Clustering of halophytic species from Cyprus based on ionic contents

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**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.

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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 lead 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); *Limbara 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<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> through a flame photometer (PFP7, Jenway, England). From the same acid-digested aliquot, Mg<sup>2+</sup> 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<sup>-</sup> contents were determined by Chloride meter (Corning-920; UK). The glycinebetaine contents were estimated according to Grieve & Gratton (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 (Sneth & 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 (TAC), 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).
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\(^{-1}\) dwt 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 Limbara 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.

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 Limbara 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 & Szaniawiski, 1981; Ozturk et al., 1986; Ashraf & Foolad, 2007; Turkylilmaz 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.

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.
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