

Clustering of halophytic species from Cyprus based on ionic contents

Ozturk M¹, S Gucl², V Altay³, MSA Ahmad⁴, MY Ashraf⁵, M Ashraf⁶

Abstract. This paper presents the work conducted on the chemical constituents of some common and widely distributed halophyte taxa from Cyprus with the aim that these studies will help in the evaluation of halophytes for different economical purposes. The plant species of *Crithmum maritimum* L., *Limbarda crithmoides* (L.) Dumort, *Atriplex portulacoides* L., *Salsola kali* L., *Atriplex halimus* L., *Limonium oleifolium* Mill., *L. meyeri* (Boiss.) Kuntze; and *Tetraena alba* (L.f.) Beier & Thulin were collected in the middle of July. The shoot tissue and leaf samples were collected from the natural habitats and left for drying under air circulation followed by placing them in oven at 60 °C for 96 hours. The material was crushed using mortar and pestle and subjected to an analysis of macro- and micro-nutrients and biochemical compounds. K⁺/Na⁺ in the leaf tissues of the dicot species showed relatively high values depicting their behavior as Na⁺ includes but very low Cl⁻ levels were recorded. Out of the species investigated here in 4 TFAA content was rather high. Values ranging from 0.5% to 1% dry weight were exhibited in one species. However, only 3 species showed very low TFAA values. Later may be due to low nitrogen availability in their environment. The phenetic analyses of eight halophyte species performed on the data matrix using Ntsys-pc program version 2.1 revealed that, cluster analysis of the overall results obtained here leads to 2 clusters. This discrimination appears to be as a result of their different abilities to accumulate either proline or glycine betaine.

Keywords: Anions; Cations; Fodder; Minerals; Organic osmolytes; Salt tolerant.

INTRODUCTION

Cyprus, is the third largest island, situated in the East Mediterranean and located within one of the major 25 biodiversity hotspots (Hadjisterkotis, 2004). The island possesses a long coastal belt abounding in halophytes, flourishing as an integral part of many marine, coastal and terrestrial ecosystems (Ozturk et al., 2011). Looking at the global distribution of these taxa reveals that there are conflicting reports on their numbers which arise from the differences in the definition of halophytes. Aronson (1989) has published a list of ~1550 salt-tolerant plants, whereas Lieth & Mochtchenko (2003) report over 2600 species. However, Flowers et al. (2010) gives a list of 345 halophytes which according to him could be regarded as euhalophytes.

These plants have developed several adaptive traits expressed at various levels of organization, which allows them to complete their life cycle under such harsh conditions (Flowers et al., 2010). In general the water movement in the different parts of plants is responsible for supplying essential nutrients, but in saline habitats the situation changes because the dissolved solutes entering a plant from the soil move to the leaves for accumulation, where recirculation or excretion occurs (Flowers et al., 2010), these may be excreted, re-circulated or accommodated.

The problem of salinity are increasing and presently over 800 million ha of land is salt-affected, i.e., nearly 6% of the global land area (Flowers et al., 2010). The reasons being over irrigation following the destruction of natural plant cover. These changes end up by raising the watertable which brings the dissolved salts to the surface. Almost 50% of irrigation schemes running all over our planet are affected

¹ Botany Department & Centre for Environmental studies, Ege University, 35040, Izmir, Turkey.

² Environmental Sciences Institute, Near East University, 99138, Turkish Republic of Northern Cyprus.

³ Biology Department, Fac. of Sciences and Arts, Hatay Mustafa Kemal University, 31060, Hatay, Turkey.

⁴ Department of Botany, University of Agriculture, 38000, Faisalabad, Pakistan.

⁵ Deputy Chief Scientist Nuclear Inst. for Agriculture and Biology (NIAB), 38000, Faisalabad, Pakistan.

⁶ Pakistan Science Foundation, 1-Constitution Avenue, G-5/2, 44050, Islamabad, Pakistan.

Address correspondence to: Munir Ozturk, e-mail: munirozturk@gmail.com

by salinity (Flowers et al., 2010). No doubt it is relatively small compared to the total global area of food production, it produces a third of the food (Barkla et al., 1999), with a huge impact on agricultural productivity. Nearly 12 billion US\$ has been estimated as the global annual cost of salt-affected land (Qadir et al., 2008; Flowers et al., 2010). Our ability to grow economically feasible plants on salt-affected lands fully relies on our future evaluation of these lands for agricultural production (Qadir et al., 2008; Flowers et al., 2010).

The halophytes are thus expected to play an important role in future bio-saline agriculture, aquaculture and habitat restoration. Therefore studies on these plants, their conservation and development fit well into UNESCO's mandate. Understanding the origin of diversity of halophytes therefore becomes a matter of paramount importance, as it will form the basis for investigating the species good for bioremediation as well as conservation of novel species (Flowers et al., 2010).

The study undertaken by Ozturk et al. (2011) for the first time in Cyprus has revealed that a total of 457 plant taxa with distinct features are distributed along a 770 km long coastline, out of these 129 taxa are typical halophytes. Many of these are gathered as vegetables since ancient times. Such uses of plants occupying saline habitats are now attracting a greater attention for the development of cash crop halophytes, as well as methods for coastal habitat restoration (Boer, 2004). They are expected to play an important role towards sustainable coastal management (Boer, 2004), using indigenous halophytes with little irrigation in agricultural practices to regain degraded pastures (Peacock et al., 2003), biosphere reserves, in ecosystem engineering projects like stabilising wetlands for revegetation programs (Fogel et al., 2004), to develop salt-tolerant species for landscape remediation and development of models for sustainable living. The revegetation programs can be used to reduce salt concentration in left over mining areas as well as movement of salts from the mine sites in runoff (Carroll & Tucker, 2000). A great care is needed in introducing species for bioremediation (Cambrolle et al., 2008), so that these do not become an environmental problem latter on, as has been reported for *Kochia* planted for forage and bioremediation in Western Australia (Qadir et al., 2008; Flowers et al., 2010).

This is especially true in these times of fresh water scarcity, population growth and increased resources consumption (Ozturk et al., 2008). The products from halophytes are believed to enable several governmental and nongovernmental organizations to generate sustainable environment-based income for people in arid coastal zones (Boer, 2004).

However, a careful analysis of the chemical composition of halophytes is needed before their utilization for different purposes (Squires & Ayoub, 2012). Some halophytes are used without any knowledge of their food value (Ozturk et al., 2008). A clear evidence for their use need to be provided. We must know that halophytes we are consuming have nutritional value and lack any harmful components (Squires & Ayoub, 2012). Some evidences also point out that high

sodium chloride levels together with some heavy metals or other elements may be accumulated by halophytes in levels not allowed in food or feed (Squires & Ayoub, 2012). Heavy industrialisation has led to tremendous increase in the concentration of heavy metals in the environment. These include Cd, Cu, Pb and Zn, which prove toxic after high accumulation in sediments or soils of marshes. For example some seagrasses and salt marsh plants can extract heavy metals from sediments which get accumulated in these plants in different tissues (Weis & Weis, 2004; Flowers et al., 2010).

This paper presented the work conducted on the chemical constituents of some common and widely distributed halophyte taxa from Cyprus with the aim that this study will help in the evaluation of halophytes as food and fodder as well as for land reclamation, landscaping and gardening. These can be also used as ornamentals (Squires & Ayoub, 2012).

MATERIALS AND METHODS

Plant material. Plants species used in this investigation were collected from the Northern part of Cyprus. The species used are: *Crithmum maritimum* L. (Apiaceae); *Limbarda crithmoides* (L.) Dumort (Asteraceae); *Atriplex portulacoides* L., *Salsola kali* L., *Atriplex halimus* L. (Amaranthaceae); *Limonium oleifolium* Mill., *L. meyeri* (Boiss.) Kuntze (Plumbaginaceae); and *Tetraena alba* (L.f.) Beier & Thulin (Zygophyllaceae).

Samples of leaves and shoot tissues were collected and dried in an oven at 70 ± 2 °C. Oven dried plant samples were used for the estimation of macro- and micro-nutrients and biochemical analyses. The leaves and twigs were ground in a micromill to pass through a 0.5 mm mesh for subsequent chemical analysis. Crude protein, crude fiber, total minerals and total carbohydrate were determined according to the method of Helrich (1990). The plant material was digested by aid-digestion method as described by Wolf (1982) and the aliquot was used for the estimation of Na^+ , K^+ and Ca^{2+} through a flame photometer (PFP7, Jenway, England). From the same acid-digested aliquot, Mg^{2+} was determined titrimetrically and P using Barton's reagent (Jackson & Barak, 2005). The concentration of micro-nutrients Mn, Cu, Fe and Zn were determined by Atomic Absorption Spectrophotometer (Analytikjena, Model novAA400, Germany). For chloride analysis, the oven-dried ground material was boiled in distilled water and Cl^- contents were determined by Chloride meter (Corning-920; UK). The glycinebetaine contents were estimated according to Grieve & Grattan (1983), proline by the method of Bates et al. (1973). The method of Hamilton & Van Slyke (1943) was used for the determination of total free amino acids (TFAA).

Statistical analysis. The data generated was statistically analyzed by one-way analysis of variance (ANOVA) and LSD values (5%) were used to compare significance of mean values. The phenetic analyses of eight halophyte species were performed on the data matrix using the Ntsys-pc program version 2.1 (Rohlf & Corti, 2000). The pairwise distances

were computed using the average taxonomic distance. The distance matrix generated was used for UPGMA cluster analysis (Sneath & Sokal, 1973) and species were grouped in clusters and sub-clusters.

RESULTS AND DISCUSSION

Table 1 presents the results of our findings on 8 species investigated during this study together with the ratios K^+/Na^+ , Cl^-/HCO_3^- as well as between K^+ and (Na^++K^+) . In addition the selectivity for K^+ over Na^+ , ratios between glycinebetaine (GB) and total amino acid content (TAA), between proline (PRO) and total free amino acids (TFAA) has also been evaluated for enlightening different aspects of these nitrogenous osmolytes.

Our investigations reveal that dicotyledonous taxa behave as Na^+ includes, which ends up with high values for K^+/Na^+ in their leaf tissues. The Cl^- content generally is low even in the chenopods. Therefore, the calculated Cl^-/HCO_3^- ratio does not go up to the value of one (Table 1), suggesting non-identified other anionic taxa take part in the ionic balance of these halophytes.

Na^+ accumulation is generally affiliated to the chloride. The relative amounts of Na^+ range between 24.20- 65.13 mg g^{-1} dwt. Internal osmotic potential and turgor maintenance are largely dependent on these amounts. The absorbance, translocation together with concentration of Na^+ and Cl^- in the shoots is dependent on these and dicotyledonous taxa growing on saline habitats behave like other halophytes

distributed in different marine ecosystems. When Na^+ and Cl^- are freely available in the soil they are the cheap as well as useful solutes at the cell level and well sequestered in vacuoles. This type of compartmentalization hinders their damaging effects in cytosol and its organelles (Tipirdamaz et al., 2006). However, such valorization points to the ability to maintain a sufficient level for K^+ especially in the cytosol compartment invaded with Na^+ , which is due to an obligate K^+ requirement for sustaining protein synthesis, as mRNA translation on ribosomal structures depends on the presence of K^+ here at 100 mM to 150 mM concentration provided Na^+ and Cl^- do not exceed 30-50 mM (Wyn Jones and Gorham, 2002; Tipirdamaz et al., 2006). This type of requirement allows facing different adaptation traits related to plant sodicity relationships, but depends on a dicotyledonous taxon. The values of internal molecular ratios $[K^+]_{int}/[Na^+]_{int}$ or $[K^+]_{int}/[K^++Na^+]_{int}$ can throw some light on this, as they are assumed to be due to the selectivity of a given plant taxon for K^+ over Na^+ . Moreover the selectivity could appear relatively low in some plants as per these ratios, being related to rather high values for $[Na^+]_{int}$ (Tipirdamaz et al., 2006). The internal Ca^{2+} level greatly varies with the species, the selectivity for K^+ over Na^+ could also be effected by this and may also be due to the Ca^{2+} availability in the external medium (Cramer, 2002; Tipirdamaz et al., 2006).

Free amino acid precursors are primarily accepted to be responsible for synthesis of PRO and GB, these compounds have been collectively determined. To some extent their amount actually reflects nitrogen availability in the natural

Table 1. Species names of halophytic dicots and the mineral and organic contents of their shoots (mg g^{-1} dwt)

	<i>Limonium meyeri</i>	<i>Salsola kali</i>	<i>Limbar da crithmoides</i>	<i>Limonium oleifolium</i>	<i>Tetraena alba</i>	<i>Crithmum maritimum</i>	<i>Atriplex portulacoides</i>	<i>Atriplex halimus</i>
Na	28.417	58.050	29.467	29.250	25.243	24.207	65.130	64.167
K	9.040	24.050	12.540	8.630	9.707	34.640	36.207	28.557
K/Na	0.322	0.416	0.427	0.296	0.386	1.434	0.556	0.445
K/Na+K	0.243	0.294	0.299	0.228	0.278	0.589	0.357	0.308
Ca	7.300	13.400	22.033	8.400	17.467	11.500	12.500	8.100
Mg	8.553	4.147	17.233	8.163	13.533	5.113	11.700	9.600
Cl⁻	2.140	1.263	3.190	2.980	3.403	3.257	6.807	5.277
HCO₃⁻	0.327	0.430	1.103	0.368	0.795	2.180	1.737	0.807
Cl/HCO₃⁻	6.562	2.939	2.892	8.091	4.283	1.494	3.920	6.536
Cl/(Cl+HCO₃⁻)	0.868	0.746	0.743	0.890	0.811	0.599	0.797	0.867
P	1.523	1.277	9.517	1.927	2.193	3.947	2.400	2.357
N	6.277	7.067	7.733	10.410	10.200	14.267	15.467	10.000
Crude Protein	59.460	44.403	48.603	67.000	63.107	88.580	90.483	62.210
Proline	0.334	0.052	0.169	0.180	0.036	0.141	0.100	0.018
GB	0.927	1.139	1.029	0.855	1.661	1.242	1.026	1.008
TFAA	1.920	0.497	1.223	1.237	0.209	1.637	0.737	0.427
Proline/TFAA	0.175	0.106	0.142	0.146	0.175	0.086	0.136	0.042
GB/TFAA	0.483	2.308	0.878	0.696	8.041	0.759	1.395	2.367
Mn	0.019	0.041	0.000	0.039	0.003	0.023	0.041	0.040
Cu	0.006	0.003	0.014	0.007	0.006	0.011	0.010	0.009
Fe	0.145	0.003	0.337	0.595	0.003	0.048	0.070	0.043
Zn	0.013	0.011	0.015	0.014	0.013	0.014	0.020	0.013

system under study together with the nitrogen status in the shoots of the plant taxa evaluated. It appears the TFAA content is rather high in four species (values higher than 1% DW). In one species values ranging from 0.5 % to 1% DW were recorded.

Only in three species have shown very low TFAA values, pointing to the low nitrogen availability in their saline habitat. In spite of this, the shoots of these species show significant amounts of the nitrogenous solute GB (*Salsola kali*, *Atriplex halimus* and *Tetraena alba*). Very high amount of free proline values ranging from 0.141 to 0.334 mg g⁻¹ dw were found in four species over the 4 dicots under study. Among 3 chenopods investigated, only 1 contained important amounts of PRO, i.e., *Atriplex portulacoides*. Apart from *Limonium meyeri*, the plant taxa accumulating high amounts of free PRO contain low to very low amount of GB (*Tetraena alba* and *Crithmum maritimum*). As against this, high to very high amounts of GB occur in the non accumulators of PRO, especially in Amaranthaceae. The abundance of GB is not restricted to the chenopods because the shoots of *Tetraena alba*, *Crithmum maritimum* and *Limbarda crithmoides* also contain high levels of betaine.

Our findings have also revealed that when a plant taxon behaves as a GB accumulator this compound quantitatively exceeds the amount of TAC, which could be associated with the presence of high amounts of free amino acids to some extent (Briens & Larher, 1982; Tipirdamaz et al., 2006).

The phenogram presented in Fig. 1 depicts that 8 halophyte plant taxa under study could be assembled into 2 main clusters.

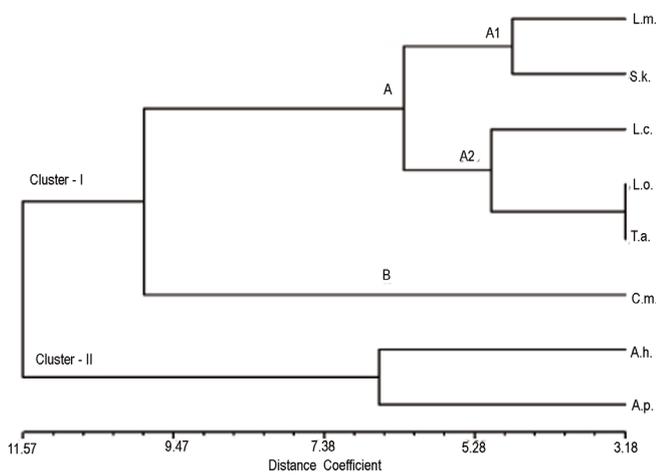


Fig. 1. Results of cluster analysis (UPGMA) of 8 halophyte species sampled from Northern Cyprus. The average taxonomic distances among 21 chemical and biochemical attributes of seeds of all species was calculated using ntsYSpc software (v 2.1). The similarity among species is indicated by distance coefficients and used to group species into remarkable clusters and sub-clusters in the phenogram

Cluster I assembles 6 species according to the two main subunits A and B. *Subunit A*: A1 group is found to be composed of *Limonium meyeri* and *Salsola kali*. *Limonium oleifolium*, *Tetraena alba* and *Limbarda crithmoides* fall in group A2. *Subunit B*: comprised *Crithmum maritimum*.

Cluster II assembles other 2 species, *Atriplex portulacoides* and *Atriplex halimus*, sampled for this study. Cluster analysis of our results points out towards the separation of 8 plant taxa investigated by us into 2 clusters, mainly originating from their diverse ability to accumulate either proline or glycine betaine. These separations agree with our other findings related to the occurrence and distribution of principal osmolytes which probably contribute towards their salt tolerance. As pointed out by Tipirdamaz et al. (2006) as well, two-ways cluster analysis performed with the values for both these variables strongly suggests that these solutes might rule each other out.

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 are a large number of plant defense mechanisms including ion homeostasis, osmoregulation, antioxidant and hormonal systems helping plants to thrive well under saline conditions (Sairam & Tyagi, 2004). The characterization of some of the mechanisms of inhibitory effect of sodium chloride on growth of higher plants has been summarized at length by Tipirdamaz et al. (2006). 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 (Jung et al., 2010).

In halophytes, proline accumulation under stress in several plant taxa has been correlated with stress tolerance. The concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants (Ozturk & Szaniawski, 1981; Ozturk et al., 1986; Ashraf & Foolad, 2007; Turkyilmaz et al., 2013). 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 (Tipirdamaz et al., 2006). Moreover, the eco-physiological studies on wide range of halophytic plant taxa inhabiting different habitats including the inland ecosystems as well can allow us to enlighten the most accurate comparative data of the diversity of strategies developed by halophytes. It will also help us to focus on most adequate plant models for studying different mechanisms related to salt tolerance under experimental as well as natural conditions.

REFERENCES

- Aronson, J.A. (1989). HALOPHa data base of salt tolerant plants of the World. Office of Arid Land Studies, University of Arizona: Tucson, AZ, USA.
- Ashraf, M., M.R. Foolad (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany* 59(2): 206-216.
- Barkla, B.J., R. Vera Estrella & O. Pantoja (1999). Towards the production of salt-tolerant crops. *Advances in Experimental Medicine and Biology* 464: 77-89.
- Bates, L.S., R.P. Waldren & I.D. Teare (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil* 39: 205-207.
- Boer, B. (2004). Halophyte development in the Gulf Arab countries-UNESCO Doha's activities 2001-2003. *Tropical Ecology* 45(1): 187.
- Briens, M. & F. Larher (1982). Osmoregulation in halophytic higher plants: a comparative study of soluble carbohydrates, polyols, betaines and free proline. *Plant, Cell & Environment* 5(4): 287-292.
- Cambrolle, J., S. Redondo-Gomez, E. Mateos-Naranjo & M.E. Figueroa (2008). Comparison of the role of two *Spartina* species in terms of phytostabilization and bioaccumulation of metals in the estuarine sediment, *Marine Pollution Bulletin* 56: 2037-2042.
- Carroll, C. & A. Tucker (2000). Effects of pasture cover on soil erosion and water quality on central Queensland coal mine rehabilitation. *Tropical Grasslands* 34: 254-262.
- Cramer, G.R. (2002). Sodium-calcium interactions under salinity stress. In: Salinity: Environment-plants-molecules, Läuchli, A. & U. Lüttge (eds.), Springer Netherlands pp. 205-227.
- Flowers, T.J., K. Hana, A.C. Galal & B.D. Lindell (2010). Evolution of halophytes: multiple origins of salt tolerance in land plants. *Functional Plant Biology* 37: 604-612.
- Fogel, B.N., C.M. Crain & M.D. Bertness (2004). Community level engineering effects of *Triglochin maritima* (seaside arrowgrass) in a salt marsh in northern New England, USA. *Journal of Ecology* 92: 589-597.
- Grieve, C.M. & S. Grattan (1983). Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant and Soil* 70(2): 303-307.
- Hadjisterkotis, E. (2004). Alien species on Cyprus. Aliens (Invasive Species Specialist Group of the IUCN Species Survival Commission) pp. 43.
- Hamilton, P.B. & D.D. Van Slyke (1943). The Gasometric analysis of free amino acids in blood filtrates by the ninhydrin-carbondioxide method. *Journal of Biological Chemistry* 150: 231.
- Helrich, K.C. (1990). Official methods of Analysis of the AOAC. Association of Official Analytical Chemists Inc.
- Jackson, M.L. & P. Barak (2005). Soil chemical analysis: Advanced course. UW-Madison Libraries Parallel Press.
- Jung, Y., J. Park, Y. Choi, J.G. Yang, D. Kim, B.G. Kim, K. Roh, D.H. Lee, C.K. Auh & S. Lee (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.
- Lieth, H. & Mochtchenko, M. (2003). Cash crop halophytes. Kluwer: Dordrecht, the Netherlands.
- Ozturk, M. & R.K. Szaniawski (1981). Root temperature stress and proline content in leaves and roots of two ecologically different plant species. *Zeitschrift für Pflanzenphysiologie* 102: 375-377.
- Ozturk, M., T. Sato & N. Takahashi (1986). Proline accumulation in shoots and roots of some ecophysiologicaly different plants under root temperature stress. *Environmental Control in Biology (Japan)* 24: 79-85.
- Ozturk, M., A. Guvensen & S. Gucl (2008). Ecology and economic potential of halophytes - A case study from Turkey. In: Crop and forage production using saline waters, Kafi, M. & M.A. Khan (eds.), Daya Publishing House, NAM S & T Centre, Delhi, India Chapter 21, pp. 255-264.
- Ozturk, M., S. Gucl, A. Guvensen, C. Kadis & C. Kounnamas (2011). Halophyte plant diversity, coastal habitat types and their conservation status in Cyprus. In: Sabkha Ecosystems, Tasks for Vegetation Science, Ozturk, M. et al. (eds.), Springer 46: 99-111.
- Peacock, J.M., M.E. Ferguson, G.A. Alhadrami, I.R. McCann, A. Al Hajoj, A. Saleh & R. Karnik (2003). Conservation through utilization: A case study of the indigenous forage grasses of the Arabian Peninsula. *Journal of Arid Environments* 54: 15-28.
- Qadir, M., A. Tubeileh, J. Akhtar, A. Larbi, P.S. Minhas & M.A. Khan (2008). Productivity enhancement of salt-affected environments through crop diversification. *Land Degradation & Development* 19: 429-453.
- Rohlf, F.J. & M. Corti (2000). Use of two-block partial least-squares to study covariation in shape. *Systematic Biology* 49(4): 740-753.
- Sairam, R.K. & A. Tyagi (2004). Physiology and molecular biology of salinity stress tolerance in plants. *Current Science* 86: 407-421.
- Sneath, P.H. & R.R. Sokal (1973). Numerical taxonomy. The principles and practice of numerical classification, Freeman. San Francisco, USA.
- Squires, V. & A.T. Ayoub (eds) (2012). Halophytes as a Resource for Livestock and for Rehabilitation of Degraded Lands. Vol. 32, Springer Science & Business Media.
- Tipirdamaz, R., D. Gagneul, C. Duhaz, A. Ainouche, C. Monnier, D. Ozkum & F. Larher (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.
- Turkylmaz Unal, B., A. Guvensen, A. Esiz Dereboylu & M. Ozturk (2013). Variations in the proline and total protein contents in *Origanum sipyleum* L. from different altitudes of Spil Mountain, Turkey. *Pakistan Journal of Botany* 45(S1): 571-576.
- Weis, J.S. & P. Weis (2004). Metal uptake, transport and release by wetland plants: Implications for

phytoremediation and restoration. *Environment International* 30: 685-700.

Wolf, B. (1982). A comprehensive system of leaf analyses and its use for diagnosing crop nutrient status. *Communications in Soil Science & Plant Analysis* 13(12): 1035-1059.

Wyn Jones, R.G. & J. Gorham (2002). Intra-and inter-cellular compartmentation of ions. In: *Salinity: environment-plants-molecules*, Läuchli, A. & U. Lüttge (eds.), Springer Netherlands, pp. 159-180.