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Supplemental Calcium Enhances Growth and Elicits Proline Accumulation in NaCl-Stressed Rice Roots

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Abstract: The present study was conducted to investigate the interactive effects of NaCl and $CaCl_2$ on the growth, ion relations and proline accumulation in seedlings of rice (***Oryza sativa***. L.). Twenty-days-old seedlings were subjected to 0, 100 and 150 mol m^{-3} NaCl concentrations with or without supplemental calcium chloride (5.0 and 0.15 mol m^{-3}). Salinity induced a reduction in growth both of Bankat and Hitomebore and Ca^{2+} decreased the effect. The proline level of root tissues was less under the higher salinity stress (150 mol m^{-3}) than at lower salinity (100 mol m^{-3}). Additional Ca^{2+} reduced the Na^{+} concentration of shoot and root tissues of both cultivars at high salinity in particular. It was concluded that the supplemental calcium decreased the detrimental effects of NaCl not only by reducing Na^{+} uptake and its translocation to shoots but also by the enhancement of proline accumulation in root tissues.

Key words: $CaCl_2$, NaCl stress, ***Oryza sativa*** L., growth, proline

Introduction

Non-halophytes cope with the detrimental effects of salt stress (osmotic effect, toxic effect and ion imbalance) by ion exclusion, extrusion, compartmentation and accumulation of organic cytosolutes (Greenway and Munns, 1980; Wyn Jones, 1985; Poljakoff-Mayber and Lerner, 1994). Salinity tolerance in rice, a non-halophyte, is not reflected in a single trait but an array of complex and potentially independent mechanisms are involved (Yeo, 1994). Proline accumulation under stress is proposed to be a part of the mechanisms of osmotic adjustment (Ahmad ***et al.***, 1981, Aspinall and Paleg, 1981; Dubey and Rani, 1989; Shah ***et al.***, 1990; Roy ***et al.***, 1992; Heuer, 1994; Misra ***et al.***, 1996; Van Heerden and de Villier, 1996), although a negative correlation have also been shown between proline accumulation and the degree of stress tolerance (Hanson ***et al.***, 1977; Perez-Alfocea ***et al.***, 1993; Lutts and Guerrier, 1995).

It has been reported that supplemental calcium have a protective effect on the growth and cellular homeostasis of non-halophytes exposed to NaCl (LaHaye and Epstein, 1969; Cramer ***et al.***, 1986; Zhong and Lauchli, 1994, Lin and Kao, 1995). To date most of the studies on calcium-salinity interactions have been focussed on the integrity of membranes and membrane transport systems (Lauchli and Schubert, 1989). There are few data on the possible role of Ca^{2+} in the osmotic

adjustment of stressed plants by the accumulation of organic cytosolutes (e.g. proline). The present experiment was designed to investigate the short-term effects of NaCl and $CaCl_2$ on growth, ion relations and proline accumulation in seedlings of two rice cultivars (Hitomebore and Bankat).

Materials and Methods

Plant culture

Seeds of rice (cv. Bankat and Hitomebore) were placed in holes of Styrofoam boards with a nylon net bottom. The boards were placed in rectangular plastic trays half filled with tap water. The germination and growth were carried out in a glasshouse under the natural day length at 32°C (day) and 28°C (night) temperature. Seven days after seeds were planted, tap water was replaced with one fourth strength nutrients solution (Mae, 1993). The nutrient solutions were renewed after every 4 days. Two-weeks-old seedlings were transferred to half strength nutrient solution and selected for their uniformity to stand and health in appearance. Ten seedlings per tray containing 7 liter medium were allowed to grow. Three-weeks-old seedlings were then subjected to salt stress.

Salt treatment and experimental design

The experiment was conducted by using a factorial design containing three concentrations of NaCl (0, 100 and 150 mol m^{-3}) and two $CaCl_2$ concentrations (0.15 and 5.0 mol m^{-3}) each with five replicates each. The 0.15 mol m^{-3} Ca^{2+} presented the standard calcium concentration of half strength Mae's (1993) medium and 5.0 mol m^{-3} Ca^{2+} was selected after a pilot study which showed that this concentration was not high enough to be toxic itself.

Measurements of growth

Before and after the treatment (3 days of NaCl stress), 12 seedlings per cultivar per treatment were harvested at random and washed for 5 seconds in running deionized water, blotted dry, separated into root and shoot tissues and fresh weights recorded. Dry weight was determined after drying the samples at 80°C for two days. The relative growth rate (RGR) was expressed on an index of tolerance (INTOL) basis (Chowdhury ***et al.***, 1996).

RGR (day^{-1})= ln (Final fresh weight)-ln (Mean initial fresh weight)/days
INTOL = RGR treatment per mean RGR control.

The index of tolerance expressed the RGR for each treatment as a proportion to the mean RGR of the appropriate control (no NaCl and same calcium).

Determination of water content

Water content of root and shoot tissue were calculated on a tissue dry weight basis, i.e. gram water per gram dry weight of tissues by the following formula:

Water = F.wt-D.wt/D.wt. Where, F.wt.=fresh weight of tissue and D.wt.= dry weight of tissue.

Chemical analysis

The experiment was harvested (Five seedling from each treatment i.e. one seedling per replication) after 3 days of NaCl stress when tips of leaves started drying.

Determination of proline

For proline estimation, 0.05 g tissue was homogenized in 3% sulfosalicylic acid and the homogenate was filtered through Whatman No. 2 filter paper. The filtrates were used for proline quantification by the method of Bates ***et al.*** (1973). The concentration of proline in each sample was calculated from a standard curve of L-proline.

Ions analyses

Dried plant samples (root and shoot) were water extracted by the method of Chowdhury ***et al.***, 1996. The concentration of Na^+ and K^+ were analyzed by atomic absorption spectrophotometer (Model AA 660, Shimadzu, Japan).

Results

Growth

The growth response (as expressed on an index of tolerance basis) of both tissues i.e. root and shoot of both cultivars (Bankat and Hitomebore) to interactive effects of NaCl and $CaCl_2$ was very similar (Fig. 1a, b, c, d). The elevation of salinity level in the medium caused gradual but significant reduction in growth rate of both the cultivars. The addition of calcium in the medium partially alleviated the adverse effects of NaCl. An ANOVA showed highly significant effects of NaCl, Ca^{2+} and their interaction on growth of rice seedlings but there was no significant difference between cultivars.

Water content

Water content of root tissues of both cultivars increased a little but significantly ($P<0.001$) with increasing salinity. Although there was no main effect of Ca^{2+} on the root water content, the interaction between NaCl and $Ca^{2+}$was found to be highly significant ($P<0.001$). In contrast, shoot water content declined with the elevation of NaCl concentration. The cultivar Bankat maintained higher water content than the cultivar Hitomebore. Statistical analysis showed a significant varietal difference ($P<0.001$) and a significant interaction between NaCl and Ca^{2+} ($P<0.001$)

Ion relations

Na^+ ion concentration in roots and shoots of both cultivars increased significantly ($P<0.001$) with increasing salt level in medium. However, raising the Ca^{2+} concentration in medium reduced the Na^+ concentration of root tissues and their translocation to shoot and the effect is highly significant at 150 mM NaCl (Table 1 and 2).

The response of K^+ accumulation under salinity to Ca^{2+} supply was greatly differing between the type of tissues. In root tissues of both cultivars K^+ content declined gradually with

Table1: The interactive effects of NaCl and $CaCl_2$ on Na^+ and K^+ content (μ moles g^{-1} dry weight) of root tissues of *Oryza sativa* L. (cvs. Bankat and Hitomebore)

Treatments (mM)		Cultivar			
		cv. Bankat		cv. Hitomebore	
NaCl	$CaCl_2$	Na^+	K^+	Na^+	K^+
0.00	0.15	114.69	494.98	152.3	637.03
		(42.15)	(28.51)	(40.05)	(69.3)
	5.00	112.42	556.07	151.84	682.45
		(30.57)	(30.50)	(50.06)	(57.7)
100	0.15	555.56	351.1	601.88	293.26
		(31.19)	(38.95)	(67.16)	(51.5)
	5.00	500.37	454.8	567.13	325.93
		(16.67)	(15.3)	(30.2)	(61.3)
150	0.15	1033.2	309.65	1020.8	230.64
		(81.89)	(43.64)	(79.5)	(32.84)
	5.00	623.87	436.3	741.87	262.03
		(23.87)	(31.75)	(114.9)	(16.27)

Table 2: The interactive effects of NaCl and $CaCl_2$ on Na^+ and K^+ content (μ moles g^{-1} dry weight) of shoot tissues of *Oryza sativa* L. (cvs. Bankat and Hitomebore)

Treatments (mM)		Cultivar			
		cv. Bankat		cv. Hitomebore	
NaCl	$CaCl_2$	Na^+	K^+	Na^+	K^+
0.00	0.15	31.38	704.06	37.02	699.3
		(2.31)	(12.98)	(6.54)	(59.3)
	5.00	32.41	703.15	36.83	663.5
		(3.34)	(30.29)	(6.23)	(53.0)
100	0.15	713.3	656.46	580.93	640.05
		(25.07)	(29.58)	(43.52)	(52.94)
	5.00	481.22	671.49	357.58	739.12
		(45.87)	(28.74)	(37.92)	(61.98)
150	0.15	1996.92	660.94	2470.65	697.45
		(1`46.9)	(30.6)	(565.45)	(91.45)
	5.00	1120.62	765.26	918.06	641.29
		(72.73)	(56.9)	(146.51)	(86.16)

The data are given as mean of 5 replicates and standard error in parenthesis

increasing salinity from 0 to 150 mol m^{-3} NaCl with little Ca^{2+} effect on maintaining the K^+ level. While there was very little decrease in K^+ content of shoot tissues of both cultivars in response to increasing medium salinity with no significant Ca^{2+} effect (Table 1 and 2). The relatively higher Na^+ content of root and shoot tissues at low Ca^{2+} level than at high Ca^{2+} resulted in higher Na^+ to K^+ ratios for both cultivars (Fig. 2).

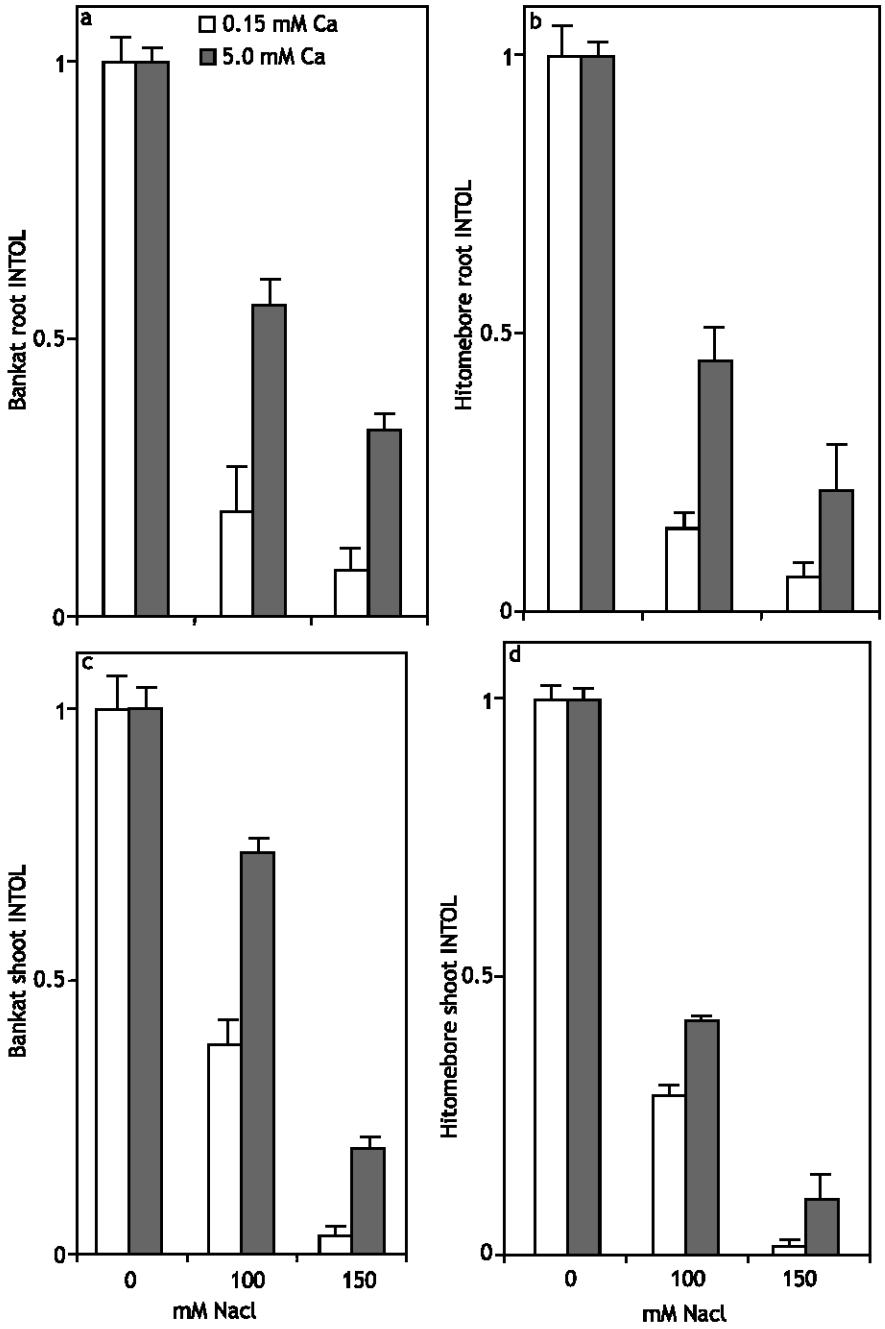

Fig. 1: The effects of NaCl and $CaCl_2$ on indices of tolerance of root and shoot tissues of Bankat and Hitomebore seedlings. (a) roots of Bankat; (b) roots of Hitomebore; © shoots of Bankat; (d) shoots of Hitomebore, Medium containing 0.15 mol m^{-3} $CaCl_2$; medium containing 5.0 mol m^{-3} $CaCl_2$. Values plotted are means±S.E.

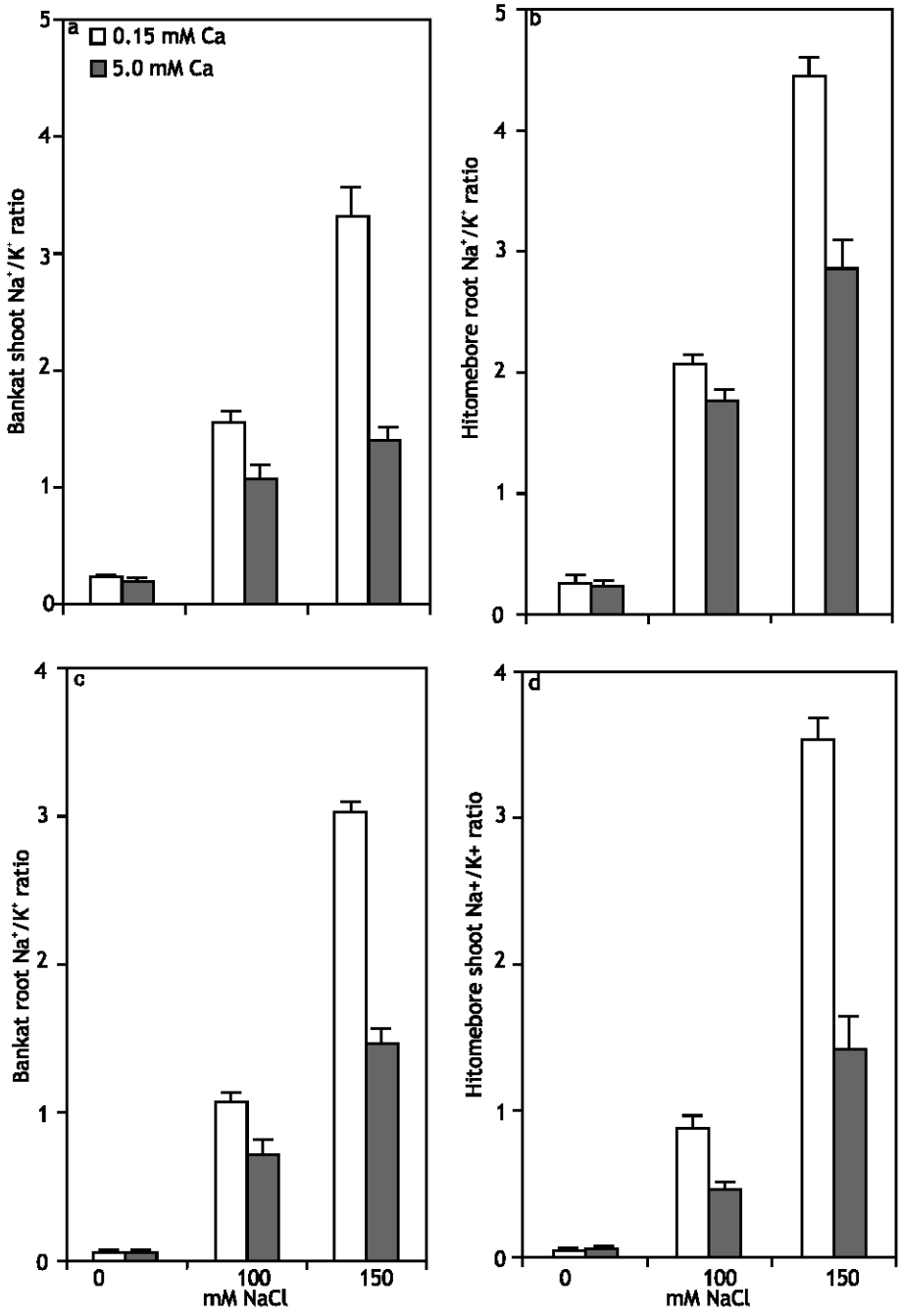

Fig. 2: The effects of NaCl and $CaCl_2$ on Na^+/K^+ ratios of root and shoot tissues of Bankat and Hitomebore seedlings. (a) roots of Bankat; (b) roots of Hitomebore; © shoots of Bankat; (d) shoots of Hitomebore, Medium containing 0.15 mol m^{-3} $CaCl_2$; medium containing 5.0 mol m^{-3} $CaCl_2$. Values plotted are means±S.E.

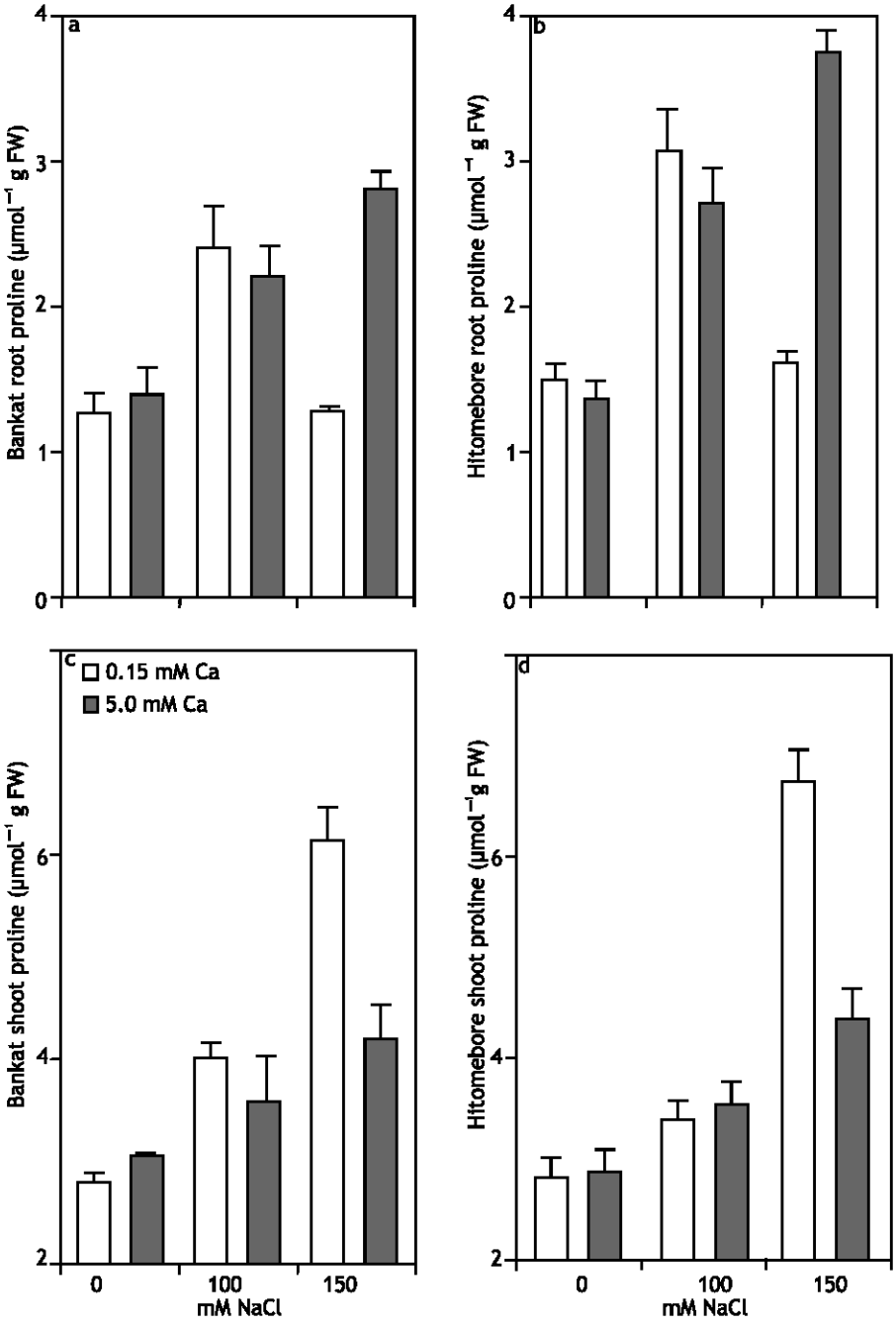

Fig. 3: The interactive effects of NaCl and $CaCl_2$ on proline accumulation in root and shoot tissues of Bankat and Hitomebore seedlings. (a) roots of Bankat; (b) roots of Hitomebore; © shoots of Bankat; (d) shoots of Hitomebore, Medium containing 0.15 mol m^{-3} $CaCl_2$; medium containing 5.0 mol m^{-3} $CaCl_2$. Values plotted are means±S.E.

Proline

NaCl treatment elicited an accumulation of proline in root and shoot tissues of both cultivars (Fig. 3a, b, c, d). Under low Ca^{2+}, root proline content of both cultivars declined at 150 mol m^{-3} NaCl after an initial increase at 100 mol m^{-3} NaCl. The proline level of roots in the presence of high Ca^{2+} showed an increase with corresponding increase in salinity. The main effects of NaCl and Ca^{2+} on the proline content in roots and their interaction were highly significant ($P<0.001$). For shoots the proline accumulation was quite similar in both cultivars. There was a gradual but significant increase in proline accumulation in response to elevating level of salinity. The highest level of proline was accumulated at the highest salinity (150 mol m^{-3} NaCl) at low Ca^{2+} as compared to the proline content at high Ca^{2+}. An ANOVA test showed a highly significant interactive effect ($P<0.001$) of NaCl and Ca^{2+} on shoot proline levels.

Discussion

Most of the data relating to salt tolerance include growth basis measurement and plants are normally categorized on this basis (Greenway and Munns, 1980). At seedling stage relative growth rate of the whole plant would seem to be the most appropriate measure to assess salt tolerance.

Results of the present study were in accordance with reports of LaHaye and Epstein (1969) that supplemental calcium had a protective effect for growth and Na^{+} exclusion and translocation in non-halophytes exposed to NaCl stress. Interestingly the present investigation also revealed that root and shoot respond very differently to NaCl and Ca^{2+} supply in accumulating proline (Fig. 3a, b, c, d). There was a gradual increase in proline content of shoots with a corresponding increase in NaCl concentration and the highest level of proline was observed at the highest salinity (150 mol m^{-3} NaCl) at low Ca^{2+}. In contrast proline level of roots decreased at the highest salinity after an increased level at 100 mol m^{-3} NaCl at low Ca^{2+} but roots treated with 5.0 mol m^{-3} Ca^{2+}accumulated the highest level of proline at highest salinity. The increased proline accumulation in roots of NaCl stressed rice seedlings in association with reduced growth had been interpreted as a symptom of injury or related to growth inhibition (Lutts and Guerrier, 1995 and Lin and Kao, 1996). This explanation did not however, seem to stand true in our case. Firstly under salt stress, supplemental Ca^{2+} enhanced the growth of root but concurrently stimulated proline accumulation under the relatively high salinity (Fig. 3a and 3b). Secondly, when the data were analyzed for the relationship between root indices of tolerance and proline accumulation within each salinity level, in non-saline media a negative correlation was found between root indices of tolerance and proline accumulation. At 100 mol m^{-3} NaCl there was observed a positive but non-significant correlation and a significant positive correlation ($r=0.693$ **, $n=10$) was found at high salinity (150 mol m^{-3}). These observations strongly suggested that elevated proline level in roots under stress could be related to continue ratio of growth and maintenance rather than the consequence of growth inhibition. The association of continued growth and enhanced proline accumulation under NaCl stress had also been observed for callus cultures of ***Medicago sativa*** L. (Shah ***et al.***, 1990) and root tips of ***Sorghum bicolor*** (Colmer ***et al.***, 1996). Recently with cell cultures of ***Oryza sativa*** Shah ***et al.***, 2001 reported that in salt stressed suspensions supplemental Ca^{2+} regulates enhanced proline accumulation at mRNA translational

level. However, the concentration of proline in shoot under salt stress was significantly higher at low Ca^{2+} in comparison with high Ca^{2+} (Figs 3c and 3d). Lauchli ***et al.*** (1994) reported similar observations in ***Sorghum bicolor*** and suggested that the accumulation of proline in salt-stressed leaves at low Ca^{2+} might be important for growth maintenance. Under high salinity and low Ca^{2+}, root Na^+ content and proline accumulation showed a poor positive correlation (r=0.174, n=10), whereas at high Ca^{2+} a significant positive correlation (r=0.641*, n=10) was found despite the tissues contained significantly lower Na^+ (Table 1). This indicated that there was no simple association between Na^+ ions concentration and proline level of root tissues. On the other hand, the coefficient of correlation analysis between shoot proline level and Na^+ content showed a significant positive correlation (r=0.626**, n=10). It was logical to view the differential response of root and shoot tissues in accumulating proline under salt stress, type of proline role in particular tissue as various tissues experiences various natures of stresses. There seemed to be a threshold level of salt stress at which proline accumulation become responsible for continued growth or growth maintenance or could be the last resort for survival. The threshold would be lower without Ca^{2+} than with Ca^{2+} supply. It could be seen from Table 1 and 2 that addition of Ca^{2+} in growth medium significantly reduced the Na^+ uptake in roots and their translocation to shoots in both cultivars. This might serve to reduce the stress by increasing the threshold level of salinity at which proline accumulation took place and maintained growth. Without Ca^{2+} addition, more ions were transported to shoots (Table 2) therefore, the shoot tissues were thought to be exposed to higher osmotic stress. The stress might induce proline accumulation in order to lower the water potential of the protoplast and to overcome the dehydration pull from apoplast and vacuole. In case of high Ca^{2+}, a similar disequilibrium would not occur or would induce ion influx, efflux and compartmentation to be in a more regulated fashion.

The responses of cultivars to interactive effects of NaCl and Ca^{2+} in maintaining the K^+ contents in both root and shoot was similar (Table 1 and 2), while a larger increase in shoot Na^+ content of Hitomebore than Bankat (Fig. 2) at 150 mol m^{-3} NaCl without supplemental Ca^{2+} shows genotypic variations between the cultivars. Better ionic regulation would be assumed in Bankat as shown in ionic ratios (Na^+ to K^+ ratio) of tissues (Fig. 2). It was clear from the Fig. 2 that Ca^{2+} improved the ionic homeostasis of stressed seedlings. Supplemental calcium under salt stress is generally known to maintain the stability, integrity and function of plasma membrane and tonoplast to internally distribute ions at the level compatible with the requirements of plants metabolic processes (Cramer ***et al.***, 1985; Lynch ***et al.***, 1987; Lauchli, 1989; Nakamura ***et al.***, 1992; Colmer ***et al.***, 1994; Lin ***et al.***, 1997 and Ballesteros ***et al.***, 1997).

When the data were further explored to investigate, whether proline level of roots played a role in Na^+ uptake and translocation under stress, a strong negative correlation (r=-0.728 **, n=10) was observed in the relationship between shoot Na^+ and root proline. This suggested that the increased proline concentration in root under salt stress partially inhibit the transport of Na^+ ion from root to shoot, in addition to being a compatible cytosolute. Similar findings had been reported by Lone ***et al.*** (1988) in cultured barley embryos grown on exogenous application of proline.

It appeared that the response of shoots in accumulating proline under NaCl stress was different from that of roots. This might possibly be due to the nature of stress organ experiences. This showed that the shoot, an aerial part of plant and responds to stress in accordance with the reception of signals or accumulation of stress agents from the roots. While roots, the organs which had in direct contact with ambient salinity and proline might had different function in different organs at different salinity levels. The accumulated proline had a role in osmoprotection and osmotic adjustment which had been related with the compartmentation of ions in plant tissues or the ratio of subcellular ionic concentration between apoplast - cytoplasm and vacuole (Bar-Nun and Poljakoff-Mayber, 1977).

All adaptive mechanisms (water uptake, selective ion uptake, translocation and confinement of toxic ions into the vacuole and biosynthesis of cytosolutes e.g. proline) of stressed plants were energy dependent. Therefore, growth retardation of non-halophytic stressed plant in comparison with the non-stressed plants should be the consequence of stress. But our results revealed that beyond the threshold level of NaCl, high level of proline accumulation in root tissue correlate positively with growth and supplemental calcium enhanced proline accumulation. Weinberg ***et al.*** (1984) also reported a similar relationship between growth and proline at high external salinity in ***Sorghum bicolor***.

Thus, it is concluded that in rice seedlings supplemental Ca^{2+} not only overcome the toxicity components of NaCl stress but also mitigate the osmotic components of NaCl stress by enhancing proline accumulation. Proline has the potential to be used as determinant of salt tolerance in rice.

References

Ahmad, I., S.J. Wainwright and G.R. Stewart, 1981. The solute and water relations of ***Agrostis stolonifera*** ecotypes differing in salt tolerance. New Phytol., 87: 615-629.

Aspinall, D. and L.G. Paleg, 1981. Proline accumulation: physiological aspects, In: L.G. Paleg and and D. Aspinall, (Ed.), The Physiology and Biochemistry of Drought Resistance in Plants, pp: 206-240.

Ballesteros, E., E. Blumwald, J.P. Donaire and A. Belver, 1997. Na^+/H^+ antiport activity in tonoplast vesicles isolated from sunflower roots induced by NaCl. Physiol. Plant, 99: 328-334.

Bar-Nun, N. and A. Poljakoff- Mayber, 1977. Salinity stress and the content of prole in roots of ***Pisum sativum*** and ***Tamarix tetragyna***. Ann. Bot., 41: 173-179.

Bates, L.S., R.P. Waldren and I.D. Tear, 1973. Rapid determination of free proline for water stress studies. Plant and Soil, 39: 205-207.

Chowdhury, M.T., S.J.Wainwright and M.J. Merrett, 1996. Comparative NaCl tolerance of Lucern plants regenerated from salt-selected suspension cultures. Plant Sci., 114: 221-232.

Colmer, T.D., T.W.M. Fan, R.H. Higashi and A. Lauchli, 1996. Interactive effects of Ca^{2+} and NaCl salinity on ionic relations and proline accumulation in the primary root tip of Sorghum bicolor. Physiol. Plant, 97: 421-424.

Colmer, C.D., T.W.M. Fan, R.H. Higashi and A. Lauchli, 1994. Interaction of Ca^{2+} and NaCl stress on the ion relations and intracellular pH of ***Sorghum bicolor*** root tips: an in vivo 31 P-NMR study. J. Exp. Bot., 45: 1037-1044.

Cramer, G.R., A. Lauchli and V.S. Polito, 1985. Displacement of Ca^{2+} by Na^{+} from the plasmalemma of root cells. A primary response to salt stress. Plant Physiol., 79: 207-211.

Cramer, G.R., A. Lauchli and E. Epstein, 1986. Effects of NaCl and $CaCl_2$ on ion in complex nutrient solutions and root growth of cotton. Plant Physiol., 81: 792-797

Dubey, R.S. and M. Rani, 1989. Influence of NaCl salinity an growth and metabolic status of protein and amino acids in rice seedlings. J. Agron. and Crop Sci., 162: 97-106.

Greenway, H. and R. Munns, 1980. Mechanisms of salt tolerance in nonhalophytes. Annu. Rev. Plant Physiol., 31: 149-190.

Hanson, A.D., C.E. Nelsen and E.H. Everson, 1977. Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. Crop Sci., 17: 720-726.

Heuer, B., 1994. Osmoregulatory role of proline in water and salt stressed plants, In M. Pessarakli (Ed.) Hand Book of Plant and Crop Stress, pp: 363-381.

LaHaye, P.A. and E. Epstein, 1969. Salt toleration by plants. Enhancement by calcium. Sci., 166: 395-396.

Lauchli, A., T.D. Colmer, T.W.M. Fan and R.M. Higashi, 1994. Solute regulation by calcium in salt stressed plants,. In J.H. Cherry (Ed.) Biochemical and Cellular. Mechanisms of Stress Tolerance in Plants, pp: 443-461.

Lauchli, A. and S. Schubert, 1989. The role of calcium in the regulation of membrane and cellular growth processes under salt stress. In J. H. Cherry (Ed.), Environmental stresses in plants Biochemical and Physiological Mechanisms, pp: 131-138.

Lin, H., S.S. Salus and K.S. Schumaker, 1997. Salt sensitivity and the activities of the H^{+}ATPases in cotton seedlings. Crop Sci., 37: 190-197.

Lin, C.C. and C.H. Kao, 1996. Proline accumulation is associated with inhibition of rice seedlings root growth caused by NaCl. Plant Sci., 114: 121-128.

Lin, C.C. and C.H. Kao, 1995. NaCl stress in rice seedlings: the influence of calcium on root growth. Bot. Bull. Acad. Sin., 36: 41-45.

Lone, M.I., J.S.H. Kueth, R.G. Wyn Jones and S.W.J. Bright, 1988. Influence of proline and glycinebetaine on salt tolerance of cultured barley embryos. J. Exp. Bot., 38: 479-490.

Lutts, S. and G. Guerrier, 1995. Peroxidase activities of two rice cultivars differing in salinity tolerance as affected by proline and NaCl. Biol. Plant, 37: 577-586.

Lynch, J., G.R. Cramer and A.Lauchli, 1987. Salinity reduces membrane associated calcium in corn root protoplasts. Plant Physiol., 83: 390-394.

Mae, T., 1993. Laboratory scale culture of rice. Plant Culture method for experiment 3: Plant Cell Tech., 5: 63-67.

Misra, A.N., B. Murmu, P. Singh and M. Misra, 1996. Growth and proline accumulation in mung bean seedlings as affected by sodium chloride. Biol. Plant, 38: 531-536.

Nakamura, Y., K. Kasamo, N. Shimosato, M. Sakata and E. Ohta, 1992. Stimulation of the extrusion of protons and H^{+}-ATPase activities with the decline in pyrophosphatase activity of the tonoplast in intact mung bean roots under high-NaCl stress and its relation to external levels of Ca^{2+} ions. Plant Cell Physiol., 33: 139-149.

Perez-Alfocea, F., M.T. Estan, M. Caro and G. Guerrier, 1993. Osmotic adjustment of Lycopersicon esculentum and L. Pennellii under NaCl and polyethylene glycol 6000 iso osmotic stresses. Physiol. Plant, 87: 493-498.

Poljakoff-Mayber, A. and H.R Lerner, 1994. Plants in saline environments In M. Pessarakli (Ed.) Hand Book of Plant and Crop Stress, pp: 65-96.

Roy, D., A. Bhunia, N. Basu and S.K. Banerjee, 1992. Effect of NaCl salinity on metabolism of proline in salt sensitive and salt resistant cultivars of rice. Biol. Plant., 34: 159-162.

Shah, S.H., S. Tobita and M. Shono, 2001. Supplemental calcium regulates proline accumulation in NaCl-stressed suspension culture of ***Oryza sativa*** L. at the level of mRNA translation. Pak. J. Biol. Sci., 4: 707-710.

Shah, S.H., S.J. Wainwright and M.J. Merrett, 1990. The interaction of sodium and calcium chlorides and light on growth, potassium nutrition and proline accumulation in callus cultures of ***Medicago sativa*** L. New Phytol., 116: 37-45.

Van Heerden, P.D.R. and O.T. De Villier, 1996. Evaluation of proline accumulation as an indicator of drought tolerance in spring wheat cultivars. S. Afr. J. Plant Soil, 13: 17-21.

Weingberg, R., H.R. Lerner and A.Poljakoff-Mayber, 1984. Change in growth and water soluble solute concentration in sorghum bicolor stressed with sodium chloride and potassium salts. Physiol. Plant, 62: 472-480.

Wyn Jones, R.G., 1985. Salt Tolerance in plants. Chemistry in Britain, pp: 454-459.

Yeo, A.R., 1994. Physiological criteria in screening and breeding In A.R. Yeo Soil Mineral Stresses, Springer-Verlage Berlin Heidelberg, pp: 37-59.

Zhong, H. and A. Lauchli, 1994. Spatial distibution of solutes, K, Na, Ca and their deposition rates in the growth zone of primary cotton roots: effects of NaCl and $CaCl_2$. Planta, 194: 34-41.