Maejo Int. J. Sci. Technol. **2011**, *5*(01), 159-168

Maejo International Journal of Science and Technology

ISSN 1905-7873
Available online at www.mijst.mju.ac.th

Communication

Salinity triggers proline synthesis in peanut leaves

Md Alamgir Hossain [1,2], Md Ashrafuzzaman [2,*] and Mohd Razi Ismail [2]

[1] Department of Crop Botany, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[2] Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
***** Corresponding author, e-mail: drashraf2007@yahoo.com

Received: 28 January 2010 / Accepted: 21 April 2011 / Published: 21 April 2011

Abstract: To investigate the magnitude of proline accumulation under different salinity levels, a salinity-imposed experiment was conducted. The peanut seedlings were cultured hydroponically for 7 days before imposing salinity and then grown further for another 7 days under salinity-loaded condition. Results showed that shoot dry matter, relative water content, chlorophyll and K^+ decreased significantly with increasing salinity. In contrast, Na^+, hydrogen peroxide and proline increased with increasing salinity level. Free proline content increased abruptly at medium and high salinity levels. Interestingly, the lowest level of dry matter (an indicator of tolerance/sensitivity) accompanied by the highest accumulation of proline at 200 mM NaCl puts a question mark on the well-documented role of proline in relation to salinity tolerance. The underlying mechanism is discussed in detail in the current study.

Keywords: salinity, proline biosynthesis, peanut leaves

INTRODUCTION

Salinity is one of the most important environmental stresses which severely limits plant growth and productivity worldwide [1-2]. The problem is ever increasing because of irrational human acts causing secondary salinisation [3] and also because of global warming with consequent rise in sea level and increase in tidal surges, particularly in coastal areas [4]. In Bangladesh, more than 1 million hectares of the coastal areas have been seriously affected by salinity [5], which is considered as one of the major problems of crop production in Bangladesh.

Salinity exerts its undesirable effects through osmotic inhibition, ionic toxicity and also by disturbing the uptake and translocation of nutritional ions [6]. Now it is widely accepted that abscisic-acid-mediated root signals limit the availability of water to the plant cells, which leads to

Maejo Int. J. Sci. Technol. **2011**, *5*(01), 159-168

slower plant growth during salinity stress [7-9]. This is known as the 'osmotic effect' of salinity [10]. This effect can disturb the physiological and biochemical functions of the plant cells, leading finally to cell death [11]. So osmotic balance is certainly crucial for the survival of a plant under salinity-stressed condition. Under various environmental stresses, plant cells have experienced the accumulation of some organic solutes such as sucrose [12], glycinebetaine [13], mannitol [14], trehalose [15-16] and proline [17-18], and these organic solutes contribute to the maintenance of turgor. Plants under salinity stress also accumulate a number of metabolites, which are termed compatible solutes because they do not interfere with the plant's metabolism even at molar concentrations [19]**.** The accumulation of such compatible osmolytes involved in osmoregulation allows additional water to be taken up from the environment, thus buffering the immediate effect of water shortage within the plant [20].

Among the accumulation of compatible osmolytes, that of proline is one of the most frequently reported modifications induced by water deficit as well as salinity stress in plants. Several functions are proposed for the accumulation of proline in tissues exposed to salinity stress: osmotic adjustment [21], C and N reserves for growth after stress relief [22-23], detoxification of excess ammonia [24], stabilisation of proteins and membranes [25], protection of macromolecules from denaturation [26], osmoprotection [27], free radical scavenging [28], antioxidation [29] and regulation of cytosolic acidity [30]. In addition, proline biosynthesis may be associated with the production of $NADP^+$ for the stimulation of the pentose phosphate pathway [31]. Now it is well documented that proline plays a predominant role in protecting plants from osmotic stress. In the present study, we aim to investigate the magnitude of proline accumulation under different levels of salinity in peanut seedlings.

MATERIALS AND METHODS

Plant Material and Treatments

Seeds of groundnut (*Arachis hypogea* L., genotype Dhaka-1) were obtained from the plant breeding division of Bangladesh Institute of Nuclear Agriculture (BINA). The seeds were surface sterilised with ethanol for 3 minutes. The surface-sterilised seeds were rinsed thoroughly with distilled water and placed on water-soaked filter paper at 25^{o}C to germinate. After germination, healthy and vigorous seedlings with uniform roots were selected and transferred to perforated plastic sieves, each of which contained 4 seedlings. The seedlings were grown hydroponically in modified half-strength Hoagland solution [5] at 25^{o}C and 60% relative humidity with a photoperiod of 16 hr (700 μmol m^{-2} s^{-1}). The seedlings were cultured for 7 days at pH 6.5 in the growth chamber under the control environment as described by Rahman et al [5]. The pH of the nutrient solution was adjusted to 6.5 using H_2SO_4 and NaOH. A salinity-imposed experiment was then conducted in full-strength Hoagland solution supplemented with 4 concentrations of salt (0, 50, 100 and 200 mM of NaCl) for 7 days. The experimental containers were laid out in completely randomised design (CRD) with 4 replicates, giving a total of 16 plots. Then the plants were harvested and seedling growth was determined by measuring the length and fresh weight of root and shoot. Plant materials were oven-dried (70^{o}C for 24 hr) and dry weights were recorded.

Maejo Int. J. Sci. Technol. **2011**, *5*(01), 159-168

Relative Water Content

A pre-dawn leaflet sample was taken from three plants for each replicate in each treatment on the 7th day after salinisation and its fresh weight immediately recorded. The leaf sample was then incubated in deionised water for 4 hr as described by Sairam et al. [32], after which the turgid weight of the leaf sample was taken. The leaf sample was then packed in a butter paper bag and oven-dried at 65°C for 48 hr and the dry weight of the sample was taken. The relative water content (RWC) was estimated as follows:

$$\text{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \text{ x } 100$$

Proline Determination

Free proline was determined according to the method of Bates et al. [33] with slight modification. Briefly, fresh leaf sample (500 mg) was homogenised in 5 ml of 3% sulphosalicylic acid by a mortar and pestle and then centrifuged at 18000 g for 10 min to remove cell debris. The resulting extract (2 ml) was taken in a test-tube and glacial acetic acid (2 ml) and ninhydrin reagent (2 ml) were added. The reaction mixture was boiled in a water bath for 60 min. After cooling of the tube in ice, toluene (6 ml) was added and mixed thoroughly. Then the upper toluene phase was separated into a glass cuvette and free proline was quantified spectrophotometrically at 520 nm. Proline concentration was calculated from proline standard (0-50 μg/ml) treated in an identical manner.

H_2O_2 Determination

H_2O_2 concentration in the leaves of peanut genotype was measured spectrophotometrically as described by Alexieva et al. [34]. Freshly harvested leaves (0.5 g) were crushed into a fine powder in a mortar under liquid N_2. Then it was centrifuged at 12000 g for 10 min at 4°C and the supernatant was collected for determination of H_2O_2. The reaction mixture consisted of 0.5 ml of 0.1% trichloroacetic acid (TCA) containing the leaf extract supernatant, 0.5 ml of 100 mM potassium phosphate buffer and 2 ml of KI reagent (1 M KI in distilled H_2O). The blank consisted of 0.1% TCA instead of the leaf extract. The reaction was allowed to run for 1 hr in the dark and the absorbance was measured at 390 nm. The amount of H_2O_2 was calculated using a standard graph of known concentrations.

Chlorophyll Determination

Fully expanded leaves (3rd and 4th from the top, photosynthetically active) were sampled for chlorophyll determination. Fresh leaf samples (0.5g) were homogenised in cold 80% acetone (4 ml) in a cold mortar and centrifuged at 3000 rpm for 10 min at 25°C. Then the supernatant was collected and the volume made up to 20 ml with 80% acetone. The absorbance reading was taken at 645 nm with a UV-VIS spectrophotometer and the chlorophyll content expressed as mg ml^{-1} [35].

Maejo Int. J. Sci. Technol. **2011**, *5*(01), 159-168

Na^+ and K^+ Content

About 0.3 g dry and ground leaves was placed in a digestion tube and 2.5 ml of digestion mixture (H_2SO_4 + $HClO_4$) was added [5]. After mixing, the tube was allowed to stand for 2 hr, then placed in a heating block and heated for 2 hr at 100°C. After cooling, three 1-ml aliquots of 30% H_2O_2 were added, the content of the tube being thoroughly mixed after each addition. The tube was then placed in an aluminium block and heated to 330°C (just below the boiling point of the digestion mixture) for about 2 hr. The cooled, clear, digested mixture was diluted to 20 ml with deionised water and filtered, and aliquots were taken for analysis. Na^+ and K^+ ion concentrations in the extract were estimated by flame photometry and expressed as % dry matter (DM).

Statistical Analysis

All data were subjected to analysis of variance by CRD method and data were expressed as mean ± SE obtained from four independent experiments. Duncan's multiple range test was applied to compare the treatment means. P value ≤ 0.05 was considered as significant.

RESULTS

Figure 1 shows a gradual decrease in shoot DM with increasing salt concentration. High salinity stress (200 mM NaCl) resulted in 50% decrease in shoot DM compared to control. RWC in the peanut leaves was also found to decrease with increasing salt concentration (Figure 2a). At 200 mM salinity a decrease of 13% compared to control can be observed.

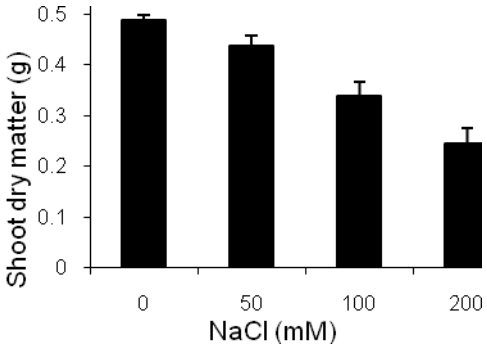

Figure 1. Effect of salinity on shoot dry matter in peanut seedlings. Vertical bars indicate means ± S.E. (n=4).

From Figure 2b, the free proline content in peanut leaves can be observed to dramatically increase with increasing salinity: a 2.5-, 10- and 18-fold increase in proline accumulation at 50, 100 and 200 mM NaCl respectively. The free proline content increased slowly at low salinity and rapidly at medium and high salinity. In response to salinity, H_2O_2 content also increased significantly with the level of salinity (Figure 2c). It increased more than threefold when the young plants were exposed to 200 mM NaCl. Conversely, the total chlorophyll content in the leaves decreased 50% from control at 200 mM NaCl (Figure 2d), indicating that the salt present in the culture solution might be involved in the damage of the chloroplasts.

When peanut seedlings were grown on hydroponic culture solutions containing various concentrations of NaCl, the sodium concentration in the leaves was always higher than that in the

Maejo Int. J. Sci. Technol. **2011**, *5*(01), 159-168

control (Table 1) and was found to increase with increasing salinity level. A reverse trend was observed for K^+ concentration. As a consequence, the K^+/Na^+ ratio decreased with increasing concentration of NaCl in the culture solution.

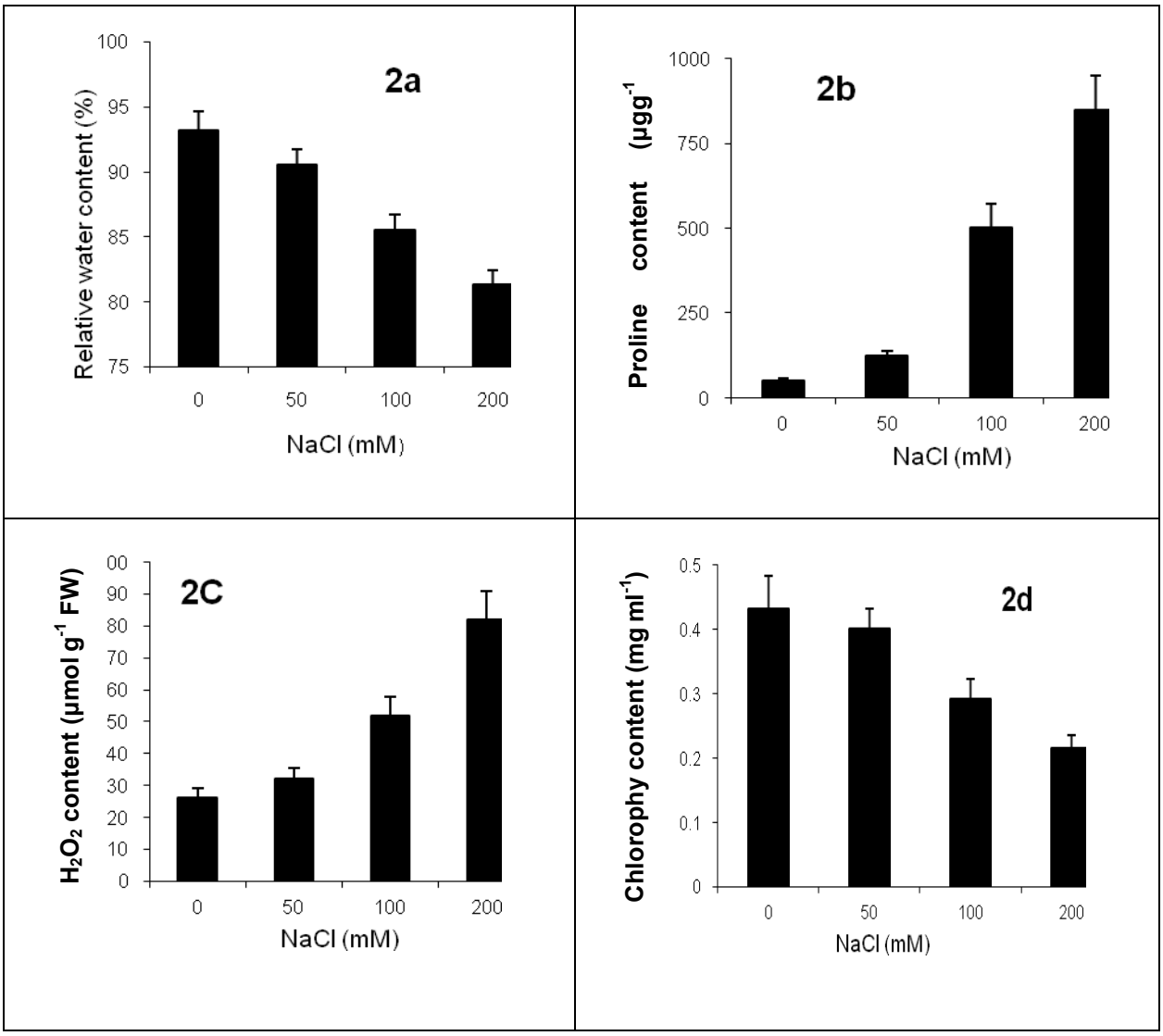

Figure 2. Effect of salinity on (a) RWC (b) proline content, (c) H_2O_2 content and (d) chlorophyll content in peanut seedlings. Vertical bars indicate means ± S.E. (n=4).

Table 1. Sodium and potassium content (% of DM) in leaves of peanut seedlings. Each value represents the mean ± S.E obtained from four independent experiments; $P \leq 0.05$.

Treatment (mM NaCl)	Na^+ content (% DM)	K^+ content (% DM)
0	0.23 ± 0.01	6.99 ± 0.08
50	0.35 ± 0.03	6.40 ± 0.06
100	9.55 ± 0.08	1.75 ± 0.24
200	13.33 ± 0.06	0.63 ± 0.01

Maejo Int. J. Sci. Technol. **2011**, *5*(01), 159-168

DISCUSSION

Proline accumulation, a common metabolic response of plants subjected to salinity stress, is considered to be involved in stress-tolerance mechanisms [20]. In the present study, a significant increase in proline content was found in peanut seedlings after 7 days of exposure to NaCl stress (Figure 2b). The results also revealed that the magnitude of proline accumulation was positively (R^2=0.98, P≤0.05) associated with the concentration of NaCl in the culture solution. These results are consistent with the findings of some earlier studies [36-37]. One distinctive feature of most plants growing in saline environments is the accumulation of proline [1] and it has been inferred that there may be a relationship between cellular proline level and cell turgidity via osmotic adjustment [23, 38]. Interestingly, such beneficial effect of elevated proline level was not reflected in the maintenance of relative water content in peanut leaves in the present study. The results could be interpreted as follows. Firstly, salinity stress limits the uptake of CO_2 [8-9], resulting in decreasing carbon reduction by Calvin cycle [39], which leads to non-availability of $NADP^+$ for acceptance of electrons during photosynthesis. In this situation, photosynthetic reducing power, $NADPH_2$, is used for proline biosynthesis and consequently $NADP^+$ is regenerated [40]. These reactions are summarised in Figure 3. The excess accumulation of proline may therefore be a result of metabolic changes induced by high salinity. The present results also agrees with the observations of Delauney and Verma [41], who stated that excess proline accumulation in response to high salinity functions by other than osmotic adjustment.

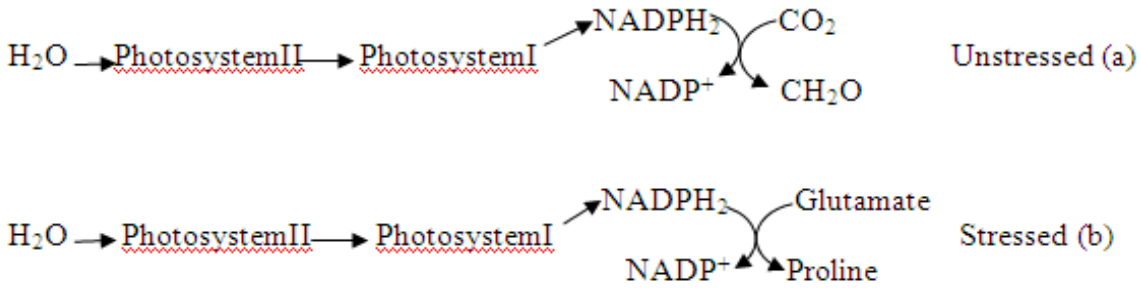

Figure 3. Schematic illustration of electron flow in : (a) normal physiological condition and (b) high-salinity stress condition, which can lead to inhibition of photosynthesis by diverting the flow of photoreductant (e^-) from the CO_2 reducing system to proline biosynthesis, leading to reduced growth and productivity of the plants.

The low shoot DM (an indicator of salt sensitivity) along with the high proline content also suggests that the increase in proline concentration may not be associated with salinity tolerance, which agrees with some previous studies [9, 42]. Thus, the maximal accumulation of proline might have occurred when plants were exposed to excessive salinity which might damage them fatally. Also, other osmolytes other than proline might be involved in leaf water content or cell turgidity. This was also reflected in our current investigation. The concentration of inorganic osmoticum (K^+) in the leaf tissues might be associated (R^2=0.97, P≤0.05) with relative water content in the leaves (Figure 2a) and actively contribute to turgor maintenance of the cells [43]. Further investigation is needed to clarify their relative importance in turgor maintenance.

Maejo Int. J. Sci. Technol. **2011**, *5*(01), 159-168

The low DM yield in high salinity level may be explained by excess accumulation of toxic ions, particularly Na^+ with concomitant reduction of K^+ ions (Table 1), an important osmolyte in the cellular environment. The degree of inhibition of K^+ uptake is associated with salinity levels in the culture solution (Table 1). This is reasonable as high Na^+ concentration negatively affects K^+ acquisition due to similar physicochemical properties of Na^+ and K^+ [44]. Moreover, low DM yield and low chlorophyll content (Figure 2d) in highly salinised leaves might be related to the elevated levels of H_2O_2 (Figure 2c), which was also stimulated by salinity [36], resulting in serious photodamage of chlorophyll [45-46].

Taken all together, it may be concluded that in the present study proline accumulation was a result of photosynthetic impairments or metabolic changes induced by high salinity whereas its function in maintaining cell turgidity/relative water content was not so strong.

REFERENCES

1. P. M. Hasegawa, R. A. Bressan, J. K. Zhu and H. J. Bohnert, "Plant cellular and molecular responses to high salinity", *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **2000**, *51*, 463-499.
2. M. Tester and R. Davenport, "Na^+ tolerance and Na^+ transport in higher plants", *Ann. Bot.*, **2003**, *91*, 503-527.
3. P. Rengasamy, "World salinization with emphasis on Australia", *J. Exp. Bot.*, **2006**, *57*, 1017-1023.
4. R. Wassmann, N. X. Hien, C. T. Hoan and T. P. Tuong, "Sea level rise affecting the Vietnamese Mekong Delta: water elevation in the flood season and implications for rice production", *Climatic Change*, **2004**, *66*, 89-107.
5. A. M. Rahman, A. K. M. Azad, M. S. A. Fakir and M. A. Hossain, "Effect of calcium chloride on seedling growth in peanut under salinity stress", *Bangladesh J. Crop Sci.*, **2007**, *18*, 373-380.
6. N. Misra and U. N. Dwivedi, "Genotypic difference in salinity tolerance of green gram cultivars", *Plant Sci.*, **2004**, *166*, 1135-1142.
7. Y. L. Li, L. F. M. Marcelis and C. Stanghellini, "Plant water relations as affected by osmotic potential of the nutrient solution and potential transpiration in tomato (*Lycopersicon esculentum* Mill.)", *J. Hort. Sci. Biotechnol.*, **2004**, *79*, 211-218.
8. J. Zhang, W. Jia, J. Yang and A. M. Ismail, "Role of ABA in integrating plant responses to drought and salt stress", *Field Crop Res.*, **2006**, *97*, 111-119.
9. R. A. James, A. R. Rivelli, R. Munns and S. von Caemmerer, "Factors affecting CO_2 assimilation, leaf injury and growth in salt-stressed durum wheat", *Func. Plant Biol.*, **2002**, *29*, 1393-1403.
10. R. Munns, R. A. James and A. Lauchli, "Approaches to increasing the salt tolerance of wheat and other cereals". *J. Exp. Bot.*, **2006**, *57*, 1025-1043.
11. L. Xiong and J. K. Zhu, "Molecular and genetic aspects of plant responses to osmotic stress", *Plant Cell Environ.*, **2002**, *25*, 131-139.
12. Y. C. Hu, H. Schnyder and U. Schmidhalter, "Carbohydrate deposition and partitioning in elongating leaves of wheat under saline soil conditions", *Aust. J. Plant Physiol.*, **2000**, *27*, 363-370.

13. T. H. Chen and N. Murata, "Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and others compatible solutes", *Curr. Opin. Plant Biol.*, **2002**, *5*, 250-257.
14. T. Abebe, A. C. Guenzi, B. Martin and J. C. Cushman, "Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity", *Plant Physiol.*, **2003**, *131*, 1748-1755.
15. H. J. Bohnert and R. G. Jensen, "Strategies for engineering water stress tolerance in plants", *Trends Biotechnol.*, **1996**, *14*, 89-97.
16. A. K. Garg, J. K. Kim, T. G. Owens, A. P. Ranwala, Y. D. Choi, L. V. Kochian and R. J. Wu, "Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses", *Proc. Natl. Acad. Sci.-USA*, **2002**, *99*,15898-15903.
17. N. Misra and A. K. Gupta, "Effect of salt stress on proline metabolism in two high yielding genotypes of green gram", *Plant Sci.*, **2005**, *169*, 331-339.
18. J. Matysik, Alia, B. Bhalu and P. Mohanty, "Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants", *Curr. Sci.*, **2002**, *82*, 525-532.
19. R. Alonso, S. Elvira, F. J. Catillo and B. S. Gimeno, "Interactive effects of ozone and drought stress on pigments and activities of antioxidative enzymes in *Pinis halpensis*", *Plant Cell Environ.*, **2001,** *24*, 905-916.
20. T. Nanjo, M. Kobayashi, Y. Yoshiba, Y. Sanada, K. Wada, H. Tsukaya, Y. Kakubari, K. Yamaguchi-Shinozaki and K. Shinozaki, "Biological functions of proline in morphogenesis and osmotolerance revealed in antisense transgenic *Arabidopsis thaliana*", *Plant J.*, **1999**, *18,*185-193.
21. G. S. Voetberg and R. E. Sharp, "Growth of the maize primary root at low water potentials. III. Role of increased proline deposition in osmotic adjustment", *Plant Physiol.*, **1991**, *96*, 1125-1130.
22. H. Hellmann, D. Funk, D. Rentsch and W. B. Frommer, "Hypersensitivity of an arabidopsis sugar signaling mutant toward exogenous proline application", *Plant Physiol.*, **2000**, *122*, 357-368.
23. F. Hayashi, T. Ichino, M. Osanai and K. Wada, "Oscillation and regulation of proline content by P5CS and ProDH gene expressions in the light/dark cycles in *Arabidopsis thaliana* L.", *Plant Cell Physiol.*, **2000**, *41*, 1096-1101.
24. D. S. Skopelitis, N. V. Paranychianakis, K. A. Paschalidis, E. D. Pliakonis, I. D. Delis, D. I. Yakoumakis, A. Kouvarakis, A. K. Papadakis, E. G. Stephanou and K. A. Roubelakis-Angelakis, "Abiotic stress generates ROS that signal expression of anionic glutamate dehydrogenases to form glutamate for proline synthesis in tobacco and grapevine", *Plant Cell.*, **2006**, *18*, 2767-2781.
25. M. M. F. Mansour, "Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against NaCl stress", *Plant Physiol. Biochem.*, **1998**, *36*, 767-772.
26. E. W. Hamilton III and S. A. Heckathorn, "Mitochondrial adaptations to NaCl. Complex I is protected by anti-oxidants and small heat shock proteins, whereas complex II is protected by proline and betaine", *Plant Physiol.*, **2001**, *126*, 1266-1274.
27. P. B. K. Kishor, Z. Hong, G. H. Miao, C. A. A. Hu and D. P. S. Verma "Overexpression of Δ^1-pyrroline-5-carboxylate synthetase increase proline production and confers osmotolerance in transgenic plants", *Plant Physiol.*, **1995**, *108*, 1387-1394.

28. C. Chen and M. B. Dickman, "Proline suppresses apoptosis in the fungal pathogen *Colletotrichum trifolii*", *Proc. Natl. Acad Sci. USA*, **2005,** *102*, 3459-3464.
29. M. A. Hoque, M. N. Banu, E. Okuma, K. Amako, Y. Nakamura, Y. Shimoishi and Y. Murata, "Exogenous proline and glycinebetaine increase NaCl-induced ascorbate-glutathione cycle enzyme activities, and proline improves salt tolerance more than glycinebetaine in tobacco Bright Yellow-2 suspension-cultured cells", *J. Plant Physiol.*, **2007**, *164*, 1457-1468.
30. P. Sivakumar, P. Sharmila and P. P. Saradhi, "Proline alleviates salt-stress-induced enhancement in Ribulose-1,5-bisphosphate oxygenase activity", *Biochem. Biophys. Res. Commun.*, **2000**, *279*, 512-515.
31. S. Lutts, V. Majerus and J.-M. Kinet, "NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings", *Physiol. Plant.*, **1999**, *105*, 450-458.
32. R. K. Sairam, K. V. Rao and G. C. Srivastava, "Differential response of wheat genotypes to long term salinity stress relation to oxidative stress, antioxidant activity and osmolyte concentration", *Plant Sci.*, **2002**, *163*, 1037-1046.
33. L. S. Bates, R. P. Waldren and I. D. Teare, "Rapid determination of free proline for water-stress studies", *Plant Soil*, **1973**, *39*, 205-207.
34. V. Alexieva, I. Sergiev, S, Mapelli and E. Karanov, "The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat", *Plant Cell Environ.*, **2001**, *24*, 1337-1344.
35. D. I. Arnon, "Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*", *Plant Physiol.*, **1949**, *24*, 1-15.
36. K. Sumithra, P. P. Jutur, B. D. Carmel and A. R. Reddy, "Salinity-induced changes in two cultivars of *Vigna radiata*: Responses of antioxidative and proline metabolism", *Plant Growth Regul.*, **2006,** *50*, 11-22.
37. S. Yokota, "Relationship between salt tolerance and proline accumulation in Australia acacia species", *J. For. Res.*, **2003,** *8*, 89-93.
38. S. G. Kumara, A. M. Reddy and C. Sudhakar, "NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance", *Plant Sci.*, **2003**, *165*, 1245-1251.
39. D. W. Lawlor and G. Cornic, "Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants", *Plant Cell Environ.*, **2002**, *25*, 275-294.
40. Z. Q.Wang, Y. Z. Yung, J. Q. Ou, Q. H. Lin and C. F. Zhang, "Glutamine synthetase and glutamate dehydrogenase contribute differentially to proline accumulation in leaves of wheat (*Triticum aestivum*) seedlings exposed to different salinity", *J. Plant Physiol.*, **2007**, *164*, 695-701.
41. A. J. Delauney and D. P. S. Verma, "Proline biosynthesis and osmoregulation in plants", *Plant J.*, **1993**, *4*, 215-223.
42. F. Moradi and A. M. Ismail, "Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice", *Ann. Bot.*, **2007**, *99*, 1161-1173.
43. S. Shabala, "Regulation of potassium transport in leaves: From molecular to tissue level", *Ann. Bot.*, **2003**, *92*, 627-634.

Maejo Int. J. Sci. Technol. **2011**, *5*(01), 159-168

44. Y. Murata, I. Obi, M. Yoshihashi, T. Ikeda and T. Kakutani, "Salt adaptation of K^+ channels in the plasma membrane of tobacco cells in suspension culture", *Plant Cell Physiol.*, **1994**, *35*, 637-644.
45. K. Asada, "The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons", *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **1999**, *50*, 601-639.
46. A. R. Yeo and T. J. Flowers, "Varietal differences in the toxicity of sodium ions in rice leaves", *Physiol. Plant.*, **1983**, *59*, 189-195.