

# COMPARISON OF SEED PROTEINS ELECTROPHORESIS OF EIGHT SPECIES OF THE GENUS *SUAEDA* (CHENOPODIACEAE) IN IRAN

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## Abstract

Seed proteins of eight species of the genus *Suaeda* (Chenopodiaceae) were compared by means of polyacrylamide disc gel electrophoresis. Different samples of the same species showed not less than 89% similarities, and geographical separation had no remarkable effect on seed protein patterns of the same species. Morphological heterogeneity, that creates difficulties in infrageneric classification, was also evident in clustering analysis of protein bands. *S. microsperma*, which is morphologically very close to *S. acuminata*, was found a great distance away from any other species.

## Introduction

The use of seed protein, especially isozymes, analysis in systematic problems of different taxonomic levels is the subject of recent investigations [2, 3, 9, 12, 14]. Some genera of the family Chenopodiaceae have also been studied by various authors in order to survey intergeneric, specific and even infrageneric relationships by means of seed protein patterns and isozymes analysis [7, 11, 13, 15].

The genus *Suaeda* belongs to the tribe Suaedeae and is a cosmopolitan halophytic genus with extraordinary polymorphism. Ungar and Bouchaud [11] and Hekmat-Shoar *et al.* [7] have shown the valuability of seed protein electrophoresis analysis in determining species relationships in this genus. The present paper describes the results of electrophoresis studies on eight species of the genus *Suaeda* based on

19 samples. Nomenclature follows according to Flora Iranica [1].

## Materials and Methods

The seeds of eight species of the genus *Suaeda* from 19 localities were collected during 1987 by H. Akhani from different parts of Iran (Table 1).

Biochemical analysis was carried out by extraction of seed proteins and their separation by electrophoresis. Protein extracts were obtained by grinding 0.5 g *Suaeda* seeds at 4°C and mixing with 3 ml 0.08 M Tris-HCl buffer containing 10% glycerol (90%) 0.6% Temed at pH 6.7. The mixture was centrifuged at 17000 g for 60 minutes. For separating the polypeptide subunits one part of sample buffer containing 0.72 g Tris, 2.34 g SDS, 10 ml glycerol, 5 ml mercaptoethanol, 1 ml of 1% bromophenolblue in 100 ml distilled water at pH 6.8. The crude protein preparations were fractionated by the vertical SDS-

**Key words:** Electrophoresis; Seed proteins; Taxonomy; Suaeda; Chenopodiaceae; Iran

**Table 1.** Localities and herbarium vouchers of the collected seeds from 27 samples of 10 species of the genus *Suaeda* in Iran for caryological studies. Abbreviations: BEHESHTI UH=Shahid Beheshti University Herbarium; MMTT = Natural History Museum of Iran; TARI=Research Institute of Forests and Rangelands

Species and sample number	Locality and herbarium voucher
1. <i>S. acuminata</i> (C. A. Mey.) Moq.	Azərbayjan: 69 km from Kaleibar to Khodaafarin, Near Safalu, 250 m, 5.10.1987, ASSADI & AKHANI, 61575 (TARI).
2."	Azərbayjan: N side of Uromieh Lake, Bandare-Sharafkhaneh, 1300 m, 4.10.1987, ASSADI & AKHANI, 61505 (TARI).
3."	Tehran: Karaj, near Mardabad, c. 1200 m, 17.11.1987, AKHANI (TARI).
4."	Fars: Arsanjan, W shores of Tashk Lake, between the villages Gomban & Katak, 1700 m, 27.11.1987, ASSADI & AKHANI, 61813 (TARI).
5."	Fars: 23 mk W of Abadeh-Tashk, border of Tashk Lake, 1700 m, 26.11.1987, ASSADI & AKHANI, 61792 (TARI).
6. <i>S. aegyptiaca</i> (Hasselq.) Zoh	Yazd: Kavire-Marvast, near Rahmatabad, 1600 m, 25.11.1987, ASSADI & AKHANI, 61772 (TARI).
7."	Yazd: Corner of Kavire-Marvast road of Mehriz towards Marvast, 1600 m, 25.11.1987, ASSADI & AKHANI, 61759 (TARI).
8."	Fars: c. 30 km W of Jahrom, 7 km after Mobarakabad towards Harm villages, 700 m, 28.11.1987, ASSADI & AKHANI, 61832 (TARI).
9."	Fars: 25 km from Ghir to Lar, near the village Abegarm, 700 m, 28.11.1987, ASSADI & AKHANI, 61833 (TARI).
10."	Bushehr: On the road from Kaki to Khur-Mouj, after the river Mond, c. 20 m, 2.12.1987, ASSADI & AKHANI, 62029 (TARI).
11."	Baluchestan: Between Chahbahar and Rash, Noobandian, c. 20 m, 21.11.1990, AKHANI, 6777 (MMTT).
12. <i>S. altissima</i> (L) PALL.	Azərbayjan: N of Uromieh Lake, Bandare-Sharafkhaneh, 1300 m, 5.10.1987, ASSADI & AKHANI, 61504 (TARI).
13."	Azərbayjan: SE of Uromieh Lake, near Chopoghlu, 1350 m, 1.10.1987, ASSADI & AKHANI, 61321-a (TARI).
14. <i>S. arcuata</i> Bunge	Semnan: Touran Protected Area, 30 km NE of Torud, Rازه, 29.10.1987, AKHANI, 4131, (MMTT).

Table 1. Continued

Species and sample number	Locality and herbarium voucher
15. <i>S. bauchistanica</i> Akhani & Podl. (in press)	Baluchestan: 6 km W Beris, sea shore, 20.11.1990, AKHANI, 6766 (MMTT).
16. <i>S. crassifolia</i> Pall.	Azərbayjan: N. of Uromieh Lake, Bandare-Sharafkhaneh, 1300 m, 4.10.1987, ASSADI & AKHANI, 61501 (TARI).
17."	Azərbayjan: E. of Uromieh Lake, 21 km SW of Azarshahr, AKHANI (TARI).
18. <i>S. fruticosa</i> J. F. Gmelin	Tehran: E. of Houze-Soltan Lake, 800 m, 14.9.1987, AKHANI, 4822 (MMTT).
19."	Hormozgan: Between Bandare-Lengeh & Bandare-Charak, after Bandare-Shenas, c.10 m, 30.11.1987, ASSADI & AKHANI (TARI).
20. <i>S. maritima</i> (L.) Pall.	Azərbayjan: 56 km E. of Maragheh towards Hashtrud, 30.9.1987, AKHANI (TARI).
21."	Arak: N. of Kavire-Meyghan, 8 km W. Davoodabad, AKHANI, 1037 (BEHESHTI UH).
22."	Tehran: Between Tehran & Qom, Rude-Shur, 1000 m, 6.9.1987, AKHANI, 4795 (MMTT).
23."	Bushehr: 9 km from Borazjan to Shiraz, 200 m, 3.12.1987, ASSADI & AKHANI, 62042 (TARI).
24. & 25. <i>S. microphylla</i> Pall.	Tehran: Karaj, c. 10 km WNW of Mardabad, c. 1200 m, 17.11.1987, ASSADI & AKHANI, 61722 (TARI).
26. <i>S. microsperma</i> (C. A. Mey.) Fenzl	Semnan: Touran Protected Area, c. 8 km NE. of Razeh, 30.10.1987, AKHANI, 4144 (MMTT).
27."	Semnan: Touran Protected Area, between Delbar & Ahmadabad, along Kal-Shur river, 30.10.1987, AKHANI, 4209 (MMTT).

PAGE method in discontinuous buffer system on different concentrations of acrylamide (10-22.5%), [4]. The electrophoresis was carried out using balanced quantities of crude protein from different specimens at 10°C in 50 mA constant current for four hours.

The gels were fixed and stained for 12 hours in a solution containing one part staining solution -0.025% coomassie blue (R-250) in 96% ethanol - and one part of 10% acetic acid. Destaining was done in four stages starting with a solution of 96% ethanol and 5% acetic acid for one to two hours. Finally the gels were incubated in water and acetic acid. Rm of protein

bands, which shows the ratio of protein migration to bromophenolblue by measuring migrated intervals, was determined. Fractionation of proteins was carried out on at least 15 gels to avoid any error.

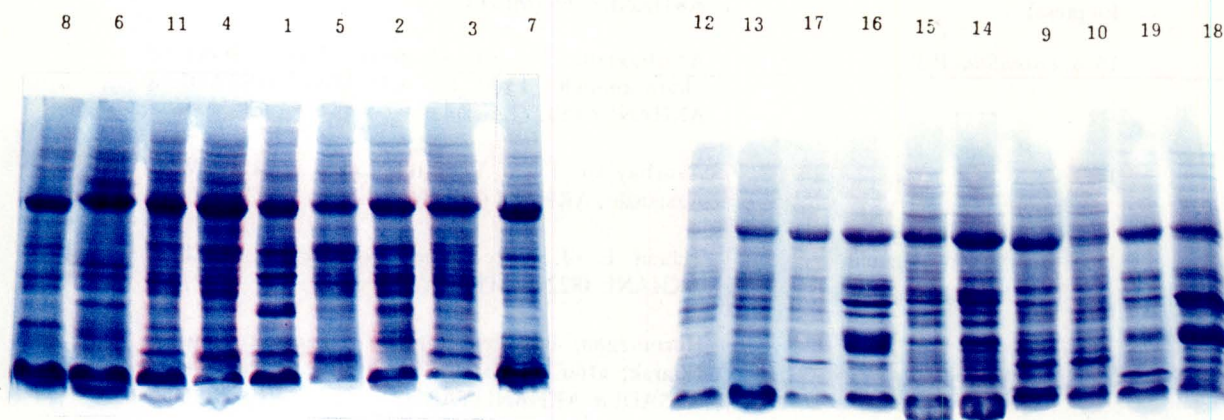
Coefficients of similarity were calculated by the formula:  $S = m / (m + u)$ , where  $m$  = the number of common bands and  $u$  = the total number of bands unique to each sample [10]. A computer program was used to prepare the dendrogram of clustering patterns.

### Results and Discussion

The seed protein profiles for each sample of *Suaeda* are presented in Table 2. They are arranged in

a matrix representing 35 different Rm values of bands. Each band has been numbered according to its position relative to the origin. The photographs of

selected gels are presented in Figure 1. Similarity coefficients for protein profiles (Table 3) and a dendrogram of clustering patterns of each sample



**Figure 1.** Photographs of selected gels of electrophoresis separation of seed proteins in eight species of the genus *Suaeda* numbered in Table 1

**Table 2.** Rm of seed protein profiles for 19 samples of eight species of the genus *Suaeda* representing 35 different bands resulting from SDS-polyacrylamide gel electrophoresis

Species	Bands															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. <i>S. acuminata</i>	.	.875	.	.125	.1875	.2125	.225	.2375	.25	.	.	.2875	.	.3375	.3625	.
2."	.	.	.	.125	.1875	.2125	.225	.2375	.25	.	.	.2875	.	.3375	.3625	.
3."	.	.	.	.125	.1875	.2125	.225	.2375	.25	.	.	.2875	.	.3375	.3625	.
4."	.	.	.	.125	.1875	.2125	.225	.2375	.25	.	.	.2875	.	.3375	.3625	.
5."	.	.	.	.125	.1875	.2125	.225	.2375	.25	.	.	.2875	.	.3375	.3625	.
6. <i>S. aegyptiaca</i>	.625	.875	.1125	.125	.1875	.2125	.225	.2375	.25	.	.275	.2875	.	.3375	.3625	.
7."	.625	.875	.1125	.125	.1875	.2125	.225	.2375	.25	.	.275	.2875	.	.3375	.3625	.
8."	.625	.875	.1125	.125	.1875	.2125	.225	.2375	.25	.	.275	.2875	.	.3375	.3625	.
9. <i>S. altissima</i>	.	.	.	.125	.1875	.2125	.225	.2375	.25	.	.	.2875	.325	.3375	.3625	.
10.	.	.	.	.125	.1875	.2125	.225	.2375	.	.	.	.2875	.325	.3375	.3625	.
11. <i>S. crassifolia</i>	.	.	.1125	.125	.1875	.2125	.225	.2375	.25	.	.275	.2875	.	.3375	.3625	.375
12. <i>S. fruticosa</i>	.625	.875	.1125	.125	.1875	.2125	.225	.2375	.25	.	.	.2875	.	.3375	.3625	.
13."	.625	.875	.1125	.125	.1875	.2125	.225	.2375	.25	.	.	.2875	.	.3375	.3625	.
14. <i>S. maritima</i>	.	.875	.	.125	.1875	.2125	.225	.2375	.25	.2625	.	.2875	.	.3375	.3625	.
15."	.	.875	.	.125	.1875	.2125	.225	.2375	.25	.	.	.2875	.	.3375	.3625	.
16. <i>S. microphylla</i>	.	.	.	.125	.1875	.2125	.225	.	.25	.	.	.	.	.3375	.3625	.
17."	.	.	.	.125	.1875	.2125	.225	.	.25	.	.	.	.	.3375	.3625	.
18. <i>S. microsperma</i>	.	.875	.	.125	.1875	.2125	.225	.2375	.	.	.	.	.325	.3375	.3625	.
19.	.	.875	.	.125	.1875	.2125	.225	.2375	.	.	.	.	.325	.3375	.3625	.

Table 2. Continued

Species	Bands																		
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1. <i>S. acuminata</i>	.4125	.	.45	.475	.5	.	.55	.575	.6	.6875	.7	.	.725	.	.7625	.8	.825	.85	.9125
2."	.4125	.	.45	.475	.5	.525	.55	.575	.6	.6875	.7	.	.725	.	.7625	.8	.825	.85	.9125
3."	.4125	.	.45	.475	.5	.525	.55	.575	.6	.6875	.7	.	.725	.	.7625	.8	.825	.85	.9125
4."	.4125	.	.45	.475	.5	.525	.55	.575	.6	.6875	.7	.	.725	.	.7625	.8	.825	.85	.9125
5."	.4125	.	.45	.475	.5	.525	.55	.575	.6	.6875	.7	.	.725	.	.7625	.8	.825	.85	.9125
6." <i>S. aegyptiaca</i>	.4125	.425	.45	.475	.5	.525	.55	.	.6	.6875	.	.	.725	.	.7625	.8	.	.85	.9125
7."	.4125	.425	.45	.475	.5	.525	.55	.575	.6	.6875	.	.	.725	.	.7625	.	.	.85	.9125
8."	.4125	.425	.45	.475	.5	.525	.	.	.6	.6875	.	.	.725	.	.7625	.8	.	.85	.9125
9. <i>S. altissima</i>	.4125	.425	.45	.475	.	.525	.55	.575	.6	.6875	.	.	.725	.	.7625	.8	.825	.85	.
10."	.4125	.425	.45	.475	.	.525	.55	.575	.6	.6875	.	.	.725	.	.7625	.8	.825	.85	.
11. <i>S. crassifolia</i>	.4125	.425	.45	.475	.5	.525	.55	.575	.6	.6875	.7	.	.725	.	.7625	.8	.825	.85	.9125
12. <i>S. fruticosa</i>	.4125	.	.45	.475	.5	.525	.55	.575	.6	.6875	.	.	.725	.	.7625	.8	.	.85	.
13."	.4125	.	.45	.475	.5	.525	.55	.575	.6	.6875	.	.	.725	.	.7625	.8	.	.85	.
14. <i>S. Maritima</i>	.4125	.	.45	.	.5	.525	.55	.575	.6	.6875	.	.	.725	.	.7625	.8	.	.85	.9125
15."	.4125	.	.45	.	.5	.525	.55	.575	.6	.6875	.	.	.725	.	.7625	.8	.	.85	.9125
16. <i>S. microphylla</i>	.4125	.	.45	.475	.5	.525	.55	.575	.6	.6875	.	.	.725	.75	.7625	.8	.	.85	.
17."	.4125	.	.45	.475	.5	.525	.55	.575	.6	.6875	.	.	.725	.75	.7625	.8	.	.85	.
18. <i>S. microsperma</i>	.4125	.	.45	.	.5	.525	.	.575	.6	.6875	.7	.7125	.	.75	.7625	.8	.825	.85	.
19."	.4125	.	.45	.	.5	.525	.	.575	.6	.6875	.7	.7125	.	.75	.7625	.8	.825	.85	.

Table 3. Similarity coefficients for protein profiles of eight species of the genus *Suaeda* from 19 samples (For localities of each number see Table 1).

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. <i>S. acuminata</i>	X																		
2."	92.3	X																	
3."	92.3	100	X																
4."	92.3	100	100	X															
5."	92.3	100	100	100	X														
6." <i>S. aegyptiaca</i>	73.3	73.3	73.3	73.3	73.3	X													
7."	73.3	73.3	73.3	73.3	73.3	92.8	X												
8."	70.	70.	70.	70.	70.	96.3	89.3	X											
9. <i>S. altissima</i>	75.	81.5	81.5	81.5	81.5	70.	70.	64.3	X										
10."	71.4	77.8	77.8	77.8	77.8	66.7	66.7	66.9	95.8	X									
11. <i>S. crassifolia</i>	80.	86.2	86.2	86.2	86.2	80.6	80.6	77.4	76.7	73.3	X								
12. <i>S. fruticosa</i>	78.6	78.6	78.6	78.6	78.6	85.7	85.7	82.1	75.	71.4	74.2	X							
13."	78.6	78.6	78.6	78.6	78.6	85.7	85.7	82.1	75.	71.4	74.2	100	X						
14. <i>S. maritima</i>	81.5	81.5	81.5	81.5	81.5	75.9	75.9	72.4	71.4	67.9	71.	81.5	81.5	X					

Table 3. Continued

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
15."	84.6	84.6	84.6	84.6	84.6	78.6	78.6	75.	74.1	70.4	73.3	84.6	84.6	95.8	X				
16. <i>S. microphylla</i>	70.4	76.9	76.9	76.9	76.9	65.5	65.5	62.1	73.1	69.2	66.7	76.9	76.9	73.1	76.	X			
17."	70.4	76.9	76.9	76.9	76.9	65.5	65.5	62.1	73.1	69.2	66.7	76.9	76.9	73.1	76.	100	X		
18. <i>S. microsperma</i>	65.5	65.5	65.5	65.5	65.5	51.5	51.5	53.1	62.1	64.3	57.6	60.	60.	62.1	64.3	63.	63.	X	
19."	65.5	65.5	65.5	65.5	65.5	51.5	51.5	53.1	62.1	64.3	57.6	60.	60.	62.1	64.3	63.	63.	100	X

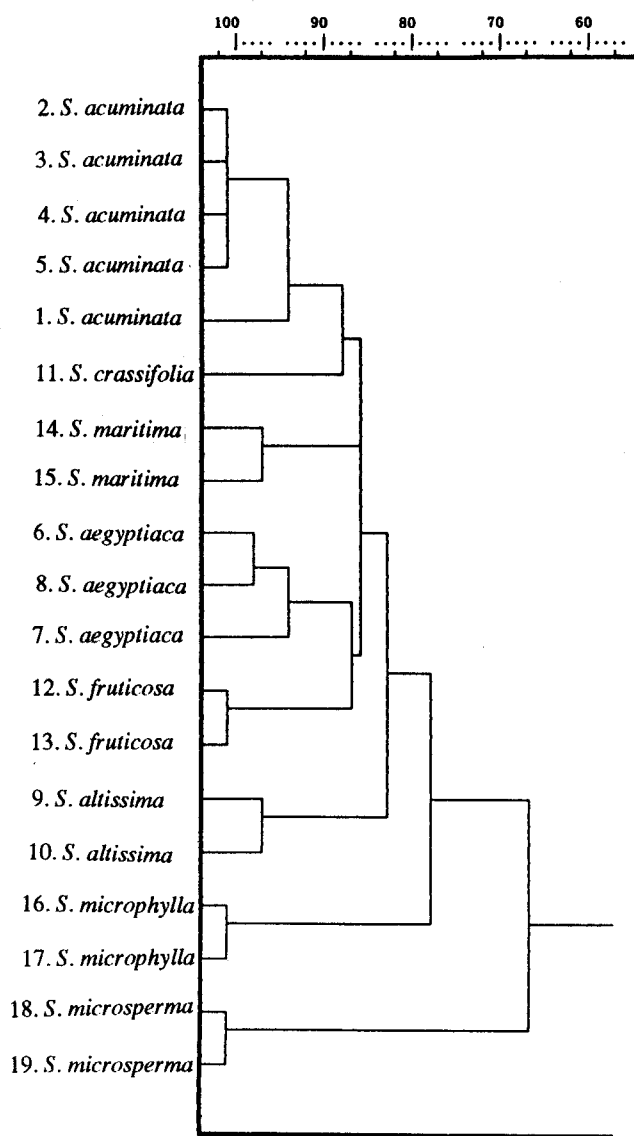


Figure 2. Cladogram of clustering patterns for 19 samples of eight species of the genus *Suaeda* in Iran based on similarity coefficients

based on coefficients of similarity (Fig. 4) are given.

All investigated bands can be categorized into three groups as: (a) common bands in all samples e.g. 4, 5, 6, 14, 15, 17, 19, 21, 25, 26, 32 & 34; (b) common bands in at least 15 samples e.g. 8, 9, 12, 20, 21, 22, 23 & 24 and (c) the remaining 13 bands were distinguished in less than 11 studied samples.

Comparison of different samples of the same species and even from remote localities shows that there is no significant difference between them in protein bands. Except for *S. crassifolia*, all other species were studied at least from two populations (in the case of *S. microphylla* two samples of the same locality were studied). The results obtained are in many cases parallel to the recent taxonomic studies of this genus in Flora Iranica and its species concept in critical groups [1]. Different samples of the same species show not less than 89% coefficient of similarity, however most cases have 100% similarity.

In *S. acuminata*, which is a widespread polymorphic species distributed in Turkey, Caucasus, Middle Asia, Central Asia, Mongolia, Afghanistan and northwest, central and south Iran, five samples from more or less remote localities were studied. Several species have been distinguished by earlier authors within this species [8], but Grobov [6] and Akhani and Podlech [1] have rejected most such species. Our results of protein analysis and chromosome studies [15] confirm that such separation in different species has no taxonomic importance. Of five studied samples, four samples show 100% similarity. Sample number 1 is a little bit different in bands with 92% coefficient of similarity. The said sample was obtained from a peculiar population in north Azarbayjan. The habitat is unique even in the genus *Suaeda*. This population was found in the *Artemisia* steppe in soil of relatively low salinity compared to other habitats of the species in other distribution ranges. Its habit and morphological characters are also rather different. The leaves are anguste-lanceolate, the colour of the plant

and leaves are light green and the plants appear leafy throughout. The chromosome data also shows that its chromosomes are smaller than other studied samples [15]. It seems that this population and possibly a more northern range of this species can be separated as a subspecies.

In *S. aegyptiaca*, three samples were studied. The similarities rank between 89.3% and 96.3%. All have 24 bands in common. Band number 24 was found only in sample number 7 and band number 32 is common in samples numbers 6 and 8. At present it is difficult to correlate such minor differences in protein bands with any morphological variation. Although this species has a wide range distribution no persistent morphological race was distinguished within their populations in the area of Flora Iranica.

In *S. maritima*, two samples from the centre of Iran were studied. Sample number 14 is diploid ( $2n=18$ ) and sample number 15 is tetraploid ( $2n=36$ ). Their similarity is 95.8%. Twenty-three bands are common between them. Band number 10 is only present in sample number 14. Taking only these two samples, it is not possible to correlate different chromosome sets with differences in protein bands and also the valuability of protein bands to help taxonomic problems that present themselves in *S. maritima* complex. Ungar and Bouchaud [11] have found evidence of valuability to correlate with three varieties in Europe.

*S. crassifolia* was studied from one sample. It is a closely related species to *S. maritima* and *S. acuminata*. Twenty-four common bands were distinguished with four similar samples of *S. acuminata* (numbers 2-5). Band numbers 3, 11, 16 and 18 which are present in *S. crassifolia* are missing in *S. acuminata*. Twenty-two bands are common with *S. maritima*. Band numbers 2 and 10 are found in one of the samples of *S. maritima* (number 14) which are absent in *S. crassifolia*. Among all studied species, band number 16 is unique in *S. crassifolia* and clustering patterns show a closer relationship between *S. crassifolia* and *S. acuminata*.

The protein bands of *S. fruticosa* were studied in two samples from the centre and south of Iran. In spite of having different chromosome numbers, no difference was noticed in the protein bands.

In *S. microsperma*, two samples from close localities were studied. This is an annual species distributed in the provinces of Khorassan and Semnan in Iran and also in Central Asia and Afghanistan. Twenty-three identical bands were distinguished in both samples. From among the species studied, only band number 28 is present in this species. Band

number 29, which is present in all other species, is missing in *S. microsperma*. Similarity percent and clustering patterns show less affinity with any other species, although its morphology and chromosome characters are close to *S. acuminata*. The prominent character that separates *S. microsperma* from *S. acuminata* is the presence of a long caducous bristle in the leaf apex and often obtuse leaves. In *S. acuminata*, leaves are acute and terminate into indistinct seta. Of course a similarity percent of 65.5% with *S. acuminata* is more than other similarities, but it is far below that expected. So far Ungar and Bouchaud [11] in *S. splendens* - a Mediterranean annual species that like *S. microsperma* has long bristles at the leaf apex - have found less similarity with other species.

Protein bands of *S. altissima* were studied from two localities around Uromieh Lake (Azarbayjan). It was found to have twenty-three bands in common, the only difference being the presence of band number 25 in sample number 9 that is absent in sample number 10. Clustering patterns show a rather isolated entity within the studied species. Morphologically, the species is also rather far from other studied species.

*S. microphylla* is a perennial Irano-Turanian species found mostly in saline and gypsum soils. The protein bands of this species were studied in two samples from the same locality near Karaj. Twenty-one bands were distinguished. According to clustering patterns, this is also a rather isolated species supported by its morphological characters. It has been placed as a close species to *S. altissima* by Iljin [8] on the basis of its flower position on the petiole-like leaf axile while it is different in other characters such as perennial habit, leaf structure and colour.

According to the present study it can be concluded that:

1-Disc gel electrophoresis of seed proteins is a useful technique to survey the relationships of the species of *Suaeda*. It is a particularly good tool for investigating the unity of different populations of the same species in complex groups. It proved that geographical distribution and ecological variations do not have profound effects on seed protein patterns.

2-Since only a few species were investigated, it is not possible to obtain a good idea about infrageneric and phylogenetic relationships within the genus *Suaeda*. Also there remains the question why *S. microsperma* - whose morphology and chromosome characters are very similar to *S. acuminata*, *S. crassifolia* and *S. maritima* - shows remote distance to them.

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