Seed 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 phnetic 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.
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 fifteen. 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 species 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 mA 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.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. strictum L.</td>
<td>Kermanshah: Javanrood, Sheikh Saleh village, Parsa H., 244.</td>
</tr>
<tr>
<td>L. tenuifolium L.</td>
<td>Azerbaijan: Arasbaran, protected region, 1050 m, Asri., 245.</td>
</tr>
<tr>
<td>L. corymbulosum Reichenb.</td>
<td>Gilan: Roodbar, 900 m, Sharifnia, 240.</td>
</tr>
<tr>
<td>L. album Ky. ex Boiss.</td>
<td>Tehran: NE of Tehran, Lashgarak, 1600 m, Sharifnia.</td>
</tr>
<tr>
<td>L. nodiflorum L.</td>
<td>Gilan: Roodbar, 900 m, Sharifnia, 241.</td>
</tr>
<tr>
<td>L. austriacum L.</td>
<td>Gilan: Roodbar, 900 m, Sharifnia, 242.</td>
</tr>
<tr>
<td>L. usitatissimum L.</td>
<td>Khozystan: Shushtar, 250 m, Sharifnia 80083.</td>
</tr>
<tr>
<td>L. bienne Mill.</td>
<td>Khozystan: Laly, 300 m, Sharifnia 80082.</td>
</tr>
<tr>
<td>L. catharticum L.</td>
<td>Tehran: NE of Tehran, Fasham, 2000 m, Sharifnia 80080.</td>
</tr>
<tr>
<td>L. bungei Boiss.</td>
<td>Mazandaran: Chalus road, Siah Bishe, 2300 m, Sharifnia 243.</td>
</tr>
<tr>
<td>L. nervosum Waldst. &amp; Kit.</td>
<td>Mazandaran: Chalus road, Siah Bishe, 2300 m, Sharifnia 80079.</td>
</tr>
</tbody>
</table>

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 separate cluster rather 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 phenoetic morphological studies (Sharifnia & Assadi 2002). The fourth cluster formed two sub-clusters 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. 2. Phenogram based on cluster analysis of seed storage protein data. st= Linum strictum, te= L. tenuifolium, cor= L. corymbulosum, no = L. nodiflorum, al= L. album, ner= L. nervosum, bu=L. bungei, cat = L. catharticum, bi=L. biene, us=L. usitatissium, gl=L. glaucum, and au=L. austriacum.

REFERENCES


