SEED PROTEIN ANALYSIS IN RELATION TO TAXONOMY OF THE IRANIAN LINUM SPECIES

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Seed storage protein analysis was carried out on twelve Iranian species of *Linum* with the aim to illustrate species inter-relationships and to evaluate the taxonomic treatments proposed for the genus. Placement of species in different sections according to cluster analysis of protein data was highly in agreement with previous phenetic morphological based studies. Grouping of species supported the proposed memberships of *Linum* species in Iran, and SDS-PAGE profile of seed proteins proved to be useful to be included together with other molecular markers in biosystematics of genus *Linum* at sub-generic level.

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Key words. Linum, seed, protein, cluster analysis, SDS-PAGE, taxonomy, Iran.

بررسی پروتئین دانه برخی گونه‌های ایرانی جنس کتان .Linum L و ارتباط تاکسونومیک آنها

فریبا شریف‌نیا و مصطفی اسدی

پروتئین‌های ذخیره‌ای دانه‌های ۱۲ گونه کتان ایرانی با هدف بررسی ارتباط بین گونه‌ای تاکسونومیک آنها مورد مطالعه قرار گرفت. جایگاه گونه‌ها در بخشه مختلف در جهت تایید مطالعات ریخت‌شناسی قبلی بود و گروه‌بندی گونه‌ها مورد تایید قرار گرفت و مشخص شد استفاده از روش SDS-PAGE برای بررسی‌های تحت جنسی مفید می‌باشد.

INTRODUCTION

The genus *Linum* L. belongs to the family *Linaceae* S. F. Gray with about 230 species distributed throughout temperate regions (Heywood, 1978).

In Flora Iranica, sixteen *Linum* species have been reported from Iran plateau and is divided into five sections (Rechinger, 1974). However, in a recent survey in the course of writing the Flora of Iran the number of species reported from Iran have been reduced to fiftheen. Moreover, *L. tenuifolium* has been removed from the section *Linum* and placed in section *Linasrum* (Planch) H. Walker in Engler & Prantl. (Sharifnia & Assadi, 2001).

The earlier studies of the genus in Iran have been based on morphological traits only (Parsa, 1951; Rechinger, 1974; Mobayen, 1995).

Seed storage protein analysis is valuable method to clarify taxonomic and phylogenetic relationships in plants (Johnson & Hall, 1965; Johnson & Thein, 1970).

In this paper, seed storage protein data was subjected to cluster analysis in order to indicate the species inter-relationships, to evaluate the previous taxonomic treatment of the genus *Linum* in Iran, and to provide the evidence for efficacy of application of protein data in taxonomic treatment of the genus *Linum* at sub-generic level.

MATERIALS AND METHODS

Plant materials

The plants and seeds of twelve *Linum* specis were collected from summer 1999 to 2000 in Iran (table 1). Vocher specimens are desposited in Central Herbarium of Iran (TARI).

Protein extraction and electrophoresis

Protein extraction and electrophoresis of samples were conducted in Laboratory Center of Tehran Science and Research Branch, Islamic Azad University.

Protein extraction was initiated according to the protocol described by Sheidai & al. (2000) with some moderations using 0. 2 g. of each seed sample and 0. 5 ml. of a buffer solution containing 77 mM Tris-HCl, (pH 6. 8), 10%C2H6O5 (2-hydroxy-1-ethanethiol), 4% C12H25NaO4S (SDS), and 3%C3H8O3 (1, 2, 3-propanetriol). The resulting mixture was placed in a 2 ml. plastic-capped tube (Eppendorf, Germany) and boiled in 80°C water bath for 10 min. and the contents of the tube were mixed and centrifuged for 5 min. at 12, 000 g. Protein electrophoresis (SDS-PAGE) was carried out following the procedure of Sheidai & al. (2000), using 10 µl. of protein of the various extracts in each lane at a constant of 45 m A for 8 hour (Akhatarian vertical electrophoresis apparatus, Model: VS-110, Iran).

Staining and de-staining of geles

Protein were stained for one hour with Coomassie Brilliant Blue G-250 (Fluka, Switzerland) in CH3OH: CH3COOH: H2O (40: 20: 40 v/v). Geles were de-stained with a solution containing CH3OH: CH3COOH: H2O (40:20:80v/v) according to Amin & al. (1990).

Cluster Analysis

To estimate the species similarities as indicated by protein electrophoresis patterns, Jaccard index was determined (Digby & Kempton, 1994). Each protein band was considered as a qualitative binary character (Carreras & al., 1997). The statistical analysis was accomplished using NTSYS-pc (Rohlf, 1990).

RESULTS AND DISCUSSION

Seed storage protein electrophoresis of twelve *Linum* species showed the presence of 60 bands in total ranging from 17 for *L. austriacum* to 25 for *L. nervosum*. Bands 11,

Table 1. List of Linum species used in seed protein analysis and their localities.

Taxon	Locality
L. strictum L.	Kermanshah: Javanrood, Sheikh Saleh village, Parsa H., 244.
L. tenuifolium L.	Azerbaijan: Arasbaran, protected region, 1050 m, Asri., 245.
L. corymbulosum Reichenb.	Gilan: Roodbar, 900 m, Sharifnia, 240.
L. album Ky. ex Boiss.	Tehran: NE of Tehran, Lashgarak, 1600 m, Sharifnia.
L. nodiflorum L.	Gilan: Roodbar, 900 m, Sharifnia, 241.
L. austriacum L.	Gilan: Roodbar, 900 m, Sharifnia, 242.
L. glaucum Boiss. & Noe.	Kordestan: Sanandaj, Abidar, 1800 m, Fatahi, 1957.
L. usitatissimum L.	Khozestan: Shushtar, 250 m, Sharifnia 80083.
L. bienne Mill.	Khozestan: Laly, 300 m, Sharifnia 80082.
L. catharticum L.	Tehran: NE of Tehran, Fasham, 2000 m, Sharifnia 80080.
L. bungei Boiss.	Mazandaran: Chalus road, Siah Bishe, 2300 m, Sharifnia 243.
L. nervosum Waldst. & Kit.	Mazandaran: Chalus road, Siah Bishe, 2300 m, Sharifnia 80079.

44 and 58 were common in all the species studied. Bands 1 and 47, bands 15 and 3, band 48 and band 51 were specific for *L. usitatissimum, L. bienne, L. corymbulosum* and *L. catharticum* respectively. Bands 14, 23, and 52 were specific for both *L. bungei* and *L. nervosum,* which could be an evidence for merging the later species (Fig. 1 A and B).

Palynological and morphological studies considered as supportive evidences (Sharifnia & Assadi, 2000 , 2001 and 2002).

Cluster analysis produced four major clusters (Fig. 2.). The first cluster separated *L. strictum* from the other species. The second and third clusters embraced *L. tenuifolium* and *L. corymbulosum*, and the fourth cluster was comprised of the other species. Presence of *L. tenuifolium* in a separate cluster far from the allies of section *Linum* may be considered as supportive evidence for placement of *L. tenuifolium* in a section other than *Linum* as treated in Flora of Europe (Tutin & al., 1968).

The presence of *L. strictum, L. corymbulosum* and *L. tenuifolium* in three close clusters was an indicative for placement of these species in a section (*Linastrum)* supporting the phenetic morphological studies (Sharifnia & Assadi 2002). The fourth cluster formed two sub-clustesrs comprising *L. nodiflorum* and *L. album* from the section *Syllinum* and the second sub-cluster comprising *L. bungei* and *L. nervosum.* The later result, as suggested by Sharifnia & Assadi (2001), showed that *L. bungei* and *L. nervosum* could be treated as synonyms.

In conclusion, protein electrophoresis phenogram was highly in agreement with morphological phenogram illustrated by Sharifnia & Assadi (2002) for Persian *Linum* species. These studies supported the proposed species memberships and efficacy of seed storage protein analysis treatment of the genus *Linum* in Iran at sub-generic level.

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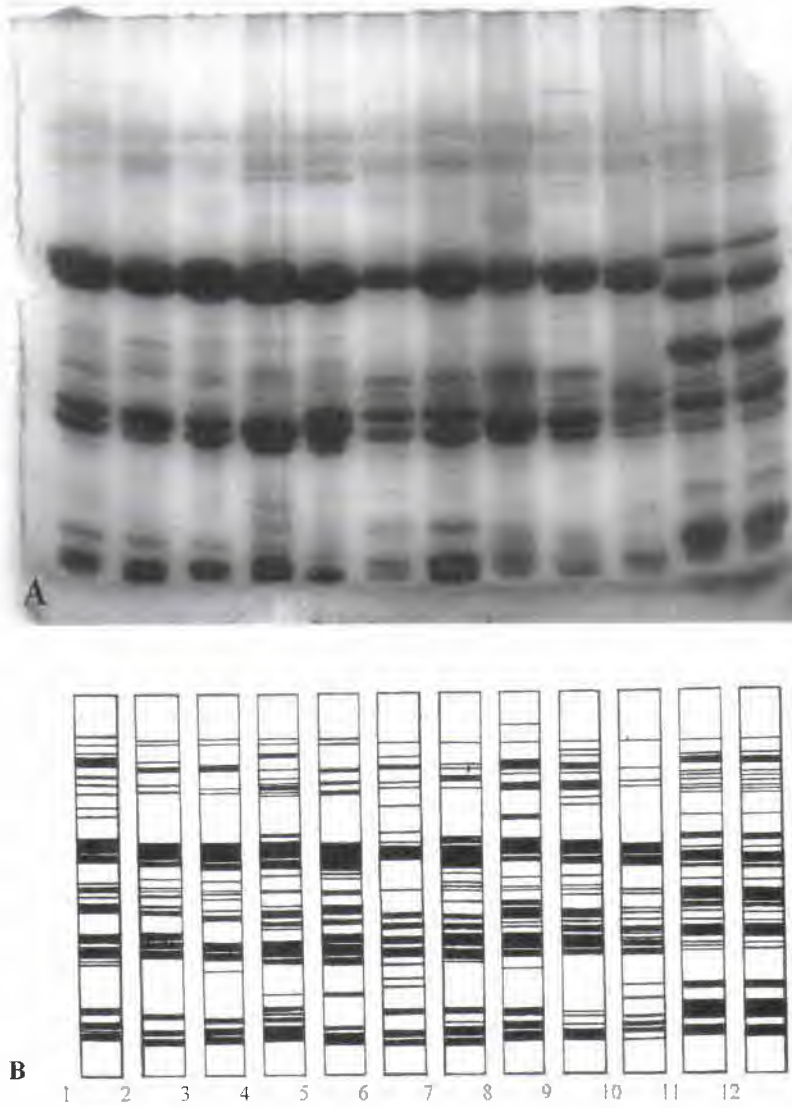

Fig. 1. SDS-PAGE protein bands of the *Linum species* studied (A). Electrophorogram of SDS-PAGE protein bands (B). 1= *L. strictum*, 2= *L. tenuifolium*, 3= *L. corymbulosum*, 4= *L. album*, 5= *L. nodiflorum*, 6= *L. austriacum*, 7=*L. glaucum*, 8= *L. usitatissimum*, 9= *L. bienne*, 10= *L. catharticum*, 11= *L. bungei*, 12= *L. nervosum*.

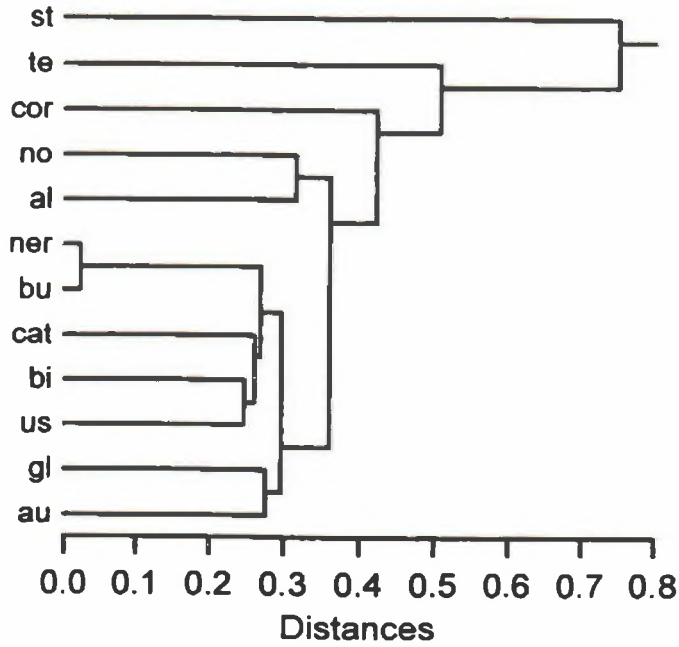

Fig. 2. Phenogram based on cluster analysis of seed storage protein data. st= *Linum strictum*, te= *L. tenuifolium*, cor= *L. corymbuolsum,* no = *L. nodiflorum*, al= *L. album*, ner= *L. nervosum*, bu=*L. bungei*, cat = *L. catharticum*, bi=*L. bienne*, us=*L. usitatissium*, gl=*L. glaucum*, and au=*L. austriacum*.

REFERENCES

Amin, E., Abdel-Halim, A., Ghazy, I. & Tahoun, M. K., 1990: The influence of Brassicca species and accessions on productivity and nutrient quality of forage rape in Egypt. -International Rapeseed congress, Canberra, Australia.

Carreras, M., Fuentes, E. & Merrino, E. F., 1997: Seed protein patterns of nine species of Cactacea. -Biochem. Syst. Ecol., 26: 43-49.

Digby, P. G. N., & Kempton, R. A., 1994: Multivariate analysis of ecological communities. -Chapman and Hall Publisher, New York.

Heywood, V. H., 1978. Flowering plants of the World. -Mayflower Books Inc., London.

Johnson, B. L., and Hall, O., 1965: Analysis of phylogenetic affinities in Triticinae by protein electrophoresis. -Am. J. Bot., 52:506-513.

Johnson, B. L., & Thein, M. M., 1970: Assessment of evolutionary affinities in Gossypium by protein electrophoresis. -Am. J. Bot., 57:1081-1092.

Mobayen, S., 1995: Flora of Vascular Plants of Iran. vol. 4. -Tehran University Press. Tehran.

Parsa, A., 1951: Flore de l'Iran. vol. 1. -Tehran University Press. Tehran.

Rechinger, K. H., 1974: Flora Iranica. no. 106. Akademische Druk, Verlagsanstalt, Austria.

Rolf, F. J., 1990: Numerical Taxonomy and Multivariate Analysis System, version 1. 80. Exeter Software, NewYork.

Sharifnia, F. and Assadi, M. 2000: Pollen morphology of Linum(Linaceae)species in Iran using SEM. -Journal of Science, Islamic Azad University. vol. 10, no. 37:667-675.

Sharifnia, F., & Assadi, M., 2001: Flora of Iran. no. 34. Research Institute of Forests and Rangelands Press, Tehran.

Sharifnia, F., & Assadi, M. 2002: A phenetic study of the genus Linum L. (Linaceae) in Iran. -Iran. Journ. Bot. vol. 9. no. 2. page:135-139.

Sheidai, M., Khatamsaz, M., & Mosallanejad, M. 2000: Numerical taxonomy and seed protein analysis of Hyocyamus species in Iran. -J. Sci. Ir., 11(2):83-91.

Sheidai, M., Noormohammadi, Z., & Ahmadi, M. R., 2001: Notes on B chromosomes and SDS-PAGE proteins in some rapeseed (Brassica napus L.) cultivars. The nucleus, 44 (3): 165-170.

Ockendon, D. J. & Walters, S. M. 1968: Linum in Tultin et al. Fl. Europaea vol. 2: 206-211. -Cambridge University Press..