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

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SDS-PAGE seed protein analysis was performed among 15 populations of 8 *Lotus* species of Iran in order to illustrate the species interrelationships. In total 29 bands were obtained some of which were common in all species and populations therefore may be considered as common bands in *Lotus* species studied. Some bands occurred only in a single species while some others differed among populations of a single species. Cluster analysis of the *Lotus* species based on protein data grouped different populations of each species in a distinct cluster indicating the species distinctness and also supported the species interrelationships observed by morphometric studies.

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

بررسی پروتئینهای ذخیره‌ای بذردر برخی جمعیتها و گونه‌های .Lotus L

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بررسی پروتئینهای ذخیره‌ای بذر در۱۵ جمعیت از ۸ گونه *Lotus* 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 گونه‌ها اختصاصی بود و تعدادی نیز در میان جمعیت‌های یک گونه گوناگونی داشتند. تجزیه خوشه‌ای اطلاعات پروتئینی، جمعیت‌های هر یک از گونه‌ها را در خوشه‌ای مجزا قرار داد که نشان دهنده متمایز بودن گونه‌ها در اختصاصات پروتئینی است. این نتایج تائید کننده جایگاه تاکسونومیکی گونه‌های مطالعه شده و ارتباطات میان گونه‌ای است که بر اساس صفات ریختی بنا شده است.

Introduction

The genus *Lotus* L. of tribe *Loteae* (*Leguminosae*) contains a heterogenous assemblage of annual and prennial species distributed widely throughout the world and is comprised of about 183-188 (Polhill 1981) or 100 species (Gunn 1983; Polhill 1994). These species are mainly distributed in Mediterranean and NW of America (Polhill & Raven, 1981).

The number of *Lotus* species growing in Iran varies according to different authors for example according to Mousavi (1974) ten species grow in Iran while Parsa (1948) and Chrtkova-Zertova (1982) reports the occurrence of only 9 species.

The genus *Lotus* is comprised of important forage plants distributed in many regions of Iran such as: *L. corniculatus*, *L. tenuis*, *L. pedunculatus* and *L. angustissimus*.

SDS-PAGE protein analysis has been widely used in biosystematic studies as seed storage proteins are less affected by environmental conditions, polyploidy level, etc. and are considered as good genetic markers for evolutionary studies (Ladizinsky, 1983, Chen & al. 1997).

Although there have been extensive reports on the biosystematic studies of the *Lotus* species from the other parts of the world (Grant, 1995), no such studies exist from Iran. Seed protein analysis has been used to show the species relationships in some *Leguminosae* taxa including *Trifolium* (Badr, 1995; Sheidai & al. 1999) while not useful in others like *Alhagi* (Sheidai & al. 2002). Therefore the present study was performed as a part of biosystematic study of the genus *Lotus* in Iran, reporting the possible use of seed proteins in *Lotus* taxonomy and revealing the species inter-relationship for the first time.

Material & Methods

Plant material

In total 15 populations of 8 Lotus species were analysed for seed proteins, the species studied are: 1- *Lotus gebelia* Vent. 2, - *L. corniculatus* L., 3- *L. tenuis* Waldst. & Serg. 4- *L. garcinii* DC., 5- *L. angustissimus* L., 6- *L. halophilus* Boiss. and 7- *L. michauxianus* Ser. (Table 1).

Seed protein extraction and electrophoresis

Three hundred mg of seeds from each sample was homogenized to obtain a fine powder. Proteins were extracted in a precooled mortar and pestle over ice with a 0.39 M Tris phosphate buffer (pH 8.3). The resulting mixture was centrifuged at 15000g for 10 min. The protein electrophoresis was carried out according to Sanchez-Yelamo & al. (1995), using 77mM Tris-Hcl (pH 6.8), 4% sodium dodecyl sulphate (SDS), 10% 2-mercaptoethanol and 3% glycerol and vertical slab gels of 1 mm thickness which were electrophoresed at a constant rate of 30 mA for 8h. Coomassie Briliant Blue G-250 was used for overnight gel staining followed by trichloroacetic acid as fixative.

To estimate species/population similarity as indicated by protein electrophoresis patterns, bands having similar relative mobility (RM) were taken as homologue and Jaccard 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. 2002). PCA was performed on protein data and the most variable protein bands were determined (Sheidai & al. 2002), finally ordination of the species was performed based on the first two PCA axis.

Statistical methods used SPSS ver. 9 (1998).

Results and discussion

The results of protein electrophoresis are presented in Table 1 and Figs. 1-3. In total 29 bands were obtained. Band 11 was common in all species and populations therefore may be considered as common bands in *Lotus* species studied. Bands 1, 2 occurred only in Marand

Table 1: Protein bands in *Lotus* species and populations.Abbreviations:b1-b29 bands.Sp.=Species,Pop.=Population. Populations locality:

1- Sanandaj-Abidar mountain, 2- Sanandaj, Naran mountain, 3- South of Sanandaj, Nashoor village, 4- Sardasht, Gardaneh Khan, 5- Ghorveh, Saalavat abad, 6- Sardasht, Khan pass, 7- Marand, 8- Ghavigol lake, 9- Khoramshahr, Moqhavemat squre, 10- Minab,Hasanlangi, 11- Bandarabass, Moqhoyeh, Charak Bandar, 12- Mazandaran, Nowshahr, 13- Karaj-Chalus road, 14- Marand.

.Band No Sp/pop	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
L. gebelia	0	0	0	0	0	1	0	0	1	1	1	0	1	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0
L. gebelia	0	0	0	0	0	1	0	0	1	1	1	0	1	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0
L. gebelia	0	0	0	0	0	1	0	0	1	1	1	0	1	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0
L. gebelia	0	0	0	0	0	1	0	0	1	1	1	0	1	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	1
L. corniculatus	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	1	0	1	1	0	1	1	0	1	1	0	1	0
L. corniculatus	0	0	1	1	0	0	0	1	1	1	1	0	0	0	1	0	1	0	1	1	0	1	1	0	1	1	0	1	0
L. corniculatus	0	0	1	1	0	0	0	1	1	1	1	0	0	0	1	0	1	0	1	1	0	1	1	0	1	1	0	1	0
L. tenuis	0	0	1	1	0	0	0	1	1	1	1	0	0	0	1	0	1	0	1	1	0	1	1	0	1	1	0	1	0
L. tenuis	0	0	1	1	0	0	0	1	1	1	1	0	0	0	1	0	1	1	0	1	0	1	1	1	0	1	1	0	0
L. laricus	0	0	0	1	0	1	1	1	1	0	1	0	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0
L. garcinii	0	0	0	1	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
L. angustissimus	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L. halophilus	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	1	0	1	0	1	0	1	1	0	0
L. michauxianus	0	0	0	0	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0
L. corniculatus	1	1	1	1	0	0	0	1	1	1	1	0	0	0	1	0	1	0	1	1	0	1	1	1	0	0	1	0	0

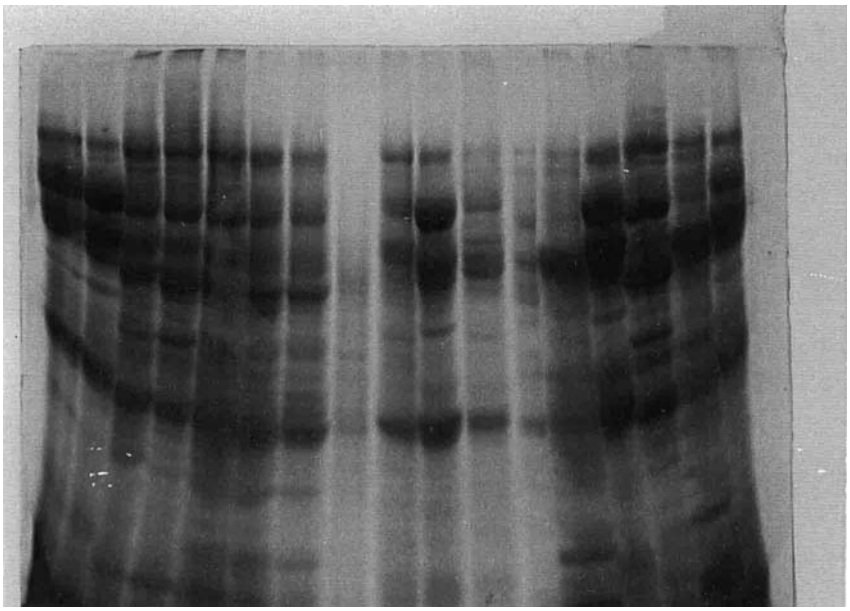

Fig. 1. Protein bands in *Lotus* species studied. Species/population code from left to right as in table 1.

population of *L. corniculatus* and Bands 5 and 14 in *L. laricus*, band 12 only in *L. garcinii* and band 16 in *L. halophilus*.

The bands 6, 9, 10, 11, 13, 20, 22, 24, 27 occurred in all populations of *L. gebelia* while band 29 was specific in Khan population of *L. gebelia*. The highest number of protein bands (16) occurred in Marand population of *L. corniculatus* while the lowest number of bands (3) occurred in *L. angustissimus.*

Grouping of the species and populations based on protein data was performed by using different methods of cluster analyses including single linkage and UPGMA as well as ordination based on PCA (Figs. 2 & 3). Both analyses revealed presence of 3 main clusters or groups which in general supports groupings suggested by Chrtkova-Zertova (1982) treating the *Lotus* species in 4 sections namely: 1- *Lotus*, 2- *Loteae*, 3- *Erythrolotus* and 4- *Ononidium*.

The first main cluster is comprised of *L. tenuis* and *L. corniculatus* populations showing similarity of these two species to each other, which have been placed in the section *Lotus*. *L. halophilus* is joined to these species with some distance. This species is placed in section *Loteae* which is also considered close to the section *Lotus* by Chrtkova-Zetova (1982).

The second main cluster or group is comprised of *L. gebelia* populations and *L. micauxianus* both of which have been also placed in section *Lotus* by Chrtkova-Zetova (1982). Although the members of these two clusters or groups show more similarity to each other, they are joined by a big distance due to their protein differences. A similar result is obtained from morphometric studies (unpublished), therefore it may be suggested the species of these two clusters or groups be considered in separate sections or series as suggested by Kupruanova (1975).

Table 2: PCA analysis of protein bands.b_1-b_{29}=protein bands

	Component			
	1	2	3	4
b1	0.355	4.461E-02	0372	0.547
b2	0.355	4.461E-02	0.372	-0.547
b3	0.743	-6.27E-02	-2.48E-0.3	-0.324
b4	0.672	-0.473	0.365	-8.41E-02
b5	-8.94E-02	-0.609	0.486	0.576
b6	-0.758	0.589	0.234	-2.41E-03
b7	-0.290	-0.938	-4.38E-02	-8.76E-02
b8	0.786	-2.00	0.450	0.124
b9	0.238	6.205E-02	0.716	-6.05E-02
b10	0.290	0.938	4.381E-02	8.765E-02
b12	-.201	-0.555	-0.153	-0.412
b13	-0.758	0.589	0.234	-2.41E-03
b14	-8.94E-02	-0.609	0.486	0.576
b15	0.948	0.195	-0.186	7.255E-02
b16	5.487E-02	6.952E-02	-0.629	0.386
b17	0.938	0.163	0.131	-0.123
b18	0.311	3.814E-02	-0.629	0.386
b19	0.810	0.149	0.148	-0.199
b20	0.275	0.657	0.408	0.526
b21	-0.213	-0.854	0.244	0.120
b22	0.290	0.938	4.381E-02	8.765E-02
b23	0.938	0.163	0.131	-0.123
b24	-0.660	0.532	0.315	-1.14E-02
b25	0.903	-0.109	5.708E-02	0.361
b26	0.785	0.176	-0.378	0.352
b27	0.948	0.195	-0.186	7.255E-02
b28	-0.758	0.589	0.234	-2.41E-03
b29	-0.317	0.268	0.195	-7.64E-03

The third major cluster is comprised of three species of *L. laricus*, *L. garcinii* and *L. angustissimus* all joined with great distance due to their protein differences.

According to Chrtkova-Zetova (1982), *L. angustissimus* belongs to the section *Lotus* along with the other species of this group which is not supported by protein analysis, however *L. laricus* is considered as a member of section *Erythrolotus* and *L. garcinii* as a member of section *Ononidium* which is supported here as they stand in distant clusters and groups (Figs. 2 & 3).

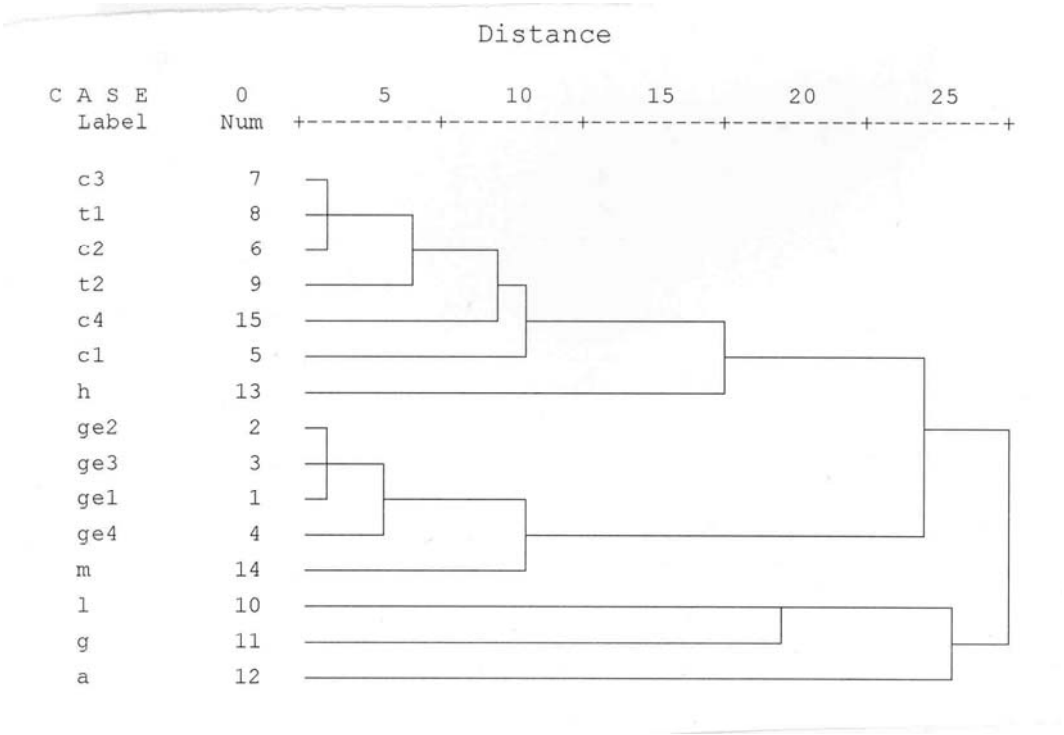

Fig. 2. UPGMA cluster analysis of protein data. Abbereviations: a =*L.angustissimus*, g =*L. garcinii*, l = *L. laricus*, m = *L. michauxianus*, ge =*L. gebelia*, h= *L. halophilus*, c=*L. corniculatus*, t= *L. tenuis* (Numbers as in Table1.).

PCA analysis at the protein bands revealed that the first 4 components comprise about 78% of total variation. In component one, which comprises about 37% of total variance, bands 3, 4, 8, 15, 17, 19, 23, 25, 26 and 27 possess the highest positive correlation (>0.60, Table 2). The first component separates mainly the species of the section *Lotus* of Chrtkova-Zertova (1982). In component 2, which comprises about 24% of total variance, bands 10, 20 and 22 possessed the highest positive correlation (>0.60). The second component separates three species of *L. garcinii, L. laricus, and L. angustissimus* from the others.

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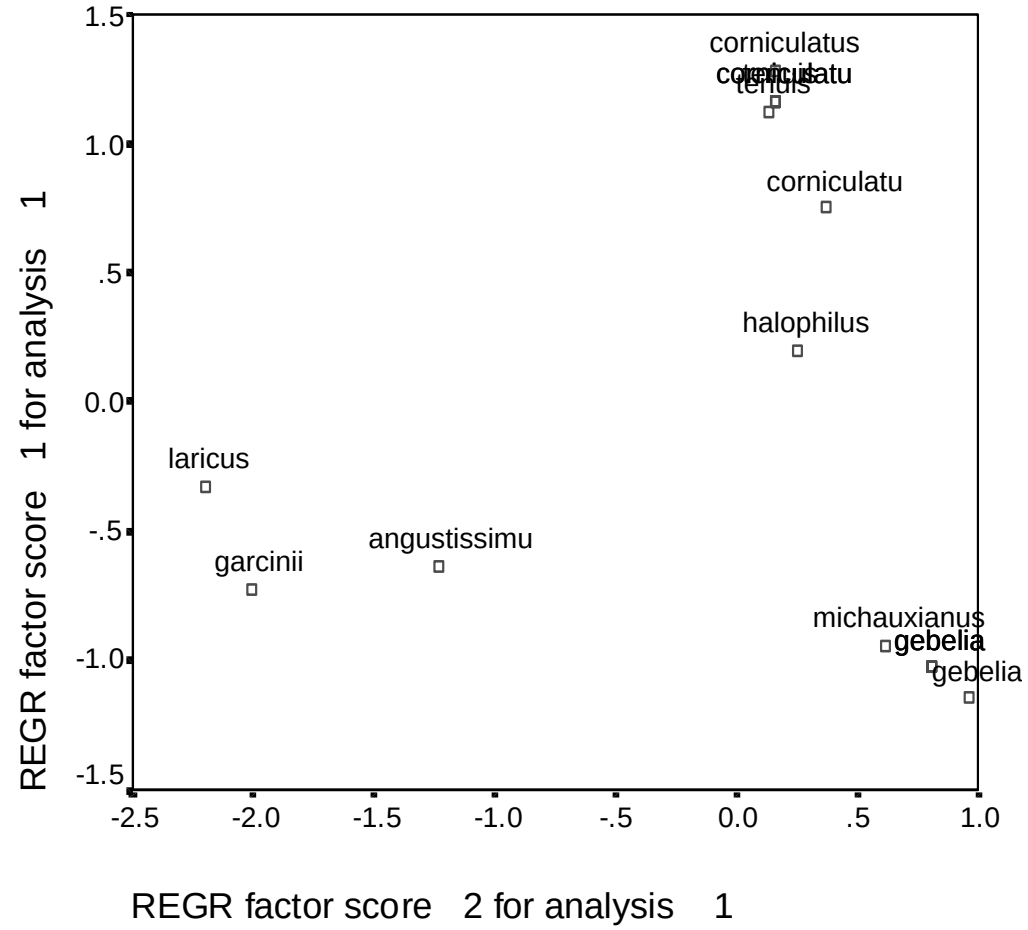

Fig. 3. PCA ordination of protein data.

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