Agricultural Journal 5 (1): 19-24, 2010
ISSN: 1816-9155

Comparative Studies in Salinity Tolerance Between New Zealand Spinach (*Tetragonia tetragonioides*) and Chard (*Beta vulgaris*) to Salt Stress

Basim S. Yousif, Li Yun Liu, Nguyen T. Nguyen, Yosikuni Masaoka and Hirofumi Saneoka
Graduate School of Biosphere Science, Hiroshima University,
1-4-4 Kagamiyama, Higashi-Hiroshima, 739-8528, Japan

Abstract: New Zealand spinach (*Tetragonia tetragonioides*) is widely cultivated throughout the world for use as a vegetable, ground cover and medicinal plant. In this study, New Zealand spinach and Chard (*Beta vulgaris*) were subjected to 0, 50, 100 and 200 mM NaCl for 14 days and tested for differences in salt tolerance. The growth of Chard was markedly inhibited by NaCl treatment, whereas, in New Zealand spinach, plant growth was increased at a low NaCl concentration (50 mM) and slightly decreased with increasing salinity. The leaf water potential and osmotic adjustment in New Zealand spinach was higher than that in Chard under salt stress. The proline content of New Zealand spinach rose with increasing salinity and the accumulation of proline was higher than in Chard on most of the salt stress treatments. Salt stress induced Na accumulation in the leaves of both species but the accumulation was lower in New Zealand spinach than in Chard. The potassium content of both species decreased with increasing salinity but the K content of New Zealand spinach was higher than in Chard. The lipid peroxidation (as measured Malondialdehyde MDA) content of Chard was significantly higher than that in New Zealand spinach at a higher salinity. The photosynthetic rate was decreased by salt stress, but that in New Zealand spinach was maintained at a higher level compared with Chard. These results indicated that New Zealand spinach is more salt tolerant than Chard.

Key words: *Beta vulgaris*, osmotic adjustment, lipid peroxidation, proline, salt tolerance, *Tetragonia tetragonioides*

INTRODUCTION

Agricultural productivity is severely affected by soil salinity and the damaging effects of salt accumulation in agricultural soils have influenced both ancient and modern civilizations (Munns, 2002; Chinnusamy *et al*., 2005). Under salt stress, plants have evolved complex mechanisms allowing them to adapt to osmotic and ionic stress caused by high salinity (Greenway and Munns, 1980; Hasegawa *et al*., 2000). These mechanisms include osmotic adjustment through the accumulation of compatible solutes such as proline and soluble sugar (Yancy, 2005) and lowering the toxic concentration of ions in the cytoplasm by the restriction of Na^+ influx or its sequestration into vacuoles and/or extrusion (Blumwald, 2000). The contributory role of proline to osmotic adjustment was reported by many researchers (Melony *et al*., 2001; Tani and Sasakawa, 2006; Ashraf and Foolad, 2007; Lee *et al*., 2008). Proline has also been considered as a carbon and nitrogen source for growth, a stabilizer for membranes and some macromolecules and also a free radical scavenger under stress conditions (Lutts and Guerrier, 1995; Mansour, 1998; Molinari *et al*., 2007). It is well-known that the free radical-induced peroxidation of lipid membranes is a reflection of stress induced damage at the cellular level (Parida and Das, 2005).

Therefore, the level of MDA, produced during the peroxidation of membrane lipids, is often used as an indicator of oxidative damage. Mandhania *et al*. (2006) reported that a salt-sensitive wheat (*Triticum aestivum*) line suffered greater damage to cellular membranes due to lipid peroxidation, as indicated by the higher accumulation of H_2O_2 and MDA compared to a salt-tolerant line. New Zealand spinach (*Tetragonia tetragonioides* (Pall.) is a member of Tetragoniaceae and is distributed widely from tropical and subtropical to temperate areas (Wilson *et al*., 2000; Matraszek, 2008). This plant is used as a vegetable, ground-cover/ornamental and medicinal source, as anti-ulcerogenic and anti-inflammatory activities have been reported indicated in compounds isolated from it (Kato *et al*., 1985); however, little information is available

Corresponding Author: Hirofumi Saneoka, Graduate School of Biosphere Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima, 739-8528, Japan

on the physiological characteristics of this plant (Wilson *et al*., 2000). Chard (*Beta vulgaris* L.) is a glycophytic member of Chenopodiaceae, is distributed all over the world and is used as a green vegetable. Chard showed a marked osmotic adjustment and accumulation of proline and inorganic ions under salt stress (Ghoulam *et al*., 2002).

The objectives of the present investigation were to study the effects of salinity stress on the accumulation of Na^+, K^+ and proline, osmotic adjustment, water relations, lipid peroxidation and photosynthetic rate in New Zealand spinach in comparison with Chard, in order to understand the adaptation mechanism of New Zealand spinach to salinity.

MATERIALS AND METHODS

Plant growth and stress treatment: This experiment was conducted at the Graduate School of Biosphere Science, Hiroshima University, Hiroshima, Japan. Seeds of New Zealand spinach and Chard were germinated in seedbeds with a soil mixture containing granite regosol soil, perlite and peat moss (2:1:1 v/v/v). Pots were kept under greenhouse conditions with natural light. Plants were irrigated with nutrient solution at each watering using an irrigation system. The basal nutrient solution contained 8.3 mM NO_3-N, 0.8 mM NH_4-N, 0.5 mM P_2O_5, 2.2 mM K_2O, 0.7 mM MgO, 2.1 mM CaO, 11 μM MnO, 5 μM B_2O_3 and 13 μM Fe. At 6 weeks after transplanting, the plants were subjected to three levels of salinity treatment through irrigation with a nutrient solution containing 0, 50, 100 and 200 mM NaCl twice (at 10:00 am and 15:00 pm) every day until water drained from the bottom of the pot. Each treatment was applied to three replicates located randomly in the greenhouse in order to avoid positional effects.

Plant harvest and measurement of growth: At 14 days after treatment, three plants were harvested and each was separated into the leaves and stems, washed gently with tap water for a few minutes, wiped with paper and the Fresh Weight (FW) was measured. The fresh samples were maintained frozen in liquid nitrogen, then freeze dried and the Dry Weight (DW) was measured. The Relative Growth Rate (RGR) was calculated according to Poorter (1989).

Measurement of leaf water relations: The leaf water potential (ψ_l) was measured using the uppermost, fully expanded leaf employing a pressure chamber (Daiki-Rika Instruments, Tokyo, Japan). After the water potential was measured, the leaves were frozen in liquid nitrogen. Leaf tissues were thawed and centrifuged at 3000x g for 25 min to extract the sap. The osmotic potential (ψ_π) of the cell sap was measured using a Wescor 5500 vapor pressure osmometer (Wescor Inc., Logan, UT. USA) and the osmotic potential ($\psi_{\pi(100)}$) at full turgor was calculated by adjusting for the Relative Water Content (RWC), as described by Wilson *et al*. (1979). RWC was measured as described by Saneoka *et al*. (1995). Turgor was calculated by subtracting the corrected osmotic and water potential. Osmotic adjustment was calculated as the difference in osmotic potential at full turgor $\psi_{\pi(100)}$ between salinized and control plants.

Measurement of photosynthesis: The photosynthetic rate was measured for the attached and uppermost, fully expanded leaves using a portable open gas exchange system (Li6400, Li-Cor, Lincoln, NE, USA). The photosynthetic photon flux density was maintained at 1,500 μmol/m^2/sec and the relative humidity was 60%. The temperature of the leaf was 25°C and the ambient CO_2 concentration was 370 μmol mol^{-1}, while measurements were being taken.

Measurement of Na^+, K^+, proline and malondialdehyde concentrations: Freeze-dried samples were ground into fine powder using a vibrating sample mill (Model T1-100, Heiko Co., Ltd., Japan). Weighed powder samples were digested by nitric acid-hydrogen peroxide and the Na^+ and K^+ contents were determined using a flame photometer (ANA 135, Eiko Instruments Inc., Tokyo, Japan). Proline was extracted using methanol and was then measured following the methods of Bates *et al*. (1973). The Malondialdehyde (MDA) content was determined by employing the Thiobarbituric Acid (TBA) reaction, as described by Fu and Hunag (2001) using frozen samples (-80°C). The concentration of MDA was calculated using a coefficient of absorbance (535 nm) of 155 mM cm^{-1}.

Statistical analysis: Data (n = 3) were examined by one-way ANOVA analysis of variance. Multiple comparisons of the means of data between different salinity treatments within the plants were performed using Duncan's test at the 0.05 significance level (all tests were performed with SPSS Version 16.0 for Windows).

RESULTS AND DISCUSSION

The dry weight of Chard significantly decreased with increasing salt stress; however, the growth of New Zealand spinach slightly increased on 50 mM NaCl treatment, was unchanged on 100 mM treatment and then decreased on 200 mM NaCl treatment (Fig. 1a, b). On the

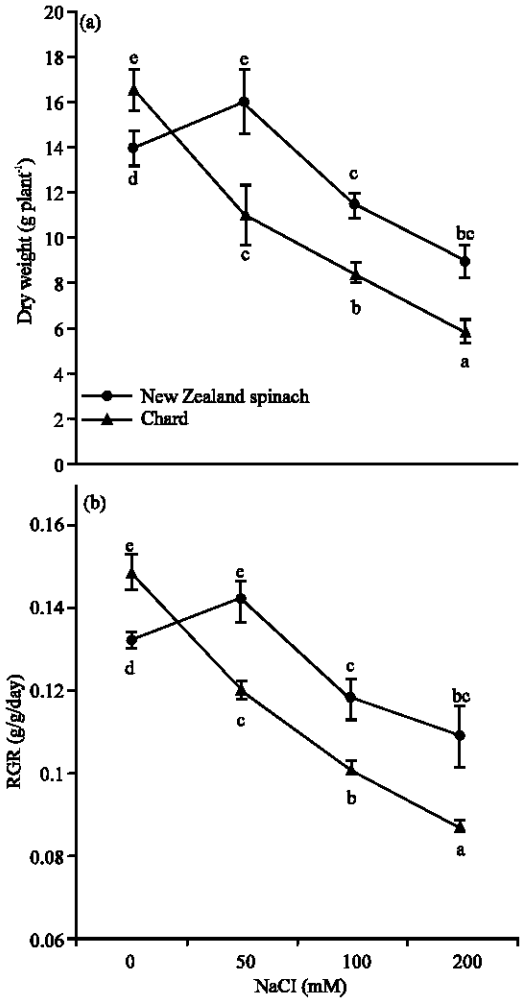

Fig. 1: Effect of salt treatment on the dry weight of shoots (a) and relative growth rate (b) of New Zealand spinach and Chard. The dry weight was measured 14 days after treatment. Values represent means±SE. Bars with different letters significantly differed at $p<0.05$

other hand that of Chard was markedly decreased by increasing salinity and the reduction of the shoot dry weight on 50, 100 and 200 mM NaCl treatments compared to control plants was 33, 49 and 58%, respectively. The Relative Growth Rate (RGR) of Chard also markedly decreased with increasing salinity, whereas the RGR of New Zealand spinach also increased at a low level of salt stress (50 mM NaCl treatment). The growth of non halophytic mesophyte species is generally reduced with increasing salinity. The growth response to salinity in this study is consistent with previous observations on the halophytes *Salicornia europaea* and *Suaeda maritina* (Moghaieb *et al*., 2004) and *Plantago coronopus* (Koyro, 2006), in which the growth of New Zealand

Table 1: The effects of salinity on the Photosynthetic rate (Po), intercellular CO_2 Concentration (Ci), stomatal conductance (gs) and Transpiration rate (Tr) of New Zealand spinach and Chard 14 days after treatment

	Treatment (NaCl mM)			
Effects	0	50	100	200
New Zealand spinach				
Po (μmol $CO_2/m^2/sec$)	31.2 ± 2.6^{d}	21.4 ± 3.5^{c}	13.9 ± 1.1^{b}	8.9 ± 2.5^{a}
Ci (μmol CO_2)	382 ± 1.2^{c}	265.3 ± 3.2^{b}	222.8 ± 0.5^{a}	236.3 ± 0.1^{a}
gs (mol $H_2O/m^2/sec$)	0.5 ± 0.1^{b}	0.4 ± 0.1^{b}	0.2 ± 0.01^{a}	0.1 ± 0.04^{a}
Tr (mol $H_2O/m^2/sec$)	6.9 ± 0.5^{d}	3.7 ± 0.8^{c}	1.8 ± 0.1^{b}	1.2 ± 0.3^{a}
Chard				
Po (μmol $CO_2/m^2/sec$)	23.4 ± 4.5^{a}	10.3 ± 2.1^{c}	6.6 ± 1.9^{b}	5.5 ± 2.4^{a}
Ci (μmol CO_2)	269.4 ± 4.6^{b}	238.3 ± 3.1^{a}	251.6 ± 2.8^{b}	236.0 ± 6.4^{a}
gs (mol $H_2O/m^2/sec$)	0.5 ± 0.2^{c}	0.1 ± 0.03^{b}	0.07 ± 0.04^{a}	0.09 ± 0.04^{ba}
Tr (mol $H_2O/m^2/sec$)	3.8 ± 1.1^{c}	1.3 ± 0.3^{b}	1.01 ± 0.2^{a}	0.9 ± 0.3^{a}

The same letter on each line indicates no significant difference ($p<0.05$)

spinach improved at low-level salinity. These growth response results indicated that New Zealand spinach is a halophyte and so the salt tolerance of this plant was higher than that of Chard.

The photosynthetic rate (Po) of both species was decreased with increasing NaCl concentration (Table 1). The reduction of the Po was lower in New Zealand spinach compared to Chard. The Po in New Zealand spinach was higher than that of Chard on each NaCl treatment. A similar tendency was observed for the stomatal conductance and intercellular CO_2 concentration, although, the impairment of the former by NaCl was more marked than that of the latter. This finding suggested that stomatal closure limited the leaf photosynthetic capacity under saline conditions. On the other hand, the Transpiration rate (Tr) of both species decreased with increasing salinity and the Tr in New Zealand spinach was higher compared to Chard at all of the treatment. New Zealand spinach still maintained transpiration under saline conditions. Based on the results obtained, it is assumed that New Zealand spinach maintained open stomata under saline conditions, which increased transpiration. Consequently, water transpiration through the stomata stimulated the translocation of water through the xylem from the roots. This water flow appeared to be regulated mainly by stomatal opening and might promote the movement of water from the roots to shoots (Moghaieb *et al*., 2006).

The Na^+ content of both species rose with increasing NaCl concentration; however, there was no difference in the Na content between the two species (Table 2). In contrast to the Na^+ content, the K^+ content in the leaves of both species was significantly decreased with increasing salinity. K^+ accumulation in New Zealand spinach was higher than that in Chard. The Na^+/K^+ ratios in the leaves of both species rose with increasing salinity; however, the Na^+/K^+ ratio in the leaves of New Zealand spinach was significantly lower than that of Chard. Na^+

Table 2: The effects of salinity on the Na^+ and K^+ contents and Na^+/K^+ ratio in the leaves of New Zealand spinach and Chard 14 days after treatment

	Treatment (NaCl mM)			
Effects	0	50	100	200
New Zealand spinach				
Na^+ (mg g^{-1} DW)	18.8±2.9^a	109.4±4.8^b	131.5±3.6^c	146.3±3.3^d
K^+ (mg g^{-1} DW)	113.4±9.2^b	96.3±4.4^a	99.5±7.0^a	91.8±4.6^a
Na^+/K^+ ratio	0.17	1.14	1.32	1.59
Chard				
Na^+ (mg g^{-1} DW)	12.8±1.4^a	123.4±3.7^b	132.6±1.3^b	156.3±2.9^c
K^+ (mg g^{-1} DW)	101.2±2.9^c	81.4±8.1^b	80.4±3.4^b	59.2±1.5^a
Na^+/K^+ ratio	0.13	1.52	1.65	2.64

Table 3: The effects of salinity on the leaf water content, leaf water potential (ψ_l), osmotic potential ($\psi_{\pi(100)}$) at full turgor, turgor and Osmotic Adjustment (OA) in New Zealand spinach and Chard 14 days after treatment

	Treatment (NaCl mM)			
Effects	0	50	100	200
New Zealand spinach				
ψ_l (-MPa)	0.51±0.01^a	0.86±0.06^b	1.06±0.10^{eb}	1.23±0.10^c
$\psi_{\pi(100)}$ (-MPa)	0.82±0.02^a	1.53±0.09^b	1.74±0.06^c	2.17±0.03^d
Turgor (MPa)	0.46±0.02^a	1.07±0.10^b	1.33±0.10^b	2.05±0.10^c
RWC	0.87±0.01^c	0.82±0.02^{bc}	0.77±0.02^{ab}	0.72±0.01^a
OA	-	0.71	0.92	1.35
Chard				
ψ_l (-MPa)	0.31±0.01^a	1.82±0.1^b	2.12±0.05^c	2.56±0.01^d
$\psi_{\pi(100)}$ (-MPa)	0.89±0.42^a	1.46±0.09^b	1.51±0.07^c	1.79±0.06^d
Turgor (MPa)	0.76±0.05^a	0.16±0.02^b	0.32±0.07^b	0.63±0.05^a
RWC	0.86±0.01^d	0.77±0.02^c	0.68±0.02^b	0.63±0.01^a
OA	-	0.57	0.62	0.90

The same letter on each line indicates no significant difference ($p<0.05$)

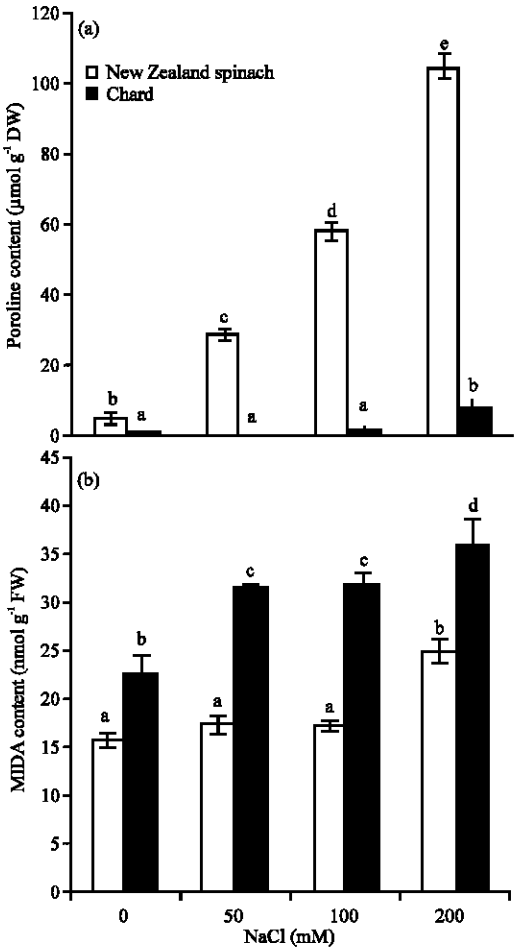

Fig. 2: Proline (a) and malondialdehyde (MDA) (b) contents of the leaves of New Zealand spinach and Chard 14 days after treatment. Values represent means±SE. Bars with different letters significantly differed at $p<0.05$

competes with K^+ for intercellular influx, since these cations are transported by common proteins. Na^+ can damage the plasma membrane and subsequently, increase K^+ efflux from intercellular stores. K^+ is also an essential cofactor for enzymes but Na^+ is not and cannot replace K^+ (Grattan and Grieve, 1999). The present study showed that New Zealand spinach possesses a much more selective mechanism for the uptake of K^+ over Na^+ than Chard under salt stress conditions.

The Relative Water Content (RWC) in both species was decreased with increasing NaCl concentration but the RWC reduction was less in New Zealand spinach than Chard (Table 3). The leaf water potential (ψ_l) in both species was also decreased by salt stress; ψ_l in New Zealand spinach was significantly higher than in Chard on all salt stress treatments. The leaf turgor was increased by salinity, but that of Chard was markedly decreased. The osmotic potential ($\psi_{\pi(100)}$) at full turgor decreased on salt stress in both species, but the decrease of $\psi_{\pi(100)}$ was higher in New Zealand spinach than in Chard. The value of osmotic adjustment, which was calculated as the difference in values of $\psi_{\pi(100)}$ between non-salinized and salinized plants, was 1.25, 1.48 and 1.58-fold higher in New Zealand spinach than Chard on 50, 100 and 200 mM salt treatments, respectively. The salt-tolerant New Zealand spinach displayed a significantly higher osmotic adjustment than salt-sensitive species Chard under salt stress conditions. The turgor was higher in New Zealand spinach than Chard. The marked increase in the turgor of the leaves of New Zealand spinach may be responsible for the promotion of growth under low salt stress conditions, since a positive turgor is required for cell elongation, stomatal opening and photosynthesis. The regulation of intercellular levels of solutes such as carbohydrates, proline and betaine is assumed to take place mainly in the cytoplasm, being important for osmotic adjustment, while Na^+ is compartmentalized in the vacuoles or distributed in both the vacuoles and cytoplasm (Parida and Das, 2005).

In the present study, both species showed an increased proline content under salt stress conditions (Fig. 2a, b). There were significant differences in the

proline content, with New Zealand spinach showing much higher levels than Chard under salt treatment.

It is well-known that compatible solutes such as proline accumulate in many plants under salt stress conditions, act as compatible solutes, an osmoprotectants and protective agents for cytosolic enzymes and cellular organelles (Bohnert and Shen, 1998; Yancy, 2005).

The accumulation of proline under salt stress in many plants has been correlated with stress tolerance (Tani and Sasakawa, 2006; Ashraf and Foolad, 2007). Petrusa and Winicov (1997) reported that the proline content was rapidly increased in salt-tolerant alfalfa plants under salt stress conditions; however in salt-sensitive plants the response was slow.

Lipid peroxidation was evaluated by the determination of MDA content in leaf tissues. The findings in the present study showed that the level of MDA accumulation significantly increased in both species subjected to salinity stress but MDA accumulation was greater in Chard than in New Zealand spinach.

These results indicated that saline conditions led to the production of less toxic reactive oxygen species in New Zealand spinach compared to Chard. Proline is an important component of plant antioxidant systems (Ashraf and Foolad, 2007). Molinari *et al.* (2007) indicated that a proline overproducing transgenic sugarcane plant showed a lowered active-oxygen species content and increased tolerance to drought.

Sumithra *et al.* (2006) also reported that salt-tolerant cultivars of *Vigna radiate* showed a greater accumulation proline and a lower level of lipid peroxidation than salt-sensitive cultivars. Proline protects plants by functioning as a cellular osmotic regulation between cytoplasm and vacuole and by detoxifying of reactive oxygen species, thus protecting membrane integrity and stabilizing antioxidant enzymes (Huang *et al*., 2009).

CONCLUSION

The growth of New Zealand spinach was promoted under saline conditions but that of Chard was markedly decreased, indicating that New Zealand spinach is halophytic. The main strategy of salt tolerance in New Zealand spinach seems to be the capacity for osmotic adjustment through the increased accumulation of proline and a more active antioxidant system under salt stress conditions.

REFERENCES

Ashraf, M. and M.R. Foolad, 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot., 59: 206-216.

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

Blumwald, E., 2000. Sodium transport and salt tolerance in plants. Curr. Opin. Cell Biol., 12: 431-434.

Bohnert, H.J. and B. Shen, 1998. Trasformation and compatible slutes. Sci. Hortic., 78: 237-260.

Chinnusamy, V., A. Jagendorf and J.K. Zhu, 2005. Understanding and improving salt tolerance in plants. Crop Sci., 45: 437-448.

Fu, J. and B. Hunag, 2001. Involvement of antioxidants and lipid peroxidation in the adaptation of two cool season grasses to localized drought stress. Environ. Exp. Bot., 45: 105-114.

Ghoulam, C., A. Foursy and K. Fares, 2002. Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. Environ. Exp. Bot., 47: 39-50.

Grattan, S.R. and C.M. Grieve, 1999. Salinity mineral nutrient relations in horticultural crops. Sci. Hortic., 78: 127-157.

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

Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert, 2000. Plant cellular and molecular responses to high salinity. Ann. Rev. Plant Physiol. Plant Mol. Biol., 51: 463-499.

Huang, Y., Z. Bie, Z. Liu, A. Zhen and W. Wang, 2009. Protective role of proline against salt stress is partially related to the improvement of water status and peroxidase enzyme activity in cucumber. Soil Sci. Plant Nutr., 55: 698-704.

Kato, M., T. Takeda, Y. Ogihara, M. Shimu, T. Nomura and Y. Tomita, 1985. Studies on the structure of polysaccharide from *Tetragonia tetragonioides*. Chem. Pharm. Bull., 33: 3675-3680.

Koyro, H.W., 2006. Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.). Environ. Exp. Bot., 56: 136-146.

Lee, G., R.N. Carrow, R.R. Duncan, M.A. Eiteman and M.W. Rieger, 2008. Synthesis of organic osmolytes and salt tolerance mechanism in *Paspalum vaginatum*. Environ. Exp. Bot., 63: 19-27.

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

Mandhania, S., S. Madan and V. Sawhney, 2006. Antioxidant defence mechanism under salt stress in wheat seedling. Biol. Plant., 50: 227-231.

Mansour, M.M.F., 1998. Protection of plasma membrane of anion epidermal cells by glycinbetaine and praline against NaCl stress. Plant Phsiol. Biochem., 36: 767-772.

Matraszek, R., 2008. Nitrate reductase activity of two leafy vegetable as affected by nickel and different nitrogen forms. Acta Physiol. Plant., 30: 361-370.

Melony, D.A., M.A. Oliva, H.A. Ruiz and C.A. Martines, 2001. Contribution of proline and inorganic solutes to osmotic adjustment in cotton under salt stress. J. Plant Nutr., 24: 599-612.

Moghaieb, R.E.A., H. Saneoka and K. Fujita, 2004. Effect of salinity on osmotic adjustment, glycinebetaine accumulation and the betaine aldehyde dehydrogenase gene expression in two halophytic plants, *Salicornia europaea* and *Suaeda maritima*. Plant Sci., 166: 1345-1349.

Moghaieb, R.E.A., N. Tanaka, H. Saneoka, Y. Murooka and H. Ono *et al*., 2006. Chracterization of salt tolerance in ectoine-transformed tobacco plants (*Nicotiana tabaccum*): Photosynthesis, osmotic adjustment and nitrogen partitioning. Plant Cell Environ., 29: 173-182.

Molinari, H.B.C., C.J. Marur, E. Daros, M.K.F. Campos and J.F.R.P. Carvalho *et al*., 2007. Evaluation of the stress inducible production of proline in transgenic sugarcane (*Saccharum* spp.): Osmtic adjustment, chlorophyll fluorescence and oxidative stress. Physiol. Plant., 130: 218-229.

Munns, R., 2002. Comparative physiology of salt and water stress. Plant Cell Environ., 25: 239-250.

Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: A review. Ecotoxicol. Environ. Safety, 60: 324-349.

Petrusa, L.M. and I. Winicov, 1997. Proline status in salt tolerant and salt sensitive alfalfa cell lines and plants in response to NaCl. Plant Physiol. Biochem., 35: 303-310.

Poorter, H., 1989. Plant growth analysis: Towards a synthesis of thge classical and the functional approach. Physiol. Plant., 75: 237-244.

Saneoka, H., C. Nagasaka, D.T. Hahn, W. Yang, G.S. Premachandra, R.J. Joly and D. Rhodes, 1995. Salt tolerance of glycinebetaine-deficient and containing maize lines. Plant Physiol., 107: 631-638.

Sumithra, K., P.P. Jutur, B.D. Carmel and A.R. Reddy, 2006. Salinity-induced changes in two cultivars of *Vigna radiate*: Responses of antioxidative and proline metabolism. Plant Growth Regul., 50: 11-22.

Tani, C. and H. Sasakawa, 2006. Proline accumulates in *Casuarina equisetifolia* seedling under salt stress. Soil Sci. Plant Nutr., 52: 21-25.

Wilson, C., S.M. Lesch and C.M. Grieve, 2000. Growth stage modulates salinity tolerance of New Zealand Spinach (*Tetragonia tetragonioides*, Pall) and red orach (*Atriplex hortensis* L.). Ann. Bot., 85: 501-509.

Wilson, J.R., M.J. Fisher, E.D. Schulze, G.R. Dolby and M.M. Ludlow, 1979. Comparison between pressure volume and dew point-hygrometry techiniques for determining the water relations characteristics of grass and legumes leaves. Oecologia, 41: 77-88.

Yancy, P.H., 2005. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. J. Exp. Biol., 208: 2819-2830.