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RESEARCH ARTICLE

Assessment of seedling salinity tolerance of Bangladeshi coastal rice landraces using morpho-physiological stress indices

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ABSTRACT

Salt stress has detrimental effects on the growth and development of rice seedlings. In the present study we assessed salt-responsiveness of three coastal rice landraces namely Nona-morchi, Kalihytta and Nara. We conducted two factors pot trial that included five rice genotypes (three candidates coastal rice landraces, Binadhan-10 as tolerant check and BRRI dhan48 as sensitive check) against three salinity levels e.g., control (tap water), 6 and 12 dSm^{-1} of sodium chloride. Study revealed that, at 12 dSm^{-1} of salinity, Binadhan-10 showed the lowest reduction in shoot length (14.62%), shoot fresh weight (30.04%) and shoot dry weight (33.71%) which was followed by Nona-morchi, Kalihytta, Nara and BRRI dhan48. Two stress tolerance indices e.g., relative water content (RWC) and electrolyte leakage (EL), also support salt-induced responses of these five rice genotypes. At the highest level of salinity (12 dSm^{-1}), the lowest RWC reduction was observed in Nona-morchi (8.20%) which is followed by Binadhan-10 (11.38%), Kalihytta (38.93%), BRRI dhan48 (36.30%) and Nara (36.51%). And, the highest EL increased in Nara (40.06%) which is followed by BRRI dhan48 (22.06%), Kalihytta (21.12%), Binadhan-10 (8.64%) and Nona-morchi (5.87%) compared with their respective controls. Importantly, photosynthetic pigments profile (chlorophyll *a*, chlorophyll *b*, total chlorophyll) were increased in Nona-morchi (24.48, 15.22 and 21.87%, respectively) and Binadhan-10 (13.75, 12.50 and 13.41%, respectively) and reduced in Kalihytta (7.89, 19.42 and 11.08%, respectively), Nara (27.76, 31.32 and 28.76%, respectively) and BRRI dhan48 (32.73, 36.97 and 34.02%, respectively) at 12 dSm^{-1} salinity compared with their respective controls. It is to noteworthy that at 6 dSm^{-1} of salt stress, shoot length significantly decreased in the sensitive check BRRI dhan48 by 23.07%, Kalihytta by 17.32% and Nara by 11.54%. While, no significant effect of 6 dSm^{-1} of salt stress was observed in Binadhan-10 and Nona-morchi in case of shoot length, root length, shoot fresh and dry weight, EL and RWC. Among the three coastal rice landraces, Nona-morchi found as highly tolerant and Kalihytta were found as moderately tolerant while Nara was identified as sensitive against salt stress (12 dSm^{-1}). In the future, these identified salt tolerant rice genotypes might be the ideal resource for breeding new salt tolerant rice varieties.

Keywords: Photosynthetic pigments; Relative water content; Rice; Salt stress

INTRODUCTION

More than fifty percent (50%) of global population consumes rice as main food which is grown throughout the world (Lou et al., 2012). It is the primary source of nutrition for Bangladeshi people, and rice agriculture accounts for the lion's share of all cultivated areas, with 34.71 million metric tonnes (MT) of overall production. Among all crops planted in Bangladesh, rice is the highest-producing food crop (BBS 2016). Although rice is a crop with a high yield, its current average yield for *indicia* rice is only 10 tha^{-1}, which is 10 to 15% less than its potential yield (IRRI 1998; Virmani et al., 1991). The optimal growth and yield of rice are frequently impeded by both biotic and abiotic stresses including high salinity, drought, heat, and cold. These stressors negatively impact the phenotypic growth, yield and pose a serious threat to global food safety (Mantri et al., 2012; Pareek et al., 2009). Among the environmental stresses, soil salinity is a critical stressor that significantly impedes global crop cultivation (Munns 2011). The main causes of salinity in arable land are flooding from seawater, poor irrigation water quality with an excess of salts, and excessive irrigation water use with poor drainage (Ismail et al., 2007). Different growth stages of rice have different

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responses to salt stress. According to Lutts et al. (1995), rice is the most susceptible to salt stress during the seedling and early vegetative stages (Somaddar et al., 2022), and thereafter during the reproductive stages (Sultana et al. 2022; Islam et al. 2020; Singh et al. 2009; Ismail et al. 2007). Studies have revealed that rice plants normally degenerate at 10 dSm^{-1} during the seedling stage (Munns et al., 2006), and that becomes worsen even at the reproductive stage. A relatively low level of salt stress can result in up to 90% yield loss at 3.5 dSm^{-1} (Rahman and Ahsan, 2001; Asch *et al.*, 2000). Salinity tolerance is a composite trait, and plant reactions to salinity is heavily influenced by environmental factors (Flowers, 2004; Gregorio and Senadhira, 1993). Rice plant responses to salt stress are complex and dependent on various factors, including the intensity of salt stress, the type of salt stress, stage of rice development, and the duration of salinity exposure (Cramer et al., 2001; Bernardo et al. 2000). Salt stress affects rice growth, causes metabolic changes, and reduces the plant's ability to absorb water and nutrients (Munns et al., 2002). Moreover, a significant reduction in rice grain yield was also caused by the poorly developed rice spikelet, particularly the inferior spikelet caused by salt stress (Zhang et al. 2015; Fu et al. 2011). It is very important to classify the tolerance and sensitivity level of rice genotypes during the early stage of seedling growth for effective crop yield in salt affected environment. Therefore, the purpose of this study was to examine the salt tolerance of coastal rice landraces at their seedling stages.

MATERIALS AND METHODS

Plant materials and experimental design

Three potential coastal rice landraces namely, Nonamorchi, Kalihytta, and Nara were collected from the Patuakhali Science and Technology University (PSTU) rice germplasm. A two factor experiment was laid out to conduct the experiment following completely randomized design (CRD) with three replications. The experiment was carried out at the field net house of the Department of Agronomy in PSTU. Briefly, the seeds were germinated in a lab setting on moist tissue paper, and on the third day, germinated seedlings were planted in perforated pots containing 3 kg of soggy rice field soil following the procedure as described by Gregorio et al (1997). For preparing growing medium, pot soil was supplemented with 50 mg urea, 25 mg TSP, and 25 mg MoP Kg^{-1} of soil as per BARC recommendation guide 2012. Four to five pre-germinated rice seeds were placed on wet soil surface of each pot. After two days of sowing, poorly settled seedlings were removed and finally three seedlings of rice were retained per pot for treatment execution. The perforated metallic pots were put in a water bath (50cm×30cm×20cm) made of stainless steel to maintain a constant water level for the growing seedlings. For soil salinization treatments, we used three (03) salinity levels of e.g., <1 dSm^{-1} (tap water as control), and 6 dSm^{-1} and 12 dSm^{-1} using NaCl solution with three independent (Three individual pot) and three biological replications (Three plants per each pot). Salinization treatments were applied to the 14-days-old rice seedlings in the water bath for three weeks.

Seedling biomass

After 21 days of salinity exposure, rice plants were kept in non-saline conditions for 7 days for recovery from salt stress. Subsequently, the plant samples (42-days old seedlings) were collected to assess comparative biomass of the salt-induced rice seedlings. All plants in each pot were harvested, and the roots and shoots were separated. Roots were gently washed with tap water for a few minutes and wiped with tissue paper and fresh weight was measured. Fresh shoots were wiped with wet tissue paper to remove the dust and weighted immediately in to record the fresh weight. Both root and shoot samples were dried in an oven at 70 °C for 72 hours and the dry weight was measured.

Photosynthetic pigment determination

Leaf chlorophyll content was analyzed following the method of Arnon (1949) and Lichtenthaler and Wellburn (1983). The freshly rice leaves (0.2 g) was homogenized in 2 mL of 80% acetone, and then centrifuged at 11,500 rpm for 12 minutes to separate the supernatant into a different tube. Subsequently, the absorbance of the supernatant was measured at 663 nm, 645 nm and 470 nm using spectrophotometer (T 60 UV-Visible Spectrophotometer, India) where 80% acetone solution was used as blank.

Determination of relative water content (% RWC)

The percent relative water content (RWC) was measured following the method as described by Weatherley (1950). Briefly, the fresh leaf samples were weighted (FW) and poured into a tube containing 20 ml distilled water. The tube was then shaken in a shaker for 6 hours at 30 °C. Following the determination of turgid weight (TW), samples were dried at 70 °C for 72 hours before determining the dry weight (DW). RWC was calculated following the following formula:

$$\frac{FW - DW}{TW - DW} \times 100$$

Where fresh weight is denoted by FW, turgid weight by TW, and dry weight by DW.

Electrolyte leakage (EL %) Determination

EL was calculated following the method described by Lutts et al. (1996) using an electrical conductivity meter (HI8733, HANNA, USA). In brief, 100 mg sample of leaf tissue was taken from the second youngest leaf of each plant after

15 days of saline treatment. Following that, the samples were washed three times with deionized water, cut into 10 mm/01 cm lengths, placed in 20 ml deionized water, and shaken at 30 °C for 6 hours. Electrical conductivity (EC1) of the sample was measured after incubation. The tubes were then placed in boiling water for thirty minutes at 100ºC, and the electrical conductivity was recorded as EC2. EL was calculated as:

$$\frac{EC\,1}{EC\,2} \times 100\,\%$$

Statistical analysis

Data from three replications were collected as means ± standard deviation (SD) and statistically analyzed using analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) test at 0.001, 0.01 and 0.05 probability level was employed to check the statistical significances of the means using statistical package JMP 16 pro from SAS Institute Inc.

RESULTS

Effect of salinity on growth parameters of rice seedlings

In the present study, we investigated several growth parameters that showed significant difference under different salinity levels except root fresh and dry weight. It was evident that plant growth has significantly affected by the progression of salinity level (6 and 12 dSm^{-1}) after 14 and 21 days after seedling (Fig. 1A, B). Shoot and root lengths significantly reduced respectively by 14.62 and 49.39% in Binadhan-10 (tolerant check), 27.20 and

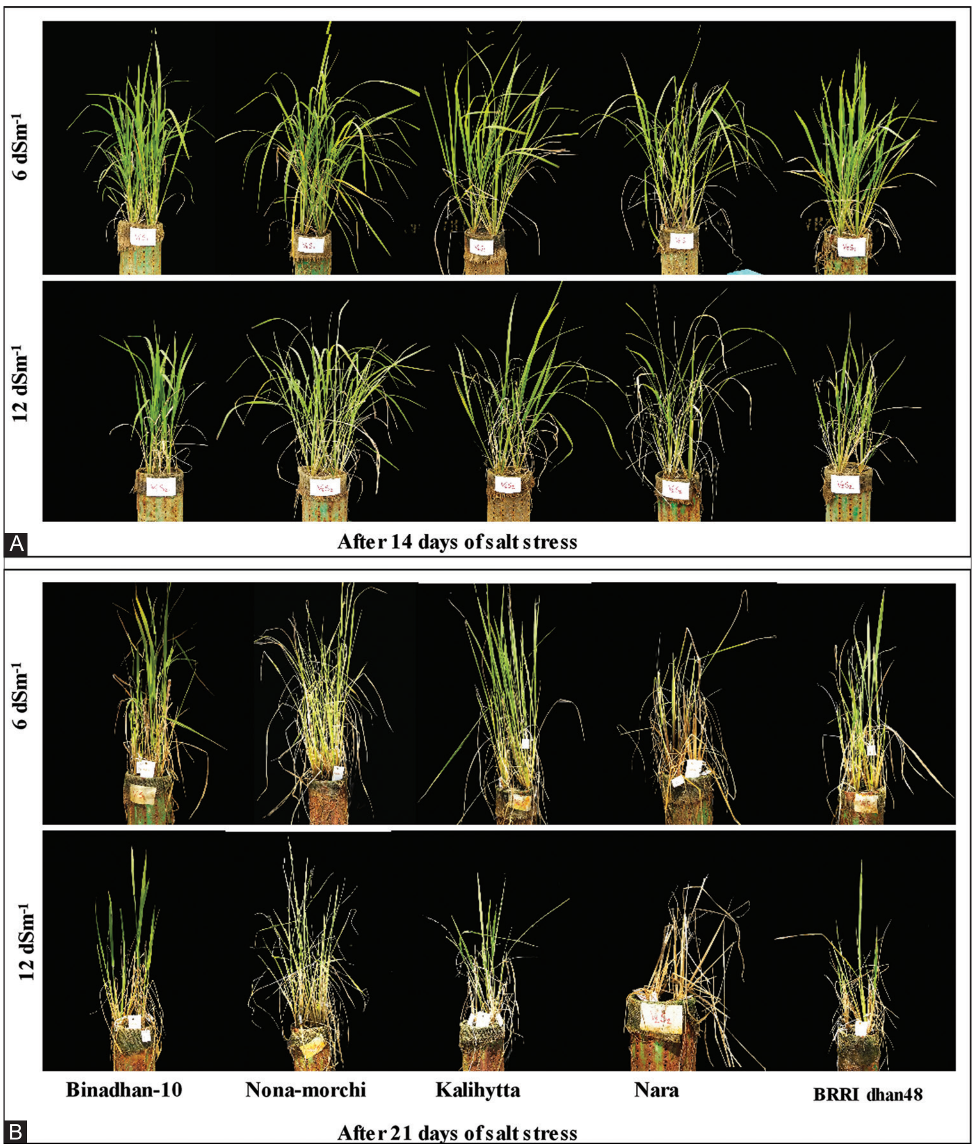

Fig 1. Effect of salinity stress on different rice cultivars. (A) Comparative phenotype of different rice seedlings after 14 days and (B) 21 days of salt stress at 6 and 12 dSm-1.

30.52% in Nona-morchi, 33.39 and 41.23% in Kalihytta, 59.69 and 35.35% in Nara and, 52.53 and 18.79% in BRRI dhan48 (sensitive check) in response to 12 dSm^{-1} salt stress compared with control (Fig. 2A, B). Besides, we observed the maximum shoot length reduction (59.69%) occurred in Nara and root length reduction (49.39%) occurred in Binadhan-10 under 12 dSm^{-1} of salt stress. On the contrary, the lowest shoot and root length (27.20 and 20.27%, respectively) were noticed in Nona-morchi and BRRI dhan48 compared with their respective controls. Interestingly, in response to 6 dSm^{-1} salt stress, Binadhan-10, Nona-morchi, Kalihytta, and Nara exhibited no significant difference; however, BRRI dhan48 showed a substantial decrease in shoot length (23.07%). While recording the root length, Binadhan-10 and Kalihytta dramatically reduced their root length by 23.58 and 26.32%, respectively as compared with non-saline control conditons (Fig. 2A and B).

In terms of shoot fresh and dry weight, a significant reduction observed with the increasing level of salt stress. Fig. 2C and 2D indicate that, 12 dSm^{-1} salt stress caused a minimum decline in shoot fresh and dry weight of the tolerant check Binadhan-10 (30 and 34%, respectively) which is followed by Nona-morchi (42 and 44%, respectively). However, the highest reduction observed in the sensitive check BRRI dhan48 (78 and 80%, respectively) followed by Nara (73 and 82%, respectively) and Kalihytta (54 and 53%, respectively).

While considering the impact of 6 dSm^{-1} salt stress, Binadhan-10 and Nona-morchi showed statistically equivalent results in shoot fresh and dry weight. In contrast, Kalihytta, Nara, and BRRI dhan48 showed a significant reduction when compared to their respective controls. Moreover, considering the salinity effect, all the rice cultivars showed significant decline in root fresh and

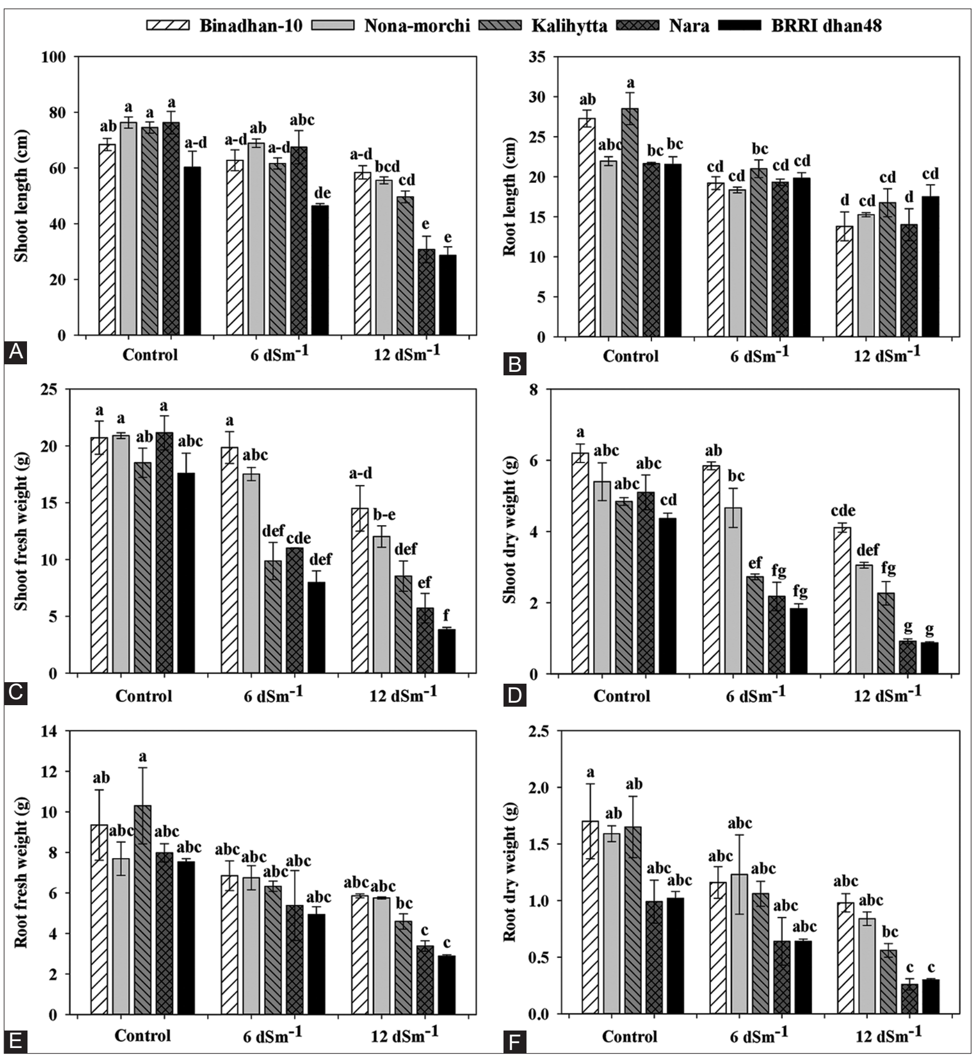

Fig 2. Effect of salinity on (A) Shoot length, (B) Root length, (C) Shoot fresh weight (D) Shoot dry weight, (E) Root fresh weight and (F) Root dry weight. Color codes above the chart defines different rice cultivars. Different lettering above the bars indicates significant difference among the rice cultivars under different levels of salinity following Tukey's HSD test.

dry weight, but no significant differences were observed in terms of interaction effect between salinity treatment and rice cultivars at both 6 and 12 dSm^{-1} levels of salinity (Table 1, Fig. 2E and F).

Effect of salinity on relative water content (RWC) and electrolyte leakage (EL)

As saline levels rose, RWC showed a noticeable reduction, as illustrated in Fig. 3A. Among all the rice cultivars, Nona-morchi showed the lowest reduction in RWC (15%) followed by the tolerant check Binadhan-10 (19%) at the maximum level of salinity (12 dSm^{-1}). Contrastingly, the highest RWC reduction was observed in Nara by 60.45%, BRRI dhan48 by 50.85% and Kalihytta by 48.73% at 12 dSm^{-1} of salt stress compared with their respective controls (Fig. 3A). However, at 6 dSm^{-1} of salinity, Binadhan-10 and Nona-morchi exhibited a statistically identical results over control whereas, a substantial reduction in RWC was recorded from Kalihytta by 10.52%, Nara by 28.49% and BRRI dhan48 by 34% compared with the respective controls. In addition, we observed a significant increment in EL with the enhancement of salt concentration (Fig. 3B). It is evident that, Binadhan-10 and Nona-morchi had statistically similar EL (less than 35%) with controls at 12 dSm^{-1} salinity. In contrast, the highest enhancement of EL was observed in Nara (250%), which is followed by Kalihytta (61.88%) and BRRI dhan48 (75.63%) as compared with their respective controls. Furthermore, at 6 dSm^{-1} of salt stress, we observed Binadhan-10, Nona-morchi and BRRI dhan48 sustained with more stable EL compared with control plants; while, a gradual increment of EL was observed in Kalihytta by 24.43%, and in Nara by 89.48% over their control plants (Fig. 3B).

Effect of salinity on chlorophyll status (chlorophyll *a*, *b* and total chlorophyll)

The result demonstrated that, chlorophyll status influenced by salt stress (6 and 12 dSm^{-1}) in rice seedlings (Fig. 4). It is obvious that, at 12 dSm^{-1} of salt stress, chlorophyll *a,* chlorophyll *b* and total chlorophyll slightly increased in Nona-morchi (24, 15 and 22%, respectively) and Binadhan-10 (14, 12 and 13%, respectively). In contrast, the sensitive check BRRI dhan48 depicted a significant drop in chlorophyll *a*, chlorophyll *b* and total chlorophyll (33, 37 and 34%, respectively), which is followed by Nara (28, 31 and 29%, respectively) and Kalihytta (8, 19 and 11%, respectively). On the other hand, no significant differences were observed in case of chlorophyll *a,* chlorophyll *b* and total chlorophyll at 6 dSm^{-1} of salinity level (Fig. 4A-C).

Principle component analysis (PCA) among rice cultivars

Principle component analysis (PCA) was performed to find out the response of different rice cultivars against different salinity level (control, 6 dSm^{-1} and 12 dSm^{-1}). The first two principal components (PCs) stated 61.1% (PC 1) and 21.1% (PC 2) of the observed variability across all rice genotypes (Fig. 5). The rice cultivars that occupy the upper right corner are regarded as tolerant cultivars, those that occupy the lower left corner as sensitive cultivars, and those that occupy both the upper left and lower right corner as moderately tolerant cultivars. Due to their combined ability to explain more than 80% of the

Table 1: Two-way ANOVA for morpho-physiological characteristics of rice seedlings under salt stress

Variables	*p* values										
	SL	RL	SFW	SDW	RFW	RDW	RWC	EL	Chl *a*	Chl *b*	T. Chl
Cultivar	0.001**	0.011**	0.001**	0.001**	0.043*	0.002**	0.004**	0.001**	0.001**	0.001**	0.001**
Treatment	0.001**	0.001**	0.001**	0.001**	0.001**	0.001**	0.001**	0.001**	0.054*	0.001**	0.002**
Cultivar×Treatment	0.003**	0.035*	0.015*	0.047**	0.580 ns	0.982 ns	0.007**	0.001**	0.001**	0.007**	0.001**

SL, shoot length; RL, root length; SFW, shoot fresh weight; SDW, shoot dry weight; RFW, root fresh weight; RDW, root dry weight; RWC, relative water content; EL, electrolyte leakage; Chl *a*, chlorophyll *a*, Chl *b*, chlorophyll *b*, T. Chl, total chlorophyll. ***p*<0.01, **p*<0.05 and ns=not significant.

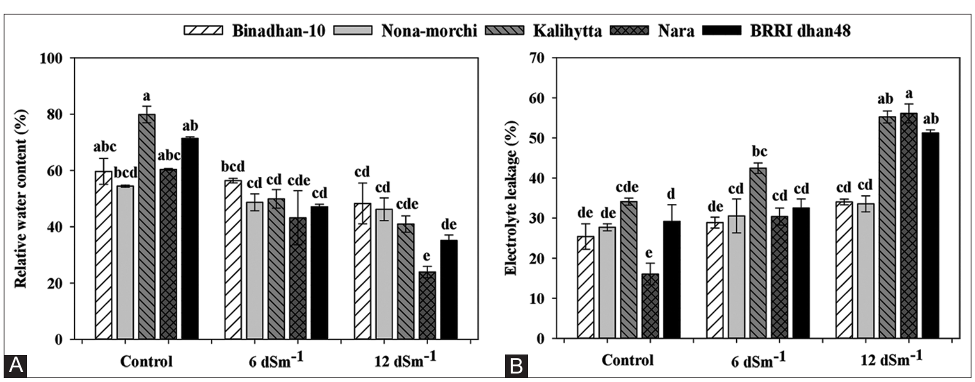

Fig 3. Effect of salinity on (A) Relative water content and (B) Electrolyte leakage. Color codes above the chart defines different rice cultivars. Different lettering above the bars indicates significant difference among the rice cultivars under different levels of salinity following Tukey's HSD test.

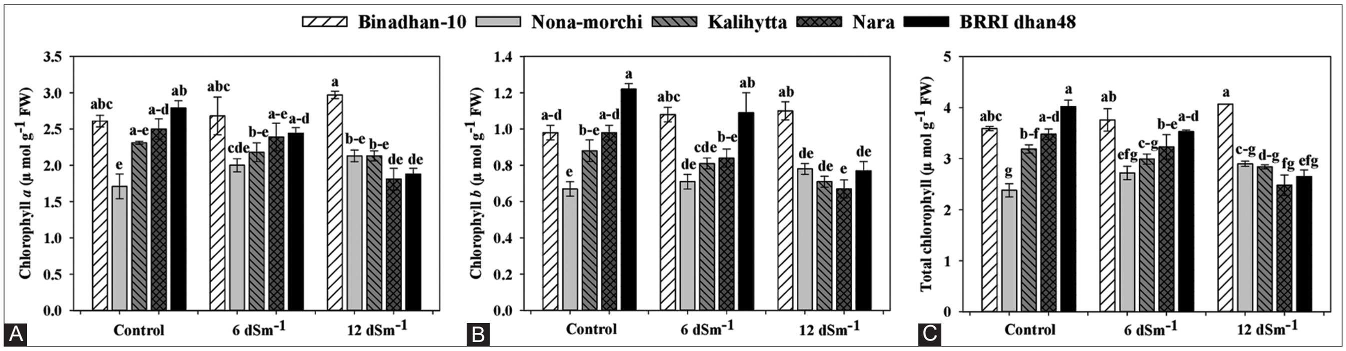

Fig 4. Effect of salinity on (A) Chlorophyll *a* (B) Chlorophyll *b* and (C) Total chlorophyll. Color codes above the chart defines different rice cultivars. Different lettering above the bars indicates significant difference among the rice cultivars under different levels of salinity following Tukey's HSD test.

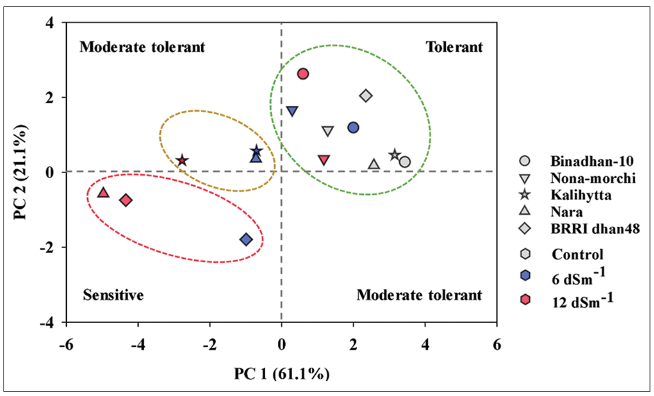

Fig 5. Principle component analysis (PCA). PCA for the first two principal component (PC) scores, PC 1 vs. PC 2, describing the classification salt response to different rice cultivars.

variation and their greater significance in the categorization of genotypes, PC1 and PC2 were the two PCs that highly contributed to this study. The result showed that, Binadhan-10 and Nona-morchi were tolerant under both salinity levels (6 and 12 dSm^{-1}) whereas, BRRI dhan48 and Nara are found most sensitive cultivars against the highest level of salinity (12 dSm^{-1}). However, Kalihytta showed moderate tolerance against both 6 and 12 dSm^{-1} salinity level and, Nara showed moderate tolerance against 6 dSm^{-1} salt stress (Fig. 5).

Correlation analysis of morpho-physiological traits

The correlation analysis was used to determine the relationships between various growth and physiological traits of different rice seedlings under salt stress (Fig. 6). The results showed that all the growth attributes and relative water content (RWC) showed a strong positive correlation ($P<0.001$) with each other while total chlorophyll (T. Chl) had positive contribution with shoot fresh and dry weight (SFW and SDW), and RWC ($P<0.05$). Contrastingly, electrolyte leakage (EL) had a significant negative relationship with almost all of the growth and physiological traits ($p< 0.01$ and $p< 0.001$), indicating the damage caused by salt stress during growth and development of different rice seedlings.

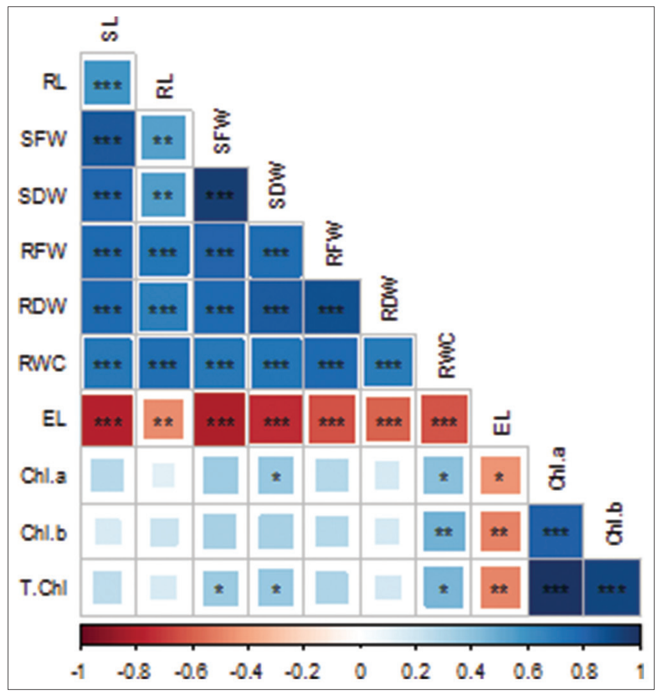

Fig 6. Pearson correlation matrix among the growth and physiological traits. SL, shoot length; RL, root length; SFW, shoot fresh weight; SDW, shoot dry weight; RFW, root fresh weight; RDW, root dry weight; EL, electrolyte leakage; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; T. Chl, total chlorophyll. Positive and negative correlations between the traits are shown in a heatmap at the bottom.

DISCUSSION

Salt stress is a critical factor in plants that has a negative impact on plant growth and physiology. Several parameters were developed in this study to evaluate salinity stress resistance in five different rice cultivars. Distinct biological processes have been thought to contribute to growth; and the literature has extensively discussed how salt treatment negatively impacted plant growth (Munns 2002). All rice cultivars showed a decreasing trend in shoot length and root length while they imposed by 6 to 12 dSm^{-1} salt stress. Among them, Nona-morchi was more stable in shoot length which is comparable to the tolerant cultivar

Binadhan-10, and Kalihytta was moderately stable. While Nara and the sensitive cultivar BRRI dhan48 showed more sensitivity under the maximum level of salinity (12 dSm^{-1}). Interestingly, in contrast with the shoot length dynamics under salt stress, there was no significant difference in root length was observed in all cultivars in response to 6 and 12 dsm^{-1} salinity. Our results indicated that the cultivars which showed sustained shoot length under salinity might have different physiological or metabolic processes to uptake water and nutrient compared with the sensitive cultivars. Kakar et al. (2019) observed a significant reduction in shoot length and root length under salt stress (6-12 dSm^{-1}). Besides, Safitri et al. (2018) conducted an experiment to test seedling salinity tolerance of 14 rice genotypes where they found the root length of all the rice genotypes reduced from 19 to 46%. Notably, they reported similar root length reduction indices among the tolerant and sensitive groups, which is consistent with our present findings (Fig. 2B). A wealth of studies also revealed that salinity adversely affected growth and development of rice seedlings in case of plant height, root length and biomass (Hussain et al., 2018; Barua et al. 2015; Ali et al. 2014). This effect was more pronounced in salt-sensitive cultivar than salt-tolerant. Besides, based on the percent reduction in total dry weight, Binadhan-10 and Komol Bhog were rated as highly salt tolerant (Tahjib-Ul-Arif et al., 2018). The result of the experiment showed that, root-shoot biomass and dry matter significantly reduced under moderate to high salinity (6 dSm^{-1} and 12 dSm^{1}); whereas, Binadhan-10 and Nona-morchi remained more stable similar as control plants; while, BRRI dhan48 and Nara had a greater drop in case of root-shoot biomass and dry mass. Similarly, Hariadi et al. (2015) reported that, rice seedlings under salt stress experienced a noticeable decline in growth. As salinity increased, rice plants' tolerance to salt stress declined, and Inpari-13, a salt-tolerant rice variety, demonstrated a lower rate of shoot length reduction than salt-sensitive rice (Sakina et al. 2016). Similar to our results, Senadhera et al. (2012) found that 50 mM NaCl stress significantly decreased seedling fresh and dry mass in the salt-sensitive cultivar IR29. In our study, the lowest reduction in RWC was observed in Nona-morchi and the tolerant check Binadhan-10. And, the highest reduction was observed in the rest two cultivars including the sensitive check. In addition, the lowest EL was noticed in Binadhan-10 and Nona-morchi (less than 10%) while the highest was found in Nara, and the rest two cultivar showed moderate EL. According to the results, the salt sensitivity (moderate to high) in rice seedlings started at 20% to 40% EL or higher (Fig. 3B). Besides, we also observed that the RWC, an important stress index, decreased significantly with high salinity. In our recently published study, we also observed rice cultivars experienced significant reductions in RWC and EL under salt stress, (Somaddar et al., 2022). In another study, Pokkali, a salt-tolerant cultivar, showed approximately 6.6% RWC reduction at 100 mm salt-stress when compared with corresponding control (Polash et al., 2018). Previous research agreed with our findings that, rice plants under salt stress had higher EL rates than their respective control plants (Hoang et al., 2014). Furthermore, Chlorophyllase activity have been reported to increase in response to salt stress, which accelerated the breakdown of chlorophyll and decreased the amount of chlorophyll in plants (Yang et al., 2011). In our present study, tolerant cultivar Binadhan-10 had the stable and highest chlorophyll *a*, chlorophyll *b*, and total chlorophyll. Likewise, Nona-morchi and Kalihytta did not show any significant reduction in chlorophyll pigment profiles under salinity stress. However, there was a greater reduction in chlorophyll content in case of Nara and the sensitive check BRRI dhan48 (Fig. 4). The similar trends of chlorophyll pigment profiles have been found in many studies where, the tolerant genotype generally showed more stability as compared with sensitive checks (Ma et al., 2018; Somaddar et al., 2022).

CONCLUSIONS

The results of the present study indicate that, salinity stress (6 and 12 dSm^{-1}) negatively affected the growth and physiological attributes three coastal rice landraces. Based on the overall results, Nona-morchi to be the best highly salt tolerant local coastal rice genotype thatshowed salt tolerance as similar as the tolerant check Binadhan-10. Besides, Kalihytta showed moderate tolerance against salt stress. And, Nara, another local rice genotype, was identified as highly sensitive among all the rice cultivars used in this study.

Author contribution

GS, UKS and MRU conceptualized and designed the research. US, SKM, HCD, and AB conducted the experiment and collected data. US performed statistical analysis, visualized the data and drafted the original manuscript. GS, UKS, MRU and US critically edited the manuscript for the final submission.

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