SEED PROTEIN ANALYSIS IN SOME IRANIAN SPECIES AND POPULATIONS OF STIPA L.

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SDS-PAGE protein analysis was performed in 16 populations of 6 *Stipa* species in order to determine the use of such data in taxonomy and elucidating the species inter-relationships. In total 29 protein bands were obtained, some of which were common to all the species while some were species specific. Intra-specific protein variation was observed in some of the species. Cluster analysis of protein data showed distinctness of the species studied and also supported the species inter-relationships revealed by morphological characters.

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Key words. SDS-PAGE protein analysis, *Stipa,* Iran.

بررسی پروتئین های ذخیره ای بذر در برخی جمعیت ها و گونه های .Stipa L

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بررسی پروتئین‌های ذخیره‌ای بذر در ۱۶ جمعیت از ۶ گونهٔ *Stipa* L. ایران با استفاده از روش SDS-PAGE انجام گرفت. بطور کلی تعداد ۲۹ باند پروتئینی مشاهده شد که تعدادی از آنها در تمامی گونه‌ها مشترک بودند. برخی از باندها برای گونه ها اختصاصی بود و تعدادی نیز در میان جمعیت‌های یک گونه گوناگونی داشتند. تجزیهٔ خوشه‌ای اطلاعات پروتئینی، جمعیت‌های هر یک از گونه ها را در خوشه‌ای مجزا قرار داد که نشان دهندهٔ متمایز بودن گونه‌ها در اختصاصات پروتئینی است. این نتایج تأیید کنندهٔ جایگاه تاکسونومیکی گونه‌های مطالعه شده و ارتباطات میان گونه‌ای است که بر اساس صفات ریختی بنا شده است.

Introduction

The genus *Stipa* L. of tribe *Stipeae* (*Poaceae*) is comprised of about 400 (Freitag 1985; Barkworth & Everrett 1987) or 300 species (Tzvelev 1989), mainly distributed in Asia but also occur in Australia, Africa and parts of the North and South America (Hartley 1973). These species are mainly adapted to temperate, dry climate and show a wide range of diversity in semiarid and arid climates.

According to different authors, the number of *Stipa* species growing in Iran varies from 15 to 19 (Parsa 1951, Bor 1970, Mobayen 1975, Freitag 1985). Discrepancy in the number of *Stipa* species points towards taxonomic problems in this genus (Vazquez & Devesa 1997).

Although there have been extensive reports on the biosystematic studies of the *Stipa* species from the other parts of the world (Johnson 1962; Renvoize 1985; Hsiao & al. 1995; Jacobs & al. 2000), no such studies exist from Iran.

The present study is a part of biosystematic study of *Stipa* species in Iran, reporting the possible use of seed proteins in *Stipa* taxonomy and revealing the species inter-relationship for the first time. Seed protein analysis has been used for indicating the species inter-relationships in several grass species (Sheidai & al. 2000).

Material & Methods

Plant material. In total 16 populations of 6 *Stipa* species were analysed for seed proteins, which are namely: 1- *Stipa arabica* Trin. & Rupr. 2- *Stipa hohenackeriana* Trin. & Rupr. 3- *Stipa iranica* Freitag. 4- *Stipa holosericea* Trin. 5- *Stipa lessingiana* Trin. & Rupr. 6- *Stipa caucasica* Schmalh. (Table 1). The voucher specimens are deposited in the herbarium of Shahid Beheshti University (HSBU).

Seed protein extraction and electrophoresis. One hundred mg. of each sample (25-50 dry seeds) was homogenized to obtain a fine powder. Proteins were extracted in a pre-cooled mortar and pestle over ice with a 0.39 M Tris phosphate buffer (pH 8.3). The protein electrophoresis was carried out according to Sanchez-Yelamo & al. (1995), using 77 mM Tris-Hcl (pH 6.8), 4 % sodium dodecyl sulphate (SDS), 10 % 2-mercaptoethanol and 3 % glycerol and vertical slab gels of 1 mm thickness.

To estimate species/population similarity as indicated by protein electrophoresis patterns, Jaccards' and simple matching indices were determined. Each protein band was considered as a qualitative character and coded as 1 (presence) versus 0 (absence). The resulting data matrix was used for cluster analysis using single linkage and average linkage methods (Sheidai & al. 2000). Statistical methods used SPSS ver. 10.1 (1999).

Results and discussion

The results of protein electrophoresis are presented in Tables 2&3 and Figs. 1-4. In total 29 bands were obtained. Bands 1, 2, 11 & 28 were common in all species and populations while, band 17 occurred only in *St. hohenackeriana* and may be considered as the species-specific band. The same is true for band 13, which occurs only in *St. iranica*, as well as band 24, which occurs only in *St. lessingiana*. Band 12 occurred in all populations of *St. arabica* while bands 7,23 & 26 were specific in *St. caucasica*.

The highest number of protein bands (18) occurred in Arasbaran population of *St. lessingiana* while the lowest number of bands (12) occurred in Gorgan population of *St. caucasica*..

Two species of *St. lessingiana* and *St. caucasica* belong to the section *Stipa*, while the other species belong to the section

Table 1. *Stipa* species, their localities and voucher numbers.

Species	Locality	Collector	Voucher No.
Stipa hohenackeriana Trin. & Rupr.	Tehran,Sohanak,Pasgahe Gardaneh Ghoochak, 1800m.	Ataei and Alijanpoor	201.421
St.hohenackeriana Trin. & Rupr.	Ardebil, Meshkinshahr, 40km. Moradlou	Ahmadzadeh	201.420
St.arabica Trin. & Rupr.	Tehran-Karaj road, Daneshkade Keshvarzy Tarbiat Modarres.	Ataei	201.417
St.arabica Trin. & Rupr.	Tehran, Sohanak, Pasgahe Gardaneh Goochak. 1800m.	Ataei and Alijanpoor	201.412
St.arabica Trin. & Rupr.	Ardebil,Meshkinshahr,40km. Moradlou	Ahmadzadeh	201.413
St.arabica Trin.& Rupr.	Arasbaran, Makidy.1900m.	Amini and Zare	200.419
St. arabica Trin.& Rupr.	Tehran, 25km.Firoozkooh→ Tehran, Aminabad, 2350m.	Ataei, Ahmadzadeh and Jalilian	201.418
St. lessingiana Trin. & Rupr.	Arasbaran, Makidy. 1900m.	Amini and Zare	200.400
St. lessingiana Trin. & Rupr.	Damavand, Dashtak village, 2200m.	Ataei, Ahmadzadeh and Fadai	201.401
St. iranica Freitag	Tehran, Sohanak, Pasgahe Gardaneh Ghoochak. 1800m.	Ataei and Ahmadzadeh	201.405
St. holosericea Trin.	Tehran,25km.Firoozkooh→ Tehran, Aminabad, 2350m.	Ataei and Ahmadzadeh	201.406
St. holosericea Trin.	Damavand, Dashtak village, 2200m.	Ataei and Ahmadzadeh	201.404
St. holosericea Trin.	Azarbaijan, 40km.Meyaneh→Khalkhal, Ahlat village, 1300m.	Ataei	201.402
St. caucasica Schmalh	Gorgan, Dashte Almeh., 1700m.	Zehzad	-
St. caucasica Schmalh	Damavand, Dashtak village, 2200m.	Ataei and Aahmadzadeh	201.408
St. caucasica Schmalh	Tehran, 25km. Firoozkooh→ Tehran, Aminabad, 2350m.	Ataei and Ahmadzadeh	201.407

Barbatae (Freitag 1985). It is interesting to note that band 4 occurs only in *St. lessingiana and St. caucasica* from the section *Stipa.* On the other hand band 3 and 9 occur in the members of the section *Barbatae* and not *Stipa.* Some of the protein bands show variation among different populations of a species. For example band 12 occurs in all populations of *St. arabica* except in Arasbaran population while band 18 occurs in all populations except in Sohanak and Karaj (Table 1).

Band 19 occurs in Damavand and Firoozkooh populations of *St. caucasica* but is absent in Gorgan population. These bands may be used in revealing the inter-populations differences.

Different methods of cluster analyses including single linkage and UPGMA, performed on Jaccard's and simple matching indices produced similar results.The phenogram

Table 2. Protein bands in *Stipa* species and populations. Abbreviations: b1-b29 bands. Sp=Species, 1-16=Species and populations names as in Fig. 1.

	b1	b2	b3	b4	b5	b6	b7	b8	b9	b10	b11	b12	b13	b14	b15	b16	b17	b18	b19	b20	b21	b22	b23	b24	b25	b26	b27	b28	b29
1	1	1	1	0	1	0	0	0	1	0	1	1	0	1	1	0	1	0	0	0	0	1	0	0	1	0	1	1	0
2	1	1	1	0	1	0	0	0	1	0	1	1	0	1	1	0	1	0	0	0	0	1	0	0	1	0	1	1	0
3	1	1	1	0	0	1	0	0	1	0	1	1	0	1	1	0	0	0	0	0	1	1	0	0	1	0	1	1	0
4	1	1	1	0	1	1	0	0	1	0	1	1	0	1	1	0	0	0	0	0	1	1	0	0	1	0	1	1	0
5	1	1	1	0	1	1	0	0	1	0	1	1	0	1	1	0	0	1	0	0	1	1	0	0	1	0	1	1	0
6	1	1	1	0	1	1	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0	1	0	0	1	0	1	1	0
7	1	1	1	0	1	1	0	0	1	0	1	1	0	1	1	0	0	1	0	0	0	1	0	0	1	0	1	1	0
8	1	1	0	1	1	1	0	0	1	1	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	1	1	1	0
9	1	1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	0	1	0	1	1	0
10	1	1	1	0	1	1	0	0	1	0	1	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0	1	1	0
11	1	1	1	0	1	1	0	0	1	0	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	1	1	0
12	1	1	1	0	1	1	0	0	1	1	1	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0	1	1	0
13	1	1	0	1	1	1	0	1	0	0	1	1	0	1	1	0	0	0	0	0	1	1	0	1	1	0	0	1	0
14	1	1	0	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0
15	1	1	0	1	1	1	1	0	0	0	1	1	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	1	1
16	1	1	0	1	1	1	1	0	0	0	1	1	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	1	1

Table 3. PCA analysis of protein bands. B1-b29=protein bands 1-29.

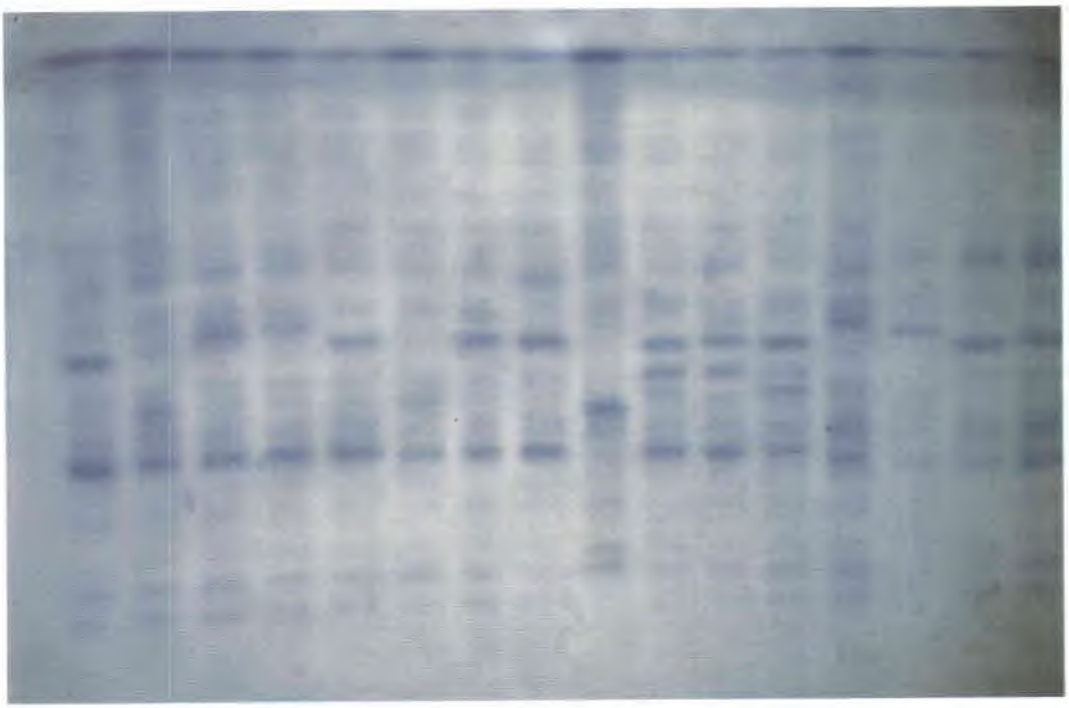
	Component			
	1	2	3	4
B23	-0.974	-7.53E-02	-0.166	-2.15E-02
B7	-0.974	-7.53E-02	-0.166	-2.15E-02
B22	0.974	7.33E-02	0.166	2.155E-02
B25	0.974	7.33E-02	0.166	2.155E-02
B27	0.900	-0.261	-0.152	0.130
B26	-0.865	7.652E-2	6.077E-02	0.314
B14	0.835	0.272	2.472E-02	0.334
B3	0.829	-0.379	-0.338	-0.189
B4	-0.829	0.379	0.338	0.189
B29	-0.812	-0.155	-0.163	9.347E-02
B19	-0.812	-0.155	-0.163	9.347E-02
B9	0.801	-5.31E-2	-0.259	0.416
B20	-0.462	8.982E-02	-4.49E-2	-0.162
B12	-0.333	0.768	-0.257	-1.12E-02
B15	0.537	0.718	-0.106	3.911E-02
B16	-0.374	-0.624	2.032E02	0.337
B8	-4.00E-02	0.589	0.539	-0.198
B18	0.472	-0.581	0.415	0.283
B17	0.239	0.362	-0.747	8.600E-02
B6	-0.239	-0.362	0.747	-8.60E-02
B24	-1.25E-02	0.620	0.670	0.291
B10	0.113	-6.06E-02	0.369	0.724
B21	0.324	7.958E-02	0.393	-0.673
B13	7.749E-02	-0.365	0.223	-0.563
B5	-0.160	-0.141	6.644E-02	0.308

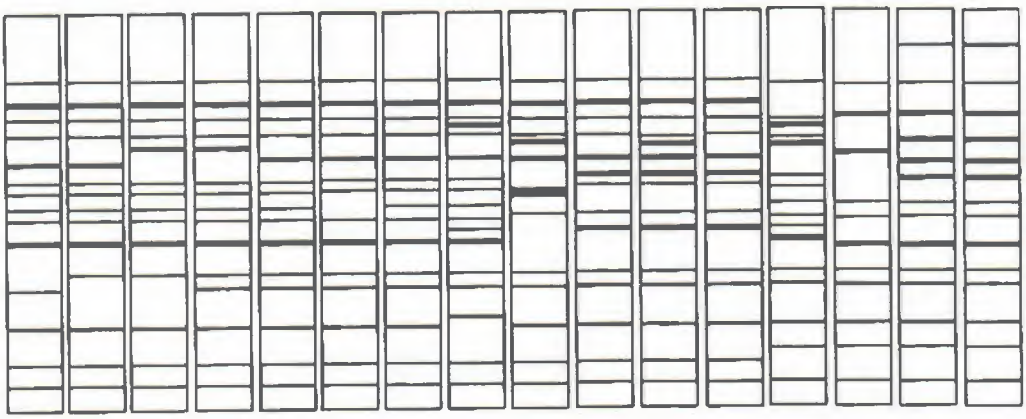
obtained from UPGMA cluster analysis is presented in Fig. 3.

Cluster analysis produced 4 major Clusters. Populations of *St. caucasica* form the first cluster while that of *St. lessingiana* form the second cluster separated from the other species. This cluster shows relationship to the cluster one, as also is evident in plot of species ordination (Fig 4). These two species have been considered close to each other based on morphological characters and have been placed in a single section (Freitag 1985).

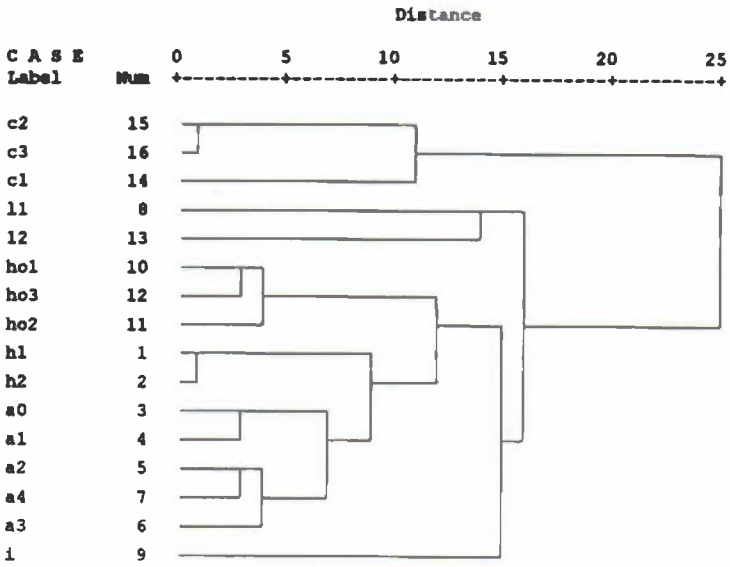
The third major cluster is comprised of two minor clusters containing 3 species of *St. holosericea, St. hohenackeriana* and *St. arabica*. The populations of *St. holosericea* comprise the first minor cluster, while the second minor cluster is formed by populations of *St. arabica* and *St. hohenackeriana*. These two species are placed a little far from each other in this cluster due to their protein differences. Based on morphological characters, Freitag (1985) also considered these two species close to each other. *St. iranica* alone forms the forth major cluster.

Therefore it seems that protein data can differentiate between the two sections suggested by Freitag for the species studied and such studies may be carried out for the other *Stipa* species too. PCA analysis at the protein bands (Table 3) revealed that the first 4





Figs. 1 & 2. SDS-PAGE protein bands in *Stipa* species and populations. From left to right: 1-2=*S. hohenackeriana* (Meshkinshahr, Sohanak populations), 3-7= *St arabica* (Karaj, Sohanak, Meshkinshahr, Arasbaran and Firoozkooh populations), 8=*St. lessingiana* (Arasbaran), 9=*St. iranica* (Sohanak), 10-12=*St. holosericea* (Firoozkhooh, Meyaneh and Damavand), 13=*St. lessingiana* (Damavand), 14-16=*St. caucasica* (Gorgan, Damavand and Firoozkhooh).



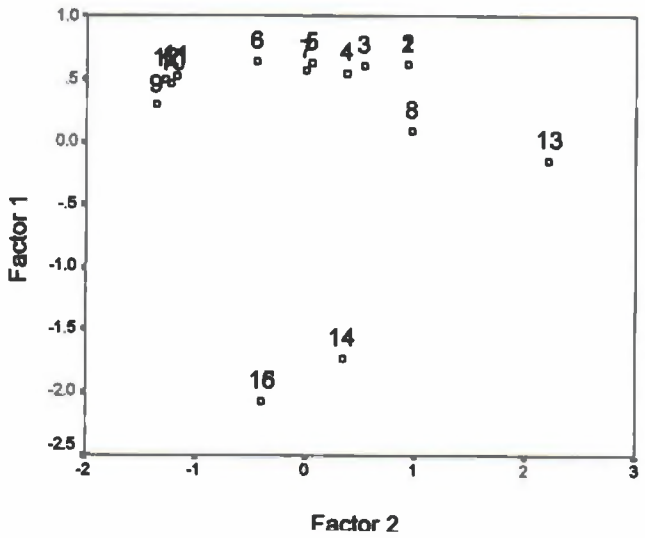


Fig. 3 & 4. UPGMA cluster analysis and ordination of *Stipa* species. Abbereviations: 1-2 *S. hohenackeriana* (Meshkinshahr, Sohanak populations), 3-7=*St. arabica* (Karaj, Sohanak, Meshkinshahr, Arasbaran and Firoozkooh populations), 8=*St. lessingiana* (Arasbaran), 9=*St. iranica* (Sohanak), 10-12=*St. holosericea* (Firoozkooh, Meyaneh and Damavand), 13=*St. lessingiana* (Damavand), 14-16=*St. caucasica* (Gorgan, Damavand and Firoozkooh).

components comprise about 78% of total variation. In component one, which comprises about 42% of total variance, bands 3, 9, 14, 22, 25 and 27 possess the highest positive correlation (>0.80) and bands 4, 7, 19, 23, 26 and 29 possessed the highest negative correlation with the component (->0.80). Therefore these are the most variable protein bands after first component. In component 2, which comprises about 14% of total variance, bands 12 & 15 possessed the highest positive correlation (>0.70). The component 1, separates two species of *St. caucasica* and *St. lessingiana* of the section *Stipa* from the others, while component 2 may differentiate the other species from each other.

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