A micro – macro morphological survey to assess the taxonomic relationship of Linm species in Iran

Sharifnia, F.[1], Assadi, M.[2]

1. Department of Biology, Faculty of Science, Islamic Azad University, North Tehran Branch

2. Research Institute of Forests and Rangelands, P.O.BOX: 13185-116 Tehran, Iran

Abstract

The present survey has been carried out on sixteen species of the genus *Linum* L. in Iran with the aim to illustrate inter specific relationships and to evaluate the taxonomic treatments proposed for the genus on the basis of morphological and palynological studies and further application of multivariate analysis to the data resulted from these studies. Grouping of species has highly supported the proposed memberships of *Linum* species in Iran and application of palynological data together with morphological data has been proven to be efficient for application at sub-generic level in taxonomic treatments of the genus *Linum* in Iran.

Key words: *Linum*, Palynology, morphology, multivariate, analysis, taxonomy, Iran

INTRODUCTION

The genus *Linum* L. (*Linaceae* S. F. Gray) has been considered to have about 230 species that are distributed throughout temperate regions of the world (Heywood, 1978). In Flora Iranica, sixteen species have been reported from Iran Plateau and the genus has been 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 has been reduced and relatively *L. tenuifolium* has been removed from the section *Linum* L., and placed in Section *Linastrum* (Planch) H. Walker (Sharifnia and Assadi, 2001). Moreover, phonetic morphologic based studies of different Persian species have been conducted in order to confirm the previous studies (Sharifnia and Albouyeh, 2002).

The previous studies of the genus *Linum* in Iran had been in forrms of classical taxonomy using only morphological traits (Parsa, 1951; Rechinger, 1974; Mobayen, 1995).

Pollen morphology is great value to clarify taxonomic and phylogenetic relationships in plants (Moore et al., 1991).

The earlier palynological studies of the genus have mainly involved the determination of general shape and the measurement of number of colpi (Moore et al., 1991). Based on pollen ornamentation, an identification key has been prepared for some *Linum* species (Moore et al., 1991). Palynological studies of some *Linum* species in Iran has been performed using SEM, but although placement of several species in their relative sections has been confirmed, pollen morphological data has not been too selective to be applied solely in sub-generic level of the genus *Linum* in Iran (Sharifnia and Assadi, 2000).

In this paper, micro morphologic (pollen characters) data together with macro morphologic data have been subjected to multivariate analysis to indicate inter specific relationships, evaluate the previous taxonomic treatments of the genus *Linum* in Iran, and to provide evidence for efficacy of application of combined micro-macro

morphologic data at sub–generic level of the genus.

MATERIALS AND METHODES

Plant Materials

The plants and pollen grains of sixteen *Linum* species were collected from summer 1999 to 2000 in Iran (Table 1). Voucher specimens were deposited in Central Herbarium of Iran (TARI).

Morphometry

In this study the same morphological characters has used as described by Sharifnia, 2002 (Table 2). The qualitative characters were coded as multi-state characters while the mean of quantitative characters were used for analysis (Sheidai et al., 2000).

Palynology

All pollen samples were prepared for SEM (LEO, Model: 440) and were coated with gold without any other treatments. Photographs were taken at the Faculty of Mechanics, Science and Research Branch, Islamic Azad University, Tehran, Iran using SEM[1]. The dimensions were based on measurements of mean of several pollen grains on each specimen. The list of pollen characters used for statistical analysis is given in Table 3.

Statistical Analysis

In order to group the species having morphological and palynological similarities cluster analysis using WARD method (Sheidai et al., 2000) and ordination of species based on the first two principal component axes (PCA) were performed and Euclidean distance was used as a dissimilarity coefficient matrices that were calculated between taxa for morphological and palynological data were plotted against each other (Rohlf, 1987). Variables were standardized (Mean=0, Variance=1) for numerical analysis (Sheidai et al., 2000).

Statistical analysis was accomplished using SPSS (Norusis, 1988) and NTSYS (Rohlf, 1987) soft wares.

RESULTS AND DISCUSSION

The study of pollen grains showed that *L. catharticum* and *L. peyronii* had the smallest and the largest pollen sizes respectively. The type of aperture was tricoplate among the species studies and all the species examined had clavate tectum ornamentation except for *L. bienne* and *L. usitatissimum* (Table 3). The later could be used in support of the theory of *L. bienne* being the ancestor of *L. usitatissimum* as suggested by Townsend and Guest (1980).

Clusters analysis of micro – macro morphological data revealed five main cluster at 7.2 linkage distance (Fig. 1) to which, the membership of different species was highly supported by ordination of species based on principal component analysis (Fig. 2).

The first cluster was comprised of a single isolated species *L. catharticum.* Sch isolation was highly in support of the placement of *L. catharticum* in the monotypic section *Cathartolinum* (Reichenb.) Planch. *L. catharticum* was not too distinct according to the groupings resulted by ordination of the species, but placement of *L. catharticum* adjacent to members of the species *Linastrum*, seen in both ordination and cluster analysis of the species studies, was in agreement with the results gained from anatomical studies of Persian *Linum* species (Sharifnia and Albouyeh, 2002).

The second cluster was composed of members of the section *Syllinum, L. nodiflorum, L. mucronatum, L. persicum,* and *L. album.* The results of cluster analysis for the section *Syllinum* not only showed agreement with the ordination of the species studies, but also represented 100% correctness for the previously proposed membership of these species to their corresponding section in Iran.

The third cluster was composed of three members of the section *Linum, L. peyronii, L. austriacum,* and *L. glaucum.* Other members of the section *Linum, L. bungei, L. nervosum,* were placed in the fourth section together with *L. densiflorum* from the monotypic section *Dasylinum* Planch., and two remaining species of the section *Linum, L. usitatissimum* and *L. bienne,* were placed in the fifth cluster together with the members of the section *Linastrum*. Placement of the allies of the section *Linum* in three clusters could be due to the composition of the section *Linum* of a more heterogeneous group of as expressed by Sharifnia and Albouyeh (2002). However, ordination of the species illustrated a more comprehensive grouping for the members of the section *Linum*, and proved to be more selective for grouping of the members of the section *Linastrum* than cluster analysis in proposed versus actual membership of

the species studied. Neither the cluster analysis, nor the ordination of species represented *L. densiflorum* as a distinct species. The later was against the results gained form anatomical studies of Persian *Linum* species (Sharifnia and Albouyeh, 2002), and the previous taxonomic treatments of the genus *Linum* in Iran, which needed to be reviewed more extensively with considering a variety of markers in biosystematics of the genus *Linum* Iran.

Close relationship of *L. bungei* and *L. nervosum* was apparent in both cluster analysis and ordination of the species studied, which could be used as an evidence for merging these species as suggested by Sharifnia & Assadi (2002). The same was also true for *L. corymbolosum* and *L. strictum*, and was again a need for a more extensive study in this regard.

As seen in cluster analysis and ordination of the species studied and as been supported by anatomical studies of the species, *L. tenuifoilum* tended to be placed more in section *Linastrum* than section *Linum*, which was a clue for placement of *L. tenuifolium* as treated in Flora Europe rather than placement in section *Linum* as treated in Flora Iranica.

In conclusion, although palynological data was not proved to be sufficient for application at sub-generic level for taxonomic treatments of the genus in Iran in previous studies (Sharifnia and Assadi, 2002), application of palynological data together with morphological data proved to be efficient to be applied at sub – generic level treatments of the genus *Linum* in Iran, as results gained from this study were highly in agreement with previously proposed taxonomic treatments for Persian *Linum* species.

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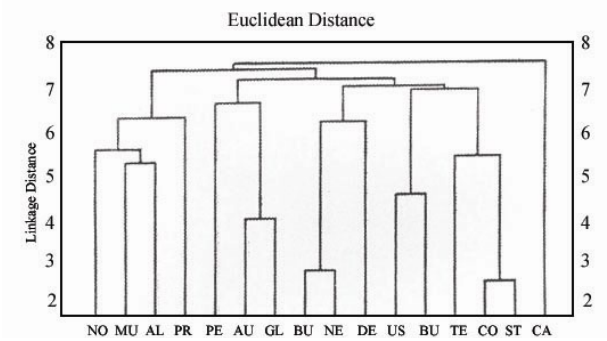

Fig. 1: cluster analysis of *Linum* species based on palynological and morphological data.
NO= *L. nodiflorum*, MU= *L. mucronatum*, AL= *L. album*, PR= *L. persicum*, PE= *L. peyronii*, AU= *L. austriacum*, GL= *L. glaucum*, BU= *L. bungei*, NE= *L. nervosum*, DE= *L. densiflorum*, US= *L. usitatissimum*, BI= *L. bienne*, TE= *L. tenuifolium*, CO= *L. corymbulosum*, ST= *L. strictum*, CA= *L. catharticum*

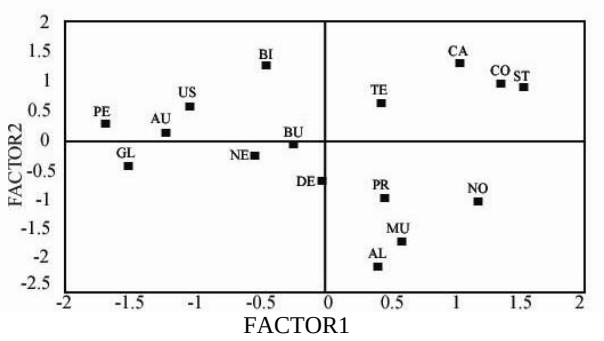

Fig. 2: ordination of *Linum* species based on palynological and morphological data.
NO= *L. nodiflorum*, MU= *L. mucronatum*, AL= *L. album*, PR= *L. persicum*, PE= *L. peyronii*, AU= *L. austriacum*, GL= *L. glaucum*, BU= *L. bungei*, NE= *L. nervosum*, DE= *L. densiflorum*, US= *L. usitatissimum*, BI= *L. bienne*, TE= *L. tenuifolium*, CO= *L. corymbulosum*, ST= *L. strictum*, CA= *L. catharticum*

Table 1: List of *Linum* species used in micro – macro morphological studies.

Taxon	Locality
L. catharticum L.	Tehran: NE. Tehran, Fasham, 2000m, Sharifnia 80080
L. strictum L.	Bushehr: Road of Taheri to Asalooyeh, 0m, Maassoumi and Abuhamzeh 51990.
L. tenuifolium L.	Azarbaijan: Arasbaran, Shabkhaneh, Asri and Hamzee.
L. corymbolusum Reichenb.	Khorasan: between maraveh tappeh and Bojnoord, 830m, Hever 3948.
L. album Ky. ex Boiss.	Tehran: NE. Tehran, Lashgarak, 1960m, Sharifnia 80081.
L. persicum Ky. ex Boiss.	Fars: Kharman kub, 2400-2650m, Mozaffarian 46886.
L. mucronatum Bertol. Spp. *mucronatum*	Kermanshah: Kuh – e Sefid, Protected region, 1500m, Fatahi and Hamzee 768.
L. nodiflorum L.	Kermenshah: Javanrood, 15km W. Javanrood, 1150m, Nemati and Roshanzadeh 3908.
L. densiflorum P.H. Davis.	Azarbaijan: Maku, Tikmeh, 2650m, 67947.
L. nervosum Waldst. & Kit.	Mazandaran: Siah Bisheh, 2300m, Sharifnia 80079.
L. bungei Boiss.	Mazandaran: 3km W. Javaherdeh, Ramsar. 2400-2600m, Rwnemark and Maassoumi, 20847.
L. glaucum Boiss. & Noe.	Kurdestan: E. Bijar, 1902m, Assadi and Shirdel poor 12258.
L. austriacum L.	Kermanshah: Hovaro mountain, 1850-1900m, Mirabdali and Heydari 2993.
L. peyronii Post.	Kermanshah: Gahvareh, 1600m, Nemati and Mirabdali 3551.
L. bienn Mill.	Khuzestan: Laly, 300m, Sharifnia 80082.
L. usitatissimum L.	Khuzestan: Shushtar, 250m, Sharifnia 80083.

Table 2: List of characters and related numerical codes used in morphological studies

No.	Character	Numerical code	No.	Character	Numerical code
1	Growth Period	0= annual, 1= perennial	19	Petal Length	mm
2	Plant Height	cm	20	Sepal length to petal length ratio	In No.
3	Leaf Arrangement	0= opposite, 1= alternate	21	Petal Color	0= white, 1= other
4	Leaf Variation	0= absence, 1= presence	22	Stigma Shape	0= non – thread form, 1= thread form
5	Leaf Shape	0= non – linear – lanceolate, 1= linear – lanceolate	23	Stigma Length	mm
6	Leaf Apex	0= non – acute, 1= acute	24	Anther Length	mm
7	Leaf Margin	0= smooth, 1= rough	25	Capsule Shape	0= almost spherical, 1= spherical
8	Leaf Length	mm	26	Capsule Length	mm
9	Leaf Width	mm	27	Capsule Diameter	mm
10	Inflorescence Length	cm	28	Sepal Length to Capsule Length ratio	In No.
11	Number of flowers	In No.	29	Seed Length	mm
12	Styles	0= homostyle, 1= heterostyled	30	Seed Width	mm
13	Sepal Shape	0= non – linear, 1= linear	31	Petiole Length	mm
14	Sepal Apex	0= non – acute, 1= acute	32	Stem Shape	0= non – lineate, 1= lineate
15	Sepal Margin	0= non – glandular, 1= glandular	33	Indumentum of the stem	0= glabrous, 1= pubescent
16	No. of Sepal Veins	In No.	34	Position of Sepals	0= free, 1= joint at the base
17	Sepal Length	mm	35	Stipule Glands of the Leaves	0= absence, 1= presence
18	Sepal Width	mm	36	Plant Color	0=green, 1= dark green

Table 3: Pollen characters used in palynological studies.

Species	Polar Axis (µm)	Equatorial diam. (µm)	P/E	۵۷ hape	Colpi Length (µm)	Ornamentation
L. catharticum	45	36	1.25	spherical	35	Clavate
L. strictum	55	43	1.27	Ellipsoid	48	Clavate
L. tenuifolium	50	39	1.28	Ellipsoid	44.5	Clavate
L. corymbolusum	49	55	0.89	Spheroidal	45	Clavate
L. album	48	40	1.2	Sub – spheroidal	43	Clavate
L. persicum	47	46	1.02	spheroidal	46	Clavate
L. mucronatum	62	43	1.44	Ellipsoid	50	Clavate
L. nodiflorum	54	40	1.35	Ellipsoid	40	Clavate
L. densiflorum	55	38	1.45	Ellipsoid	32	Clavate
L. nervosum	55	44	1.25	Sub -spheroidal	47	Clavate
L. bungei	55	43.5	1.26	Sub - spheroidal	49	Clavate
L. glaucum	64	56	1.14	Sub - spheroidal	53	Clavate
L. austriacum	62.5	54	1.15	Sub -spheroidal	53	Clavate
L. peryonii	65.5	45	1.21	Sub - spheroidal	47	Clavate
L. bienne	49	55	0.89	Spheroidal	35	F lat – topped Gemmae
L. usitatissimum	48	53	0.90	Spheroidal	33	F lat – topped Gemmae

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بررسی میکرو- ماکرو مورفولوژی جهت ارزیابی ارتباط تاکسونومیکی گونه‌های کتان در ایران

فریبا شریف‌نیا[۱]، مصطفی اسدی[۲]

۱- گروه زیست شناسی، دانشکده علوم پایه، دانشگاه آزاد اسلامی واحد تهران شمال.
۲- مؤسسه تحقیقات جنگلها و مراتع، تهران، ایران.

چکیده

تحقیق حاضر بر روی ۱۶ گونه کتان ایرانی با هدف نشان دادن ارتباط تاکسونومیکی بین گونه‌ای انجام گرفت. این ارزیابی شامل مطالعه همزمان مورفولوژی و گرده شناسی بر روی گونه‌های جنس مذکور می‌باشد. همچنین آنالیز چند متغیره برای نتیجه گیری از داده‌های این مطالعات انجام شد. بکارگیری اطلاعات مورفولوژی و گرده شناسی بطور قوی گروهبندی گونه‌های جنس کتان را تائید می‌کند و نشان داد به کارگیری با هم این دو نوع مطالعه کارایی لازم را در سطح تحت جنس کتان را دارد.

واژه‌های کلیدی: کتان ـ گرده‌شناسی ـ مورفولوژی ـ آنالیز چند متغیره ـ تاکسونومی ـ ایران