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Seed Protein Variations of *Salicornia* L. and Allied Taxa in Turkey

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Abstract: Electrophoretic seed protein patterns of a number of accessions of *Salicornia europaea* L. sl., *S. prostrata* Palas, *S. fragilis* P.W. Ball and Tutin, *Sarcocornia fruticosa* (L.) A. J. Scott, *Sarcocornia perennis* (Miller.) A. J. Scott, *Arthrocnemum glaucum* (Del.) Ung.-Sternb., *Microcnemum coralloides* (Loscos and Pardo) subsp. *anatolicum* Wagenitz and *Halocnemum strobilaceum* (Pall.) Bieb. were electrophoretically analysed on SDS-PAGE. In total 48 different bands were identified. The obtained data have been treated numerically using the cluster analysis method of unweighted pair group (UPGMA). Finally it was determined that all species separated according to seed protein profiles. And the cladogram obtained studied taxa have been given.

Key words: Arthrocnemum, chenopodiaceae, SDS-PAGE, seed proteins, *Salicornia*, *Sarcocornia*

INTRODUCTION

Salicornia L. (Chenopodiaceae) is a genus of annual, apparently leafless halophytic herbs that have articulated, succulent stems. World wide there are 13 species with innumerable variants (Scott, 1977). The number of European species varies between seven (Scott, 1977) and eight (Ball and Akeroyd, 1993). There is no revision for Asian Salicornia, most of the floras cited only *Salicornia europaea* L. sensu lato (Iljin, 1936; Grubov, 2000; Hedge, 1997; Bolous, 1996). Freitag *et al*. (2001) recorded *S. perennans* Wild. From northern Caspian and Akhani (2003) described *S. persica* Akhani from central Iran. There are three *Salicornia* species known in Turkey *S. europaea* L., *S. prostrata* Pallas, *S. fragilis* P.W. Ball and Tutin, each are known from other parts of Europe (Ball, 1967a, b). In *Salicornia*, a combination of inbreeding, which allows the development of locally differentiated populations, considerable phenotypic plasticity, a much reduced morphology of the plant and the inadequacy of herbarium material in representing the succulent growth form has created great taxonomic difficulties (Ball, 1960, 1964; Davy *et al*., 2001).

Arthrocnemum is a genus of succulent halophytic subshurbs or shrubs closely related to *Salicornia*. There are three species known from Europe, Middle East and Turkey; *A. perenne* (Mill.) Moss., *A. fruticosum* (L.) Moq. and *Arthrocnemum glaucum* (Del.) Ung.-Sternb. (Ball, 1967a, b, 1993). The limits of the genera *Salicornia*, *Arthrocnemum* and *Sarcocornia* are not clear. *Arthrocnemum perenne* and *A. fruticosum* are assigned to three different genera (*Salicornia*, *Arthrocnemum* and *Sarcocornia*) by different authors (Ball, 1993, 1967a, b; Scott, 1977; Freitag, 2000). The distinctness of *Arthrocnemum perenne* and *A. fruticosum* is not clear (Freitag, 2000).

It is therefore difficult to find convincing species delimitations on the basis of morphological characters only. Molecular studies have shown that even morphologically hardly distinguishable species are genetically distinct (Wolff and Jefferies, 1987; Jefferies and Gottlieb, 1982). The high stability of seed proteins makes them a powerful tool in elucidation of the origin, evolution and relationship of taxa (El Naggar, 2001; Bhargava *et al*., 2005; Vladova *et al*., 2000). Therefore, we tried to distinguish the Turkish *Salicornia* L. species and allied taxa with the aid of seed protein markers in this study. Salicornia species have seed dimorphism except *S. pusilla* J. Woods (König, 1960; Dalby, 1962; Ungar, 1979). Central seeds were used for the study. The obtained data were analyzed by numerical analysis (cluster analysis) based on Jaccard's coefficient (Sneath and Sokal, 1973).

MATERIALS AND METHODS

First specimens to be identified have been collected from the cited localities (Table 1) in flowering time in 70% alcohol (Ball, 1960). After identification of the specimens, seed specimens have been collected from the same localities. Five individual plants with seed per populations have been sampled for Salicornia, one per others. Voucher specimens of studied taxa are deposited in Herbarium ANK.

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Table 1: Localities of the studied taxa

Taxon	Locality
Salicornia europaea sl.	Ankara-Sereflikoçhisar, 24.11.00
Salicornia europaea sl.	Aksaray- Aksaray-Ulukişla arasi, 25.11.00
Salicornia europaea sl.	Konya-Kirkişla, 31.10.00
Salicornia europaea sl. MT-1	Çorum-Sungurlu, 27.10.01
Salicornia europaea sl. MT-2	Çorum-Sungurlu, 27.10.01
Salicornia prostrata (1)	Samsun-Bafra Cernek Gölü, 28.10.01
Salicornia prostrata (2)	Samsun-Bafra Cernek Gölü, 28.10.01
Salicornia europaea sl. (1)	Icel-Tarsus Alifakhi Çorağı, 26.11.00
Salicornia europaea sl. (2)	Icel-Tarsus Alifakhi Çorağı, 26.11.00
Salicornia fragilis	Izmir-Menemen Çamalti tuzlasi, 23.11.00
Sarcocornia perennis	Samsun-Bafra Çernek Golu, 28.10.01
Sarcocornia fruticosa	Izmir-Menemen Çamalti tuzlasi, 23.11.00
Arthrocnemum glaucum	Izmir-Menemen Çamalti tuzlasi, 02.11.00
Microcnemum coralloides subsp.anatolicum	Aksaray- Aksaray-Ulukişla arasi, 25.11.00
Halocnemum strobilaceum	Ankara-Şereflikochişar, 24.11.00

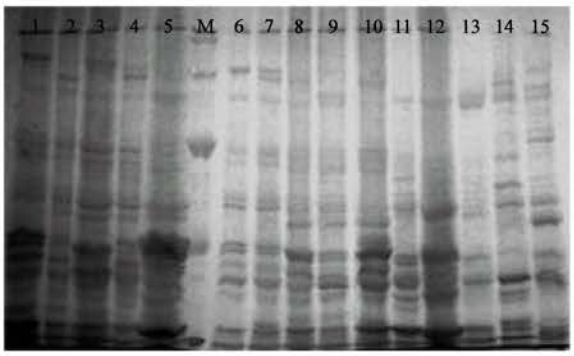

Fig. 1: The photograph of electhrophoretic banding patterns of the studied taxa. M is Molecular Weight Marker, Numbers are representing taxa in same order in Table 1

Seeds of each individual plant were ground separately to fine flour in a prechilled mortar and pestle. Several extraction methods have been tried but the best results could be obtained as follows; Proteins were extracted (0.3 g seed flour to 150 µL extract) in the 0.5 M Tris/HCI buffer, pH 6.8. The extract was centrifuged at 13000 rpm for 3 min and the 75 µL supernatant was decanted and 25 µL concentrated sample buffer (4X) added and boiled for 5 min and aliquots used in PAGE.

Five individual per *Salicornia* sp. populations and one individual per each of other taxa have been examined electrophoretically. Electrophoresis was carried out in the modified discontinuous SDS-PAGE system of Laemmli (1970) using 10% acrylamide resolving gel (pH 8.6) and 4% stacking gel (pH 6.8). Gels were stained by 0.1% Coomassie Brilant Blue R-250 and destained in destaining solution (25% isopropyl alcohol, 10% glacial acetic acid and 65% distilled water). After decolorization, gels were photographed. The electhrophoretic banding patterns of the studied taxa are shown in Fig. 1 and the drawing of it is in Fig. 2.

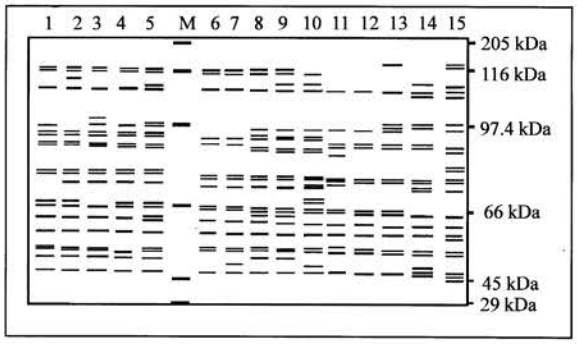

Fig. 2: The drawing of electhrophoretic banding patterns of the studied taxa. M is Molecular Weight Marker, Numbers are representing taxa in the same order as in Table 1

In total, 48 different bands were identified. Forty eight protein characters scored for each of the 15 Operational Taxonomic Units (OTU's). Each character was classified as presence (1) and absence (0). The Jaccard's coefficient $S_j = a/(a+b+c)$, (where a is the number of characters shared by a pair of samples, b is the number of characters found in one of a pair only and c is the number of characters found only in the other one of a pair) was used a measure of similarity of pattern (Sneath and Sokal, 1973). The matrix of Jacard's coefficients was used in a pair-wise cluster analysis using the unweighted pair group method (UPGMA) using arithmetic average to produce a phenogram of similarities.

RESULTS AND DISCUSSION

Two different morph of *S. europaea* sl. has been observed in Çorum, Sungurlu. Both morph included to study and treated as different taxon. In total, 48 different bands were identified (Fig. 1 and 2). Seed protein variation in population level has been observed only in the Tarsus population of *S. europaea* sl and Samsun population of *S. prostrata*. Each variant have been run in final gel and treated as different OTUs for statistical analysis (Fig. 3).

Cladogram has distinguished *Halocnemum strobilaceum* in the other taxa, Papini *et al*. (2004) also found similar results for *Halocnemum* in their ITS based study. The cladogram showed that genus Sarcocornia and *Arthrocnemum* are sister taxa. In ITS based cladogram of Papini *et al*. (2004) genus *Sarcocornia* is not distinguished in genus Arthrocnemum and they advocate threating them in genus *Arthrocnemum*. Kadereit *et al*. (2005), Davy *et al*. (2006) support distinctions between *Arthrocnemum* and *Sarcocornia* in their DNA based studies. Genus *Sarcocornia* was closer to *Arthrocnemum*

Table 2: Matrix of similarity between studied taxa. Numbers are representing taxa in the same order as in Table 1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.00														
2	8.50	1.00													
3	8.50	8.10	1.00												
4	8.95	8.50	8.50	1.00											
5	7.73	7.39	7.39	7.73	1.00										
6	6.50	7.00	6.19	6.50	6.36	1.00									
7	6.19	6.67	5.91	6.19	6.09	9.38	1.00								
8	9.00	8.57	8.57	9.00	7.83	6.67	6.36	1.00							
9	8.57	8.18	8.18	8.57	7.50	6.36	6.09	9.52	1.00						
10	6.67	6.40	6.40	6.67	6.54	5.42	5.83	6.80	7.20	1.00					
11	5.00	4.17	4.17	4.35	4.40	5.00	4.76	4.58	4.40	4.80	1.00				
12	5.50	4.55	4.55	4.76	4.78	5.56	5.26	5.00	4.78	4.58	8.67	1.00			
13	5.00	4.17	4.17	4.35	4.40	5.00	4.76	4.58	4.40	4.23	7.65	8.67	1.00		
14	3.10	3.45	3.45	3.10	3.23	4.00	4.40	3.33	3.23	4.00	5.22	5.00	5.91	1.00	
15	3.23	3.13	3.55	3.23	3.33	3.57	3.45	3.44	3.33	3.24	3.57	3.85	4.07	4.83	1.00

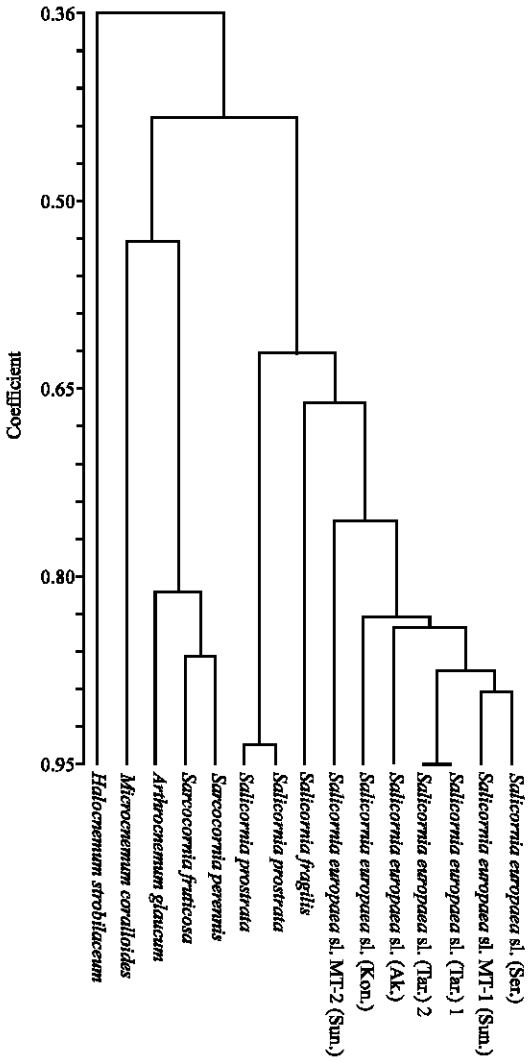

Fig. 3: Dendrogram shows the relationships between the taxa based on protein characters

than *Salicornia* in the cladogram, Papini *et al.* (2004) also found similar results while Kadereit *et al.* (2005) found *Sarcocornia* and *Salicornia* closer. Actually, morphologically Salicornia and Sarcocornia are closer except former is annual and the latter is perennial. *Microcnemum corraloides* subsp. *anatolicum* is closer to *Arthrocnemum* and *Sarcocornia* than *Salicornia* in the cladogram.

Cladogram has distinguished the known species of *Salicornia*, *S. europaea*, *S. prostrata*, *S. fragilis* from each other. On the other hand it showed that *S. europaea* clade is so variable in seed proteins. *S. europaea* sl. is also so variable morphologically therefore it needs to be investigated in details.

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