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Proline Accumulation in Some Coastal Zone Plants of the Aegean Region of Turkey

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ABSTRACT

In this study, the proline contents of ecophysiologically different plants were determined on a seasonal basis. The plant species investigated were *Cakile maritima*, *Medicago marina*, *Pancratium maritimum* and *Suaeda prostrata* (psammophyte-from sand dunes); *Arthrocnemum fruticosum*, *Halimione portulacoides*, *Halocnemum strobilaceum*, *Limonium bellidifolium* and *Salicornia europaea* (halophytes-from saline habitats). The results showed that proline content in all taxa was highest in autumn and lowest in spring. The accummulation in general was higher in halophytes (0.104 ± 0.004 μmol g^{-1} (f.m.) to 55.138 ± 6.138 μmol g^{-1} (f.m.)), as compared to psammophytes (0.242 ± 0.118 μmol g^{-1} (f.m.) to 38.307 ± 1.455 μmol g^{-1} (f.m.)). This can be attributed to both salinity and water stress in the halophytes, and water stress alone in the psammophytes.

Keywords: halophyte, osmolyte, psammophyte, salinity, tolerance, water stress

INTRODUCTION

The plant diversity of coastal habitats consists of a relatively small number of species that are able to survive environmental stress of salinity and drought. In halophytes, there is a large number of plant defence mechanisms including ion homeostasis, osmoregulation, antioxidant and hormonal systems helping plants to thrive well under saline conditions (Sairam and Tyagi 2004; Flowers and Colmer 2008). Several studies have been undertaken to understand if proline accumulation in plants would be a possible criterion to identify the tolerance of plants to salt. Proline accumulation is said to be specific to species and also to varieties, with the amount changing according to the stress (Singh and Rai 1981; Ball *et al*. 1984; Pandey and Ganapathy 1985; Rhizopoulou *et al.* 1990; Venkatesan and Chellappan 1998; Kavi Kishore *et al.* 2005; Noreen and Ashraf 2009). However, the importance of proline accumulation among the diverse species of inland saline and coastal habitats as an adaptation to the adverse environmental conditions has not been investigated much (Cavalieri and Huang 1979; Tipirdamaz *et al.* 2006). Turkey possesses 8333 km of coast extending from Georgia to Syria, with a rich plant diversity. Until now, no work has been done on proline accumulation in the naturally growing plant taxa along the coast. In this paper we present the results covering a survey of the major psammophytes (plants growing on sandy habitats in nature) and halophytes (plants growing on saline habitats in nature) of the Aegean coastal zone in Turkey.

MATERIALS AND METHODS

Whole plant samples of *Cakile maritima* Scop., *Pancratium maritimum* L., *Suaeda prostrata* Pall. and *Medicago marina* L. (psammophyte-from sand dunes); *Arthrocnemum fruticosum* (L.) Moq., *Halimione portulacoides* Aellen., *Halocnemum strobilaceum* (Pallas) Bieb., *Limonium bellidifolium* (Gouan) Dumort. and *Salicornia europaea* L. (halophytes-from saline habitats) were collected in the months of April, August and October (2003) from the Aegean coast in Turkey. The collection time lasted 2 days in each case and species selection was done on the basis of dominance in the locality. The material was transported to the laboratory in a freezer at -4°C. These were separated into aboveground and belowground parts after brought to the laboratory and immediately frozen with liquid nitrogen, again left in a -80°C freezer until use. Proline levels were determined according to Bates *et al.* (1973) on a fresh weight basis. Leaf samples were homogenized in 3% (w/v) sulfosalicylic acid solution and then centrifuged. The supernatant was taken into a test tube to which glacial acetic acid and acid ninhydrin solution were added. Tubes were incubated in a boiling water bath for 1 h and then allowed to cool to room temperature. After adding toluene, the mixture was vortexed and allowed to separation of toluene and aqueous phase. The absorbance of toluene phase was measured at 520 nm in a spectrophotometer. The concentration was calculated from a proline standard curve and expressed as μmol/g FW. Samples were selected in random, 4 samples were separated as above- and belowground parts and analysed for each plant. The mean values obtained were compared by Tukey's test and analyzed using SPSS software.

RESULTS AND DISCUSSION

Proline is an important osmolyte involved in the control of osmotic pressure in cells (Ashraf and Foolad 2007). Proline accumulates normally in the cytosol in high concentrations in response to drought or salinity stress in plants, thus contributing in the stabilization of sub-cellular structures, scavenging of free radicals, controlling of osmotic pressure and buffering of cellular redox potential (Cha-um and Kirdmanee 2010). Proline is also known to act as a major reservoir of energy and nitrogen for utilization upon exposure to salinity (Tawfik 2008). Moreover, its rapid breakdown upon release of stress may provide sufficient reducing agents for recovery from stress and repairing stress-induced damage. Proline also induces expression of salt-stress-responsive genes, which possess proline-responsive elements (e.g. PRE, ACTCAT) in their promoters (Satoh *et al.* 2002; Oono *et al.* 2003; Chinnusamy *et al.* 2005). Proline accumulation in plants is mediated by both ABA-dependent and ABA-independent signalling pathways (Zhu 2001, 2002).

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Research Note

Table 1 Proline accumulation fluctuations of psammophytes relative to Spring (n = 4, ± standard deviation of the mean).

	Spring		Summer		Autumn	
	Below ground	**Above ground**	**Below ground**	**Above ground**	**Below ground**	**Above ground**
Cakile maritima	0.544 ± 0.007 b	1.430 ± 0.218 a	2.548 ± 1.146 ab	18.229 ± 4.474 a	4.989 ± 0.823 ab	27.420 ± 2.731 a
Medicago marina	4.178 ± 0.363 abd	3.197 ± 0.957 abc	15.714 ± 0.557 bcd	4.084 ± 0.190 ac	11.624 ± 0.409 ad	7.553 ± 0.407 ac
Pancratium maritimum	2.064 ± 0.009 cd	0.242 ± 0.118 cde	2.155 ± 0.007 ad	1.164 ± 0.411 ad	6.118 ± 0.587 af	4.980 ± 1.448 ae
Suaeda prostrata	1.425 ± 0.275 cd	2.360 ± 0.116 be	3.867 ± 0.573 adc	8.838 ± 0.577 abcd	30.703 ± 1.420 bcdefg	38.307± 1.455 ag

The same letter within a column indicates significant differences ($P < 0.05$) according to Tukey's test.

Table 2 Proline accumulation fluctuations of halophytes relative to Spring (n = 4, ± standard deviation of the mean).

	Spring		Summer		Autumn	
	Below ground	**Above ground**	**Below ground**	**Above ground**	**Below ground**	**Above ground**
Arthrocnemum fruticosum	2.260 ± 0.495 b	1.154 ± 0.003 a	7.931 ± 1.742 ab	5.227 ± 1.087 a	55.138 ± 6.138 ab	33.287 ± 0.950 a
Halimione portulacoides	0.234 ± 0.001 d	0.104 ± 0.004 c	0.921 ± 0.189 adb	0.543 ± 0.114 acb	2.911 ± 0.713 abd	2.167 ± 0.263 abc
Halocnemum strobilaceum	4.599 ± 0.279 acdef	0.463 ± 0.003 e	5.064 ± 1.092 bcdf	4.167 ± 0.363 bcde	42.765 ± 4.122 abcdef	4.896 ± 0.769 abe
Limonium bellidifolium	5.334 ± 2.451 abcdegh	0.988 ± 0.177 fg	9.493 ± 1.391 acdefgh	3.351 ± 0.122 bcg	42.975 ± 3.689 abcde	41.047 ± 4.052 bcdefg
Salicornia europaea	3.323 ± 0.305 cde	0.632 ± 0.001 fhi	5.473 ± 0.443 cdhi	1.041 ± 0.139 abefhi	13.693 ± 3.662 abcdfghi	2.988 ± 0.176 abfghi

The same letter within a column indicates significant differences ($P < 0.05$) according to Tukey's test.

The variations of proline contents of halophytes and psammophytes from the study area are presented in **Tables 1** and **2**.

The proline concentration in all taxa was highest in autumn and lowest in spring. The accumulation in general was higher in halophytes (0.104 ± 0.004 μmol g^{-1} (f.m.) to 55.138 ± 6.138 μmol g^{-1} (f.m.)), as compared to psammophytes (0.242 ± 0.118 μmol g^{-1} (f.m.) to 38.307 ± 1.455 μmol g^{-1} (f.m.)).

The values are higher for belowground than aboveground parts in *Arthrocnemum fruticosum*, *Halimione portulacoides*, *Halocnemum strobilaceum*, *Salicornia europaea* and *Limonium bellidifolium*. In the case of the psammophytes, the concentrations of proline are higher in the belowground parts in *Medicago marina*, *Pancratium maritimum* but higher in the aboveground parts of *Cakile maritima* and *Suaeda prostrata*.

Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants (Melony *et al.* 2001; Tani and Sasakawa 2006; Ashraf and Foolad 2007; Lee *et al.* 2008). For example, in salt-tolerant alfalfa plants proline concentration in the root rapidly doubled under salt stress, while in salt-sensitive genotypes, the response was slow (Petrusa and Winicov 1997). Similarly, salt-tolerant ecotypes of *Agrostis stolonifera* accumulated higher concentration of proline in response to salinity than salt-sensitive ecotypes (Ahmad *et al.* 1981). Also a higher free proline content in varieties of tolerant bean compared to other two sensitive varieties showed that his amino-acid may be highly involved in a plant adaptation mechanism to water and salinity stress (Cárdenas-Avila 2006). However, assessment of proline accumulation and distribution during shoot and leaf development in two *Sorghum* genotypes contrasting in salt tolerance suggested that proline accumulation was a reaction to salt stress and not a plant response associated with tolerance (de Lacerda *et al.* 2003). In halophytes, high levels of proline are present in *Tamarix tetragyna* and in several other species (Stewart and Lee 1974). Proline can be used to determine plant genotypes and ecotypes (Claussen 2005; Kant *et al.* 2006; Ferreira and Lima-Costa 2006; Megdiche *et al.* 2007; Flowers and Colmer 2008). It is important to understand the physiological mechanism of salinity, in order to conserve natural vegetation in saline environments and to allow the adaptation of new plant species to the areas.

REFERENCES

Ahmad I, Wainwright SJ, Stewart GR (1981) The solute and water relations of *Agrostis stolonifera* ecotypes differing in their salt tolerance. *New Phytologist* **87**, 615-629

Ashraf M (2004) Some important physiological selection criteria for salt tolerance in plants. *Flora* **199**, 361-376

Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany* **59 (2)**, 206-216

Ashraf M (2009) Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology Advances* **27**, 84-93

Ball AR, Quadar A, Joshi YC, Rana RS (1984) Free proline accumulation under salt stress in wheat and barley. *Journal of Current Agriculture* **8**, 91-95

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant and Soil* **39**, 205-207

Cárdenas-Avila ML, Verde-Star J, Marti RK, Foroughbakhch-PR, Gámez-González H, Martinez-Lozano S, Núñez-González MA, Garcia DG, Hernández-Piñero JL, Morales-Vallarta MR (2006) Variability in accumulation of free proline on invitrocalli of four bean (*Phaseolus vulgaris* L.) varieties exposed to salinity and induced moisture stres. *Phyton (Buenos Aires)* **75**, 103-108

Cavalieri AJ, Huang AHC (1979) Evaluation of proline accumulation in the adaptation of diverse species of marsh halophytes to the saline environment. *American Journal of Botany* **66 (3)**, 307-312

Chinnusamy V, Jagendorf A, Zhu JK (2005) Understanding and improving salt tolerance in plants. *Crop Science* **45**, 437-448

Cha-um S, Kirdmanee C (2010) Effects of water stress induced by sodium chloride and mannitol on proline accumulation, photosynthetic abilities and growth characters of eucalyptus (*Eucalyptus camaldulensis* Dehnh.). *New Forests* **40 (3)**, 349

Claussen W (2005) Proline as a measure of stres in tomato plants. *Plant and Soil* **168**, 241-248

de Lacerda CF, Cambraia J, Oliva MA, Ruiz HA, Prisco JT (2003) Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. *Environmental and Experimental Botany* **49**, 107-120

Ferreira AL, Lima-Costa ME (2006) Metabolic responses to salt stress in cell suspension cultures of sensitive and resistant *Citrus*. *Journal of Horticultural Science and Biotechnology* **81 (6)**, 983-988

Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. *New Phytologist* **179**, 945-963

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology* **51**, 463-499

Hsu SY, Hsu YT, Kao CH (2003) The effect of polyethylene glycol on proline accumulation in rice leaves. *Biologia Plantarum* **46**, 73-78

Jung Y, Park J, Choi Y, Yang JG, Kim D, Kim BG, Roh K, Lee DH, Auh CK, Lee S (2010) Expression analysis of proline metabolism-related genes from halophyte *Arabis stelleri* under osmotic stress conditions. *Journal of Integrative Plant Biology* **52 (10)**, 891-903

Kant S, Kant P, Raveh E, Barak S (2006) Evidence that differential gene expression between the halophyte *Thellungiella halophila* and *Arabidopsis thaliana* is responsible for higher levels of the compatible osmolyte proline and tight control of Na^+ uptake in *T. halophila*. *Plant Cell and Environment* **29 (7)**, 1220-1234

Kavi Kishore PB, Sangam S, Amrutha RN, Laxmi PS, Naidu KR, Rao KRSS, Rao S, Reddy KJ, Theriappan P, Sreenivasulu N (2005) Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Current*

Science **88**, 424-438

Lee C, Carrow RN, Duncan RR, Eiteman MA, Rieger MW (2008) Synthesis of organic osmolytes and salt tolerance mechanism in *Paspalum vaginatum. Environmental and Experimental Botany* **63**, 19-27

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses. An overwiew. *Archives of Biochemistry and Biophysics* **444 (2)**, 139-158

Mansour MMF (2000) Nitrogen containing compounds and adaptation of plants to salinity stress. *Biologia Plantarum* **43**, 491-500

Megdiche W, Amor NBİ Debez A, Hessini K, Ksouri R, Zuliy-Folid Y, Abdelly C (2007) Salt tolerance of the annual halophyte *Cakile maritima* as affected by the provenance and the developmental stage. *Acta Physiologiae Plantarum* **29 (4)**, 375-384

Melony DA, Oliva DA, Ruiz HA, Martines CA (2001) Contribution of proline and inorganic solutes to osmotic adjustment in cotton under salt stress. *Journal of Plant Nutrition* **24**, 599-612

Nawaz K, Ashraf M (2009) Exogenous application of glycinebetaine modulates-activities of antioxidants in maize plants subjected to salt stress. *Journal of Agronomy and Crop Science* **196 (1)**, 28-37

Nayyar H, Walia DP (2003) Water stress induced proline accumulation in contrasting wheat genotypes as affected by calcium and abscisic acid. *Biologia Plantarum* **46**, 275-279

Noreen Z, Ashraf M (2009) Changes in antioxidant enzymes and some key metabolites in some genetically diverse cultivars of radish (*Raphanus sativus* L.). *Environmental and Experimental Botany* **67**, 395-402

Oono Y, Ooura C, Rahman A, Aspuria ET, Hayashi K, Tanaka A, Uchimiya H (2003) p-Chlorophenoxyisobutyric acid impairs auxin response in *Arabidopsis* root. *Plant Physiology* **133**, 1135-1147

Ozturk L, Demir Y (2002) *In vivo* and *in vitro* protective role of proline. *Plant Growth Regulation* **38**, 259-264

Pandey RM, Ganapathy PS (1985) The proline enigma: NaCl-tolerant and NaCl-sensitive callus lines of *Cicer arietinum*. *Plant Science* **40**, 13-17

Petrusa LM, Winicov I (1997) Proline status in salt tolerant and salt sensitive alfalfa cell lines and plants in response to NaCl. *Plant Physiology and Biochemistry* **35**, 303-310

Rhizopoulou S, Diamantogloy S, Passiakou L (1990) Free proline accumulation in leaves, stems and roots of four Mediterranean native *Phrygana* species. *Acta Oecologia* **11**, 585-593

Satoh R, Nakashima K, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2002) ACTCAT, a novel *cis*-acting element for proline- and hypoosmolarity-responsive expression of the *ProDH* gene encoding proline dehydrogenase in *Arabidopsis*. *Plant Physiology* **130**, 709-719

Sairam RK, Tyagi A (2004) Physiology and molecular biology of salinity stress tolerance in plants. *Current Science* **86**, 407-421

Serraj R, Sinclair TR (2002) Osmolyte accumulation: Can it really help increase crop yield under drought conditions? *Plant Cell and Environment* **25**, 333-341

Shao H-B, Chu LY (2005) Plant molecular biology in China: Opportunities and challenges. *Plant Molecular Biology Reporter* **23 (4)**, 345-358

Shao HB, Chen XY, Chu LY, Zhao XN, Wu GY, Yong B, Zhao CX, Hu ZM (2006) Investigation on the relationship of proline with wheat anti-drought under soil water deficits. *Colloids and Surfaces B: Biointerfaces* **53 (1)**, 113-119

Singh G, Rai VK (1981) Proline accumulation and drought resistance *Cicer arientinum* L. *Biologia Plantarum* **23**, 86-90

Song W-Y, Zhang Z-B, Shao H-B, Guo X-L, Cao H-X, Zhao HB, Fu Z-Y, Hu X-J (2008) Relationship between calcium decoding elements and plant abiotic stress resistance. *International Journal of Biological Sciences* **4 (2)**, 116-125

Stewart GR, Lee JA (1974) The role of proline accumulation in halophytes. *Planta* **120**, 279-289

Tani C, Sasakawa H (2006) Proline accumulates in *Casuarina equisetifolia* seedlings under salt stress. *Soil Science and Plant Nutrition* **52 (1)**, 21-25

Tawfik KM (2008) Evaluating the use of *Rhizobacterin* on cowpea plants grown under salt stress. *Research Journal of Agriculture and Biological Sciences* **4 (19)**, 26-33

Tipirdamaz R, Gagneul D, Duhaz C, Ainouche A, Monnier C, Ozkum D, Larher F (2006) Clustering of halophytes from an inland salt marsh in Turkey according to their ability to accumulate sodium and nitrogenous osmolytes. *Environmental and Experimental Botany* **57**, 139-153

Venkatesan A, Chellappan KP (1998) Accumulation of proline and glycine betaine in *Ipomoea pescaprae* induced by NaCl. *Biologia Plantarum* **41**, 271-276

Yancey PH (2005) Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *Journal of Experimental Biology* **208**, 2819-2830

Yousif BS, Liu YL, Nguyen NT, Masaoka Y, Saneoka H (2010) Comparative studies in salinity tolerance between New Zealand spinach (*Tetragonia tetragonoides*) and chard (*Beta vulgaris*) to salt stress. *Agricultural Journal* **5 (1)**, 19-24

Zhu JK (2001) Plant salt tolerance. *Trends in Plant Science* **2**, 66-71

Zhu JK (2002) Salt and drought stress signal transduction in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **53**, 247-273