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Effect of Sodium Chloride Salinity on Seed Germination and Early Seedling Growth of Rice (*Oryza sativa* L.)

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Abstract: Nipponbare (typical japonica type), Gorishita and Pangri (indica type; cultivated in the southern districts of Bangladesh) were compared with emphasis on seed germination and early seedling growth characters. Seeds were placed for germination and the seedlings were allowed to grow for 7 days at 0, 0.01, 0.03, 0.06 and 0.1% NaCl concentrations in water culture and for 14 days at 0, 0.1, 0.3, 1.0 and 3.0% NaCl concentrations in soil culture. Salinities up to 0.3% delayed germination but did not reduce final germination percentage; it was reduced significantly at ≥1.0% NaCl. Rice cultivars at the stage of seed germination were more tolerant to salinity than at the early seedling stage. Lower levels of salinity were not much inhibitory to the length, fresh and dry weights, reduction in moisture content of root and shoot but under higher levels of salinity, almost all these parameters were significantly affected in all cultivars. Among the cultivars, Nipponbare performed well by producing highest root length, fresh and dry weights of root and shoot, total dry matter and with a relatively high root:shoot length, fresh and dry weight.

Key words: Germination index, NaCl salinity, Rice (*Oryza sativa* L.), Seedling growth characters

Introduction

Salinity is a growth reducing factor for most of the plant species. The major inhibitory effect of salinity on plant growth and development had been attributed to osmotic inhibition of water availability, toxic effects of salt ions responsible for salinization and nutritional imbalance caused by such ions but the specific effects of soil salinity largely differ depending upon the predominant ions, their concentrations and the physiological stage of growth at which the plants were subjected to salinity (Greenway and Munns, 1980). The sensitivity of various crops also varies at seedling emergence and further at various growth stages with respect to salinity. Early stages of plant growth are most critical as later development and crop production depends on effective germination and seedling emergence (Pearson and Bernstein, 1959). Salinity results in poor plant stand due to the decrease in the germination rate of seeds and seedling survival for most of the agricultural crops (Karim *et al*., 1992). Germination ability of seeds under saline conditions differ from one crop to another and even a significant variation is recorded amongst the different cultivars of the same crop (Asana and Kale, 1965; Kumar and Bharadwaj, 1981; Maliwal and Paliwal, 1967).

Rice is an important grain crop worldwide and is very sensitive to salinity since a NaCl concentration as low as 50 mM could be considered as lethal at the seedling stage (Yeo *et al*., 1990). Heenan *et al*. (1988) have already reported changes in salt resistance during rice development and showed that the young seedling and flowering stages are the most sensitive. It has also been reported that cultivars of the same species show remarkable differences in their salt tolerance (Maas and Hoffman, 1977). However, since there is much variability in salt susceptibility between varieties of cultivated glycophytes, another approach is possible by studying the salt responses of varieties differing in salt resistance. The cultivars Gorishita and Pangri used in this experiment are locally grown in the southern districts of Bangladesh. But to our knowledge, there is yet no research reports regarding their responses to salinity. The present study was thus conducted to investigate the potential for differences in salt tolerance of these two rice cultivars including Nipponbare with emphasis on seed germination and on early root and shoot growth which are important factors for seedling establishment.

Materials and Methods

Germination (Experiment 1): Seeds of three different cultivars of rice namely Nipponbare (japonica type) and Gorishita and Pangri (indica type) were surface sterilized by immersion for 5 minutes in sodium hypochlorite solution, then repeatedly washed with deionized water. Twenty seeds of uniform size in each treatment for each cultivar were allowed to germinate on a filter paper in 9cm diameter petridishes. Each filter paper was moistened with a water solution at five different NaCl concentrations, 0.1, 0.3, 1.0, 1.5 and 3.0% and with distilled water *as* the control. The treatments were replicated three times. The petridishes were placed in a growth chamber for 6 days at 28°C under a 16 h light/8 h dark photoperiodic conditions with a light intensity of 60 μmol m^{-2} s^{-1}. The number of seeds that sprouted and germinated was counted daily. Seeds that sprouted were able to produce at least one visible plumule or radicle, whereas seeds were considered to have germinated when shoot extended to more than 2 mm from the seeds. After final germination, the germination index (GI) was calculated by the following equation:

$$GI = \frac{\text{\% Germination in each treatment}}{\text{\% Germination in the control}} \times 100$$

Young seedling (Experiment 2): After surface sterilization as stated above, the seeds of each variety were allowed to imbibe in petridishes containing distilled water in the culture room at 24±2°C till the appearance of the white tip of the coleoptile. Then the seedlings were raised using the following two methods.

a) Water culture: After imbibition the seeds were sown on plastic nets placed above the surface of 300 ml NaCl solution of different concentrations (0.01, 0.03, 0.06 and 0.1%) and on distilled water (as control) contained in tall beakers (500 ml). Seedlings were grown in a culture room at 24±2°C and were illuminated with fluorescent lamps which provided 40/2 μmol m^{-2} s^{-1} at the plant level. Then the root and shoot lengths of 9 seedlings grown in each concentration were taken at 7 days after seeding (DAS).

b) Soil culture: After imbibition the seeds were planted in holed plastic pots filled with soil. Seedlings were grown in a phytotron at 25°C and they were supplied with excess amount of 1000-fold diluted Hyponex solution without or with NaCl of different concentrations (0.1, 0.3, 1.0 and 3.0%) almost everyday. Fourteen day-old seedlings in batches of 10 plants grown in each concentration were harvested carefully without giving any physical injuries and brought to the laboratory keeping inside the polythene bags to prevent the transpiration loss. Then the component parts representing the root and shoot were separated and weighed for fresh weights. The components were then oven dried for 48 hours at 80°C and their corresponding dry weights were determined by a sensitive balance. The moisture content was calculated as $(FW-DW)/FW \times 100$.

Data on the seedling characteristics for each NaCl treatment were compared with those for seedlings treated with 0% NaCl in each cultivar and were expressed as percentage values to the control. Statistical analyses were performed by Duncan's Multiple Range Test (DMRT). All the percentage data of seed germination were transformed to arc sine values before statistical analysis.

Results

Germination and Germination Index (GI): The mean values of germination and the germination index of rice cultivars at different levels of salinity are presented in Table 1. There was no germination at 3.0% level of salinity. NaCl treatment delayed seed germination. Cultivar differences were observed in relation to time required for germination under saline condition. In the control treatment germination started on 2nd day after the seeds were placed for germination in all cultivars. At 1.5% NaCt, Nipponbare took shorter time than the other two. Germination index decreased significantly at 1.0% NaCl treatment. Cultivar differences were not observed in this character.

Root and shoot length in water culture: In water culture, root length of all cultivars was remarkably suppressed over shoot length in all concentrations with an exception at 0.01% of NaCl. Root length was significantly increased at 0.01% of NaCl and thereafter decreased (Fig. 1). Significant decrease was observed at 0.03% NaCl and at higher levels in cultivar Gorishita and Pangri but the value was insignificant in Nipponbare at 0.03% of NaCl. Nipponbare produced the longest root length in all concentrations of NaCl treatment and it was more than double of the other two at 0.03, 0.06 and 0.1% NaCl treatments.

Increase in shoot length in all cultivars was observed at 0.01% NaCl and thereafter decreased (Fig. 2). Significant decrease was observed at 0.06% and at higher level of NaCl treatment. The shoot length of cv. Pangri increased significantly at 0.01% NaCl but that of the other two cultivars increased insignificantly. At 1.0% NaCl concentration, the root lengths were 9.0, 4.9 and 3.5% and the shoot lengths were 54.7, 54.1 and 52.7% of the control in Nipponbare, Gorishita and Pangri respectively.

Fresh weight of root and shoot in soil culture: In soil culture, the pattern of fresh weight of root and shoot were almost identical in nature. The fresh weight of root and shoot of Nipponbare and Pangri were declined significantly by $\geq$0.3% but that of Gorishita was by z0.1% NaCl (Fig. 3, 4). Increase in fresh weight of both root (112.3% of the control) and shoot (107.5% of the control) of Nipponbare were observed in 0.1% of NaCl treatment although not significant. As far as fresh weight of root and shoot was concerned, inhibition was the lowest in cv. Nipponbare followed by Gorishita with an exception at 0.1% of NaCl treatment where cv. Pangri was found superior to Gorishita although the result was not significant. At highest level of salinity, fresh weights of root were 20.7, 14.5 and 13.4% and that of shoot were 9.2, 9.6 and 7.2% of the control in Nipponbare, Gorishita and Pangri respectively.

Dry weight of root and shoot in soil culture: The pattern of dry weight of root (Fig. 5) almost resembles to that of the shoot (Fig. 6). There was an increase in dry weight of both root and shoot of Nipponbare at 0.1% of NaCl treatment although not significant and thereafter it decreased significantly. Dry weight of root and shoot of cultivars Gorishita and Pangri decreased with the increase in salinity levels. Significant decrease in root dry weight was observed at $\geq$0.1% and at $\geq$0.3% of NaCl in Gorishita and Pangri respectively but dry weight of shoot of both the cultivars decreased significantly at $\geq$0.3% of NaCl treatment. Nipponbare produced the highest dry weight of both root and shoot at all concentrations of NaCl treatment. At highest salinity level, root dry weights were 26.5, 18.5 and 18.6% and shoot dry weights were 15.2, 12.3 and 11.9% of the control in Nipponbare, Gorishita and Pangri respectively.

Total dry matter accumulation: Total dry matter accumulation was significantly suppressed by NaCl of 0.3% and by higher level in all the cultivars (Fig. 7). There was an increase in dry matter.production in Nipponbare at 0.1% of NaCl treatment although it was not statistically significant. Significant decrease was observed at 0.1% and 0.3% of NaCl in Gorishita and Pangri respectively. At highest level of salinity total dry weights were 17.2, 13.4 and 13.1% of the control in Nipponbare, Gorishita and Pangri respectively. At all concentrations of NaCl treatment, Nipponbare depicted the maximum dry weight.

Ratio analyses of root/shoot: The analyses of root:shoot growth under salinity are shown in Fig. 8-10. The root:shoot length at 0.01% NaCl increased significantly over control values in all cultivars and thereafter decreased (Fig. 8). Among the cultivars, Nipponbare depicted the highest root:shoot length at all concentrations of NaCl treatment. At 0.03% NaCl, root:shoot length of cv. Nipponbare decreased but it was statistically non-significant. Both the root:shoot fresh weight (Fig. 9) and dry weight (Fig. 10) of all cultivars increased significantly at 1.0% NaCl. However, they were occasionally decreased at 0.1 and 0.3% NaCl treatment. There was an increase in root:shoot fresh weight in Nipponbare and in Pangri and dry weight in Gorishita, but they were statistically non-significant. At highest level of salinity, Nipponbare depicted the maximum root:shoot fresh weight and dry weight over the control values.

Moisture content: At 1.0% NaCl, significant decrease in shoot moisture content was observed in all cultivars but that in root was observed in Nipponbare and Gorishita (Fig. 11, 12). There was an increase in water content of both root (101.1% of the control) and shoot (100.1% of the control) in Nipponbare only at 0.1% NaCl although not significant. Root moisture content was always higher in Nippponbare than the other two cultivars but shoot moisture content was lower in Nipponbare than that of the other two at 0.3 and 1.0% of NaCl treatment.

Table 1: Germination percentage and germination index of rice cultivars at different levels of salinity

Cultivars	NaCl%				
	0	0.1	0.3	1.0	1.5
	G	G1	G1	G1	G1
Nipponbare	100	98.3^{ns}	96.7^{ns}	73.3**	18.3**
Gorishita	96.7	100^{ns}	98.3^{ns}	67.3**	17.2**
Pangri	98.3	98.3^{ns}	98.3^{ns}	69.4**	15.3**

G: Germination percentage; GI, Germination index;
** Significant at the 1% level;
ns: not significant at the 5% level

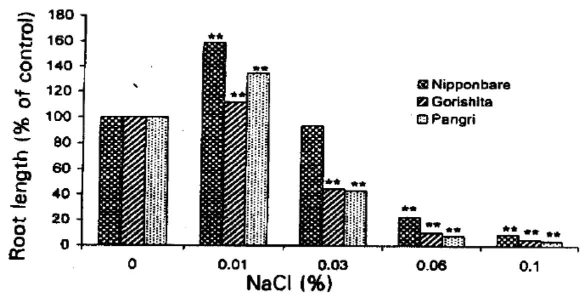

Fig. 1: Salinity effect on relative root length of rice in water culture (Nipponbare: 100% = 4.65±0.13 cm, Gorishita: 100% = 12.09±0.27 cm and Pangri: 100% = 9.46±0.37 cm). * *Indicate significant at the 1% level

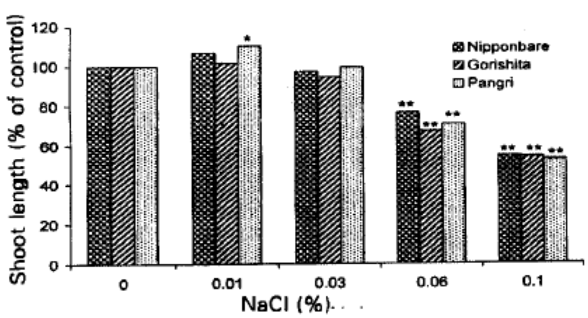

Fig. 2: Salinity effect on relative shoot length of rice in water culture (Nipponbare: 100% = 3.84±0.08 cm, Gorishita: 100% = 7.93±0.08 cm and Pangri: 100% = 6.57±0.16 cm). * and **indicate significant at the 5 and 1% level respectively

Discussion

The result agrees with the work of Mondal *et al*. (1988) and Karim *et al*. (1992) that salinity delays germination process. In our experiment, rice at the stage of seed germination was found more tolerant to salinity than at the early seedling stage. The higher tolerance of rice at germination compared to the early seedling stage is similar to that of other crops such as barley, wheat and triticale (Ayers, 1953; Donovan and Day, 1969; Karim *et al*., 1992; Maas and Hoffman, 1977). Rhoades (1990) has also reported that some plants are generally relatively tolerant during germination, but become sensitive during emergence and early seedling stage.

In water culture, the length of root and also of shoot was increased at 0.01% of NaCl, but the magnitude was higher in root. This might be due to low osmotic potential and/or low

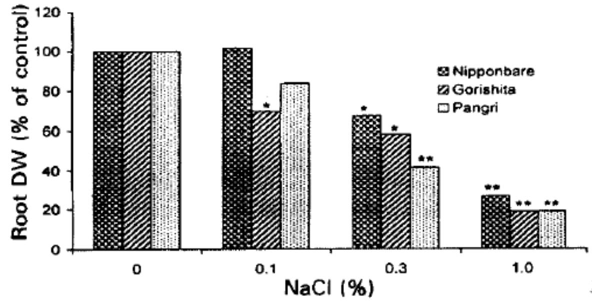

Fig. 3: Salinity effect on relative fresh weight of root in soil culture (Nipponbare: 100% = 114.7±13.7 mg, Gorishita: 100% = 158.4±12.0 mg and Pangri: 100% = 149.0±19.9 mg). * and **Indicate significant at the 5 and 1% level respectively

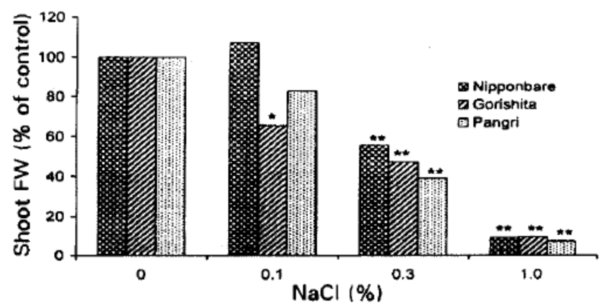

Fig. 4: Salinity effect on relative fresh weight of root in soil culture (Nipponbare: 100% = 356.9±26.3 mg, Gorishita: 100% =524.8±38.5 mg and Pangri: 100% = 481.1 ±48.8 mg). *and **Indicate significant at the 5 and 1% level respectively

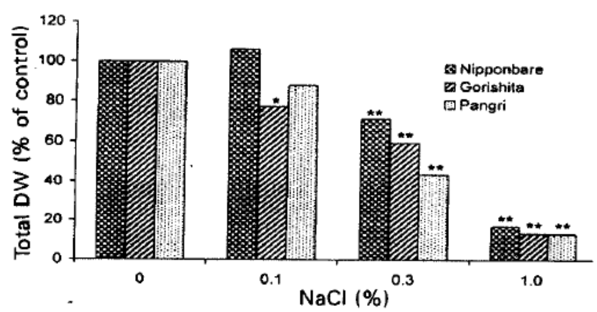

Fig. 5: Salinity effect on relative dry weight of root in soil culture (Nipponbare: 100% = 11.7 ±1.2 mg, Gorishita: 100% = 14.6±1.8 mg and Pangri: 100% = 15.6±2.1 mg). * and **indicate significant at the 5 and 1% level respectively

ionic concentrations of the control (Munns *et al*., 1995). Salama *et al*. (1994) have also reported the stimulated growth in wheat under low concentrations of NaCl (25 mM). In our experiment, root growth was relatively more sensitive to salt than the shoot in water culture. This is in confirmatory with the findings of Asana and Kale (1965) and Ansari *et al*. (1980) who reported that NaCl and $CaCl_2$ salts are more inhibitory to root growth compared to that of shoot growth of wheat plants.

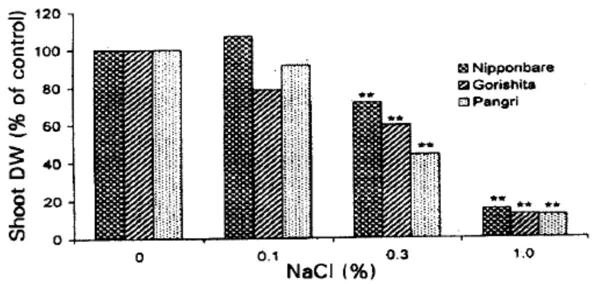

Fig. 6: Salinity effect on relative dry weight of shoot in soil culture (Nipponbare: 100% = 56.4 ±3.9 mg, Gorishita: 100% = 68.9±8.3 mg and Pangri: 100% = 71.5±7.2 mg). **indicate significant at the 1% level

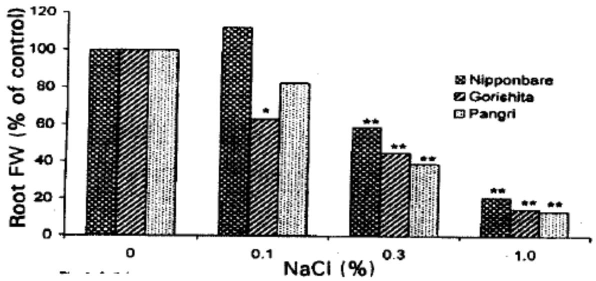

Fig. 7: Salinity effect on relative total dry weight of rice in soil culture (Nipponbare: 100% = 68.2±5.0 mg, Gorishita: 100% = 83.5 ± 10.1 mg and Pangri: 100% = 87.1±9.1 mg). * and **indicate significant at the 5 and 1% level respectively

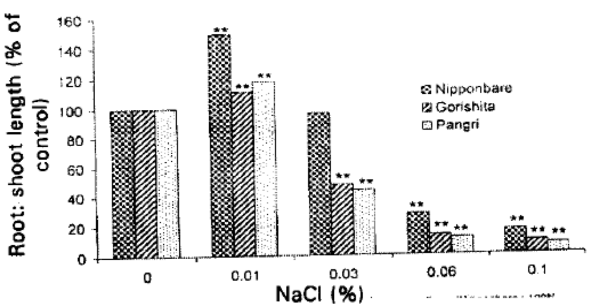

Fig. 8: Salinity effect on relative root:shoot length of rice in water culture (Nipponbare: 100% = 1.21 ±0.02, Gorishita: 100% =1 .52 ±0.03 and Pangri: 100% = 1.44 ±0.04). **indicate significant at the 1% level

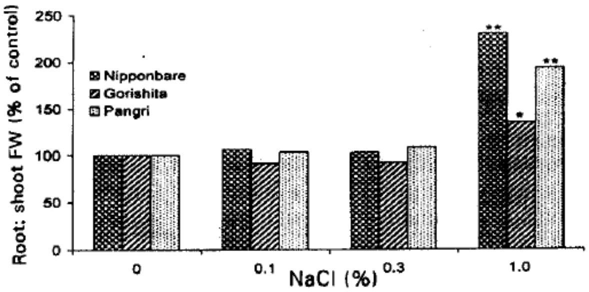

Fig. 9: Salinity effect on relative root:shoot fresh weight of rice in soil culture (Nipponbare: 100% = 0.31 ± 0.02, Gorishita: 100% =0.31 ±0.01 and Pangri: 100% = 0.30±0.02). *and** indicate significant at the 5 and 1% level respectively

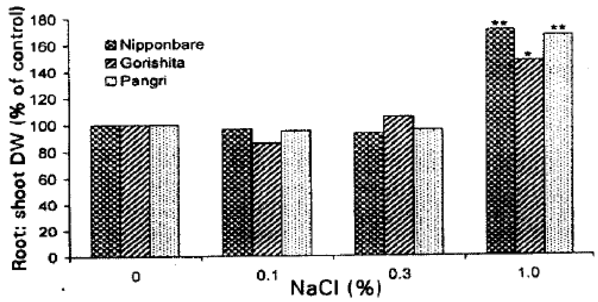

Fig. 10: Salinity effect on relative root:shoot dry weight of rice in soil culture (Nipponbare: 100% = 0.21±0.01, Gorishita: 100% = 0.21±0.01 and Pangri: 100% = 0.21±0.01). *and** indicate significant at the 5 and 1% level respectively

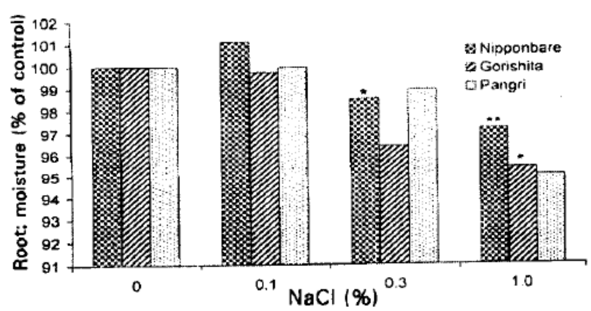

Fig. 11: Salinity effect on relative moisutre content in root in soil culture (Nipponbare: 100% = 89.6±0.4, Gorishita: 100% = 89.9±0.5 and Pangri: 100% = 89.4±0.4). *and** indicate significant at the 5 and 1% level respectively

In soil culture, the suppressing effect of NaCl on shoot fresh weight was more pronounced than on root fresh weight. Moreover, fresh weight of shoot and root was more affected by salt than dry weight of shoot and root indicating a salt derived effect on the water content of the shoot and root tissue rather than on the synthesis of shoot and root biomass (Lechno *et al.*, 1997). In our experiment, the enhancements in the root and shoot growth as well as total biomass accumulation at lower levels of salinity are in agreement with the reports reviewed by Munns and Termaat (1986).

In our experiment, the root:shoot length in water culture was increased at 0.01% NaCl over control values in all cultivars and thereafter decreased. Among the cultivars, Nipponbare depicted the highest root:shoot length at all concentrations of NaCl treatment. The initial increase in root:shoot length might be due to low osmotic potential and low ionic concentrations of the control (Munns *et al.*, 1995). Munns and Termaat (1986) reported that at low salinity levels root growth may not decrease at all; it can even increase resulting in an increase in root:shoot length. The gradual decrease in root:shoot length with the increase in salinity as observed in our experiment might be due to more inhibitory effect of NaCl salt to root

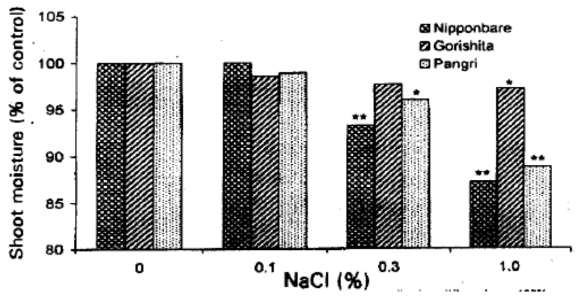

Fig. 12: Salinity oeffect on relative moisture content in shoot in soil culture (Nipponbare: 100% = 84.2±0.2, Gorishita: 100% =85.4±0.5 and Pangri: 100% = 85.1±0.3). *and ** indicate significant at the 5 and 1% level, respectively

growth compared to that of shoot growth (Ansari *et al.*, 1980; Asana and Kale, 1965). In our experiment, the increase in root:shoot fresh and dry weights with the increase in salinity is in good agreement with the earlier reports (Bernstein, 1975; Nejad and Najafi, 1990; Munns and Termaat, 1986; Nieman *et al.*, 1988). Higher root/shoot ratio may be favourable but root growth in saline media may be at the expense of shoot growth resulting in a larger relative to control reduction in shoot growth.

In conclusion, the results in this study reveal that rice at the stage of seed germination is more tolerant to salinity than at the early seedling stage. The results further suggest that lower level of salinity (up to 0.03% in water culture and 0.1% in soil culture) was not much inhibitory to plant height, root length, fresh and dry weights of root and shoot, reduction in moisture content but under higher level of salinity almost all the above mentioned parameters were significantly affected by NaCl treatment in all cultivars. However, among them Nipponbare performed well by producing highest root length, fresh and dry weights of root and shoot resulting the highest dry matter and with a relatively high root:shoot length, fresh and dry weight. It was our assumption that Gorishita and Pangri might be tolerant to salinity as they were grown in the southern coastal areas of Bangladesh. But our findings indicate that Nipponbare is more resistant to NaCl salinity at initial growth stages. The better performance of Nipponbare than the other two might be linked to the absorption of higher quantity of water (specially in root), thus diluting the toxicity of ions and allowing rapid germination followed by a higher biomass production. Salt tolerance at germination and early seedling stage may not correspond with that at advanced growth stages (Maas and Hoffman, 1977). Therefore, their further detailed physiological and biochemical analyses including studies of the advanced stages of development are required to ascertain these facts.

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