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Physiological Response of Rice (*Oryza Sativa L.*) Genotypes under Different Salinity Levels at Early Seeding Stage

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Abstract Salinity is one of the major threats facing the cultivation of field crops. The response of six rice *(Oryza sativus* L.*)* genotypes to different sodium chloride levels; 0, 40, 60, 80 and100 mM were investigated at early seedling stages. Among the tested genotypes 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, 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 under saline environment. Furthermore it is concluded that the rice genotypes adjust their osmotic potential by accumulating both types of solutes (organic and inorganic ions), and their better performance might be correlated to the selective uptake of K^+ over Na^+ and high accumulation of proline.

Keywords: physiological response, solute accumulation, rice, salinity stress

Introduction

Abiotic stress is the main factor 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 and food security and adversely impact the social-economic fabric in many developing countries, affecting about 95 million hectares worldwide (Ghassemi-Golezani *et al.,* 2010). Salinity is an important problem affecting irrigated agriculture of Pakistan and 40,000 hectares of arable land is lost annually due to salinity (Ahmad *et al.,* 2006; Ashraf *et al.,* 2008; Mehmood *et al.,* 2009).

Improper irrigation practices and lack of drainage have generally led to accumulation of salts in the soil in concentrations, which are harmful to the

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crops. There is a major imbalance in the amount of salt entering and leaving the soil in `Pakistan. Each year about 120 million tonnes of salts are added to the land in canal water and brackish underground water. Only about 1/5th of this salt finds its ways to the sea. The remainder accumulates in the soil; it continues to decrease the growth and survival of crops (Alam *et al.,* 2000).

Rice *(Oryza sativa L.)* is one of the most important crops in the world and is the primary stable food for over two billion peoples. 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.

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.

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).

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. The objective of the current study was to evaluate the effect of induced salinity on physiological and biochemical behavior of rice genotypes.

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.

Seed source

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.

Experimental details

Approximately 400 healthy seeds of each rice genotype 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 Yoshida 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.

Pigments analysis

The samples for pigments contents (chlorophyll Ch-a, Ch-b, total chlorophyll and carotenoids) were extracted in 80% acetone and the absorbance of the centrifuged extract was recorded at 470, 646.8, and 663.2nm using a spectrophotometer. Chlorophyll contents were calculated according to Lichtenthaler (1987).

Proline analysis

The proline content of the shoot samples was determined according to Bates *et al*. (1973). Approximately 0.5 g of plant material was ground in liquid nitrogen, homogenized in 10 mL of sulfosalicylic acid, and filtered through

Whatman #2 filter paper. Then 2 mL of the filtrate was incubated with 2 mL of acid ninhydrine and 2 mL of glacial acetic acid for 1 h at 100 ℃. The reaction was stopped by transferring the samples into an ice bath. The reaction mixture was extracted with 4 mL of toluene and the absorbance of the aqueous phase was measured at 520 nm. Proline concentration was calculated on a fresh weight basis.

Sodium (Na) and Potassium (K) analysis

Sodium and potassium contents were measured in percentage (% g^{-1} fresh weight) using method elaborated by Flowers (1986). For the determination of Na^+ and K^+ in fresh grinded leaf shoot, the plant material was treated with 0.2 mM acetic acid (CH_3COOH) in water bath for 1 hour pre heated at 95^{o}C. The extracted solution was filtered and suitable dilution was made. Na^+ and K^+ concentration were determined by flame photometer (jenway, Model PFP7).

Statistical analysis

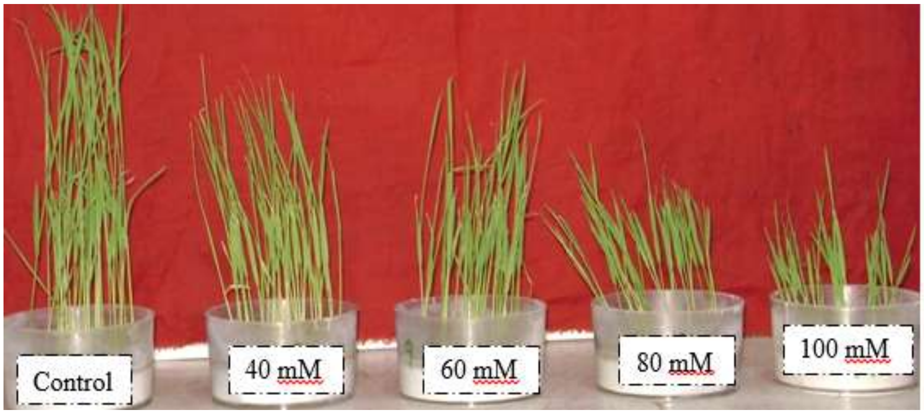
The experiment was conducted in a completely randomized design (CRD) with three replications. The treatments means were compared by Least Significant Difference (LSD) test at 0.05 probability level (Steel and Torrie, 1984).

Result and discussions

Chlorophyll-a content (mg g^{-1} fresh weight)

There was decrease in chlorophyll-a content with increase in salinity treatment in all rice genotypes. The decrease was more at highest salinity level 100mM (NaCl) as compare to control treatment (Figure 1).

The values for chlorophyll-a content at highest salinity treatment (100 mM NaCl), was observed maximum in genotype IR 84675-58-4-1-B-B (i.e. 0.43 mg g^{-1} fresh weight), followed by HHZ5-SAL10-DT2-DT1 (0.24), IR 66946-3 R-178-1-1 (0.35), IR 84675-7-3-2-B-B (0.21), IR 84675-25-7-3-B-B (0.22 mg g^{-1} fresh weight), while minimum chlorophyll-a content under high salinity treatment was recorded in genotype Sarshar (i.e.0.10 mg g^{-1} fresh weight).



Chlorophyll-b content (mg g^{-1} fresh weight)

The results for chlorophyll-b showed significant decrease in chlorophyll-b content with increase in salinity treatment in all rice genotypes (Figure 2.). The decrease was more at highest salinity level 100 mM (NaCl) as compare to control treatment.

The values for chlorophyll-b content at highest salinity treatment (100 mM NaCl), were observed maximum in genotype IR 66946-3 R-178-1-1 (i.e. 0.39 mg g^{-1} fresh weight), followed by, HHZ5-SAL10-DT2-DT1 (0.24), Sarshar (0.20), IR 84675-58-4-1-B-B (0.25 mg g^{-1} fresh weight), while minimum chlorophyll-b content under high salinity treatment was recorded in genotype IR 84675-25-7-3-B-B (0.12) and IR 84675-7-3-2-B-B (i.e.0.12 mg g^{-1} fresh weight).

Total Chlorophyll content (mg g^{-1} fresh weight)

The total chlorophyll content was declined as salinity treatment increased in all rice genotypes (Figure-3).

The values for total chlorophyll content at highest salinity treatment (100 mM NaCl), was observed maximum in genotype IR 84675-58-4-1-B-B (i.e. 0.68 g^{-1} fresh weight), followed by, HHZ5-SAL10-DT2-DT1 (0.48), IR 84675-7-3-2-B-B (0.33), Sarshar (0.30), IR 84675-25-7-3-B-B (0.34 mg g^{-1} fresh weight), while minimum chlorophyll-a content under high salinity treatment was recorded in genotype IR 66946-3 R-178-1-1 (0.21mg g^{-1} fresh weight), respectively.

Carotenoids content (mg g^{-1} fresh weight)

There was significant decline was recorded with respect of carotenoids content with increase in salinity treatment in all rice genotypes. The decrease was more at highest salinity level 100 mM (NaCl) as compare to control treatment (Figure-4).

The values for Carotenoids content at highest salinity treatment (100 mM NaCl), was observed maximum in genotype IR 66946-3 R-178-1-1 (i.e. 1.90 mg g^{-1} fresh weight), followed by, Sarshar (1.26), IR 84675-7-3-2-B-B (0.78), IR 84675-25-7-3-B-B (0.74), IR 84675-58-4-1-B-B (1.26 mg g^{-1} fresh weight), while minimum Carotenoids content under high salinity treatment was recorded in genotype HHZ5-SAL10-DT2-DT1 (i.e. 0.60 mg g^{-1} fresh weight).

Proline content (μg/g fresh weight)

There was increase in proline with increase in salinity treatment in all rice genotypes. The increase was more at highest salinity level (100mM NaCl) as compare to control treatment (Figure-5).

The values for Proline accumulation at highest salinity treatment (100 mM NaCl), were also observed maximum in genotype IR 66946-3 R-178-1-1 (i.e. 11.51μg/g fresh weight), followed by HHZ5-SAL10-DT2-DT1 (8.76), Sarshar (6.86), IR 84675-7-3-2-B-B (9.77), IR 84675-25-7-3-B-B (9.77 μg/g fresh weight), while the minimum Proline was recorded in genotype IR 84675-58-4-1-B-B (4.98 μg/g fresh weight) respectively.

Shoot sodium (Na^+) content (%)

It was observed that increasing concentration of sodium chloride (NaCl), in the rooting medium have increased sodium (Na^+) content in plant shoot (Figure-6). Maximum increase in (Na^+) contents was observed at highest salt treatment 100 mM NaCl. Mean values for Na^+ content in five treatments were observed as 0.25, 0.66, 0.93, 0.98, 1.08% in control, 40, 60, 80 and 100 mM NaCl, respectively.

The data revealed that the maximum sodium (Na^+) content was recorded in genotype Sarshar (1.78%) at highest salinity treatment I00 mM NaCl, followed by IR 66946-3 R-178-1-1 (1.28), HHZ5-SAL10-DT2-DT1 (1.25), IR 84675-7-3-2-B-B (0.83), IR 84675-25-7-3-B-B (0.82%), while, minimum values for Na^+ contents was recorded the genotype IR 84675-58-4-1-B-B i.e. (0.50%) respectively.

Shoot potassium (K^+) content (%)

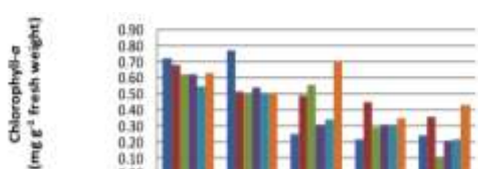
The results pertaining to the potassium (K^+) uptake by rice genotypes showed that K^+ content increased in three genotypes while potassium (K^+) uptake in remaining genotypes, decreased with increasing salinity level (Figure-7). Mean values for potassium contents were recorded as 0.45, 0.47, 0.58, 0.51 and 0.46% in control, 40, 60, 80 and 150 mM NaCl, respectively. It is also observed that the genotypes having better growth performance i.e. IR 66946-3 R-178-1-1, IR 84675-7-3-2-B-B, and Sarshar had increasing trend for K^+ uptake under saline environment, which is good indication to cope the toxic effects of Na. Maximum reduction in potassium uptake was observed at highest NaCl treatment i.e.100 mM NaCl.

At highest salinity level, the genotype IR 66946-3 R-178-1-1 had the maximum K^+ content (0.63%), while the genotype IR 84675-58-4-1-B-B had the lowest potassium values (0.32%).

Shoot Potassium and Sodium (K^+/Na^+) ratio

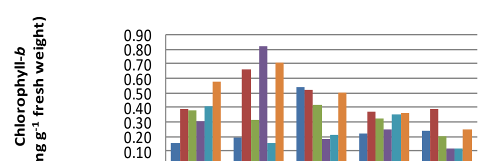
There was decrease in K^+/Na^+ ratio with the increasing in salinity treatment in all rice genotypes (Figure 8). The decrease was more in 100 as compared to control treatment. Mean value for K^+/Na^+ ratio in five treatments was observed as 1.86, 0.98, 0.76, 0.68, and 0.47% in control, 40, 60, 80 and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

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).



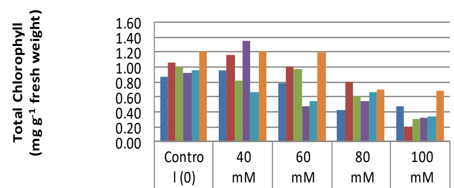
	Control (0)	40 mM	60 mM	80 mM	100 mM
HHZ5-SAL10-DT2-DT1	0.73	0.78	0.25	0.21	0.24
IR 66946-3 R-178-1-1	0.69	0.51	0.49	0.45	0.35
Sarshar	0.63	0.50	0.56	0.29	0.10
IR 84675-7-3-2-B-B	0.63	0.54	0.31	0.31	0.21
IR 84675-25-7-3-B-B	0.55	0.51	0.34	0.31	0.22
IR 84675-58-4-1-B-B	0.63	0.51	0.70	0.35	0.43

Fig. 1. Effect of different salinity levels on shoot Chlorophyll-*a* content in rice genotypes



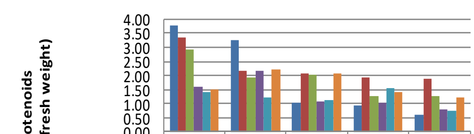
	Control (0)	40 mM	60 mM	80 mM	100 mM
HHZ5-SAL10-DT2-DT1	0.15	0.19	0.54	0.22	0.24
IR 66946-3 R-178-1-1	0.39	0.66	0.52	0.37	0.39
Sarshar	0.38	0.32	0.42	0.32	0.20
IR 84675-7-3-2-B-B	0.30	0.82	0.18	0.25	0.12
IR 84675-25-7-3-B-B	0.41	0.15	0.21	0.35	0.12
IR 84675-58-4-1-B-B	0.58	0.71	0.51	0.36	0.25

Fig. 2. Effect of different salinity levels on shoot Chlorophyll-*b* content in rice genotypes



	Control (0)	40 mM	60 mM	80 mM	100 mM
HHZ5-SAL10-DT2-DT1	0.88	0.97	0.78	0.43	0.48
IR 66946-3 R-178-1-1	1.07	1.17	1.01	0.81	0.21
Sarshar	1.01	0.82	0.97	0.61	0.30
IR 84675-7-3-2-B-B	0.93	1.36	0.48	0.56	0.33
IR 84675-25-7-3-B-B	0.96	0.66	0.55	0.66	0.34
IR 84675-58-4-1-B-B	1.21	1.22	1.21	0.71	0.68

Fig. 3. Effect of different salinity levels on shoot Chlorophyll content in rice genotypes



	Control (0)	40 mM	60 mM	80 mM	100 mM
HHZ5-SAL10-DT2-DT1	3.80	3.24	1.02	0.94	0.60
IR 66946-3 R-178-1-1	3.33	2.16	2.09	1.93	1.90
Sarshar	2.91	1.94	2.01	1.25	1.26
IR 84675-7-3-2-B-B	1.59	2.18	1.07	1.02	0.78
IR 84675-25-7-3-B-B	1.41	1.22	1.14	1.58	0.74
IR 84675-58-4-1-B-B	1.52	2.23	2.07	1.39	1.25

Fig. 4. Effect of different salinity levels on shoot Cartenolds content in rice genotypes

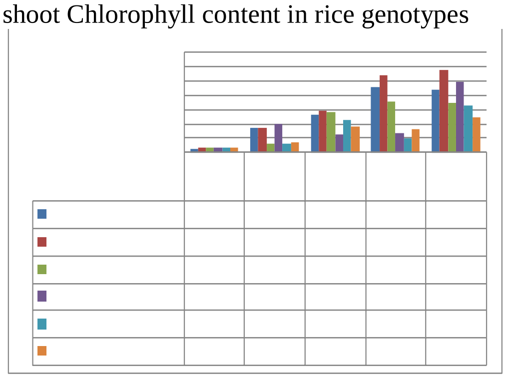
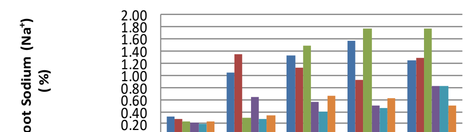
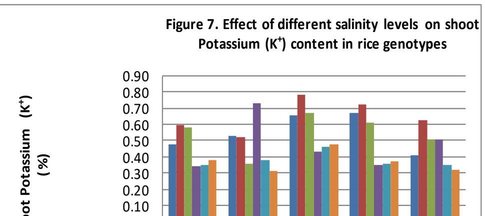


Fig. 5. Effect of different salinity levels on shoot Proline content in rice genotypes



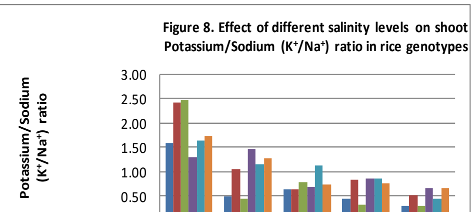
	Control (0)	40 mM	60 mM	80 mM	100 mM
HHZ5-SAL10-DT2-DT1	0.32	1.04	1.32	1.57	1.25
IR 66946-3 R-178-1-1	0.28	1.35	1.13	0.93	1.28
Sarshar	0.25	0.31	1.48	1.78	1.78
IR 84675-7-3-2-B-B	0.22	0.64	0.56	0.51	0.83
IR 84675-25-7-3-B-B	0.20	0.28	0.41	0.47	0.82
IR 84675-58-4-1-B-B	0.23	0.34	0.67	0.63	0.50

Fig. 6. Effect of different salinity levels on shoot Sodium (Na^+) content in rice genotypes



	Control (0)	40 mM	60 mM	80 mM	100 mM
HHZ5-SAL10-DT2-DT1	0.48	0.53	0.66	0.67	0.41
IR 66946-3 R-178-1-1	0.60	0.52	0.78	0.72	0.63
Sarshar	0.58	0.36	0.67	0.61	0.51
IR 84675-7-3-2-B-B	0.34	0.73	0.43	0.35	0.51
IR 84675-25-7-3-B-B	0.35	0.38	0.46	0.36	0.35
IR 84675-58-4-1-B-B	0.38	0.31	0.48	0.37	0.32

Fig. 7. Effect of different salinity levels on Potassium (K^+) content in rice genotypes



	Control (0)	40 mM	60 mM	80 mM	100 mM
HHZ5-SAL10-DT2-DT1	1.60	0.49	0.63	0.44	0.30
IR 66946-3 R-178-1-1	2.41	1.06	0.63	0.83	0.51
Sarshar	2.47	0.45	0.77	0.32	0.29
IR 84675-7-3-2-B-B	1.29	1.46	0.69	0.86	0.65
IR 84675-25-7-3-B-B	1.65	1.16	1.13	0.85	0.44
IR 84675-58-4-1-B-B	1.74	1.27	0.73	0.75	0.65

Fig. 8. Effect of different salinity levels on Potassium/Sodium (K^+/ Na^+) content in rice genotypes

Discussion

Physiological parameters play a vital role for the identification of salt tolerant behavior in crops. Selective accumulation of ions (organic and inorganic), is mainly responsible for the adjustment of osmotic potential in the plant cell.

Pigment contents

Our finding revealed that pigments contents chlorophyll (a, b, total chlorophyll) and carotenoids reduced due to salinity in all the rice genotypes by increasing salinity in growing medium. The reduction in chlorophyll contents is to be expected under stress; being membranous bound, its stability is dependent on membrane stability, which under saline condition seldom remains intact (Ashraf *et al*., 2005). The decrease in chlorophyll contents under saline conditions is reported by Iqbal *et al*. (2006); Ashraf *et al.* (2005). There is however some reports where an increase in chlorophyll contents was observed in genotypes of rice (Alamgir and Ali, 1999).

Solute accumulations

Solute accumulations play a vital role for osmotic adjustment of plant under stress conditions. There are two types of solute in plant (i.e. organic and inorganic). Among the organic solute proline is well documented, whereas among inorganic, sodium and potassium and their ratio i.e. (K^+/Na^+ ratio), are important.

According to Muhammad and Aslam (1998) higher K^+ and lower Na^+ in the leaf sap are the criteria for salt tolerance. In the present study there was an overall increase in Na^+ contents in all the rice genotypes. Comparatively higher Na^+ accumulation was observed in genotypes HHZ5-SAL10-DT2-DT1, IR 66946-3 R-178-1-1 and Sarshar. Similarly higher contents of potassium was observed at 80 mM salinity in growing medium resulting in high K^+/Na^+ ratio, while the under high salinity levels100 mM NaCl resulting in low K^+/Na^+ ratio. This showed the selective uptake of K^+ over Na^+ by these genotypes. According to Sharma (1986), the selective up take of K^+ over Na^+, active compartmentation of Na^+ and K^+ exclusion are the major processes involved in salt tolerance.

On the other hand the genotypes HHZ5-SAL10-DT2-DT1 and Sarshar which showed comparatively poor performance had also showed higher accumulation of Na^+ content at I00 mM NaCl salinity resulting in low K^+/Na^+ ratio. It is reported that with increasing soil salinity, the concentrations of Na^+

increased and that of K^+ decreased in leaf sap, which led to a decreased in the K^+/ Na^+ ratio (Rajpar *et al*., 2006). Promila *et al*. (2000) have the opinion that the re-establishment of membrane integrity may be indicated by development of K^+ uptake capacity. On the other hand, Li Qing Song *et al.* (2009) observed no significant correlation between transpiration rate and sodium accumulation in the shoots. The results regarding to proline accumulation showed that genotype IR 66946-3 R-178-1-1 had increased accumulation of proline in shoot (i.e. 6.429 μg/g Fresh wt.) indicating that one genotype was adjusting their osmotic potential through the accumulation both types of solute (organic and inorganic ions). On the other hand the genotype IR 84675-58-4-1-B-B had minimum proline accumulation (i.e. 2.784 μg/g fresh wt.). which indicates that this genotype might adjusting its osmotic potential through higher uptake of K^+, resulting in higher K^+/Na^+ ratio. Salinity index of proline showed strong positive relationship with salinity index of yield and is thus a promising index for deploying in breeding programmes for evolving salt tolerant rice reported by Pandey and Srivastava (1989); Summart *et al.* (2010).

Conclusion

As it is manifest from present data that genotypes, treatments and their interaction were highly significant for pigment contents, organic solute (proline) and inorganic solute (Na^+ and K^+) accumulation in plants. 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, 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 both types of solutes (organic and inorganic ions), and their better performance might be related to the selective uptake of K^+ over Na^+ and high accumulation of proline.

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