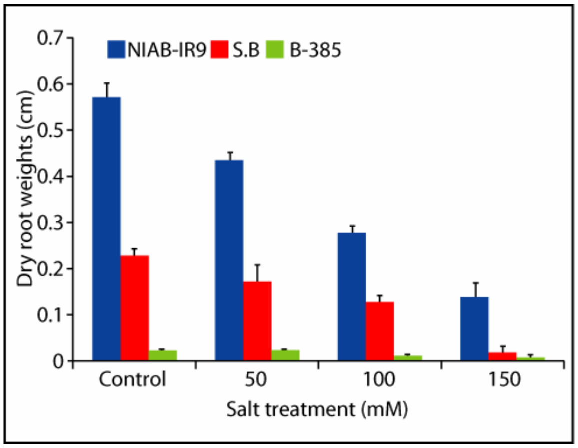

Fig. 7. Effect of different concentration of salt on dry root weight of NIAB-IR 9, S.B (Shaheen Basmati) and Basmati-385. Each value is expressed as mean + S.D. (n=5) statistically significant at $p<0.05$.

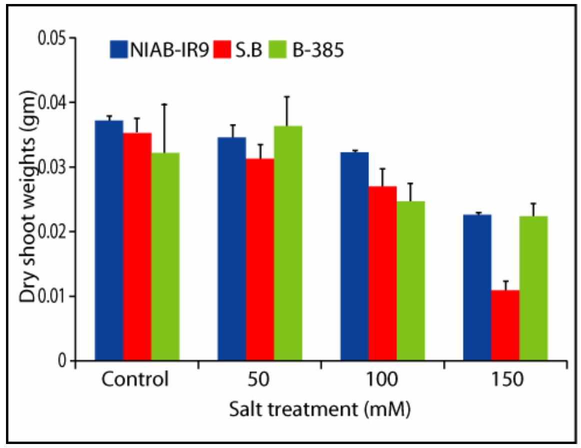

Fig. 8. Effect of different concentration of salt on dry shoot weight of NIAB-IR 9, S.B (Shaheen Basmati) and Basmati-385. Each value is expressed as mean + S.D. (n=5) statistically significant at $p<0.05$.

Na^+, K^+ and Ca^{2+} content: NaCl salinity exhibited significant effects on ion concentrations of rice plants. Sodium (Na) ion accumulation in rice plants was directly enhanced relating to NaCl salt concentrations in the Hoagland solution. In contrast, potassium (K) ion and calcium (Ca) ion in salt-stressed plants were significantly reduced. Higher sodium (Na) ion accumulation was observed in Basmati-385 while similar kind of intake was measured in NIAB-IR-9 and Shaheen Basmati. Higher reduction of K Concentration was observed in Basmati-385. Leakage of Ca was more pronounced in Shaheen Basmati than other (Fig. 9). This implied a competition between Na and K absorption in rice plants under stress, resulting in a Na / K antagonism. The reduction in K uptake caused by Na is likely to be the result of the competitive intracellular influx of ions.

Total chlorophyll content and carotenoids: Chl a, Chl b and total carotenoid content in salt stressed plants were significantly decreased depending on NaCl concentration. When grown without salt stress, the sand culture rice plants significantly showed higher concentrations of major pigments as compared to plants under salt stress. After salt stress, the pigment concentrations of the rice plants were several folds lower than plants grown without salt-stress (Fig. 10). Results indicated that salt stress affected Chl a more than Chl b (Fig. 10). The affect was more severe on the Chl a of Basmati-385 as compared to NIAB-IR 9 and Shaheen Basmati. The carotenoid content was less in Shaheen Basmati at higher NaCl concentration than Basmati-385 and NIAB-IR 9 (Fig. 11).

Total protein content: It was high in control treated plants and significantly diminished in plants grown under salt stress. At 150 mM salt stress, protein content was reduced in all varieties; it showed that rice plants were severely affected at high salt concentration (Fig. 12). The affects was more severe in Shaheen Basmati than other two varieties in term of protein content (Fig. 12).

Discussion

Rice plants are relatively susceptible to soil salinity as an abiotic stress (Flowers & Yeo, 1989; Gao *et al*., 2007). A decrease in both percentage and rate of seed germination (Fig.1) was observed, when exposed to increased salinity level (Figs.1 & 2). Rice seedlings growth was significantly influenced by the levels of salt it contained (Figs. 3-8). Seed germination and early seedling growth of rice has been shown to be highly sensitive to salt stress because of marked effects of osmotic stress and specific ion toxicity on these growth stages in this plant species (Mohammad & Sen, 1990; Duan *et al.,* 2004). Salt causes inhibition of seed germination which could be attributed to specific ion toxicity (Huang & Redmann, 1995). Salinity reduces shoot and root growth by reducing turgor in expanding tissues resulting from lowered water potential in root growth medium (Alam *et al*., 2004). A disturbance in mineral supply, either on excess or deficiency, induced by changes in concentrations of specific ions the growth medium might have directly affected growth (Lazof & Bernstein, 1998).

The increase in Na+ ion content and decrease in K+ ion uptake disturbs ionic imbalance as observed in most species exposed to salt stress (Fig. 9). The diminution of K+ concentration in tissue may also be due to direct competition between K+ and Na+ at plasma membrane, inhibition of Na+ on K+ transport process in xylem tissues and/or Na+ induced K+ efflux from the roots. K+ and Ca+ + have been reported to be the major cautions in cell organization as well as the major contributors to osmotic adjustment under stress conditions in several plant species (Santos-Diaz & Alejo-Ochoa, 1994; Hirschi, 2004). In the present study, the level of K+ and Ca+ + in the salt-stressed rice cells gradually decreased while that of Na+ was dramatically increased (Fig. 9). The decrease in K+ and Ca+ + content under stress condition has been previously reported in other species particularly in the salt-sensitive lines (Lutts *et al*., 1996, 2004). According to Weimberg (1987), high levels of Na+ inside the cells inhibit the K+ uptake and as a result it causes an increase in the Na+/K+ ratio. Many of the deleterious effects of Na+ seem to be related to the structural and functional integrity of membranes (Kurth *et al.,* 1986).

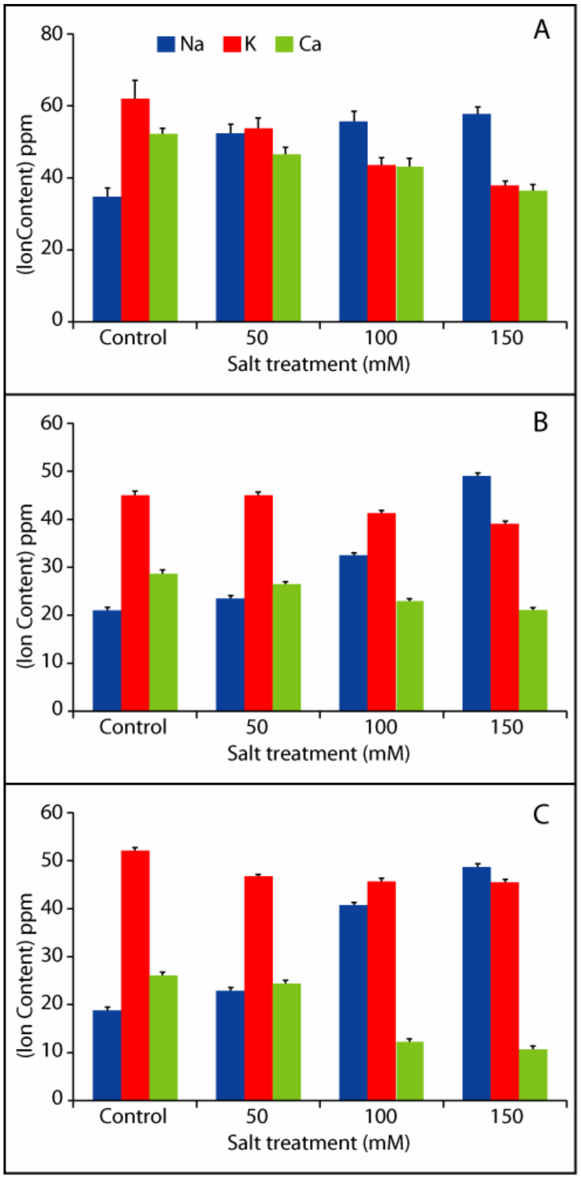

Fig. 9. Effect of different concentration of salt on ion content of Basmati-385(A), NIAB-IR 9(B) and S.B (Shaheen Basmati) (C). Each value is expressed as mean +S.D. (n=3) statistically significant at $p < 0.05$.

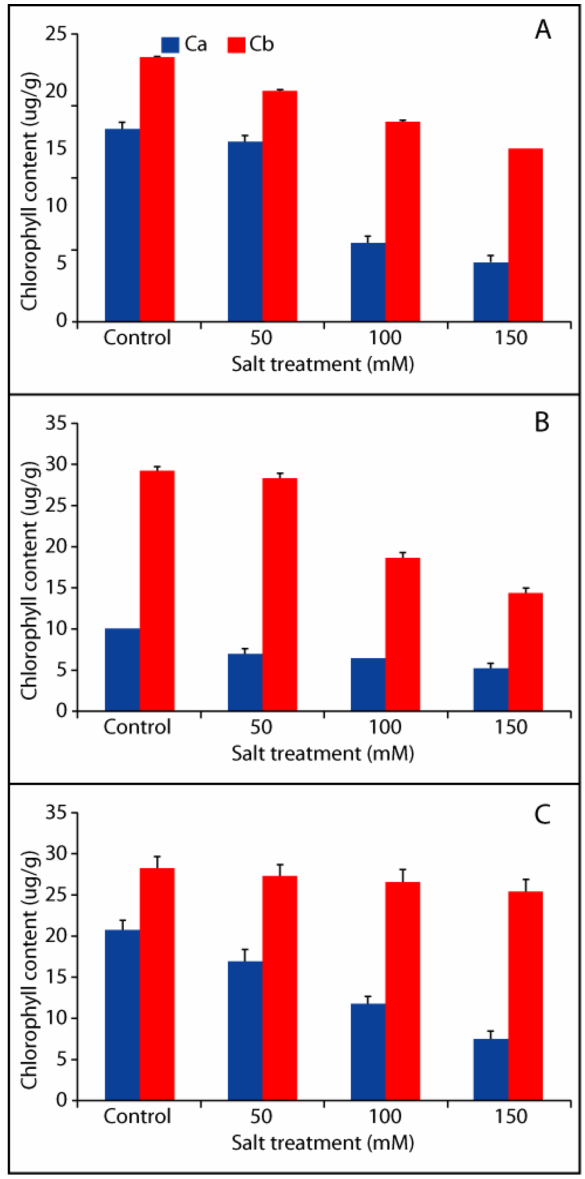

Fig. 10. Effect of different concentration of salt on chlorophyll content of Basmati-385 (A), NIAB-IR 9 (B) and S.B (Shaheen Basmati) (C). Each value is expressed as mean + S. D. (n=3) statistically significant at $p<0.05$.

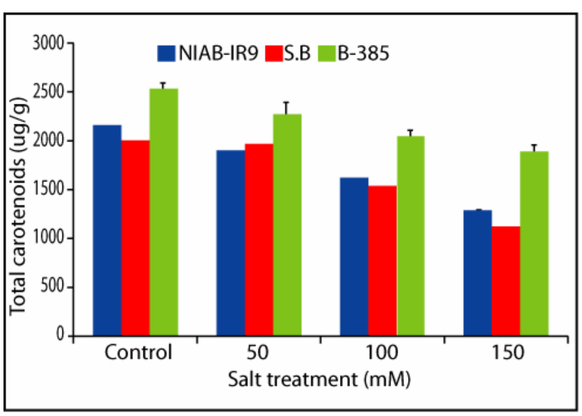

Fig. 11. Effect of different concentration of salt on total carotenoids of Basmati-385 (A), NIAB-IR 9 (B) and S.B (Shaheen Basmati) (C). Each value is expressed as mean + S. D. (n=3) statistically significant at $p<0.05$.

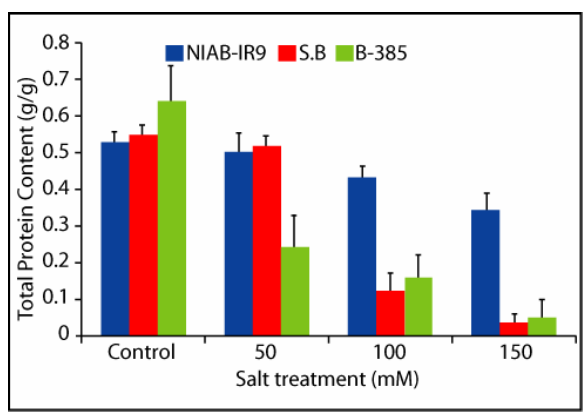

Fig. 12. Effect of different concentration of salt on total protein content of NIAB-IR 9, S.B (Shaheen Basmati) and Basmati-385. Each value is expressed as mean + S. D. (n=3) statistically significant at $p<0.05$.

This study shows that photosynthetic pigments (Figs. 7 & 8) and protein concentrations (Fig. 9) of leaves were reduced by salinity. A decrease in chlorophyll concentration in salinized plants could be attributed to increased activity of the chlorophyll-degrading enzyme chlorophyllase (Reddy & Vora, 1986). Ion accumulation in leaves also adversely affected chlorophyll concentration (Dubey, 1994)**.** The decrease in carotenoids under salt stress leads to degradation of β-carotene and formation of zeaxanthins, which are apparently involved in protection against photoinhibition (Sharma & Hall, 1991). A decrease in Chl and protein content with the increase of NaCl was also reported by Khan (2003), Azooz *et al*., (2004) and Dager *et al*., (2004). Water deficit impedes protein synthesis perhaps at the ribosomal level; some proteins are apparently formed and inactivated quickly whereas others appear to be relatively stable (Lutts *et al.*, 1996). In many plant tissues a reduced water potential causes a reduction of total protein synthesis and a rapid dissociation of polyribosomes (Jones, 1996; Bardzik *et al.*, 1971).

It was concluded that salinity effect physiological and biochemical characteristics of different rice varieties but the response was different at different stages of plant growth. Shaheen Basmati was better at germination but poor at seedling stage and other biochemical parameters. . It was also concluded on the basis of above mention results that Shaheen Basmati is more sensitive to salt stress as compared to Basmati-385 and NIAB-IR 9.

Acknowledgements

Financial support for this study was generously provided by the Higher Education Commission, Pakistan through Research Grant#1348.

References

Alam, M.Z., T. Stuchbury, R.E. Naylor and M.A. Rashid. 2004. Effect of salinity on growth of some modern rice cultivars. *J. Agron.*, 3: 1-10.

Ashraf, M. 2010. Inducing drought tolerance in plants: some recent advances. *Biotechnol. Adv*., 28: 169-183.

Awan, J.A. and U.R. Salim. 1997. Food analysis manual. *Vet. Ag. Publication*., 5: 2-7.

Azooz, M., M.A. Shaddad and A.A. Abdel-Latef. 2004. The accumulation and compartmentation of proline in relation to salt tolerance of three sorghum cultivars. *Indian Journal of Plant Physiology*, 9: 1-8.

Bardzik, J., H.V. Marsh and J.R. Harvis. 1971. Effects of water stress on the activities of three enzymes in maize seedlings. *Plant Physiol*., 47: 828-831.

Bashir, M.U., N. Akbar, A. Iqbal and H. Zaman. 2010. Effect of different sowing dates on yield and yield components of direct seeded coarse rice (*Oryza sativa* L). *Pak. J. Agri. Sci*., 47: 361-365.

Basu, S., G. Gangopadhyay and B. Mukherjee. 2002. Salt tolerance in rice *in vitro*: Implication of accumulation of Na+, K+ and proline. *Plant Cell Tiss. Org. Cult*., 69: 55-64.

Berstein, N., W.K. Silk and A. Lauchli. 1995. Growth and development of sorghum leaves under conditions of NaCl stress: possible role of some mineral elements in growth inhibition. *Planta*, 196: 699-705.

Cha-um, S., K. Supaibulwatana and C. Kirdmanee. 2007. Glycinebetaine accumulation, physiological characterizations and growth efficiency in salt-tolerant and salt-sensitive lines of indica rice (*Oryza sativa* L. ssp. *indica*) in response to salt stress. *J. Agron. Crop Sci*., 193: 157-166.

Dagar, J.C., H. Bhagwan and Y. Kumar. 2004. Effect on growth performance and biochemical contents of Salvadora persica when irrigated with water of different salinity. *Indian Journal of Plant Physiology*, 9: 234-238.

Duan, D., X. Liu, M.A. Khan and B. Gul. 2004. Effects of salt and water stress on the germination of *Chenopodium glaucum* L., seed. *Pak. J. Bot*., 36: 793-800.

Dubey, R.S. 1997. Photosynthesis in plants under stressful conditions In: Pessarakli, M. (Ed.) Handbook of Photosynthesis, New York: Marcel Dekker, 1997. 859-875.

Flowers, T.J. and A.R. Yeo. 1989. Effects of salinity on plant growth and crop yield. In: Environmental Stress in Plants, (Ed.): J.H. Cherry. Springer Verlag: Berlin. pp. 101-119.

Gao, J.P., D.Y. Chao and H.X. Lin. 2007. Understanding abiotic stress tolerance mechanisms: recent studies on stress response in rice. *Journal of Integrative Plant Biology*, 49 : 742-750.

Harinasut, P., S. Srisunak, S. Pitukchaisopol and R. Charoensataporn. 2000. Mechanisms of adaptation to increasing salinity of mulberry: proline content and ascorbate peroxidase activity in leaves of multiple shoots. *Sci Asia*., 26: 207-11.

Hilal, M., A.M. Zenoff, G. Ponessa, H. Moreno and E.D. Massa. 1998. Saline stress alters the temporal patterns of xylem differentiation and alternative oxidative expression in developing soybean roots. *Plant Physiol*., 11: 695-701.

Hirschi, D. 2004. The calcium conundrum, both versatile nutrient and specific signal. *Plant Physiol*., 136: 2438-2442.

Hong, C.Y., Y.T. Hsu, Y.C. Tsai and C.H. Kao. 2007. Expression of ascorbate peroxidase 8 in roots of rice (*Oryza sativa L.*) seedlings in response to NaCl. *J. Exp. Bot.*, 58: 3273-3283.

Huang, J. and R.E. Redmann. 1995. Physiological response of canola and wild mustard to salinity and contrasting calcium supply. *Journal of Plant Nutrition,* 18: 1931-1949.

Islam, M.S., J.H. Hur and M.H. Wang. 2008. The influence of abiotic stresses on Expression of zinc finger protein gene in rice. *Russian Journal of Plant Physiology*. 56: 695-701.

Jamil, M., W. Iqbal, A. Bangash, S. Rehman, Q.M. Imran and E.S. Rha. 2010. Constitutive expression of OSC3H33, OSC3H50 and OSC3H37 genes in rice under salt stress. *Pak.J. Bot*., 42: 4003-4009.

Jones, G.H. 1996. Plants and microclimate. *Cambridge USA. Ed*. 2: 72-108.

Khan, G. 1998. Soil Salinity/Sodicity Status in Pakistan. Soil Survey of Pakistan, Lahore. 59.

Kurth, E., G.R. Cramer, A. Lauchli and E. Epstein. 1986. Effects of NaCl and CaCl2 on cell enlargement and cell production in cotton roots. *Plant Physiol*., 82: 1102-1106.

Lazof, D.B. and N. Bernstein. 1998. In: *Advances in Botanical Research*. (Ed.): J.A. Callow. The NaCl induced inhibition of shoot growth: The case of disturbed nutrition with special consideration of calcium. pp. 113-189.

Li, C.C. 1964. *Introduction to Experimental statistics.* McGraw Hill Book Company, New York, USA.

Lichtenthaler, H.K. and A.R. Wellburn. 1985. Determination of total carotenoids and chlorophylls A and B of leaf in different solvents.*Biol. Soc. Trans*., 11: 591-592.

Lutts, S., J.M. Kinet and J. Bouharmont. 1996. Effects of various salts and of mannitol on ion and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) callus cultures. *J. Plant Physiol*., 149: 186-195.

Lutts, S., M. Almansouri and J.M. Kinet. 2004. Salinity and water stress have contrasting effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. *Plant Sci*., 167: 9-18.

Mansour, M.M. and K.H. Salama. 2004. Cellular basis of salinity tolerance in plants. *Environ. Exp. Bot*., 52: 113-122.

Mohammad, S. and D.N. Sen. 1990. Germination behavior of some halophytes in Indian desert. *Int. J. Exp. Biol*., 28: 545-549.

Osakabe, Y., S. Kajita and K. Osakabe. 2011. Genetic engineering of woody plants: current and future targets in a stressful environment. *Physiologia Plantarum*, 142: 105-117.

Reddy, M.P. and A.B. Vora. 1986. Changes in pigment composition, hill reaction activity and saccharides metabolism in bajra (Pennisetum typhoides S&H) leaves under NaCl salinity. *Photosynthica*, 20: 50-55.

Sahi, C., A. Singh, K. Kumar, E. Blumwald and A. Grover. 2006. Salt stress response in Rice: genetics, molecular biology, and comparative genomics. *Functional and Integrative Genomics*, 6: 263-284.

Santos-Diaz, M.S. and N. Alejo-Ochoa. 1994. PEG-tolerant cell clones of chili pepper (*Capsicum annum* L.): growth, osmotic potentials and solute accumulation. *Plant Cell Tiss. Organ. Cult.*, 37: 1-8.

Sharma, P.K. and D.O. Hall. 1991. Interaction of salt stress and photoinhibition on photosynthesis in barley and sorghum. *J. Plant Physiol*., 138: 614-619.

Sinclair, T.R. and J.E. Sheehy. 1999. Erect Leaves and Photosynthesis in Rice. *Science*, 283: 1455.

Thiyagarajan, T.M. and R. Selvaraju. 2001. Water saving in rice cultivation in India. Proceedings of an international workshop on water saving rice production systems. Nanjing University, China. 15-45.

Weimberg, R. 1987. Solute adjustments in leaves of two species of wheat at two different stages of growth in response to salinity. *Physiol. Plant*., 70: 381-388.

Yeo, A.R. 1998. Molecular biology of salt tolerance in the context of whole-plant physiology. *J. Exp. Bot*., 49: 915-929.

Yeo, A.R. and T.J. Flowers. 1983. Varietal differences of sodium ions in rice leaves. *Physiol. Plant.*, 59: 189-195.

Zeng, L. and M.C. Shannon. 2000. Salinity effects on seedling growth and yield Components of rice. *Crop Science*., 40: 996-1003.

Zia, M.S., M. Aslam, M.B. Rehmatullah, M. Khan, B. Baig and A. Ali. 1998. Assessment Of nitrogen availability in calcareous soil under rice based cropping system. *Pakistan J. Soil Sci*., 15: 166-73.

(Received for publication 12 February 2011)