



SALINITY STRESS EFFECT ON BIOCHEMICAL CHANGES IN LEAVES OF RICE GENOTYPES DIFFERING IN SALT TOLERANCE

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Abstract

The impact of salinity stress on biochemical changes of salt tolerant genotypes PVSB9, PVSB19, PNR381, PNR519, Iratom24 and salt sensitive genotype NS15 along with one standard check salt tolerant rice cultivar Pokkali were assessed in two factors [7 rice genotypes in combination with 5 levels of salinity (0, 3, 6, 9 and 12 dS m⁻¹)] in pot culture. The biochemical aspects studied include chlorophyll, free amino acids and soluble proteins of the selected rice genotypes in view to evaluate their response at different salinity levels. The results of biochemical changes indicated that salinity seriously affected the biosynthesis of leaf chlorophyll of all the selected genotypes at different days after transplanting (DAT). At 35, 65 and 75 DATs, chlorophyll a and chlorophyll a/b ratio increased with increase in salinity levels but total chlorophyll and chlorophyll b decreased with increasing the levels of salinity. The soluble protein and free amino acids content in leaves of seven selected rice genotypes increased significantly with increase in salinity levels at 35 DAT but at 60 and 75 DAT, this increasing pattern continued up to 6 and 9 dSm⁻¹, respectively.

Key Words: *Oryza sativa*; Sodidity; Plant metabolism; Chlorophyll.

Introduction

Rice (*Oryza sativa* L.) is the grain that has shaped the cultures, diets and economies of billions of Asians. For them, rice is more than food; rice is life. About 120,000 varieties are grown across the world in an extensive range of climatic soil and water condition. Rice breeders have used genetic variability to produce cultivars that have high yield potential and that resist disease and insect damage and that tolerate cold, drought, and salinity. Salinity is a major threat to crop productivity in the southern and south-western part of Bangladesh, where it is developed due to frequent flood by sea water of the Bay of Bengal and on the other hand introduction of irrigation with saline waters. The huge lands of these coastal and offshore areas are affected by varying degrees of salinity. Osmotic adjustment in plants subjected to salt stress can occur by the accumulation of high concentration of either inorganic ions or low molecular weight organic solutes. The compatible

osmolytes generally found in higher plants are of low molecular weight sugars, organic acids, amino acids, proteins and quaternary ammonium compounds. The accumulation of soluble carbohydrates in plants has been widely reported as response to salinity or drought, despite a significant decrease in net CO₂ assimilation rate [1]. Proteins that accumulate in plants grown under saline conditions may provide a storage form of nitrogen that is neutralized when stress is over and may play a role in osmotic adjustment [2]. A higher content of soluble proteins has been observed in salt tolerant than in salt sensitive cultivars of rice [3]. Pareek *et al.* [3] also suggested that stress proteins could be used as important molecular markers for improvement of salt tolerance using genetic engineering techniques. Amino acids have been reported to have accumulated in higher plants under salinity stress [4]. It was reported by many scientists that salinity greatly affected the growth and

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physiology of plants [5, 6]. This research work has been, therefore, planned with the aim of finding out the strategies for selecting salt tolerant rice genotypes adaptable in coastal belt of Bangladesh notably during *boro* (winter) season; detailed information needs to be gathered on the changes in physiological and biochemical aspects due to salt stress.

Materials and Methods

The research work was conducted at the glasshouse of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during the period from December, 2005 to June, 2006. The experiment was laid out in two factorials CRD (Completely Randomized Design) with four replications. There were two factors viz. Factor-1: 7 Rice genotypes and Factor-2: 5 salinity level (0, 3, 6, 9 and 12 dS m⁻¹). Among the seven rice genotypes-PVSB9, PVSB19, PNR381, PNR519, Iratom24 were salt tolerant and NS15 was salt sensitive along with one standard check salt tolerant rice cultivar Pokkali. Soil for this pot experiment was collected from the field of BINA Farm, which was non-calcareous Dark Grey Floodplain having loamy texture and belonging to the Agro-Ecological-Zone of Old Brahmaputra Flood Plain. The soil was dried under the sun followed by crushing, mixed thoroughly and 8 (eight) kg soil was put in each pot. The pot soil was fertilized with urea, triple super phosphate (TSP), muriate of potash (MOP) and gypsum as sources of N, P, K and S at the rate of 100 kg N, 60 kg P₂O₅, 75 kg K₂O and 20 kg S ha⁻¹, respectively. The whole amount of TSP, MP, gypsum and 1/3rd of urea was applied prior to final preparation of the pots. Thereafter, the soil in pots was moistened with water and commercial NaCl salt was added to salinize pots to develop salinity upto the level of 3 dSm⁻¹. Six weeks-old single seedling of selected rice genotypes per hill and three hills per pot were transplanted in the respective pot. Two weeks after transplanting the remaining salt solutions were applied in each pot according to the treatments. To avoid osmotic shock, salt solutions were added in three equal installments on alternate days until the expected conductivity was reached. Salt solutions were collected every 24 hours from each pot and electric conductivity (EC) was measured with a conductivity meter and necessary adjustments were made. The remaining 2/3rd urea were top dressed in two equal installments at 25

and 50 days after transplanting. Weeds grown in the pots and visible insects were removed time to time by hands in order to keep the pots neat and clean. Watering was done in each pot to hold the soil water level and salt concentration constant when needed.

Determination of biochemical parameters

The biochemical parameters were determined in 3 times at 35, 60 days after transplanting (DAT) from leaf blade of 3rd leaf from the top and at 75 DAT from leaf blade of flag leaf.

i) Total chlorophyll content: Chlorophyll content such as chlorophyll-a, chlorophyll-b and total chlorophyll were determined from the leaf samples using the method of Coombs *et al.* [7].

ii) Free amino acid content: Extraction and estimation of total free amino acid contained in leaf samples were performed following the procedure outlined by Yemm and Cocking [8].

v) Soluble protein content: Soluble protein content in the leaf samples was determined according to the method of Lowry *et al.* [9].

Statistical analyses

The collected data were analyzed statistically following Completely Randomized Design by MSTAT-C computer package programme developed by Russel [10]. The mean differences were adjudged by Duncan's Multiple Range Test (DMRT) and regression lines were developed as and when required.

Results and Discussion

The genotypes PVSB9, PVSB19, PNR519, PNR381 were found tolerant while Iratom24 was moderately tolerant and NS15 as susceptible and Pokkali is a standard check tolerant cultivar. These rice genotypes have been selected for this study in order to simplify some biochemical data presentation and interpretation of the results to study the mechanism of salinity tolerance in rice plant system.

Chlorophyll content

The content of leaf chlorophylls viz. chlorophyll a, chlorophyll b, total chlorophyll and also the chlorophyll a/b ratio of seven selected rice genotypes were affected due to different salinity levels. At 35 days after

transplanting (DAT) the chlorophyll a increased with increase in salinity upto the highest level (12 dSm⁻¹). But with the age of the rice plants (60 and 75 DATs) the content of chlorophyll a decreased after 6 dSm⁻¹ level. The content of chlorophyll b increased with the age of the

plants but decreased with increase in salinity levels at all DAT studied (Table 1). These differences in content of chlorophyll a and b reflected on the results of total chlorophyll content and on the ratio of chlorophyll a/b.

Table 1. The effect of different salinity levels on chlorophyll content in leaves of seven selected rice genotypes at different days after transplanting (DAT) (each value is a mean of 7 genotypes)

| Salinity level (dSm ⁻¹) | Chlorophyll a (mg g ⁻¹ fw) | | | Chlorophyll b (mg g ⁻¹ fw) | | | Total chlorophyll (mg g ⁻¹ fw) | | | Chlorophyll a/b ratio | | |
|-------------------------------------|---------------------------------------|--------|--------|---------------------------------------|--------|--------|---|--------|--------|-----------------------|--------|--------|
| | 35 DAT | 60 DAT | 75 DAT | 35 DAT | 60 DAT | 75 DAT | 35 DAT | 60 DAT | 75 DAT | 35 DAT | 60 DAT | 75 DAT |
| 0 | 1.62 d | 1.58 c | 2.57 b | 0.95 a | 0.98 a | 1.49 a | 2.57 a | 2.56 a | 4.06 a | 1.72 e | 1.63 d | 1.84 e |
| 3 | 1.66 c | 1.62 b | 2.58 b | 0.78 b | 0.81 b | 1.24 b | 2.44 b | 2.43 b | 3.81 b | 2.16 d | 2.01 c | 2.45 d |
| 6 | 1.70 b | 1.68 a | 2.93 a | 0.63 c | 0.61 c | 0.59 c | 2.33 c | 2.29 c | 3.53 c | 2.71 c | 2.83 b | 5.34 b |
| 9 | 1.73 a | 1.50 d | 2.49 c | 0.46 d | 0.40 d | 0.37 d | 2.19 d | 1.90 d | 2.86 d | 3.98 b | 3.42 a | 5.90 a |
| 12 | 1.75 a | 0.76 e | 1.27 d | 0.28 e | 0.15 e | 0.14 e | 2.03 e | 0.91 e | 1.41 e | 6.89 a | 2.24 c | 4.13 c |
| CV(%) | 1.61 | 2.86 | 4.34 | 5.48 | 13.58 | 10.17 | 1.61 | 4.43 | 3.99 | 9.73 | 19.76 | 12.60 |

Values having same letter (s) in a column do not differ significantly at 1% level of probability.

Table 2. Genotypic effect on chlorophyll content in leaves of seven selected rice genotypes at different days after transplanting (DAT) [each value is a mean of 5 salinity levels (0, 3, 6, 9, & 12 dSm⁻¹)]

| Genotype | Chlorophyll a (mg g ⁻¹ fw) | | | Chlorophyll b (mg g ⁻¹ fw) | | | Total chlorophyll (mg g ⁻¹ fw) | | | Chlorophyll a/b ratio | | |
|----------|---------------------------------------|--------|--------|---------------------------------------|---------|--------|---|--------|--------|-----------------------|---------|---------|
| | 35 DAT | 60 DAT | 75 DAT | 35 DAT | 60 DAT | 75 DAT | 35 DAT | 60 DAT | 75 DAT | 35 DAT | 60 DAT | 75 DAT |
| Pokkali | 1.67 b | 1.63 c | 2.60 b | 0.70 a | 0.63 b | 0.89 b | 2.37 a | 2.25 b | 3.49 b | 2.66 e | 2.99 ab | 5.34 a |
| PVS B19 | 1.68 b | 1.71 a | 2.54 b | 0.58 cd | 0.66 ab | 0.91 b | 2.26 e | 2.35 a | 3.45 b | 3.67 b | 3.19 a | 4.18 bc |
| PVS B9 | 1.72 a | 1.66 b | 2.80 a | 0.64 b | 0.69 a | 0.96 a | 2.35 ab | 2.37 a | 3.76 a | 2.94 de | 2.71 b | 4.43 b |
| PNR519 | 1.68 b | 1.34 d | 1.97 d | 0.65 b | 0.54 c | 0.74 c | 2.33 bc | 1.88 d | 2.71 d | 3.28 c | 2.28 c | 2.95 d |
| PNR381 | 1.74 a | 1.33 d | 1.99 d | 0.60 c | 0.52 c | 0.73 c | 2.33 bc | 1.85 d | 2.72 d | 3.92 b | 2.40 c | 2.72 d |
| NS15 | 1.68 b | 0.98 e | 2.31 c | 0.59 cd | 0.47 d | 0.41 d | 2.28 de | 1.45 e | 2.71 d | 4.71 a | 1.53 e | 3.92 c |
| Iratom24 | 1.66 b | 1.34 d | 2.35 c | 0.60 c | 0.63 b | 0.71 c | 2.27 e | 1.97 c | 3.06 c | 3.23 cd | 1.90 d | 3.98 c |
| CV(%) | 1.61 | 2.86 | 4.34 | 5.48 | 13.58 | 10.17 | 1.61 | 4.43 | 3.99 | 9.73 | 19.76 | 12.60 |

Values having same letter(s) in a column do not differ significantly at 1% level of probability.

Table 3. The effect of different salinity levels on free amino acid and soluble protein content in leaves of seven selected rice genotypes (each value is a mean of 7 genotypes)

| Salinity level (dSm ⁻¹) | Free amino acid (mg g ⁻¹ fw) | | | Soluble protein (mg g ⁻¹ fw) | | |
|-------------------------------------|---|---------|---------|---|---------|---------|
| | 35 DAT | 35 DAT | 35 DAT | 35 DAT | 60 DAT | 75 DAT |
| 0 | 0.53 d | 29.32 d | 29.32 d | 29.32 d | 31.82 d | 37.25 d |
| 3 | 0.57 d | 31.26 c | 31.26 c | 31.26 c | 34.08 b | 38.13 c |
| 6 | 0.73 c | 32.37 b | 32.37 b | 32.37 b | 34.84 a | 42.64 a |
| 9 | 1.22 b | 33.37 a | 33.37 a | 33.37 a | 32.96 c | 40.06 b |
| 12 | 2.05 a | 33.72 a | 33.72 a | 33.72 a | 16.35 e | 22.06 e |
| CV(%) | 16.89 | 3.35 | 3.35 | 3.35 | 3.21 | 3.78 |

Values having same letter(s) in a column do not differ significantly at 1% level of probability.

Table 4. Genotypic effect on free amino acid and soluble protein content in leaves of selected rice genotypes [each value is a mean of 5 salinity levels (0, 3, 6, 9, & 12 dSm⁻¹)]

| Genotype | Free amino acid (mg g ⁻¹ fw) | | | Soluble protein (mg g ⁻¹ fw) | | |
|----------|---|---------|---------|---|---------|----------|
| | 35 DAT | 60 DAT | 75 DAT | 35 DAT | 35 DAT | 35 DAT |
| Pokkali | 0.88 cd | 13.33 a | 19.68 c | 31.91 c | 34.52 b | 42.62 b |
| PVS B19 | 1.30 a | 10.98 c | 22.43 b | 31.20 c | 35.21 a | 43.59 a |
| PVS B9 | 1.12 b | 11.82 b | 24.16 a | 31.74 c | 34.43 b | 42.85 ab |
| PNR519 | 0.65 e | 7.43 d | 15.98 d | 33.99 a | 28.97 c | 31.06 d |
| PNR381 | 1.40 a | 7.72 d | 14.52 e | 33.00 b | 28.96 c | 33.06 c |
| NS15 | 0.81 d | 3.56 e | 12.70 f | 30.36 d | 20.22 e | 26.37 e |
| Iratom24 | 0.98 c | 7.26 d | 14.55 e | 31.87 c | 27.81 d | 32.64 c |
| CV(%) | 16.89 | 10.79 | 6.33 | 3.35 | 3.21 | 3.78 |

Values having same letter(s) in a column do not differ significantly at 5% level of probability

When the mean effect of five levels of salinity was considered it was observed that the content of chlorophyll a, chlorophyll b and total chlorophyll as well as chlorophyll a/b ratio significantly differed due to salinity at different DAT (Table 2). At 35 DAT, the highest chlorophyll a content (1.74 mg g⁻¹fw) was found in PNR381, which was statistically identical with PVSB9 and the lowest amount of chlorophyll a (1.66 mg g⁻¹fw) was obtained in Iratom24, which was statistically similar to that of Pokkali, PVSB19, PNR519 and NS15. At 60 DAT the genotype PVSB19 contained the highest amount of chlorophyll a (1.71 mg g⁻¹fw) and NS15 contained the lowest amount of chlorophyll a (0.98 mg g⁻¹fw). But at 75 DAT, the highest amount of chlorophyll a (2.80 mg g⁻¹fw) was present in PVSB9 and the lowest amount (1.99 mg g⁻¹fw) was in PNR519, which was statistically similar with that of PNR381. The highest amount of chlorophyll b and total chlorophyll were present in Pokkali at 35 DAT and in PVSB9 at 60 and 75 DAT. The lowest amount of chlorophyll b and total chlorophyll were contained in NS15 and Iratom24, which was statistically similar to that of PVSB19 at 35 DAT. But at 60 and 75 DAT the lowest amount of chlorophyll b and total chlorophyll were recorded in NS15. In case of chlorophyll a/b ratio, the highest values were observed in NS15 at 35 DAT, in PVSB19 at 60 DAT and in Pokkali at 75 DAT; the lowest value of chlorophyll a/b ratio was found in Pokkali at 35 DAT, in NS15 at 60 DAT and in PNR381 at 75 DAT which was statistically identical with that of PNR519.

The effect of different salinity levels on leaf chlorophylls content in selected seven rice genotypes significantly differed at different DAT. The total chlorophyll (a + b) content decreased with the increasing salinity levels in all genotypes at different DAT (Fig. 1). The total chlorophyll content was highest at 75 DAT in all the genotypes under different salinity levels. The very high values (2.91, 2.79 and 5.27 mg g⁻¹fw) of total chlorophyll content were found in NS15 under control treatment at 35, 60 and 75 DAT respectively, while it was minimum (1.68, 1.99 and 3.10 mg g⁻¹fw) in the genotypes NS15 at 12 and 6 dSm⁻¹ levels and in PNR519 at 9 dSm⁻¹ level of salinity at 35, 60 and 75 DAT respectively. The effect of salinity levels on chlorophyll a/b ratio of different rice genotypes was found to increase with increasing the salinity levels in all genotypes at different DAT (Fig.1). At 35 DAT, the highest value (11.20) of chlorophyll a/b ratio was in genotype NS15 at 12 dSm⁻¹ level and the lowest

ratio (1.60) was in the same genotype at control treatment which was statistically identical with that of other genotypes at control. At 60 DAT, the highest value of chlorophyll a/b ratio (6.32) was recorded in PVSB19 at 12 dSm⁻¹ level and it was lowest in Iratom24 (1.49) at control. But at 75 DAT, the highest chlorophyll a/b ratio (12.32) was obtained in Pokkali at 12 dSm⁻¹ level of salinity and the lowest value (1.29) was found in PVSB9 at control treatment. These results corroborate with those of Islam [11] who stated that salinity decreased chlorophyll b content more than chlorophyll a and thus increased the chlorophyll a/b ratio. Abdullah *et al.* [12] studied the salinity stress (50 mM) on the performance of IR28 and observed a significant reduction in both chlorophyll a and chlorophyll b content in different parts of the rice leaves at saline condition. Reddy and Vora [13] reported that a decrease in chlorophyll concentration in salinized plants could be attributed to increased activity of the chlorophyll-degrading enzyme chlorophyllase.

Free amino acid

The content of free amino acid in rice leaves of seven selected rice genotypes significantly increased with increasing the salinity levels upto the highest salinity levels (12 dSm⁻¹) at 35 DAT but at 60 and 75 DAT, this increase continue up to 9 dSm⁻¹ level of salinity and then again it gradually reduced (Table 3). The application of different levels of salinity increased the accumulation of free amino acid in leaves of rice genotypes and this increase was prominent with the increase in age of plants.

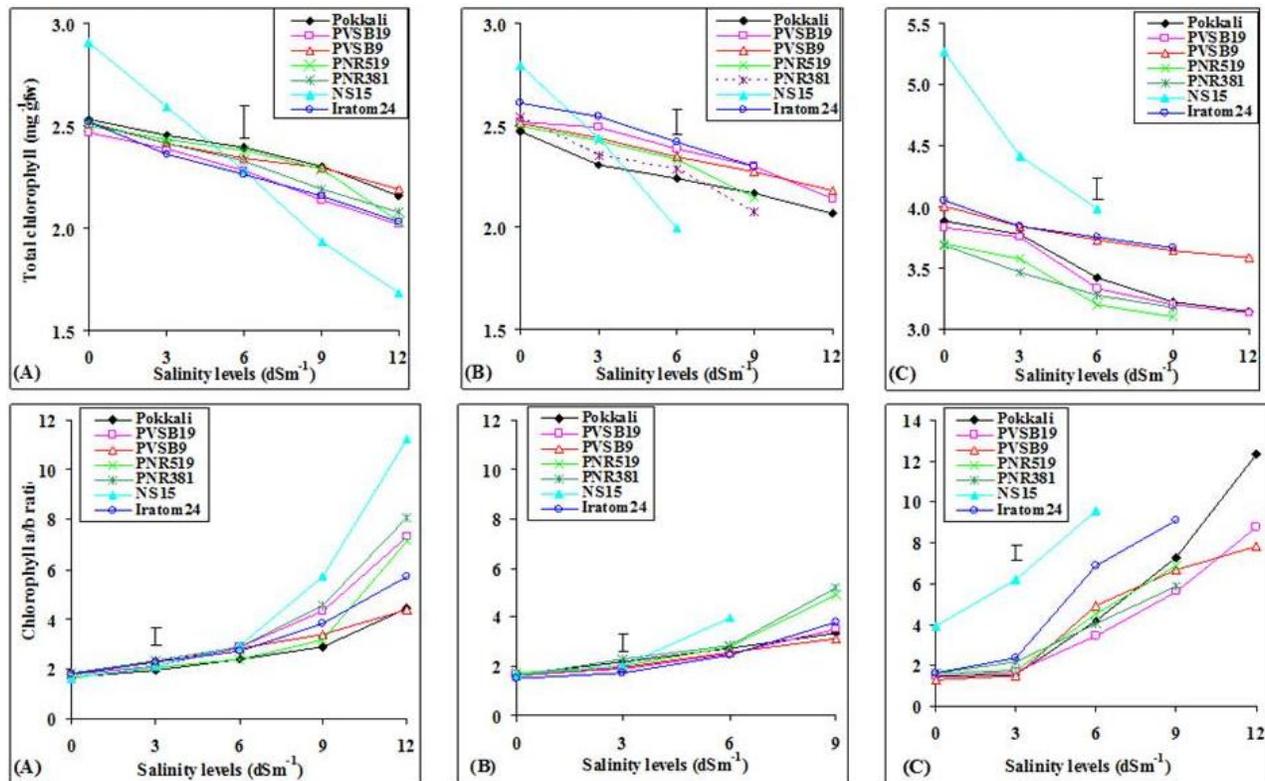
The results presented in Table 4 show that the content of free amino acid in the seven selected rice genotypes varied significantly due to the mean effect of salinity levels at different DAT. The highest free amino acid was observed in all the genotypes at 75 DAT followed by 60 and 35 DAT respectively. At 35 DAT, the highest amount of free amino acid (1.40 mg g⁻¹fw) was obtained in the leaves of PNR381, which was statistically identical with PVSB19 and it was lowest (0.65 mg g⁻¹fw) in PNR519. But at 60 and 75 DAT, the highest amount of free amino acids (13.33 and 24.16 mg g⁻¹fw) was found in tolerant genotypes Pokkali and PVSB9 and the lowest amount (3.56 and 12.70 mg g⁻¹fw) was recorded in NS15, respectively.

The different salinity levels had significant effect on free amino acid content in leaves of seven selected rice genotypes at different DAT (Fig. 2). The content of free amino acid increased in leaves of tolerant rice genotypes

Pokkali, PVSB19, PVSB9, PNR519, PNR381 and Iratom24 with increase in salinity levels and it decreased in susceptible genotype NS15 with increasing salinity levels at different DAT. The highest amount of free amino acids (3.43 and 22.33 mg g⁻¹fw) was obtained in PVSB19 under 12 dSm⁻¹ level of salinity at 35 and 60 DAT

respectively and that was the lowest (0.38 and 2.09 mg g⁻¹fw) in the same genotype under control condition at 35 and 60 DAT respectively. Though at control treatment the leaves of rice genotype NS15 contained highest amount of free amino acids but with increase in salinity level it reduced dramatically (Fig. 2).

Fig. 1. The effect of different salinity levels on total chlorophyll content and chlorophyll a/b ratio in leaves of seven selected rice genotypes at 35 (A), 60 (B) and 75 (D) DAP (vertical bars represent LSD at 0.05 level of significance)



The Fig. 2 clearly showed that the content of amino acid increased in tolerant genotypes Pokkali, PVSB19, PVSB9, PNR519, PNR381, Iratom24 and it decreased in susceptible genotype NS15 with increasing the salinity levels at different DAT. These results are in conformity with the findings of Jha and Singh [14] who reported that the accumulations of osmolytes like soluble sugar and total amino acids were higher in leaf tissues under stress condition in tolerant rice genotypes. Amino acids have been reported to accumulate in higher plants under salinity stress [4].

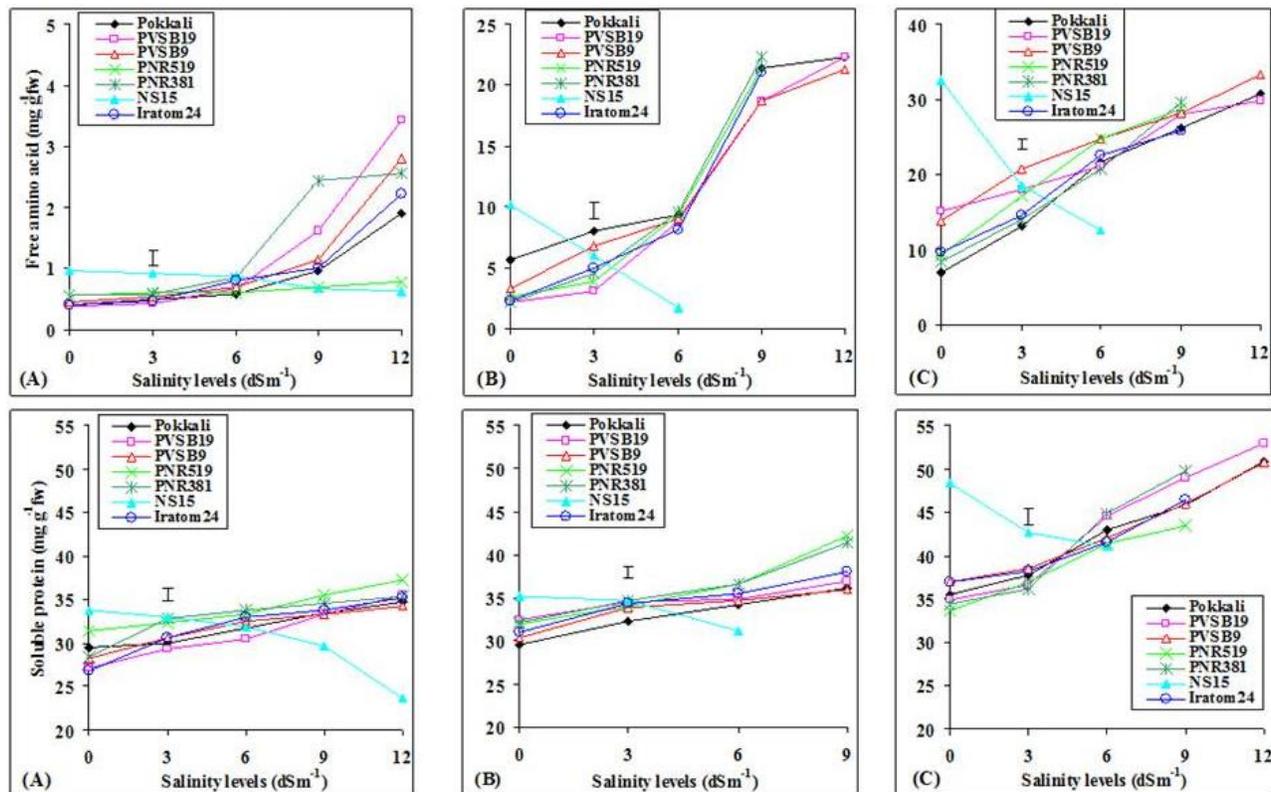
Active accumulation of solutes in the cell sap led to osmotic adjustment which helped to maintain leaf turgor of living cells [15]. Our results indicate that the content of higher free amino acids in leaves of selected rice genotypes was in the order of 75 DAT (flowering stage),

60 and 35 DAT at all the salinity levels under study (Fig.2.14a). To minimize the osmotic potential at flowering stage, the rice plant synthesized higher amount of organic solutes [16].

Soluble protein

The content of soluble protein in rice leaves of seven selected rice genotypes varied significantly due to variation in salinity levels at different DAT (Table 3). At early stage (35 DAT) the increase of soluble protein in leaves was found to continue up to the highest salinity level used (12 dSm⁻¹) while in later stages (60 and 75 DAT) it continued up to 6 dSm⁻¹ level of salinity, though the concentration of soluble protein in rice leaves was more and more higher at older stages of growth.

Fig. 2. The effect of different salinity levels on free amino acid and soluble protein content in leaves of seven selected rice genotypes at 35 (A), 60 (B) and 75 (D) DAP (vertical bars represent LSD at 0.05 level of significance)



While considering the mean effect of all the salinity levels it was observed that soluble protein content in leaves of selected rice genotypes varied significantly at different DAT (Table 4) and the concentration of the soluble protein increased progressively with the age of the plants and was in the order of 75, 60 and 35 DAT. At 35 DAT, the highest soluble protein content (33.99 mg g⁻¹fw) was found in PNR519 and it was lowest (30.36 mg g⁻¹fw) in NS15. But at 60 and 75 DAT, the highest soluble protein (35.21 and 43.59 mg g⁻¹fw) content was found in tolerant genotype PVSB19 and the lowest (20.22 and 26.37 mg g⁻¹fw respectively) was in NS15. It was further noted that the soluble protein content in genotypes PNR519, PNR381, NS15 and Iratom24 decreased considerably at older ages (60 and 75 DAT) of growth as compared to early stage (35 DAT).

The content of soluble protein in leaves of seven selected rice genotypes varied significantly at different DAT due to the influence of different levels of salinity (Fig.2). From the results it appeared that except NS15 in all the genotypes the content of soluble protein increased progressively with the age of plant and it reached the highest level at 75 DAT. These results are in conformity

with the findings of Pareek *et al.* [3]. Yoshida [16] opined that to minimize the osmotic potential in flowering stage, the rice plant synthesized higher amount of organic solutes. Proteins that accumulate in plants grown under saline conditions might provide a storage form of nitrogen that would be neutralized when stress would over [2]. Cheng *et al.* [17] showed that second generation transgenic rice plants accumulated higher amount of protein due to dehydration or salt stress, which were correlated with increased tolerance. Abdullah *et al.* [12] stated that under salinity stress, total protein concentration in flag, second and third leaves were higher than in control plants.

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