

SEASONAL VARIATIONS IN PLANT WATER STATUS OF FOUR DESERT HALOPHYTES FROM SEMI-ARID REGION OF KARACHI

IRFAN AZIZ, BILQUEES GUL, SALMAN GULZAR AND M. AJMAL KHAN*

*Institute of Sustainable Halophyte Utilization,
University of Karachi, Karachi-75270.*

*Corresponding author E-mail: majmalk@uok.edu.pk

Abstract

Halophytes in arid and semi arid zones of the world are often subjected to extremely variable drought, salinity and temperature. These fluctuations may bring about changes in their osmoregulation and gas exchange responses besides other physiological and biochemical processes. The purpose of this study was to detect temporal changes in plant water status and osmotic adjustment in four desert halophytes viz., *Suaeda fruticosa*, *Heliotropium curassavicum*, *Haloxylon stocksii* and *Atriplex stocksii* from an inland community at Karachi University Campus. During the dry period (November to January) water and osmotic potentials of all test species increased with higher values in *A. stocksii* (salt secretor) than those of *S. fruticosa* and *H. stocksii* (salt includer) and *H. curassavicum* (salt excluder). Proline increased substantially and was highest in *H. curassavicum* followed by *A. stocksii* in comparison to the two salt includers. The lowering of osmotic potential corresponded to an increase in Na and Cl, lower stomatal conductance and chlorophyll content indicating reduced gas exchange during the dry period. The increase in proline may have little role in osmoregulation but could contribute in scavenging reactive oxygen species.

Introduction

Arid and semi-arid regions typically receive low annual rainfall with long dry spells over consecutive years which could be highly stressful for the natural vegetation. Karachi experiences dry weather for most part of the year due to a low average annual rainfall of less than 220 mm. A combination of water deficit, high evapo-transpiration, irradiance, temperatures and salinity allow few plant species to survive. Besides scanty rainfall and high soil salinity, spatial heterogeneity is the main cause of uneven and patchy distribution of plants mostly dominated by perennial shrubs in Karachi (Chaudhri, 1961). Prolonged drought has severe consequences for crop productivity in arid regions (Reddy *et al.*, 2003) while the situation is further aggravated due to the heterogeneous habitat conditions which could also inflict ecosystem level vegetation processes (Neilson, 2003). Recharge of soil moisture resulting from either a single heavy downpour or a series of rainfall events during monsoon improve biochemical responses among key organisms (Reynolds *et al.*, 2004). Various plant species may not use environmental resources such as precipitation and light in the same manner during the growing season (Ehleringer, 1993) due to differences in their physiological and biochemical adaptive responses (Villar *et al.*, 1999).

Plant strategies like improved or maintained water use efficiency, net carbon gain and osmotic adjustment of plants respond to temporal changes in the net radiation, temperature, salinity and availability of water and are genetically controlled (Bohnert *et al.*, 2006; Ezawa & Tada, 2009; Laüchli & Grattan, 2007). Lowering of water potential (Aziz, 2007), stomatal closure, protection of endogenous enzymes and photosystems, leaf shedding, leaf rolling, accumulation of compatible solutes and organic acids (Munns, 2002) are among common responses against Na and Cl accumulation which reduce plant

growth (Ben-Hassine *et al.*, 2008). Lowering of leaf water potential causes stomatal closure (Nardini & Salleo, 2001) and helps in maintaining turgor (Laüchli & Grattan, 2007). Turgor is maintained by osmotic adjustment by both a net accumulation of inorganic solutes (Bohnert *et al.*, 2006) and osmolytes (Munns & Tester, 2008). These osmolytes facilitate water uptake and protect and stabilize enzymes in hyper-saline conditions (Ben-Hassine *et al.*, 2008; Bohnert *et al.*, 2006; McNiel *et al.*, 2000). In low concentrations organic solutes are reported to enhance the activity of antioxidant enzymes in some cases by directly scavenging ROS (Banu *et al.*, 2009; Miller *et al.*, 2010 and Parida & Jha, 2010).

The present study explores seasonal variations in water relations and osmotic adjustment of four halophytic shrubs to understand their ecophysiological strategies to cope with the natural stressful environments.

Materials and Methods

The study site was located in Karachi University campus about 15 km away from the coast. The area is classified as a subtropical maritime desert (latitude 24° 48' N, longitude 65° 55' E) and receives an average annual rainfall of 220 mm. During this study water relations and stomatal conductance were measured twice a month at about noon throughout the study period and for ions and proline determination, leaf samples were collected in pre-weighed plastic bags and immediately brought to the laboratory.

Leaf water potential (Wp) of plants was measured in the field using a dew point microvoltmeter (Model HR-33 T, Wescor Inc., Logan, Utah) on 5 mm diameter leaf disks, placed in a C-52 sample chamber. Osmotic potential (Op) was measured by freeze killing leaf disks in liquid nitrogen and then inserted in a sample chamber for equilibration. Stomatal conductance was measured on abaxial surface of leaves with the help of A-P4 Porometer (Delta-T Devices, U.K).

Hot water extracts were prepared by boiling finely ground oven dried material in distilled water in a water bath for 1 h. Dilutions of this extract were used to determine Na, K and Cl by different electrodes using Radiometer (Ion-85) analyzer.

Hot water extracts prepared as described above were used to estimate proline using the method of Bates *et al.*, (1973). Chlorophyll a and b were determined in chopped fresh leaves and boiled in 80% acetone for 1 h. The extract was filtered using glass wool, diluted with acetone and pigment concentrations calculated using the method of Machlachlam & Zalik (1963).

Results

Water potential increased during the dry months from November to February in all species (Fig. 1). A two way ANOVA showed a significant ($p < 0.01$) individual effect of time (months), species and their combined effect ($p < 0.001$, Table 1) on plant water relations. Plants maintained higher turgor during the wet (monsoon) period (July to September) by lowering their osmotic potential (Fig. 1). *Heliotropium curassavicum* (salt excluder) had the highest osmotic potential followed by *A. stocksii* (leaf secretor) (Fig. 1) with lower values in *S. fruticosa* (leaf succulent) and *H. stocksii* (stem succulent). Proline increased significantly ($p < 0.001$, Table 1, Fig. 2), along with a significant ($p < 0.001$) decrease in stomatal conductance (Table 1, Fig. 2), gas exchange and transpiration during the dry period.

Table 1. Two-way ANOVA of characteristics by month (M), plant species (Sp) and their interactions (M x Sp).

Dependent variables	Independent variables		
	M	Sp	M x Sp
Water potential	33.1**	39.7**	4.7***
Stomatal conductance	41.8**	81.7*	4.5***
Proline	63.4**	53.1**	9.8***
Sodium	28.6*	19.7*	5.6**
Chloride	34.5*	21.4*	6.2**
Chlorophyll 'a'	1.8*	2.1*	1.1**
Chlorophyll 'b'	1.1*	1.8 ^{n.s}	0.9**

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; n.s = non-significant

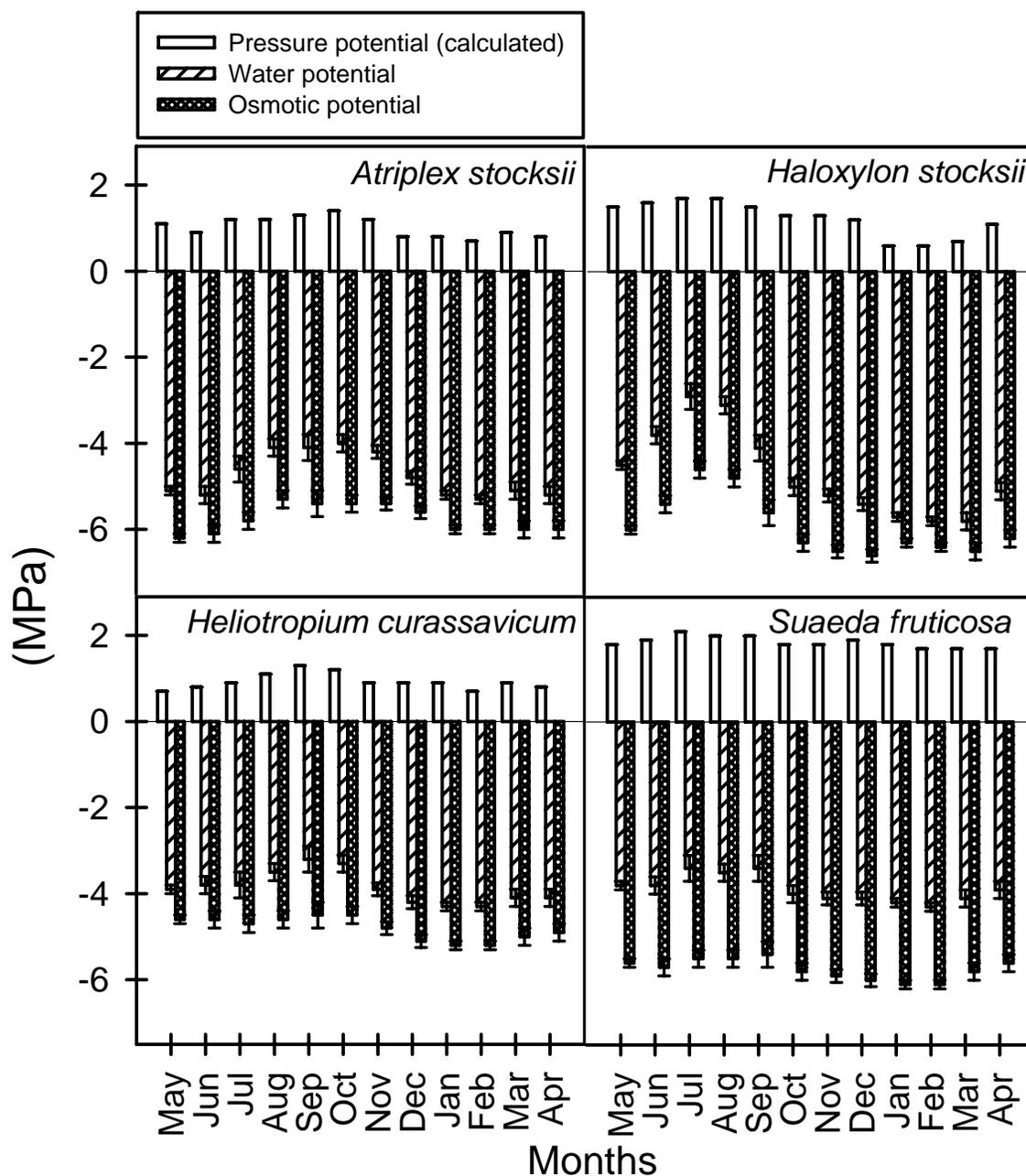


Fig. 1. Seasonal changes in water and osmotic potentials in *Atriplex stocksii*, *Haloxylon stocksii*, *Heliotropium curassavicum* and *Suaeda fruticosa*. Bars represent means (\pm S.E.): $n = 5$.

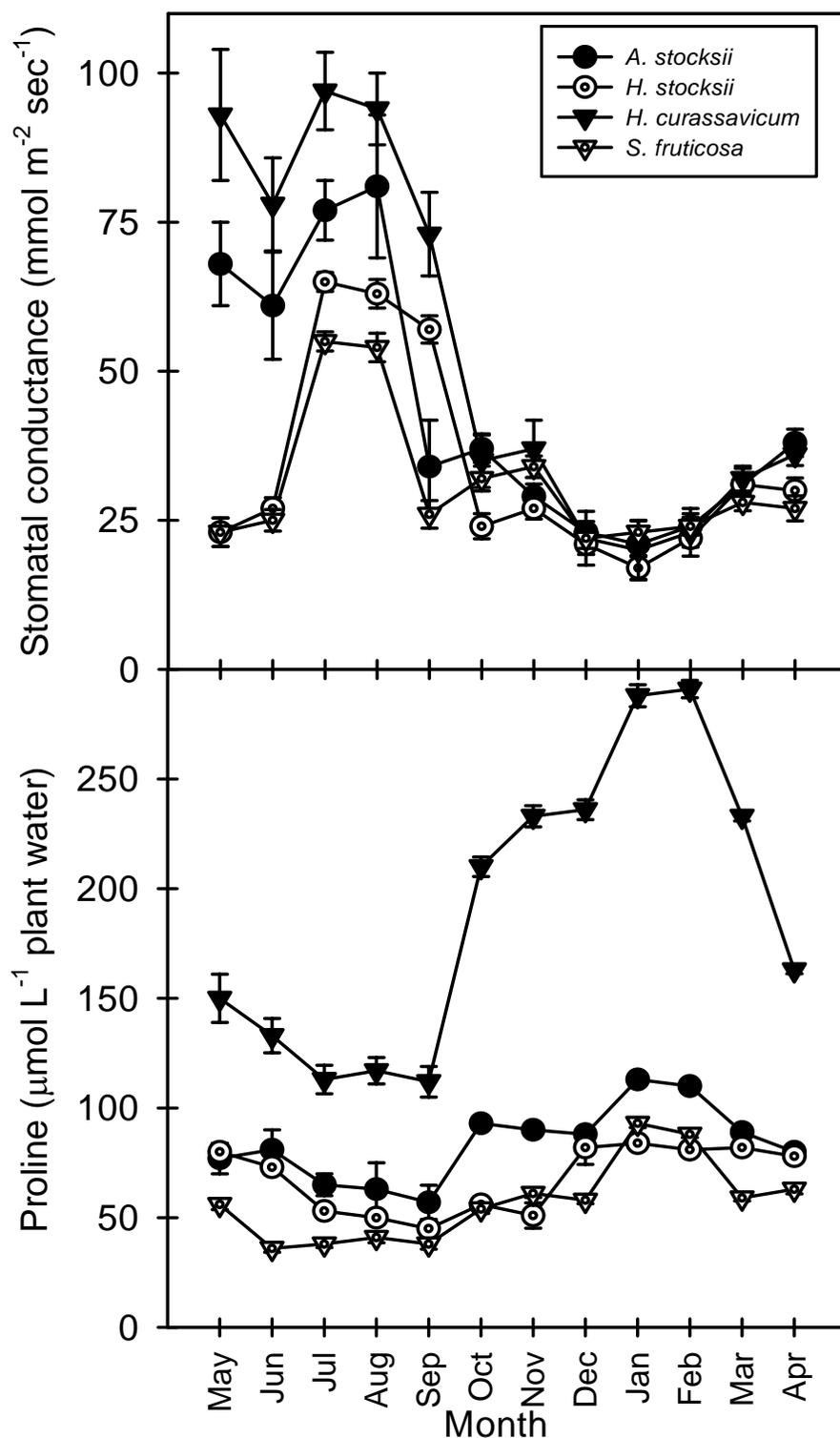


Fig. 2. Seasonal changes in stomatal conductance and proline in *Atriplex stocksii*, *Haloxylon stocksii*, *Heliotropium curassavicum* and *Suaeda fruticosa*. Symbols represent means (\pm S.E.): n = 5.

Sodium and chloride accumulation showed a similar trend in all test species (Fig. 3) with highest levels of accumulation in *S. fruticosa* (salt accumulator), followed by *A. stocksii*. A two way ANOVA showed significant individual effects of months, species and their interaction ($p < 0.05$; Table 1). Chlorophyll a and b increased significantly ($p < 0.001$) following monsoon rains (Table, 1; Fig. 4). Chlorophyll a and b remained higher in *S. fruticosa* during entire wet period, however, in *H. curassavicum* higher values for chlorophyll were observed in August only (Fig. 4).

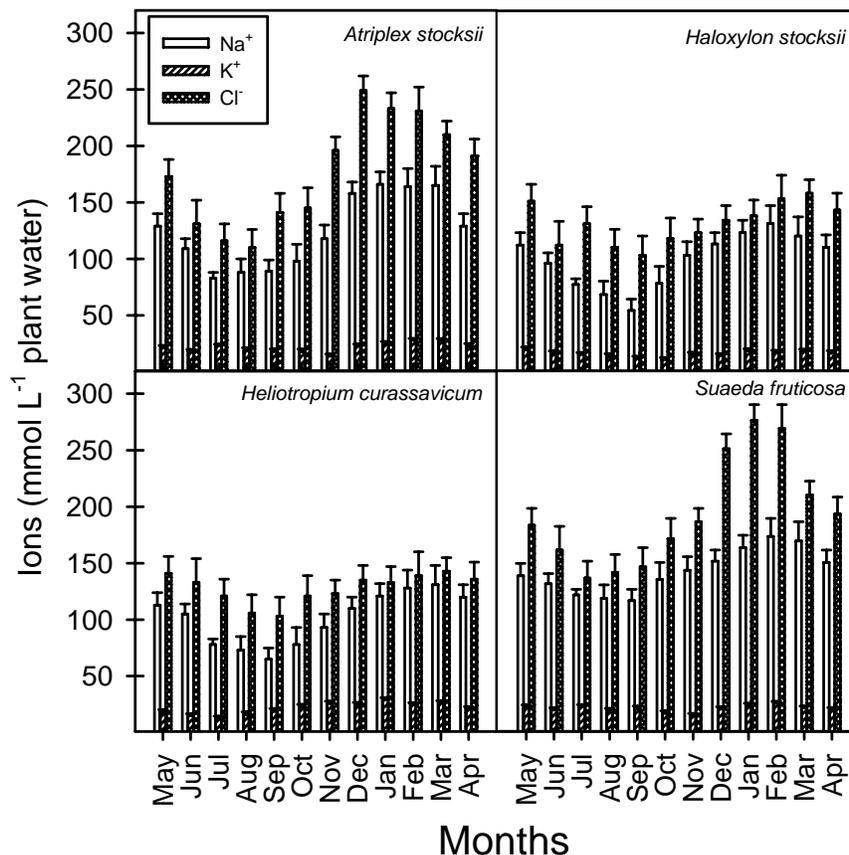


Fig. 3. Seasonal changes in leaf Na, K, and Cl in *Atriplex stocksii*, *Haloxyylon stocksii*, *Heliotropium curassavicum* and *Suaeda fruticosa*. Symbols represent means (\pm S.E.): n = 5.

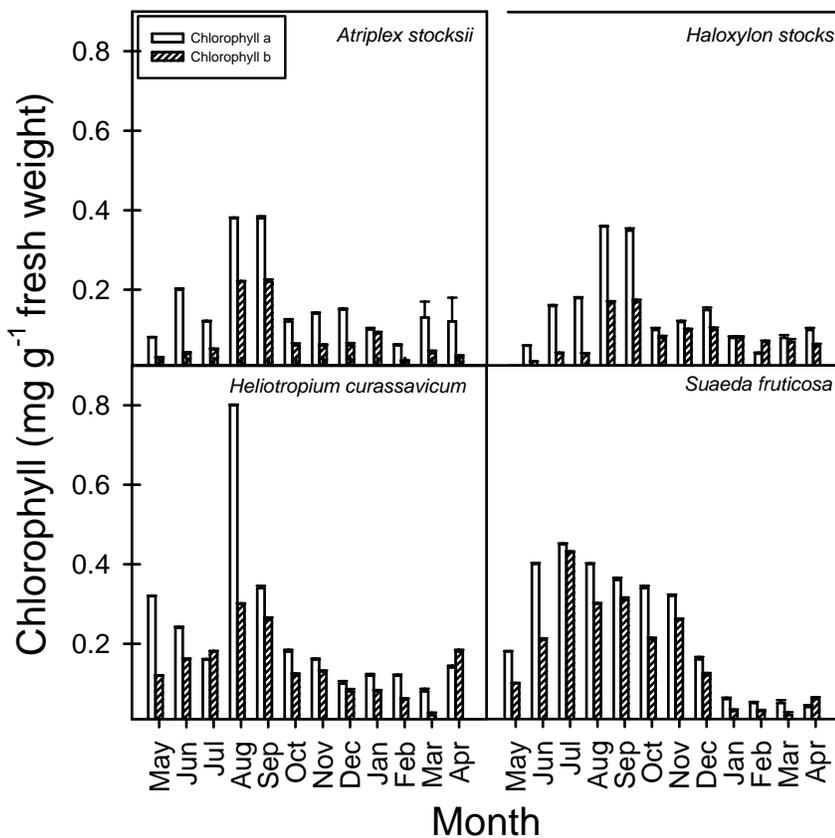


Fig. 4. Seasonal changes in chlorophyll a and b in *Atriplex stocksii*, *Haloxyylon stocksii*, *Heliotropium curassavicum* and *Suaeda fruticosa*. Symbols represent means (\pm S.E.): n = 5.

Discussion

Drought and salinity interact in influencing physiological traits such as increase in osmolyte concentration, decreased water potential, improved Na, K and water use efficiencies in high stress tolerant species (Kang *et al.*, 2003; Nunes *et al.*, 2008; Slama *et al.* 2008). The amplitude, frequency and duration of a stress also determine plant responses to the stress event (Dobrota, 2006; Koyro, 2006). In the natural environmental conditions low soil moisture corresponded to low or more negative plant water potentials and lower turgor which decreased during the dry period along with a substantial decrease in leaf water potential. Similar seasonal trends were reported for few xerophytes and xerohalophytes (Aziz, 2007; Beena & Khan, 2002) but with higher proline content in the xerophytes. Accumulation of compatible solutes such as glycinebetaine and proline during salinity and drought are reported to improve PS-II efficiency and antioxidant enzyme activity (Ben-Hassine *et al.*, 2008). Most Amaranthaceae (Chenopodiaceae) members characteristically accumulate glycinebetaine as the chief osmotica (Rhodes & Hanson, 1993) to cope with high leaf Na and Cl concentrations while many halophytes also accumulate proline however, species with higher proline usually maintain low glycinebetaine (Tipirdamaz *et al.*, 2006). Green house experiments on salt tolerance mechanisms of *Atriplex stocksii*, *Haloxylon stocksii* and *Suaeda fruticosa* (Khan *et al.*, 2000a,b&c) revealed constitutively high glycinebetaine levels. Similar results were reported for *Heliotropium kotschyi* (Laurie *et al.* 1994). *Atriplex stocksii* and *S. fruticosa*, the two succulent species accumulated higher amounts of sodium and chloride during the dry period and this strategy could be related to the ionic adjustments of the plants to achieve osmotic balance. Among the four species in the present study *A. stocksii*, *S. fruticosa* and *H. stocksii* displayed higher osmotic potential and lower proline content in comparison to *H. curassavicum* but with higher Na and Cl in the former two species. More negative osmotic potential in *H. stocksii* could be attributed to possibly higher glycinebetaine under similar soil salinities (Khan *et al.*, 2000a). Although proline was substantially higher in *Heliotropium curassavicum* it was not sufficiently high to act as a compatible solute (Rhodes & Hanson, 1993). Its role as osmoprotectant for enzymes such as catalase and ascorbate peroxidase could be more important to avoid oxidative stress (Chen & Murata, 2002; Hoque *et al.*, 2007; Miller *et al.*, 2010; Raymond & Smirnov, 2002).

Decrease in chlorophyll content may result in lower rates of photosynthesis under extreme stress (Li *et al.*, 2010). Chlorophyll content substantially decreased in all test species with subsequent decrease in stomatal conductance during the drier months suggesting a possible influence on net photosynthetic rates (Koyro, 2006; Moorthy & Katherisan, 1999; Naidoo, 2009). *Heliotropium curassavicum* had the highest stomatal conductance during the monsoon period reflected by equally large chlorophyll values in August. Photosynthetic pigments were generally higher in *S. fruticosa* during monsoon period despite lowest stomatal conductance values throughout the year as a possible regulatory mechanism to maintain photosynthetic carbon assimilation at a minimum level for sustainable growth and survival in variable environmental conditions. *Atriplex stocksii* and *H. stocksii* showed comparative values and seasonal trends of stomatal conductance and photosynthetic pigments despite differences in other physiological attributes.

The seasonal variations in water relations and ionic composition of the test species reveal different ecophysiological strategies to grow under similar drought and salinity conditions. However, further studies on antioxidant defense system, gas exchange parameters, fluorescence and compatible osmolytes are needed to better evaluate the

stress perception of local species in the natural environment. Such studies could provide information to grow halophytes as non-conventional crops on saline soils for various economic purposes (Gul *et al.*, 2010).

References

- Aziz, I. 2007. Seasonal flux in water potential, proline and chlorophyll content in desert shrubs at Ziarat valley, Balochistan, Pakistan. *Pak. J. Bot.*, 39: 1995-2002.
- Aziz, I., S. Gulzar, M. Noor and M.A. Khan. 2005. Seasonal variation in water relations of *Halopyrum mucronatum* (L.) Stapf., growing near Sandspit, Karachi. *Pak. J. Bot.*, 37: 141-148.
- Banu, N.A., N. Watanabe-Sugimoto, K. Matsuoka, Y. Nakamura, Y. Shimoishi and Y. Murata. 2009. Proline and glycinebetaine induce antioxidant defense gene expression and suppress cell death in cultured tobacco cells under salt stress. *J. Plant Physiol.*, 166: 146-156.
- Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant and Soil*, 39: 205-207.
- Beena, N. and M.A. Khan. 2002. Seasonal variation in water relations of desert shrubs from Karachi, Pakistan. *Pak. J. Bot.*, 34: 329-340.
- Ben-Hassine, A., M.E. Ghanem, S. Bouzid and S. Lutts. 2008. An inland and a coastal population of the Mediterranean xero-halophyte species *Atriplex halimus* L., differ in their ability to accumulate proline and glycinebetaine in response to salinity and water stress. *J. Exp. Bot.*, 1: 1-12.
- Bohnert, H.J., Q. Gong, P. Li and S. Ma. 2006. Unraveling abiotic stress tolerance mechanisms – getting genomics going. *Curr. Opin. Plant Bio.*, 9: 180-188.
- Chaudhri, I.I. 1961. The vegetation of Karachi. *Vegetatio*, 10: 229-246.
- Chen, T.H.H. and N. Murata. 2002. Enhancements of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. *Curr. Opin. Plant Biol.*, 5: 250-257.
- Dobrota, C. 2006. Energy dependant plant stress acclimation. *Rev. Environ. Sci. Biotechnol.*, 5: 243-251.
- Ehleringer, J.R. 1993. Carbon and water relations in desert plants: An isotopic perspective. In: *Perspectives of Plant Carbon and Water Relations from Stable Isotopes* (Eds.): J.R. Ehleringer, A.E. Hall & G.D. Farquhar, pp. 155-172. Academic Press, San Diego, California.
- Ezawa, S. and Y. Tada. 2009. Identification of salt tolerance genes from the mangrove plant *Bruguiera gymnorrhiza* using *Agrobacterium* functional screening. *Plant Sci.*, 176: 272-278.
- Gul, B., R. Ansari, I. Aziz and M.A. Khan. 2010. Salt tolerance of *Kochia scoparia*: A new fodder crop for highly saline arid regions. *Pak. J. Bot.*, 42: 2479-2487.
- Hoque, M.A., M.N.A. Banu, E. Okuma, K. Nakamura and Y. Shimoishi. 2007. 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.*, 164: 1457-1468.
- Kang, S., W.J. Davies, L. Shan and H. Cai. 2003. *Water-Saving Agriculture and Sustainable Use of Water and Land Resources*, Vols. 1 & 2. Shaanxi Science and Technology Press.
- Khan, M.A., I.A. Ungar and A.M. Showalter. 2000a. Effects of sodium chloride treatments on growth and ion accumulation of the halophyte *Haloxylon recurvum*. *Comm. Soil Sci. Plant Nut.*, 31: 2763-2774.
- Khan, M.A., I.A. Ungar and A.M. Showalter. 2000b. Growth, water, and ion relationships of a leaf succulent perennial halophyte, *Suaeda fruticosa* (L.) Forssk. *J. Arid Environ.*, 45: 73-84.
- Khan, M.A., I.A. Ungar and A.M. Showalter. 2000c. Salt tolerance in the subtropical perennial halophyte *Atriplex griffithii* Moq. var. *stocksii* Boiss. *Ann. Bot.*, 85: 225-232.
- 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: 136-142.
- Lauchli, A. and S.R. Grattan. 2007. Plant growth and development under salinity stress. In: *Advances in Molecular Breeding Towards Drought and Salt Tolerant Crops*, M.A. Jenks *et al.*, (Eds.), p. 1-32. Springer.

- Laurie, Li, R., F. Shi and K. Fukuda. 2010. Interactive effects of various salt and alkali stresses on growth, organic solutes and action accumulation in a halophyte *Spartina alterniflora* (Poaceae). *Env. Exp. Bot.*, 68: 66-74.
- Maclachlam, S. and S. Zalik. 1963. Extraction and estimation of chlorophyll. *Can. J. Bot.*, 41: 1053.
- Miller, G., N. Suzuki, S. Cifti-Yilmaz and R. Mittler. 2010. Reactive oxygen species homeostasis and signaling during drought and salinity stresses. *Plant Cell Environ.*, 33: 453-467.
- Moorthy, P. and K. Katherisan. 1999. Effects of UV-B radiation on photosynthetic reactions in *Rhizophora apiculata*. *Plant Growth Regul.*, 28: 49-54.
- Munns, R. 2002. Comparative Physiology of Salt and water stress. *Plant Cell and Environ.* 25: 239-250.
- Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. *Ann. Rev. Plant Biol.*, 59: 651-681.
- Naidoo, G. 2009. Differential effects of nitrogen and phosphorus enrichment on growth of dwarf *Avicennia marina* mangroves. *Aquat. Bot.*, 90: 184-190.
- Nardini, A., M.T. Tyree and S. Salleo. 2001. Xylem cavitation in the leaf of *Prunus laurocerasus* L. and its impact on leaf hydraulics. *Plant Physiol.*, 125: 1700-1709.
- Neilson, R.P. 2003. The importance of precipitation seasonality in controlling vegetation distribution. In: *Changing precipitation Regimes and Terrestrial Ecosystems: A North American Perspective*. (Eds.): J.F. Weltzin and G.R. McPherson, p. 47-71. The University of Arizona Press.
- Nunes, C., S. deSousa, C. Araújo, J.M. da Silva, M. Pedro, F. Fevereiro and A.B. da Silva. 2008. Physiological responses of the legume model *Medicago truncatula* C.V. Jemalong to water deficit. *Environ. Exp. Bot.*, 63: 289-296.
- Parida A.K., A.B. Das and P. Mohanty. 2004 Defense potentials to NaCl in a mangrove, *Bruguiera parviflora*: differential changes of isoforms of some antioxidative enzymes. *J. Plant Physiol.*, 161: 531-542.
- Parida, A.K. and B. Jha. 2010. Salt tolerance mechanisms in mangroves: A review. *Trees*, 24: 199-217.
- Raymond, M.J. and M. Smirnov. 2002. Proline metabolism and transport in maize seedlings at low water potential. *Ann. Bot.*, 89: 813-823.
- Reddy, T.Y., V.R. Reddy and V. Anbumozhi. 2003. Physiological responses of groundnut (*Arachis hypogaea* L.) to drought stress and its amelioration: a critical review. *Plant Growth Regul.*, 41: 75-88.
- Reynolds, J.F., P.R. Kemp, K. Ogle and R.J. Fernandez. 2004. Modifying the “pulse-reserve” paradigm for deserts of North America: Precipitation pulses, soil water, and plant responses, *Oecologia.*, 141: 194-210.
- Rhodes, D. and A.D. Hanson. 1993. Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Ann. Rev. Plant Physiol. & Plant Mol. Biol.*, 44: 357-384.
- S., M. Bradbury and G.R. Stewart. 1994. Relationship between leaf temperature, compatible solutes and antitranspirant treatment in some desert plants. *Plant Sci.*, 100: 147-156.
- Slama, I, T. Ghnaya, A. Savoure and C. Abdelly. 2008. Combined effect of long term salinity and soil drying on growth, water relations, nutrient status and proline accumulation of *Sesuvium portulacastrum*. *Plant Biol. & Pathol.*, 331: 442-451.
- Tipirdamaz, R., D. Gagneul, C. Duhazé, A. Ainouche, C. Monnier, D. Özkum and F. Larher. 2006. Clustering of halophytes from an inland salt marsh in Turkey according to their ability to accumulate sodium and nitrogenous osmolytes. *Environ. Exp. Bot.*, 57: 139-153.
- Villar, P., L. Ocana, J.L. Penuelas and I. Carrasco. 1999. Effect of water stress conditioning on the water relations, root growth capacity and the nitrogen and non-structural carbohydrate concentration of *Pinus halepensis* Mill. (Allepo pine) seedlings. *Ann. For. Sci.*, 56: 459-465.