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The use of seed proteins as revealed by SDS-PAGE in the taxonomy of some *Astragalus* species (Fabaceae)

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Total seed proteins of 20 *Astragalus* L. species belonging to 16 sections were electrophoretically analyzed using SDS-PAGE technique. Multivariate analysis (cluster and principal coordinate analysis) of the electrophoretic data showed heterogeneity between some taxa. This heterogeneity was discussed on the light of the controversy over the taxonomic position of *Astragalus* L. species. The relationships among the examined taxa have been demonstrated as cluster tree using SYSTAT-pc software. This tree illustrated that the studied taxa were separated into two major groups, the New World and the Old World species. The New World group include the species *Astragalus wootoni, A. allochrous, A. palmeri, A. layneae, A. lentiginosus, A. nothoxys, A. minthorniae* and *A. nuttallianu.* However, *Astragalus coccineus, A. pectinatus* and *A. didymocarpus* that considered from the New World, but separated in the Old World group. *Astragalus layneae* separated from the rest taxa in the New World group. This species was located in a separate section layneani. The Old World group divided into three subgroups and discussed on the light of earlier classification.

Keywords: *Astragalus* L., seed protein electrophoreses, multivariate analysis.

Introduction

The genus *Astragalus* L. (Fabaceae) is one of the largest genera of flowering plants comprising a world total of 2500 species mostly perennial species, grouped in over than 150 sections, from which 2000 are in Old World (Podlech, 1986). The genus is cosmopolitan

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being distributed almost in all continents except Australia. A number of species of *Astragalus* from Southwest and Southcentral Asia (e.g. *A. gummifer* Lab., Iran) are the source of "gum tragacanth" - a substance tapped from roots or stems with hydrophilic and colloidal properties valuable in ice creams, lotions, pharmaceuticals, used since the time of ancient Greece. A few species are edible (*A. canadensis* L., N. America) or have medicinal uses, and some are used for livestock forage (*A. cicer* L., USA), but a large number of North American species are poisonous (e.g., *A. mollissimus* Torr., N. America), especially to livestock and wild life, a property due to the accumulation of selenium from soils or synthesis of toxic levels of certain nitrotoxins and alkaloids in the foliage - hence the name "locoweed" ("loco" is Spanish for crazy) given to many species (Wojciechowski *et al.*, 1999). The genus *Astragalus* L. has been surveyed in several parts of the world. For example in Yugoslavia (Micevsky and Mayer, 1984) in America (Isely, 1984, 1986) in Mongalia (Ulziikhutag, 1986) in south west Asia (Podlech, 1986) in the Arabian Peninsula (Hedge and Podlech, 1987) in Iran (Maassoumi,1988) and Ghahremani-nejad (2003, 2005) and in Egypt (Boulos, 1999).

Jones (1923) initiated a new era in the systematic of *Astragalus* L., by the search for evolutionary relationships between species based on similarities in a small arbitrarily chosen set of morphological characters. He revised the genus in North America; that comprised 273 species delimited in 30 sections. Rydberg (1929), on the other hand, classified the genus in North America into 3 subgenera and 82 sections. Because of the great number of species there has been a controversy about the number of subgenera, sections and species. The delimitation of almost all these categories had been confronted with difficulties when vegetative and floral characters were considered. For this reason, the subgeneric categories of *Astragalus* L. have been subjected to both nomenclatural and taxonomic changes. Moreover, most of the sections were not completely typified (Hedge and Podlech, 1987) and this led to undesired new description of subgeneric taxa. Also, the phylogenetic status of taxa in *Astragalus* L. has been continuously subjected to alterations because of the different evaluation rate of characters in the same taxon (Podlech, 1986).

Comprehensive studies on its biogeography showed that *Astragalus* L. has continental distribution with amazing ecological variation for adaptation in different habitats. This fact promoted

number of scholars to more studies through different disciplines. These studies comprised Cladistic analysis (Lavin and Marriott 1997 and Wojciechowski *et al.*, 1999), orthodox taxonomy (Valesecchi, 1994; Liston *et al.*, 1997 and Podlech and Aytac, 1998), anatomy (Sukhova, 1990; Engel, 1992; Kandemir *et al*., 1996; Zarrinkamar, 1996), cytology (Cartier, 1979; Liston, 1990; Dopchiz *et al.*, 1995) and morphology of pollen grains (Saad and Taia, 1988 and Abou-El-Naga and Rizk, 1997). Podlech (1983) concluded that only two subgenera of *Astragalus*, can be maintained. They are subgenus *Astragalus* which contains all species with basifixed hairs, and subgenus Crcidothrix with medifixed hairs. Other characters that had been used for the recognition of subgenera by previous authors e.g. life duration and enlargement of calyx were considered as polyphyletic characters that can not be used for the establishment of subgenera. Podlech (1986) has pointed out that division into sections is also full of problems. He considered the delimitation of many of about 150 sections that have been described within the Old World *Astragali,* to be uncertain. In fact most of the sections weren't typified. A consequence of these problems is that nobody can be certain about the divisions into sections in the genus *Astragalus* L.

Seed proteins which were first detected in legumes were composed of four different classes namely albumin, globulin (legumin, vicilin and convicillin) prolamin and glutein (Debyshire *et al*., 1976; Jensen and Buttner, 1981). Gel electrophoresis of seed protein produces reproducible band pattern when its components were prepared in a standard method. The presence or absence of these bands was used as diagnostic characters for a group of taxa or for a certain taxon (Ladizinsky and Hymowitz, 1979; Jensen and Lixue, 1991). The use of seed proteins in systematic is supported by the fact that mature seeds possess the same protein components and thus provide valid evidence for relationships of plants (Crawford, 1990). Electrophoretic patterns of total seed proteins, as revealed by polyacrylalmide gel electrophoresis (PAGE) provide valid evidence for addressing taxonomic and evolutionary problems in plants (Ladizinsky and Hymowitz, 1979; Crawford, 1990).

The major storage seed proteins in plants have also been utilized to provide an understanding of the relationships in some genera of Fabaceae. For example, in *Trifolium* (Badr, 1995 and Sammour, 1999),

Amaranthus (Sammour *et al*., 1993), *Sesbania* (Badr *et al*., 1998), *Lathyrus* (El-Shanshoury, 1997 and Badr *et al*., 2000). Seed proteins in some Egyptian *Astragalus* L. species were examined by El-Rabey (1992). He noted that seed protein electrophoresis revealed high level of similarity between *A. stella* and *A. tribulordes*, particularly under non-reducing conditions. This data was congruent with the morphological study (Ahmed *et al*., 1989). Otherwise, the result of the electrophoretic study on six species of *Astragalus* L. agreed with the traditional taxonomic relationships based on morphological criteria (El-Rabey, 1992 and Khafagi, 1995). However, the electrophoretic study distinguished closely related species.

The objective of the present work is to assess the contribution of seed protein electrophoretic evidence to the taxonomy of some *Astragalus* L. species.

Materials and Methods

Twenty species representing 16 sections were studied; their sections, accession number, locality and country of origin were shown in Table 1. The seeds were obtained from Desert Legume Program, 2120 East Allen Road Tucson, AZ 85719. Systematic treatment that adopted in this study was according to Podlech (1986).

Seed protein was extracted in buffer Tris-HCl (Tris, SDS, β-mercaptoethanol at pH=7.6). For extraction, 0.2 g seed were milled to fine powder and mixed with 2 ml buffer for l h at room temperature. The mixture was centrifuged for 10 min at 12,000 rpm and 5µl of supernatant (protein extract) of each accession was analyzed on SDS-PAGE under reducing conditions. Protein concentration was determined according to Bradford (1976). Six µl of a marker protein mixture containing 6 different protein subunits with known molecular weight were loaded side by side with samples. Consort vertical slab gel apparatus was used for electrophoresis. Gel was stained in sufficient amount of Coomassie blue 250 (Serva) for 30 minutes and de-stained in a 2:1(v/v) mixture of methanol and acetic acid for 2-3 days.

Table 1. Sections, species, accession number and country of origin of 20 studied accessions of *Astragalus* L. species.

Sections	Species and Code Number	Accession number	Locality	Country
1- Inflati	1-*Astragalus palmeri Gray* **A18**	920094	Hwys2, just N of MP 31. 32°58'34"	California -USA
	2-*Astragalus wootoni* sheld **A7**	910104	Tubac	Arizona-USA
	3-*Astragalus allochrous* Gray **A1**	DLEG910521	N.side of Ina Road.	Arizona-USA
2-Haematodes	4-*Astragalus annularis* Foressk. **A2**	950095		Palestine
3-Hypoglottidei	5-*Astragalus cicer* **A3**	950106		Turkey
4- Argophyllii	6-*Astragalus coccineus* Brand **A4**	910025	150 Color Cave Rd.,Sedona	Arizona-USA
5- Microlobium	7-*Astragalus didymocarpus* H.&A. **A5**	900541	Avra Valley,E of Ragged Top Mt.	Arizona-USA
6- Astracantta	8-*Astragalus microcephalus* **A13**	880049	Badga,e of Shiraz	Iran
	9-*Astragalus echidnae* Formis **A6**	٨٨٠٠٤٥	Feridan	Iran
7- Sesamei	10-*Astragalus filicaulis* Foressk. **A8**	950116		Afghanistan
8- Buceras	11-*Astragalus hamosus* L. **A9**	950120		Iran
9- Ankylobus	12-*Astragalus hispidulus* DC. **A10**	890384		Palestine
10- Diphysi	13-*Astragalus lentiginosus* Dougl.ex Hook. **A11**	٩١٠١٢٢	Taylor Residence,Tucson	Arizona-USA
11- Layneani	14-*Astragalus layneae* Greene **A12**	50060٩	Hidden Hills Road area SE of Granite	California -USA
12- Malaci	15-*Astragalus minthorniae* (Rydb.) Jepson **A14**	950063	Molycorp Picnic Area, Clark	California -USA
13-Leptocarpi	16-*Astragalus nothoxys* Gray **A15**	910105	6010 N Canyon Tucson.Elevation 2500 ft	California -USA
	17-*Astragalus nuttallianus* A.DC. **A16**	910115	Hwy 89,ca.8 m.	Arizona-USA
14-Uliginosi	18-*Astragalus odoratus* A.DC. **A17**	950129		Iran
15-Pectinati	19-*Astragalus pectinatus* Gray **A19**	900284		Colorado-USA
16-Trichopodi	20-*Astragalus trichopodus* (Nutt.)Gray **A20**	910508		Mexico-MEX

The banding profile of the 20 examined samples was photographed using Agfa pan film, and prints were made using Kodak photographic paper. The number of bands was scored by critical observation of gel records. The best observations were achieved by placing the gel against white background. The bands produced by each sample were counted and their relative motilities' compared with those of the standard marker proteins. The presence or absence of each band was treated as a binary character in a data matrix (coded 1 and 0 respectively) for computation using the program SYSTAT Pc. The data was subjected to both cluster and principal coordinate analysis. Quantitative variation expressed as difference in intensity and thickness of bands, was also observed, but since this type of variation is often associated with the genotype, it was not taken into consideration when coding for the numerical analysis. The method applied is based on cluster analysis and expresses the relationships of the studied taxa as percent similarity in dendrogram. The bands scored from electropherograms produced were used as sets of data for computer analysis, to produce a cluster tree.

Results and Discussion

The analysis of seed proteins by SDS-PAGE revealed that seeds of *Astragalus* L. are rich in storage proteins with a large number of stable bands in the electrophrogram produced. This reflects a number of genetic and phylogenic relationships which could be used as criteria for the classification of species in this genus (Gazer, 1993). The electropherograms of the examined taxa revealed a total number of 44 bands. The minimum number of bands was recorded in *Astragalus pectinatus*, while the maximum number of bands was recorded in *Astragalus wootoni* and *A. allochrous*. The electrophoretic patterns of the total seed proteins of studied taxa produced by SDS-PAGE analysis were shown in Figure 1(a, b & c)**.** The total seed proteins of each species exhibited distinctive electrophoretic patterns. However, the degree of variation in the electrophoretic patterns between the taxa of each section was less, pronounced. The greatest variation was found between the taxa of sections Inflati and Leptocarpi.

The dendrogram produced from the cluster analysis based on the protein bands derived from the electrophoretic analysis of the total seed protein analyzed on SDS-PAGE (Fig. 2) showed that the studied 20 taxa have an

average taxonomic distance about 0.55. At this level the taxa were separated into two major groups; The New World and the Old World species. The New World group included *Astragalus wootoni, A. allochrous A. palmeri, A. layneae, A. lentiginosus, A. nothoxys, A. minthorniae* and *A. nuttallianu,* whereas the Old World species group included the rest of taxa . In the first group, *Astragalus layneae* separated from the other taxa, at taxonomic distance 0.38. This species is located in a separate section layneani (Podlech, 1991). *Astragalus nothoxys* is delimited from *A. nuttllianus* at taxonomic distance 0.34 which seems to be in contradiction with their previous sectional delimitation in section Leptocarpi (Podlech, 1991). *Astragalus lentiginosus* was delimited from the group at taxonomic distance 0.27. This is in agreement with its delimitation in a separate section Diphysi (Sharawy *et al.,* 2003). The dendogram also displayed a great similarity between species *Astragalus wootoni, A. allochrous* and *A. palmeri* which were grouped together in section Inflate that seems to be in agreement with their previous sectional delimitation by El-Rebey (1992) and Al-Nowaihi *et al*. (2002).

The second major group has a taxonomic distance of 0.37. At this point, the second group separated into three subgroups: the first included *Astragalus microcephalus* and *A. echidnae* which support their classification into one section Astracantta (Podlech, 1986). The second one included the species *Astragalus trichopodus, A. pectinatus, A. odoratus, A. Filicaulis, A. hamosus* and *A. hispidulus* and the third subgroup included *A. annularis*, *A. cicer*, *A. didymocarpus* and *A. coccineus.* These species separated from each other at different taxomonic distance as shown in the dendrogram.

Seed protein electrophoretic analysis distinguished *A. hamosus* from *A. hispidulus* and *A. odoratus* at taxonomic distance 0.23; that is in agreement with the morphological characters. Ahmed and Mohamed (1988) separated *Astragalus hamosus* from the other species using some anatomical characters, that supported by the results of analysis based on the seed protein characters used in this study. Whereas, Ahmed *et al.* (1989) grouped *Astragalus hamosus* with *A. annularis* based on similarities in leaf and flower characters. El- Rabey (1992) and Al-Nowaihi *et al.* (2002) also found that the seed protein electrophoresis manifested a high level of similarity between *Astragalus hamosus* and *A. annulari.* This seems to be in agreement with this study. *Astragalus hamosus, A. hispidulus* and *A. tripcho*

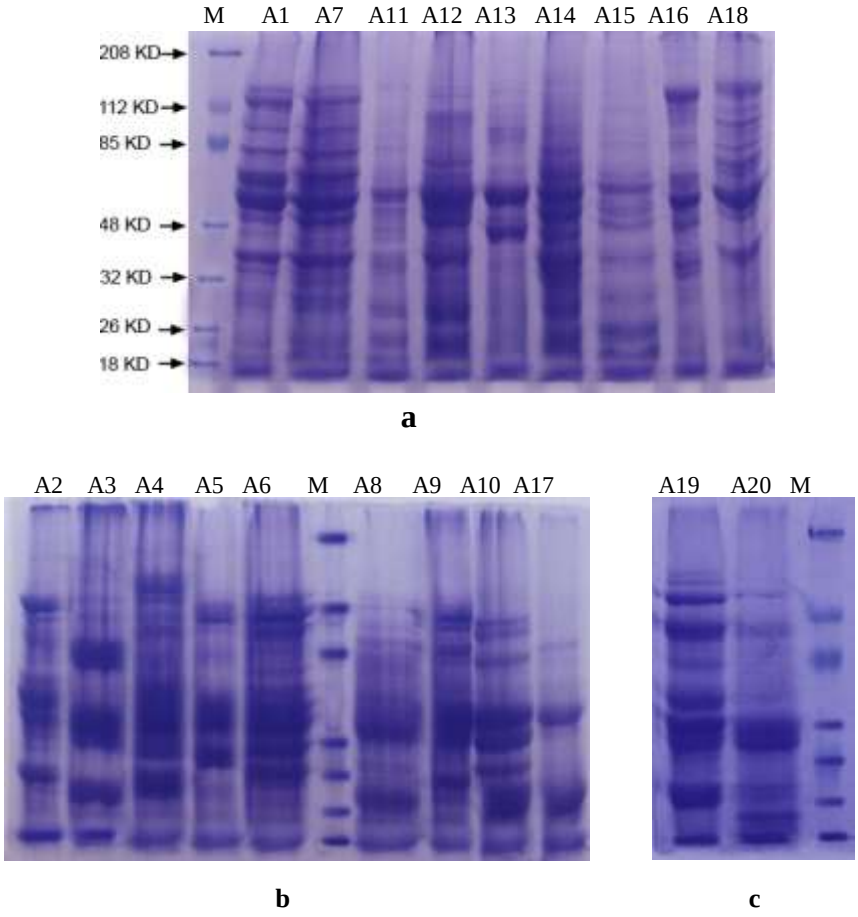

Fig. 1 (a, b, c). Electropherograms produced by SDS-PAGE seed proteins of *Astragalus* L. samples M= marker protein.

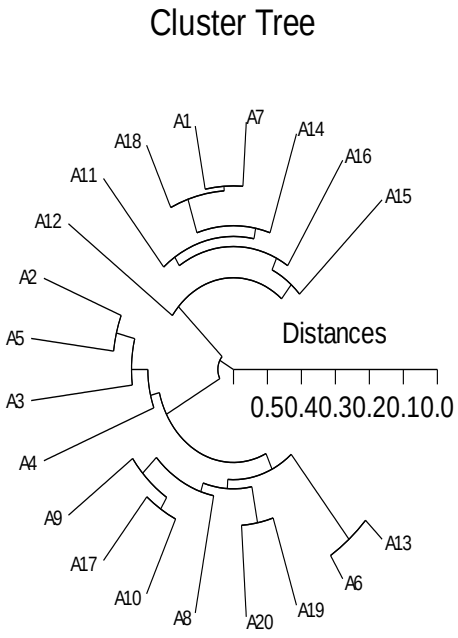

Fig. 2. Dendogram showing relationships among the studied *Astragalus* species based on SDS-PAGE data.

podus were separated into three different subgroups, although Sharawy *et al*. (2003) recognized them in one group on the basis of morphological and anatomical characters. The variations in protein criteria among these species appear to contradict the morphological similarities between them. In this respect, it may be assumed that the morphological criteria were not paralleled by changes in genes coding for the storage proteins of the seed (Al-Nowaihi *et al*., 2002). *Astragalus annularis* was distinguished from the other species confirming its taxonomic delimitation in section Haematodes. This species was characterized by a few numbers of leaflet pairs (2-4 pairs) and red blotched fruits (Hedge and Podlech, 1987). Anatomically this species was characterized by the presence of idioplast cells and this is in agreement with the observation of Ahmed and Mohamed (1988). Compared to other species, *A. annularis* is also cytologically different from other species (Badr *et al.,* 1996).

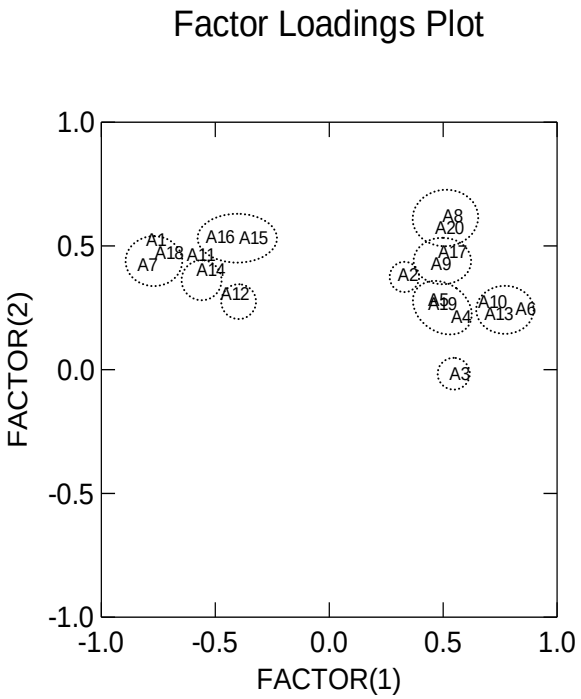

Fig. 3. Principal coordinate analysis showing relations of *Astragalus* L. species indicated by SDS-PAGE data.

The principal coordinate analysis (PCoA) of the total seed protein banding patterns showed that the first two factors accounted for 42.549 of the total variance, they were projected into a two dimensional graphic (Figure3). The New World and the Old World species were more or less delimited as two separate groups, accounted for 25.751% and 16.798% of the total seed protein banding pattern variations, respectively. Variables which contributed most to the second principal coordination were *Astragalus allochrous, A. palmeri , A. wootoni , A. lentiginosus, A. layneae, A. minthorniae, A. nothoxys* , *A nuttallianus*. All these taxa belonged to section Inflati, Diphysi, Layneani, Malaci and Leptocarpi. *Astragalus cicer* had a negative correlation to the two factors. This indicated that *Astragalus cicer* was distantly related to the other species. The accessions belonged to

the Old World were *Astragalus microcephalus, A. annularis, A. cicer, A. echidnaeformis, A. filicaulis, A. hamosus, A. hispidulus* and *A. odoratus.* However *Astragalus coccineus, A. pectinatus* and *A. didymocarpus* were from the New World but separated with the Old World group species.

In conclusion, the present results confirm the validity of seed protein as source of taxonomic criteria. It is also conclude that the relationships within and between groups produced by the analysis of seed protein electrophoretic profile does not thoroughly agree with Podlech (1991) and Gazer (1993) classification of *Astragalu*s L. species.

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