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Research Article

Differential Responses of Rice (*Oryza sativa L.*) Cultivars to NaCl in Relation to Physiological and Biochemical Parameters at Seedling Stage

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Abstract

Rice is one of the most important staple food crops in world and relatively susceptible to salinity. Effects of NaCl were studied on six rice cultivars viz. Ratnagiri-24, Ratnagiri-711, Ratnagiri-4, Palghar-2, Karjat-2 and Karjat-184 under control (0 mM) and increasing levels of salinity (50 mM, 100 mM, 150 mM and 200 mM) at seedling level. Differential responses of rice cultivars were observed in relation to salinity levels. As the level of salinity increases, an apparent reduction in germination %, shoot length, root length, root/shoot ratio, seed vigor index and photosynthetic pigments in all the cultivars were revealed. Of all the cultivars, these parameters were less affected in Ratnagiri-24 and Ratnagiri-711. The levels of proline were high under salt stress (200 mM), however, it remained exceptionally higher in Karjat-2 followed by Palghar-2 as compared to rest of the cultivars. Proline exhibited highly significant correlations with most of the physiological parameters ($p < 0.05$). Based on these parameters, rice cultivars can be classified into three groups. Group I includes Ratnagiri-24 and Ratnagiri-711 as highly salt tolerant while group II consist of Ratnagiri-711, Palghar-2 and Karjat-2 as moderately salt sensitive. Group III comprises highly salt sensitive cultivar Karjat-184.

Keywords: Proline; Rice; Root Length; Salinity; Shoot Length

Abbreviations

Chl: Chlorophyll; Cm: Centimeter; DAS: Day After Sowing; S.E.: Standard Error; SVI: Seed Vigor Index

Introduction

Rice (*Oryza sativa L.*) is one of the most important staple foods in the world, and Asia alone accommodates approximately two billion rice consuming populace [1]. Several rice-growing countries, both in tropics and in temperate regions, are facing high soil salinity as a major problem [2] and rice is relatively susceptible to it [3]. Salinity alters a wide array of metabolic processes in rice plants and induces changes in constituents and activities of several enzymes [4]. The detrimental effects of salinity such as decrease in plant's water uptake, specific ion toxicities and induction of oxidative damage to plant cells is catalyzed by reactive oxygen species3 results into reduction in growth and yield of rice plants [5].

Salt-tolerant species have many defense mechanisms to cope with stress [6]. In rice, salinity tolerance is predominantly associated with the maintenance of ion homeostasis, particularly low Na^+/K^+ ratio, through exclusion, compartmentation, and partitioning of Na^+ [7]. In addition to ion homeostasis strategies, a variety of protective mechanisms have evolved in plants to allow them to acclimatize to salinity conditions for survival and growth by accumulating amino acids, sugars, polyols, betaines and proline [4,8]. It is well known that proline protects plants from stress through different processes, including the adjustment of cellular water, detoxification of ROS, protection of membrane integrity, and stabilization of enzymes and proteins, thus, it can be beneficial to plants in adapting to stress [9-11].

Increased salt tolerance of crops including rice is needed to sustain food production in various regions of the world [12]. As salinity problems are increasing around the world, great effort has been devoted to understand the physiological aspects of tolerance to salinity in plants, as a basis for plant breeders to develop salinity-tolerant genotypes. In spite of this great effort, only a small number of cultivars, partially tolerant to salinity have been developed [13]. Therefore, screening of cultivars using salt tolerance indices may help to assist in the evaluation of relative field performance of different rice genotypes and characterization of contributing physiological traits that may be employed as reliable indicators for breeding and selection for salt tolerance. Germination and seedling development are very important for early establishment of plants under stress condition and therefore selecting cultivars for rapid and uniform germination under saline conditions can contribute towards early seedling establishment [14]. Therefore, the objective of this study was to generate information on the variability of the physiological and biochemical parameters that can be used to classify rice cultivars for salinity tolerance.

Materials and Methods

Plant material and NaCl stress treatment

Six cultivars of rice viz. Ratnagiri-24, Ratnagiri-711, Ratnagiri-4, Palghar-2, Karjat-2 and Karjat-184 were procured from Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli, India. The seeds were soaked in water for 24 hours and allowed to germinate in petriplates (Borosil, India) lined with germination papers. Seeds were treated with 10 mL NaCl (50 mM, 100 mM, 150 mM and 200 mM) and without NaCl (0 mM – control – 10 mL distilled water).

The plates were incubated at 25°C for two days in dark and after initiation of rooting, the plates were transferred in light. The experiment was performed in triplicate and each replicate contained 20 seeds.

Physiological parameters

Germination percentage was calculated after two days at radicle emergence while the observations on root length (cm) and shoot length (cm) of seedlings were recorded from 10 seedlings on 15th day after sowing (DAS). Seed Vigor Index (SVI) was calculated as SVI = (germination percentage × means of total seedling length in cm)/100.

Chlorophyll determination

Chlorophyll concentration (Chl-a, Chl-b and Total Chl) was determined according to Arnon [15]. The leaves (0.1g) were crushed in 10 ml 80% Acetone and filtered through muslin cloth. The samples were centrifuged at 5000 rpm for 15 minutes at 4°C (Bioera, High speed refrigerated programmable centrifuge, India). The absorbance of extract recorded at 663 and 645 nm on UV-VIS spectrophotometer (Systronics PC based double beam spectrophotometer 2202, India).

Proline determination

The proline was determined according to Bates L [16]. The whole seedling (0.5g) was ground and extracted with 10 mL of 3% sulfosalicylic acid. The extract was filtered through a Whatman filter paper (No. 2). The reaction mixture contains 2 mL filtrate, 2 mL acid ninhydrin and 2 mL glacial acetic acid. The reaction mixture was heated in boiling water bath for 60 minutes. The reaction was terminated by placing the tubes on ice and the chromophore was extracted using 4 mL of toluene. The absorbance of the chromophore phase was read at 520 nm using a spectrophotometer (Systronics PC based double beam spectrophotometer 2202, India). Reference standards of proline (100 to 1000 $\mu g\ mL^{-1}$) were prepared in 2N sulfuric acid. The amount of proline is expressed as $\mu moles\ g^{-1}$ fresh weight.

Statistical analysis

The data were subjected to analysis of variance (ANOVA), Duncan's Multiple Range Test (DMRT), Pearson Correlation analysis and Hierarchical clustering analysis using Minitab 17. The values were expressed as mean ± standard error (S.E.) of three replications. Differences were considered significant at $p < 0.001$, $p < 0.01$ and $p < 0.05$ level of probability. Graphical representations were performed in Microsoft office excel 2007.

Results

Effect of NaCl stress on germination and physiological parameters

Differential responses of rice cultivars were noticed in relation to physiological parameters at seedling stage. As the concentration of NaCl increased (50 - 200 mM), an apparent significant decrease in seed germination was noticed in all the cultivars (Table 1). Likewise, significant reduction in shoot length (cm) was observed with increasing NaCl concentration in all the cultivars. On the contrary, root lengths (cm) of Ratnagiri-24, Ratnagiri-4 and Karjat-2 were increased at 50 mM and then decreased at higher levels of salinity. Compared to other varieties, rice cultivars Ratnagiri-24 and Ratnagiri-711 exhibited lesser reduction in root length, shoot length and seed vigor index along with decreased root/shoot ratio at high salinity levels (Table 1).

Cultivar	NaCl (mM)	Germination (%)	SL (cm)	RL (cm)	R/S ratio	SVI
Ratnagiri-24	0 (Control)	100.00 ± 0.0a	5.25 ± 0.1a	5.19 ± 0.2ab	0.99 ± 0.1c	10.28 ± 0.2a
	50	96.67 ± 1.7a	4.59 ± 0.2a	5.95 ± 0.3ab	1.29 ± 0.1bc	10.05 ± 0.2a
	100	95.00 ± 0.0ab	3.43 ± 0.2b	6.06 ± 0.1a	1.77 ± 0.1ab	8.77 ± 0.1b
	150	93.33 ± 1.7ab	2.48 ± 0.4bc	4.30 ± 0.7bc	1.73 ± 0.0ab	5.39 ± 0.0c
	200	91.67 ± 5.8b	1.60 ± 0.1c	3.12 ± 0.2c	1.95 ± 0.2a	4.40 ± 0.1d
Ratnagiri-711	0 (Control)	98.33 ± 1.7a	4.30 ± 0.1a	6.00 ± 0.2a	1.29 ± 0.0bc	10.31 ± 0.2a
	50	98.33 ± 1.7a	3.92 ± 0.1a	5.57 ± 0.8a	1.42 ± 0.0ab	8.96 ± 0.3ab
	100	96.67 ± 3.3ab	3.21 ± 0.3b	5.68 ± 0.3a	1.77 ± 0.0ab	9.00 ± 0.7ab
	150	91.67 ±1.7ab	2.86 ± 0.2b	5.01 ± 0.2a	1.75 ± 0.1abc	7.34 ± 0.2b
	200	90.00 ± 1.7a	1.33 ± 0.0c	2.81 ± 0.2b	2.11 ± 0.1a	3.85 ± 0.1c
Ratnagiri-4	0 (Control)	98.33 ± 1.7a	6.15 ± 0.2a	8.37 ± 0.9ab	1.36 ± 0.1b	15.40 ± 1.3a
	50	95.00 ± 2.9a	5.61 ± 0.1a	9.01 ± 0.8a	1.61 ± 0.1ab	13.31 ± 1.1ab
	100	91.67 ± 1.7ab	3.82 ± 0.5b	5.92 ± 0.3bc	1.55 ± 0.2ab	9.85 ± 0.9bc
	150	90.00 ± 5.8ab	2.77 ± 0.2b	5.37 ± 0.4cd	1.94 ± 0.1ab	8.09 ± 1.0cd

	200	83.33 ± 6.0b	1.34 ± 0.2c	2.92 ± 0.3d	2.18 ± 0.3a	4.11 ± 0.9d
Palghar-2	0 (Control)	98.33 ± 1.7a	5.68 ± 0.2a	6.47 ± 0.3a	1.14 ± 0.1a	11.95 ± 0.4a
	50	96.67 ± 1.7a	4.56 ± 0.2ab	5.98 ± 0.1a	1.31 ± 0.0a	10.18 ± 0.2a
	100	91.67 ± 4.4ab	4.28 ± 0.2b	6.67 ± 0.0a	1.56 ± 0.1a	10.06 ± 0.7a
	150	88.33 ±1.7ab	2.21 ± 0.4c	4.03 ± 0.3b	1.82 ± 0.4a	5.50 ± 0.5b
	200	76.67 ± 6.0b	0.77 ± 0.1d	1.94 ± 0.3c	2.53 ± 0.6a	2.08 ± 0.3c
Karjat-2	0 (Control)	93.33 ± 1.7a	5.86 ± 0.1a	7.11 ± 0.1ab	1.21 ± 0.0b	12.11 ± 0.3a
	50	93.33 ± 1.7a	4.04 ± 0.2b	7.35 ± 0.3a	1.82 ± 0.0b	10.61 ± 0.8a
	100	91.67 ± 3.3a	2.51 ± 0.2c	5.40 ± 0.2bc	2.15 ± 0.0b	7.25 ± 0.2b
	150	88.33 ± 6.0a	1.98 ± 0.1cd	3.84 ± 0.3cd	1.94 ± 0.1b	5.09 ± 1.0c
	200	71.67 ± 3.3b	0.85 ± 0.2d	2.72 ± 02d	3.21 ± 0.3a	3.08 ± 0.5c
Karjat-184	0 (Control)	96.67 ± 1.7a	6.14 ± 0.2a	7.88 ± 0.1a	1.28 ± 0.0b	13.56 ± 0.3a
	50	95.00 ± 2.9ab	3.79 ±0.3b	6.92 ± 0.3a	1.82 ± 0.2ab	10.16 ± 0.1b
	100	93.33 ± 1.7ab	2.69 ± 0.6b	7.87 ± 0.8a	2.93 ± 0.7a	9.83 ± 0.6b
	150	93.33 ± 1.7ab	2.72 ± 0.3b	5.92 ± 0.4a	2.18 ± 0.2ab	8.09 ± 1.1b
	200	86.67 ± 3.3b	0.60 ± 0.2c	2.03 ± 0.4b	3.38 ± 0.3a	2.41 ± 0.5c

Table 1: Effects of NaCl stress on physiological parameters in rice cultivars.

The values are means of three replicates ± SE. Different letters show significant difference at $P < 0.05$ by Duncan's Multiple Range Test. SL: Shoot Length; RL: Root Length; R/S ratio: Root/shoot ratio and SVI: Seed Vigor Index.

Ratnagiri-24 and Ratnagiri-711 displayed lesser reduction in shoot length (3.65 fold decrease) and root length (2.07 fold decrease) as compared to Ratnagiri-04 (4.81 and 5.45 fold decrease), Palghar-2 (4.91 and 4.52 fold decrease), Karjat-2 (5.02 and 4.32) and Karjat-184 (5.54 and 5.85 fold decrease) (Table 1). Similarly, rice cultivars Ratnagiri-24 and Ratnagiri-711 exhibited lesser root/shoot ratio and seed vigor index at higher salinity levels as compared to other rice cultivars (Table 1).

Chlorophyll content

The contents of Chl-a, Chl-b and total Chl (mg^{-1} g fresh weight) in leaves were significantly decreased with increasing NaCl concentrations in all the cultivars (Figure 1). The rate of decrease at high levels of salinity (200 mM) was more in Ratnagiri-4, Karjat-2 and Karjat-184 compared to other cultivars (Figure 1). Moreover, amount of Chl-a (Figure 1a) was found to be more damaging as compared to Chl-b (Figure 1b). Percent reduction in Chl-a (26.20) (Figure 1a) was more in Ratnagiri-04 while Chl-b (Figure 1b) was found to be more damaging in Karjat-2 (31.23% reduction) at higher level of salinity (200 mM) when compared with rest of the cultivars. Similarly, the reduction in total Chl was more in Ratnagiri-04 (32.34%) at higher levels of salinity as compared to rest of the cultivars (Figure 1c).

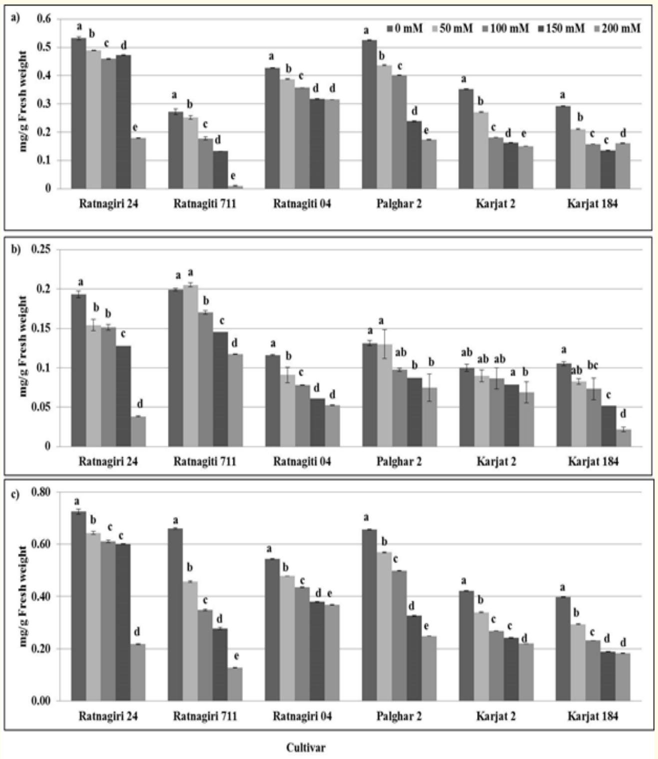

Figure 1: Effects of NaCl on physiological parameters in rice varieties.

a) Chlorophyll a, b) Chlorophyll b and c) Total Chlorophylls. Bars represent standard error from three replications. Means followed by the same letter(s) are not significantly different at $P = 0.05$ according to Duncan test. Note the scale difference.

Proline content

Increment of salinity levels generally results in a reduction of seedling growth and physiological parameters with concomitant increase in osmolytes. Proline contents were substantially different in rice cultivars. Initially, in control, the levels of proline were at par in Ratnagiri-24 and Ratnagiri-711. While it was low in Karjat-184, comparably, Palghar-2 exhibited high proline content followed by Karjat-2 (Figure 2). As the levels of NaCl elevated, significant increase in leaf proline (µmoles-1gm Fresh weight) was noticed in all the cultivars. A slight increment in proline accumulation was noted in Ratnagiri-24 (4 fold) and Ratnagiri-711 (2 fold) at 200 mM NaCl over control (Figure 2). Remarkably it was 5.5, 7.0 and 7.5 folds higher in Palghar-2, Ratnagiri-4 and Karjat-2 than that observed for the control (Figure 2). Notably, Karjat-184 exhibited a dramatic increase in proline accumulation (16 folds) while it was marginally fluctuated in Ratnagiri-24 and Ratnagiri-711.

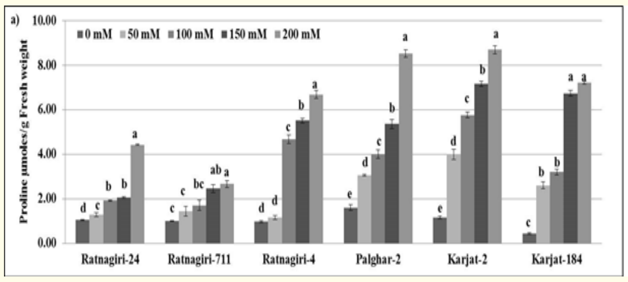

Figure 2: Effects of NaCl on proline concentration in rice varieties.

a) Proline. Bars represent standard error from three replications. Means followed by the same letter(s) are not significantly different at P = 0.05 according to Duncan test. Note the scale difference.

Pearson's correlations

Pearson Correlation coefficient analysis revealed very high significant correlations among most of the physiological parameters and osmolyte concentrations (Table 2).

Parameters	**Parameters**								
	G	SL	RL	RL/SL	SVI	Chl-a	Chl-b	TChl	Pro-line
SL	0.98^{***}	1.0							
RL	0.98^{***}	0.97^{***}	1.0						
RL/SL	-0.97^{***}	-0.94^{ns}	-0.92^{ns}	1.0					
SVI	0.98^{***}	0.99^{**}	0.99^{**}	-0.92^{ns}	1.0				
Chl-a	0.98^{***}	1.0^{*}	0.98^{***}	-0.94^{ns}	0.99^{**}	1.0			
Chl-b	0.99^{***}	0.99^{**}	0.99^{**}	-0.94^{ns}	0.99^{**}	0.99^{**}	1.0		
TChl	$0.98s^{***}$	0.99^{**}	0.98^{***}	-0.94^{ns}	0.99^{**}	1.0^{*}	1.0^{*}	1.0	
Proline	-0.96^{***}	-0.99^{**}	-0.97^{***}	0.91^{ns}	-0.99^{**}	-0.99^{**}	-0.99^{**}	-0.99^{**}	1.0
Glybet	-0.82^{ns}	-0.92^{ns}	-0.84^{ns}	0.80^{ns}	-0.88^{ns}	-0.91^{ns}	-0.89^{ns}	-0.90^{ns}	0.93^{ns}
GB/Pro	0.93^{ns}	0.98^{***}	0.93^{ns}	-0.90^{ns}	0.96^{***}	0.98^{***}	0.97^{***}	0.97^{***}	-0.99^{**}

Table 2: Pearson's correlation coefficients among physiological parameters from six rice cultivars exposed to 50 to 200 mM NaCl.

Note: Correlations were determined by Pearson Correlation coefficient (r) analysis. Pearson Correlation coefficient (r) indicates the strength of a relationship between two variables. *, ** and *** indicate significance at $p < 0.001$, $p < 0.01$ and $p < 0.05$ respectively whereas N.S.: not significant at 0.05 level of probability. Where, G: Germination; SL: Shoot Length; RL: Root Length; R/S: Root to Shoot Ratio; SVI: Seed Vigor Index; Chl a: Chlorophyll a, Chl-b: Chlorophyll-b, TChl: Total Chlorophylls.

Hierarchical cluster analysis

Hierarchical cluster analysis (HCA) based on physiological parameters and osmolytes was conducted to classify cultivars for their salinity tolerance (Figure 3). The six rice cultivars were classified into three main clusters. Cluster I represented the salt-tolerant group Ratnagiri-24 and Ratnagiri-711. Ratnagiri-4, Palghar-2 and Karjat-2 were classified into Cluster II as moderately sensitive while Cluster III denoted the highly sensitive group, includes Karjat-184.

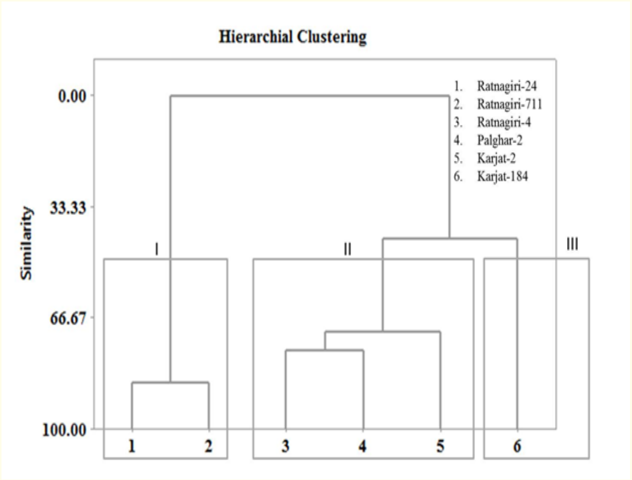

Figure 3: Cluster analysis of six rice cultivars based on physiological parameters.

Cluster analysis of the six rice cultivars based on physiological parameters in salt stress condition by Hierarchical cluster analysis of the Minitab 17 package.

Discussion

Increasing soil salinity is vulnerable to crops and therefore screening of cultivars for salt tolerance at seedling stage may be employed as reliable indicators for breeding and selection for salt tolerance. Detrimental effects of salinity on various physiological processes of crops such as seed germination, seedling growth and vigor, vegetative growth, flowering and fruit set causes reduced economic yield and quality of produce [17]. Reduction in growth parameters such as germination percentage, root length, shoot length, root shoot ratio at increasing salinity levels (Table 1) were consistent with previous researchers [18,19] who reported that physiological parameters were inversely related to level of salt concentration at the seedling stage in rice. This is mainly due to due to the fact that Na^+ and Cl^- sequestered in the vacuole could decrease the internal osmotic potential and cause partial dehydration of cytoplasm. As a result, dehydration impairs the cellular metabolism and ultimately reduced the growth of the seedlings [20]. Therefore, understanding the responses of plants at these stages is particularly important for elucidating the mechanisms of salt resistance or sensitivity in plants and their survival [18].

Photosynthetic pigments in salt-stressed seedlings were significantly reduced, in all the cultivars which are in agreement with previous results [19,21] who also reported significant reduction in chlorophyll contents at increasing levels of salinity. The photosynthetic pigments in Ratnagiri-24 and Ratnagiri-711 seedlings grown under salt stress were found to be stabilized better than those in Ratnagiri-4, Palghar-2, Karjat-2 and Karjat-184 (Figure 1). The results on variability in stability of photosynthetic pigments under stress conditions in different cultivars (Figure 1) are consistent with [6] who demonstrated that salt tolerant genotypes maintained better photosynthetic pigments stability as compared to salt sensitive genotypes under salt stress. Therefore, chlorophyll concentration can be used as a sensitive indicator of the cellular metabolic state; thus, its decrease signifies toxicity in tissues due to the accumulation of ions [6].

High levels of proline may display a salt defensive response mechanism in terms of pigment stabilization, protecting photosynthetic machinery of the plant organelles by stabilizing ultrastructure of the chloroplast [12]. Proline concentration has been shown to be generally higher in stress-tolerant plants (Ratnagiri-24 and Ratnagiri-711) than in stress-sensitive plants when under stress [6]. Conversely, proline in Ratnagiri-4, Palghar-2, Karjat-2 and Ratnagiri-184 (Figure 2) may be too low to prevent plant cells, organelles and tissues from being damaged by prolonged exposure to salt-stress conditions from reactive oxygen species. Based on the findings, it is interesting to note that increased levels of proline in these cultivars did not correspond to the extent of improved salinity resistance (Figure 2). Excessive levels of proline in salt sensitive cultivars (Figure 2a, b) may be a response of leaf damage or may be a symptom of stress is in agreement with the results presented by [22] who demonstrated that over-accumulation of proline was related to a symptom of salt injury rather than an indicator of salt resistance. Increased proline accumulation did not contribute to improved salt tolerance in rice were consistent with [8] who reported that under salt stress the highly susceptible rice cultivars accumulated the highest level of proline than the tolerant cultivars. Proline might be most effective in alleviating degradation of photosynthetic pigments probably because the high ratio of osmolytes function in protecting macromolecules and stabilizing protein structures [23].

Proline showed significant negative correlation with all the physiological parameters except with the ratio of root length to shoot length. In a number of studies a positive correlation between the proline with stress tolerance has been recorded [4,24]. These results indicate that proline help plant cells against the ravages of salt stress by stabilizing the structure of key proteins such as Rubisco and thereby promoting the photosynthetic capacity during salt stress [11,25]. Several reports indicated that enhanced accumulation of Proline in cytosol is positively correlated with higher tolerance to salt stress [24,26,27]. Recently, Hasanuzzaman., *et al.* [28] showed that the exogenous application of Proline resulted into increased tolerance to salt-induced oxidative damage in salt sensitive and salt tolerant varieties by upregulating their antioxidant defense.

The multivariate cluster analysis for salt tolerance has been utilized to classify the group of salt-tolerant rice (Figure 3) [6,29]. The results suggest that cluster analysis is a useful tool for classification of rice cultivars into different salt tolerance groups based on physiological and biochemical parameters in response to salinity stress.

Rice cultivars placed in Cluster I were however, more tolerant to salinity in terms of relatively low damage of physiological traits. Clusters II and III, indicated moderately salt tolerant and sensitive groups. These results suggest that cluster analysis and multivariate analysis are useful tools for classification of rice cultivars into different salt tolerance groups based on their changes in physiological and biochemical characteristics in response to salinity stress.

Conclusions

In summary, the results presented above indicated that increasing levels of salt stress are toxic to germinating seeds and hampers the growth parameters of rice cultivars. Accumulation of compatible solute such as proline may help to alleviate the detrimental effects of NaCl. Understanding of variability and correlation between salinity profiles at seedling stage will be an important step for salinity tolerance genotype in Indian rice cultivars. Salt tolerant cultivars can be further explored in field studies and the manipulation of specific candidate gene using molecular techniques may be a powerful tool for enhanced salinity tolerance in rice cultivars.

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Conflict of Interest

Authors declare that they have no conflict of interest.

Author's Contribution

All the authors contributed equally to this manuscript.

Bibliography

1. Rao AN., *et al*. “Weed management in direct seeded rice”. *Advances in Agronomy* 93 (2007): 153-255.

2. Lee KS., *et al*. “Salinity tolerance of japonica and indica rice (Oryza sativa L) at the seedling stage”. *Planta* 216.6 (2003): 1043-1046.

3. Gao JP., *et al*. “Understanding abiotic stress tolerance mechanisms: Recent studies on stress response in rice”. *International Journal of Plant Biology* 49.6 (2007): 742-750.

4. Chunthaburee S., *et al*. “Physiological and biochemical parameters for evaluation and clustering of rice cultivars differing in salt tolerance at seedling stage”. *Saudi Journal of Biological Sciences* 23.4 (2016): 467-477.

5. Tatar O., *et al*. “Physiological responses of two Turkish rice (Oryza sativa L) varieties to salinity”. *Turkish Journal of Agriculture* 34 (2010): 451-459.

6. Chutipaijit S., *et al*. “High contents of proline and anthocyanin increase protective response to salinity in Oryza sativa L spp indica”. *Australian Journal of Crop Science* 5 (2011): 1191-1198.

7. Blumwald E. “Sodium transport and salt tolerance in plants”. *Current Opinion in Cell Biology* 12.4 (2000): 431-434.

8. Demiral T and Türkan I. “Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance”. *Environmental and Experimental Botany* 53.3 (2005): 247-257.

9. Heuer B. “Influence of exogenous application of proline and glycinebetaine on growth of salt-stressed tomato plants”. *Plant Science* 165.4 (2003): 693-699.

10. Maggio A., *et al*. “Does proline accumulation play an active role in stress-induced growth reduction?” *Plant Journal* 31.6 (2002): 699-712.

11. Trovato M., *et al*. “Multiple roles of proline in plant stress tolerance and development”. *Rendiconti Lincei* 19.4 (2008): 325-346.

12. Munns R., *et al*. “Approaches to increasing the salt tolerance of wheat and other cereals”. *Journal of Experimental Botany* 57.5 (2006): 1025-1043.

13. Jogeswa G., *et al*. “Antioxidative response in different sorghum species under short term salinity stress”. *Acta Physiologia Plantarum* 28.5 (2006): 465-475.

14. Kazemi IK and Eskandari H. “Effects of salt stress on germination and early seedling growth of rice (Oryza sativa) cultivars”. *African Journal of Biotechnology* 10.77 (2011): 17789-17792.

15. Arnon DI. “Copper enzymes in isolated chloroplasts. Polyphenol oxidase in Beta vulgaris”. *Plant Physiology* 24.1 (1949): 1-15.

16. Bates L., *et al*. “Rapid determination of free proline for water stress studies”. *Plant and Soil* 39.1 (1973): 205-207.

17. Sairam RK and Tyagi A. “Physiology and molecular biology of salinity stress tolerance in plants”. *Current Science* 86.3 (2004): 407-421.

18. Tambhale D., *et al*. “Differential response of two scented Indica Rice (Oryza sativa) cultivars under salt stress”. *Journal of Stress Physiology and Biochemistry* 7.4 (2011): 387-397.

19. Deivanai SR., *et al*. “Role of exogenous proline in ameliorating salt stress at early stage in two rice cultivars”. *Journal of Stress Physiology and Biochemistry* 7 (2011): 157-174.

20. Le Rudulier D. “Osmoregulation in rhizobia: The key role of compatible solutes”. *Grain Legumes* 42 (2005): 18-19.

21. Kumar V., *et al*. “Differential response of indica rice genotypes to NaCl stress in relation to physiological and biochemical parameters”. *Archives of Agronomy and Soil Science* 53.5 (2007): 581-592.

22. Lutts S., *et al*. “NaCl induced senescence in leaves of rice (Oryza sativa L.) cultivars differing in salinity resistance”. *Annals of Botany* 78.3 (1996): 389-398.

23. Hayat S., *et al*. “Role of proline under changing environments”. *Plant Signaling Behavior* 7.11 (2012): 1456-1466.

24. Cha-um S., *et al*. “Screening upland rice (Oryza sativa L. ssp. indica) genotypes for salt-tolerance using multivariate cluster analysis”. *African Journal of Biotechnology* 9.30 (2010): 4731-4740.

25. Makela P., *et al*. “Effect of glycine betaine on chloroplast ultrastructure, chlorophyll and protein content, and Rubisco activities in tomato grown under drought or salinity”. *Biologia Plantarum* 43 (2000): 471-475.

26. Ashraf M and Foolad MR. “Roles of glycine betaine and proline in improving plant abiotic stress resistance”. *Environmental and Experimental Botany* 59.2 (2007): 206-216.

27. Hoque MA., *et al*. “Exogenous proline and glycinebetaine increase NaCl-induced ascorbate-glutathione cycle enzyme activities, and proline improve salt tolerance more than glycinebetaine in tobacco Bright Yellow-2 suspension-cultured cells”. *Journal of Plant Physiology* 164.11 (2007): 1457-1468.

28. Hasanuzzaman M., *et al*. “Exogenous Proline and Glycine Betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (Oryza sativa L.) varieties”. *BioMed Research International* (2014).

29. Cha-Um S and Kirdmanee C. “Effect of glycinebetaine on proline, water use and photosynthetic efficiencies and growth of rice seedlings under salt stress”. *Turkish Journal of Agriculture and Forestry* 34 (2010): 517- 527.

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