

Use of anatomical characteristics for taxonomical study of some Iranian *Linum* taxa

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Abstract

Linum is considered as the largest genus of Linaceae family, containing more than 180 species. The wide range of diversity within the genus *Linum* continues to challenge its taxonomical investigations. In present study anatomical features of vegetative organs, stem and leaf, of fourteen species, subspecies or varieties of three sections, were described with aim to improve the infrageneric classification of this genus. Plant samples were collected from natural populations of the studied taxa during 2011–2012. Embedded materials were used for microscopic investigation. Transverse hand sections of the lamina and stem were made from the middle part of fully-grown leaves and stems. Forty five qualitative and quantitative anatomical characteristics were examined in both stem and leaf. Principal Correspondence Analysis (PCA) of stem and leaf anatomical traits showed that some of these features were the most variable traits among the studied taxa. Analysis of variance showed significant differences for many of quantitative characteristics between taxons. A PCA biplot of anatomical features showed that some of the studied taxa had stable anatomical traits, which was confirmed by box and whisker plots. Cluster analysis of the studied taxa using the Unweighted Paired Group using Average method tree, as well as PCA and Principal Coordinate Analysis plots did not conform to the species classification in the traditional sections in references such as Flora Iranica and Flora of Iran and, with the exception of section *Syllinum*, the members of the other taxa did not occur together. Our results suggested that stem and leaf anatomical features were useful for infrageneric classification of the genus *Linum*.

Key words: anatomy, leaf, *Linum*, Iran, stem, taxonomy.

Abbreviations: ANOVA, analysis of variance; HSBU, herbarium of Shahid Beheshti University Tehran, Iran; PCA, principal correspondence analysis; PCoA, principal coordinate analysis; UPGMA, unweighted paired group using average method.

Introduction

Classification of plants has continuously been discussed among plant systematists and taxonomists. Plant taxa are classified and reclassified as soon as novel approaches appear and these processes have been a persistent practice throughout the history of plant taxonomy. Many plants are classified on the basis of external morphological structures, such as flowers and fruits. Morphological structures are not always available for observation, as they are produced seasonally. Other means of classification such as anatomical investigations (Davis, Heywood 1963) and molecular markers need to be involved (Sheidai et al. 2014a).

Different aspects of internal structure followed by anatomical features have been used in taxonomical studies for more than one hundred years (Radford et al. 1974). Many anatomical traits of some dicotyledonous families were summarized by Solereder (1908). Plant anatomy, the study of the internal structure of plants, has been a source of fascination and a field of scientific inquiry since the time of the earliest microscopists. Dickison (2000) said that the plant anatomy focuses on the various faces of internal

structures that can be seen with the light microscope, such as: the spatial arrangements of the dermal, ground, and vascular tissue systems within vegetative as well as reproductive organs, tissue and cell patterning and types in the mentioned systems; and the nature of individual specialized cell kinds. In general, plant anatomy combines the fields of morphology and cell biology; although, the borders between these sciences tend to be obscure.

Linum is considered as the largest genus of Linaceae family, containing over than 180 species growing in different temperate and subtropical regions of the world (Rogers 1982; Muir, Westcott 2003; McDill et al. 2009).

Various species of *Linum* have been used by man as a source of fiber (*Linum usitatissimum* L.), seed oils that are important components of paints, varnishes, and other products, as fodder for animals, and as ornamentals. Flax seed oils contain ω -3 fatty acids and its seed has become a popular 'health food' for human consumption. Moreover, potential anti-cancer compounds have been isolated from the seeds of several *Linum* species (Rogers 1982), and lignans such as podophyllotoxin and 6-methoxy podophyllotoxin, which have antiviral and anticancer properties, have been

identified in flowering aerial parts of some species such as *Linum album* (Schmidt et al. 2010; Sheidai et al. 2014b).

The infrageneric classification of the genus *Linum* using morphological, cytological (Chennaveeraiah, Joshi 1983; Muravenko et al. 2010), palynological (Talebi et al. 2012a), seed micromorphological (Talebi et al. 2012b) and biochemical characteristics is still controversial; this genus is usually divided into variable infrageneric groups, sometimes referred to as subgenera, sometimes as sections. However, none of the systems proposed can be regarded as satisfactory (Velasco, Goffman 2000).

The existence of wide range of diversity within the genus *Linum* continues to challenge its systematic classification. Ockendon and Walters (1968) subdivided the genus *Linum* into five sections: *Linum*, *Linastrum*, *Syllinum*, *Dasylinum* and *Cathartolinum* in Flora Europaea. According to the recently established molecular phylogeny of the Linaceae, the genus *Linum* has two main clusters, one mainly consisting of the sections *Linopsis* and *Syllinum* and the other containing section *Linum* (McDill et al. 2009).

Very few anatomical studies have been carried out on the species of this genus. The only existing study is the work of Sharifnia, Albouyeh (2002), who investigated anatomical structure of twelve Persian *Linum* species. In the present study anatomical features of vegetative organs, stem and leaf, of fourteen species, subspecies or varieties of three sections, were described for infrageneric classification of this genus. Literature survey showed that statistical analyses of anatomical characteristics of this genus have not been published previously.

Materials and methods

Plant material preparation

In this study 45 qualitative and quantitative anatomical

characteristics of stem and leaf of 84 randomly collected plants were described in 14 *Linum* species, subspecies or varieties of three sections. All of the studied specimens were at the same developmental stage. The specimens included: six taxa (e.g. *Linum nervosum* var. *nervosum*, *Linum nervosum* var. *bungei*, *Linum glaucum*, *Linum usitatissimum* var. *usitatissimum*, *Linum bienne* and *Linum austriacum*) from section *Linum*, five taxa (e.g. *Linum album*, *Linum mucronatum* subsp. *armenum*, *Linum mucronatum* subsp. *mucronatum*, *Linum mucronatum* subsp. *orientale* and *Linum mucronatum* subsp. *assyriacum*) from section *Syllinum*, and three taxa (e.g. *Linum strictum* var. *strictum*, *Linum strictum* var. *spicatum* and *Linum corymbulosum*) from section *Linastrum*.

Details of localities and herbarium voucher numbers are given in Table 1. For each taxon one population was chosen randomly and in each population two independent groups of three individuals each were selected from central as well as marginal parts of its habitat. One measurement were taken per each flowering stem for each trait. Taxa were identified based on the descriptions provided in Flora Iranica (Rechinger 1974) and Flora of Iran (Sharifnia, Assadi 2001). The vouchers were deposited in the herbarium of Shahid Beheshti University Tehran, Iran (HSBU).

Embedded materials were used for microscopic investigation. Transverse hand sections of the lamina and stem were made from the middle part of fully-grown leaves and stems using commercial razor blades. Embedded materials were prepared as follows: adult plant samples were fixed with FAA (formalin 5%: acetic acid 5% and 50% ethanol 90%) for 48 to 72 h, then dehydrated in a graded ethanol series and embedded in 70% ethanol. The sections were stained with methyl blue and carmine and mounted on the slides using Canada balsam. Forty five (ten qualitative and thirty five quantitative) anatomical characteristics were

Table 1. Locality and herbarium voucher numbers of the studied taxa

Taxa	Locality	Voucher number
Section <i>Linastrum</i> (Planch) H.Winkler		
<i>L. corymbulosum</i> Reichenb.	Guilan, Rodbar, Darestan Jungle, 654 m	HSBU2011127
<i>L. strictum</i> L. var. <i>spicatum</i> Pers.	Hormozgan, Bandar Abbas, Geno, 1700 m	HSBU2011193
<i>L. strictum</i> L. var. <i>strictum</i>	Hormozgan, Bandar Abbas, Geno, 1600 m	HSBU2011198
Section <i>Linum</i>		
<i>L. nervosum</i> Waldst & Kit. var. <i>nervosum</i>	Mazenderan, 90 km Karaj to Chalous, 2193 m	HSBU2011130
<i>L. bienne</i> Miller	Guilan, Rasht, Saravan Park, 150 m	HSBU2011128
<i>L. nervosum</i> Waldst & Kit. var. <i>bungei</i> (Boiss.) Sharifnia	Mazenderan, 90 km Karaj to Chalous, 2193 m	HSBU2011129
<i>L. usitatissimum</i> L. var. <i>usitatissimum</i>	Markazi, 20 km Saveh to Salafchegan, 1320 m	HSBU2011165
<i>L. austriacum</i> L.	Saveh to Hamedan, after Nobaran, 1761m	HSBU2011103
<i>L. glaucum</i> Boiss. & Nöe	Kurdistan, 25 km Baneh to Saghez, 1623 m	HSBU2011161
Section <i>Syllinum</i> Griseb		
<i>L. album</i> Ky.ex Boiss.	Kurdistan, Sanandaj to Kamyaran, 1329 m	HSBU2011114
<i>L. mucronatum</i> Bertol. subsp. <i>mucronatum</i>	Hamedan, Avaj, 2350 m	HSBU2011196
<i>L. mucronatum</i> subsp. <i>orientale</i> (Boiss.) P.H.Davis	Zanjan, 90 km Abhar to Zanjan, 1839 m	HSBU2011132
<i>L. mucronatum</i> subsp. <i>assyriacum</i> P.H.Davis	Khuzestan, Izeh, Atabaki Park 350 m	HSBU2011164
<i>L. mucronatum</i> subsp. <i>armenum</i> (Bordzil) P.H.Davis	Azerbaijan, Salmas, Ghoshchi, 1557 m	HSBU2011140

selected and examined in both stem and leaf. Details of the studied traits are given in Table 2.

Statistical analysis

The mean and standard deviation of the studied quantitative traits were determined. Data were standardized (mean = 0, variance = 1) for use in the multivariate analyses including Unweighted Paired Group with Average (UPGMA), Principal Coordinate Ordination (PCO) and Principal Coordinate Analysis (PCA) (Podani 2000). Box and whisker plots were used for demonstration of variation in anatomical features among the studied taxa. One-way Analysis of Variance (ANOVA) was employed to assess the significance of differences in quantitative anatomical features difference between individuals and taxa. NTSYS ver. 2 (Rohlf 1998) and SPSS ver. 9 softwares was used for statistical analyses.

Results

Stem anatomy

Twenty four qualitative and quantitative anatomical traits of the stem were investigated (Table 2). Stem shape in width cross-section was polygonal (e.g. *L. album* and *L. mucronatum* subsp. *mucronatum*), stellate (e.g. *L. corymbulosum* and *L. mucronatum* subsp. *orientale*), polygonal-stellate (e.g. *L. mucronatum* subsp. *assyriacum* and *L. mucronatum* subsp. *armenum*) or circular (in the rest). Stem surface was glabrous in taxa such as *L. album*, *L. mucronatum* subsp. *armenum*, *L. nervosum* var. *nervosum*, *L. nervosum* var. *bungei*, *L. glaucum*, *L. strictum* var. *strictum*, *L. strictum* var. *spicatum*, *L. austriacum* and *L. usitatissimum* var. *usitatissimum*.

Cortex sclerenchyma tissues were composed of fiber cells in the shape of joined rings (in *L. mucronatum* subsp. *armenum*, *L. nervosum* var. *nervosum*, *L. glaucum*, *L. strictum* var. *strictum*, *L. strictum* var. *spicatum* and *L. usitatissimum* var. *usitatissimum*) or separated groups (in the others). In some taxa such as *L. nervosum* var. *nervosum*, *L. nervosum* var. *bungei*, *L. strictum* var. *strictum*, *L. strictum* var. *spicatum*, *L. glaucum*, *L. mucronatum* subsp. *armenum* and *L. bienne*, stem pith was hollow, while in the other taxa it was present and consisted of different-shaped cells. In the PCA ordination of stem anatomical features, the second PCA axis (Fig. 1) was correlated with xylem features ($r > 0.87$) and fiber cell width ($r > 0.67$), indicating that these stem anatomical traits were the most variable characters among the studied taxa.

ANOVA showed significant difference ($p < 0.01$) for all quantitative anatomical characteristics, with the exception of number of epidermal cell layers. A PCA biplot (Fig. 2) of anatomical traits showed that some of the studied taxa had consistent anatomical traits. For example, width of epidermal cells and dimensions of fiber cells were diagnostic traits for *L. austriacum*, and dimensions of parenchyma as well as pith cells were important characteristics for *L.*

Table 2. Qualitative and quantitative anatomical traits of stem and leaf in the studied taxa

Code	Characteristic
Stem characteristics	
1	Epidermal layer no.
2	Epidermal cell width
3	Epidermal cell length
4	Parenchyma cell width
5	Parenchyma cell length
6	Status of fiber groups in cortex
7	Number of fiber cell in group in cortex
8	Number of fiber group
9	Pith cell width
10	Pith cell length
11	Absence/presence of pit cell
12	Pit / cortex ratio
13	Cortex fiber cell width
14	Cortex fiber cell length
15	Phloem tissue width
16	Xylem cell width
17	Xylem cell length
18	Xylem cell no in rows
19	Xylem tissue width
20	Xylem distance
21	Fiber cell width
22	Fiber cell length
23	Absence/ presence of trichomes
24	Stem shape in cross section
Leaf characteristics	
25	Epidermal cell width
26	Epidermal cell length
27	Palisade layer no.
28	Palisade width
29	Palisad length
30	Absence/presence of collenchyma
31	Collenchyma cell number
32	Collenchyma cell width
33	Collenchyma cell length
34	Parenchyma layer No.
35	Parenchyma cell width
36	Parenchyma cell length
37	Xylem width
38	Xylem length
39	Phloem width
40	Phloem length
41	Absence/ presence of trichomes
42	Absence/ presence of trichomes in midrib
43	Xylem length/ width ratio
44	Phloem length/ width ratio
45	Phloem length/ xylem length ratio

mucronatum subsp. *orientale*. This was confirmed by box and whisker plots (Fig. 3), which also showed that width of xylem and fiber cells were distinct features for *L. album*.

Table 3. Some important stem and leaf anatomical characteristics in the studied taxa (all measurement values are in $\mu\text{m} \pm \text{SE}$)

Taxa	No.	Stem shape	Epiderm width	Epiderm length	Parenchyma width	Parenchyma length	Pith cell width	Collenchyma width	Xylem cell width	Fiber width	Epiderm width	Epiderm length	Palisade width	Parenchyma width	Parenchyma length	Tri-cho-sade layer No.
<i>L. austriacum</i>	1	circular	2.14 ± 0.64	0.97 ± 0.17	1.44 ± 0.45	1.08 ± 0.14	3.08 ± 0.94	2.25 ± 0.46	2.21 ± 1.08	40.83 ± 2.42	3.93 ± 0.56	3.70 ± 1.44	4.34 ± 1.31	3.35 ± 1.13	3.19 ± 0.97	1
	2	circular	2.97 ± 0.17	1.39 ± 0.37	2.16 ± 0.22	1.03 ± 0.31	4.50 ± 1.79	3.52 ± 0.92	2.48 ± 0.67	39.00 ± 9.26	3.49 ± 0.85	3.43 ± 0.68	3.88 ± 0.63	3.30 ± 1.39	3.19 ± 0.80	1
<i>L. glaucum</i>	1	circular	1.29 ± 0.05	0.86 ± 0.12	0.97 ± 0.04	0.82 ± 0.10	1.55 ± 0.69	1.61 ± 0.39	1.09 ± 0.07	17.42 ± 5.41	3.16 ± 1.13	2.55 ± 0.49	3.14 ± 0.33	2.61 ± 0.41	2.47 ± 0.77	2
	2	circular	1.61 ± 0.54	0.83 ± 0.08	0.71 ± 0.04	0.64 ± 0.17	1.58 ± 0.30	1.19 ± 0.33	1.30 ± 0.41	13.50 ± 4.41	3.46 ± 0.68	3.11 ± 0.19	3.21 ± 0.58	2.91 ± 1.04	2.89 ± 1.00	2
<i>L. bienne</i>	1	circular	1.68 ± 0.15	1.67 ± 0.22	1.69 ± 0.66	1.54 ± 0.44	2.49 ± 0.76	2.02 ± 0.69	1.65 ± 0.36	18.31 ± 1.73	4.51 ± 1.01	2.91 ± 0.93	5.00 ± 0.75	3.05 ± 1.05	2.66 ± 1.16	1
	2	circular	2.27 ± 0.45	1.36 ± 0.20	1.42 ± 0.32	1.31 ± 0.34	2.46 ± 0.91	1.70 ± 0.50	1.70 ± 0.16	14.11 ± 5.14	3.64 ± 0.54	3.44 ± 0.41	4.65 ± 0.56	3.49 ± 0.47	2.47 ± 0.35	1
<i>L. nervosum</i>	1	circular	1.52 ± 0.17	1.22 ± 0.17	1.50 ± 0.44	1.08 ± 0.38	2.19 ± 1.07	1.80 ± 0.19	1.11 ± 0.25	20.14 ± 4.04	2.12 ± 0.2	1.89 ± 0.50	3.53 ± 0.81	2.06 ± 0.59	1.83 ± 0.62	2
var. <i>bungei</i>	2	circular	1.43 ± 0.31	1.51 ± 0.19	1.58 ± 0.46	1.41 ± 0.38	2.19 ± 0.87	2.25 ± 0.17	1.16 ± 0.46	19.29 ± 3.50	3.30 ± 0.92	2.91 ± 0.62	3.03 ± 0.83	1.87 ± 0.25	1.39 ± 0.37	2
<i>L.</i>	1	circular	2.03 ± 0.25	1.01 ± 0.20	1.39 ± 0.12	1.30 ± 0.21	3.47 ± 1.02	1.97 ± 0.45	1.75 ± 0.43	14.50 ± 3.12	3.25 ± 0.66	2.97 ± 0.17	3.80 ± 0.78	1.00 ± 0.17	0.94 ± 0.23	2
<i>usiatissimum</i>	2	circular	1.79 ± 0.42	1.19 ± 0.36	0.94 ± 0.04	0.83 ± 0.25	4.35 ± 1.20	1.86 ± 0.29	1.72 ± 0.44	11.64 ± 1.93	3.00 ± 0.84	2.72 ± 0.40	3.64 ± 0.36	1.11 ± 0.12	1.02 ± 0.21	2
var. <i>usiatissimum</i>																
<i>L. nervosum</i>	1	circular	1.53 ± 0.39	1.07 ± 0.18	1.38 ± 0.07	0.95 ± 0.26	1.72 ± 0.67	1.41 ± 0.44	1.26 ± 0.19	11.34 ± 5.34	2.81 ± 0.83	2.69 ± 0.71	3.40 ± 0.42	1.98 ± 0.14	1.57 ± 0.26	2
var. <i>nervosum</i>	2	circular	1.89 ± 0.45	1.53 ± 0.50	1.47 ± 0.50	1.39 ± 0.55	2.08 ± 0.70	1.41 ± 0.22	1.40 ± 0.34	12.01 ± 4.92	2.46 ± 0.63	2.15 ± 0.54	2.59 ± 0.40	1.90 ± 0.66	1.59 ± 0.15	2
<i>L. mucronatum</i>	1	polygonal-stellate	3.77 ± 1.16	3.05 ± 0.97	1.94 ± 0.41	1.83 ± 0.41	4.38 ± 1.50	1.08 ± 0.19	1.86 ± 0.17	27.03 ± 3.06	3.79 ± 0.56	3.61 ± 0.53	5.38 ± 0.64	1.70 ± 0.58	1.42 ± 0.68	1
subsp. <i>armenum</i>	2	polygonal-stellate	3.34 ± 1.32	2.94 ± 0.53	2.26 ± 0.13	2.11 ± 0.33	6.30 ± 1.99	1.50 ± 0.33	1.83 ± 0.46	23.67 ± 1.36	3.27 ± 0.86	3.03 ± 0.61	5.07 ± 0.88	1.42 ± 0.25	1.28 ± 0.34	1
<i>L. mucronatum</i>	1	polygonal-stellate	2.47 ± 0.46	2.19 ± 0.73	2.58 ± 0.66	2.46 ± 0.57	4.47 ± 1.06	1.52 ± 0.08	2.29 ± 0.71	19.90 ± 8.96	4.45 ± 0.57	4.40 ± 0.60	2.81 ± 0.70	1.33 ± 0.25	1.11 ± 0.20	1
subsp. <i>assyriacum</i>	2	polygonal-stellate	3.09 ± 0.30	2.54 ± 0.17	2.95 ± 1.77	2.66 ± 0.62	4.03 ± 0.83	1.39 ± 0.33	1.98 ± 0.56	21.75 ± 8.80	3.29 ± 1.12	3.17 ± 1.13	3.88 ± 0.46	1.22 ± 0.24	1.05 ± 0.21	1
<i>L. mucronatum</i>	1	polygonal	1.53 ± 0.34	1.30 ± 0.21	1.86 ± 0.70	1.42 ± 0.42	4.41 ± 1.66	2.00 ± 0.52	1.39 ± 0.54	18.58 ± 8.37	4.27 ± 1.06	3.59 ± 0.17	4.42 ± 0.35	1.61 ± 0.20	1.16 ± 0.30	2
subsp. <i>mucronatum</i>	2	polygonal	2.81 ± 0.62	2.31 ± 0.38	1.80 ± 0.67	1.58 ± 0.38	3.97 ± 1.10	2.08 ± 0.38	1.50 ± 0.54	33.06 ± 18.47	3.96 ± 0.61	3.44 ± 0.09	4.22 ± 0.93	1.91 ± 0.59	1.66 ± 0.14	2
<i>L. mucronatum</i>	1	stellate	2.57 ± 0.38	2.17 ± 0.11	1.92 ± 0.24	1.65 ± 0.59	4.89 ± 2.72	2.22 ± 0.29	0.97 ± 0.17	19.94 ± 6.45	4.23 ± 1.08	3.85 ± 1.13	5.21 ± 1.61	1.52 ± 0.26	1.30 ± 0.12	1
subsp. <i>orientale</i>	2	stellate	2.25 ± 0.68	1.72 ± 0.57	1.80 ± 0.31	1.50 ± 0.17	5.66 ± 2.35	1.53 ± 0.31	1.13 ± 0.39	22.22 ± 6.37	4.32 ± 1.16	4.16 ± 1.53	4.22 ± 0.92	3.02 ± 0.89	2.89 ± 0.84	1
<i>L. album</i>	1	polygonal	2.64 ± 0.24	2.05 ± 0.04	1.97 ± 0.29	1.87 ± 0.49	5.44 ± 1.50	1.20 ± 0.33	2.27 ± 0.60	20.00 ± 7.88	4.58 ± 0.91	4.53 ± 0.91	3.46 ± 0.18	2.30 ± 0.60	1.95 ± 0.58	1
	2	polygonal	1.91 ± 0.54	1.86 ± 0.31	2.89 ± 0.96	2.27 ± 0.29	4.72 ± 1.67	1.00 ± 0.08	3.08 ± 0.88	18.54 ± 12.83	4.36 ± 0.95	2.63 ± 0.41	4.00 ± 0.71	2.50 ± 0.25	2.19 ± 0.31	1
<i>L.</i>	1	stellate	2.74 ± 0.76	2.55 ± 0.54	2.25 ± 0.62	2.13 ± 0.58	3.91 ± 1.25	2.03 ± 0.45	1.91 ± 0.43	19.44 ± 4.86	4.44 ± 0.85	3.55 ± 0.55	5.44 ± 0.16	1.25 ± 0.08	1.22 ± 0.26	2
<i>corymbulosum</i>	2	stellate	3.13 ± 1.06	2.28 ± 0.76	2.61 ± 0.41	2.16 ± 0.71	4.68 ± 1.81	1.58 ± 0.08	1.60 ± 0.27	17.64 ± 7.04	4.03 ± 0.48	3.91 ± 0.60	5.17 ± 0.49	1.94 ± 0.56	1.74 ± 0.44	2
<i>L. strictum</i> var. <i>spicatum</i>	1	circular	2.11 ± 0.26	1.00 ± 0.38	0.86 ± 0.17	0.83 ± 0.08	2.80 ± 1.42	1.47 ± 0.38	1.16 ± 0.42	17.75 ± 8.74	2.15 ± 0.49	1.86 ± 0.58	3.00 ± 0.79	1.83 ± 0.57	1.72 ± 0.72	2
	2	circular	2.50 ± 0.71	1.47 ± 0.29	1.50 ± 0.42	1.19 ± 0.41	2.97 ± 1.08	1.47 ± 0.12	1.30 ± 0.43	15.58 ± 4.07	2.82 ± 0.79	2.41 ± 1.09	3.59 ± 0.44	1.64 ± 0.53	1.55 ± 0.21	2
<i>L. strictum</i> var. <i>strictum</i>	1	circular	1.44 ± 0.39	1.02 ± 0.17	1.39 ± 0.40	0.97 ± 0.17	1.83 ± 0.45	1.66 ± 0.66	0.92 ± 0.25	16.51 ± 4.84	2.00 ± 0.23	1.86 ± 0.32	4.05 ± 0.54	1.88 ± 0.60	1.89 ± 0.50	2
	2	circular	1.45 ± 0.33	1.05 ± 0.33	1.17 ± 0.24	1.08 ± 0.16	2.11 ± 0.92	1.78 ± 0.78	0.92 ± 0.24	19.73 ± 4.41	2.96 ± 0.76	2.78 ± 0.75	4.55 ± 0.35	1.64 ± 0.25	1.39 ± 0.25	2
<i>L. mucronatum</i>	1	polygonal	2.91 ± 0.80	2.40 ± 0.34	2.44 ± 0.17	1.58 ± 0.25	3.14 ± 1.07	1.47 ± 0.25	2.16 ± 0.33	23.88 ± 6.58	4.66 ± 0.52	3.97 ± 0.60	4.25 ± 0.92	2.28 ± 0.38	2.14 ± 0.46	2
subsp. <i>mucronatum</i>	2	polygonal	4.19 ± 0.56	3.97 ± 0.59	2.50 ± 0.58	2.25 ± 0.29	2.97 ± 0.54	1.86 ± 0.25	1.58 ± 0.14	25.44 ± 7.80	4.09 ± 0.86	3.72 ± 0.68	3.23 ± 0.99	2.03 ± 0.17	1.97 ± 0.63	2

strictum clustered together with *L. corymbulosum* separate from them. *Linum* section members were scattered in the UPGMA. For example, *L. austriacum* occurred separate from other members and even two varieties of *L. nervosum* were placed far from the others.

Discussion

Although, morphological traits are very important features for all taxonomical studies and are the basis for plant identification, in many cases these traits are not sufficient for plant classifications and use of other features like anatomical traits are necessary. Botanists (such as Edeoga, Okoli 1998; Edeoga, Eboka 2000; Edeoga, Ikem 2001; Güvenç, Duman 2010; Ranjbar et al. 2010; and Güvenç et al. 2011) believed that anatomical traits are not always as useful as morphological characters for plant identification; however, these features are well-established criteria and can offer significant assistance in plant taxonomy and biosystematical investigations of various taxa.

In this study, both stem and leaf anatomical characteristics were used for taxonomical investigation of the genus *Linum*. The general usefulness of leaf and stem anatomical studies in plant taxonomy were noted by Metcalfe and Chalk (1950). Although Sharifnia and Albouyeh (2002) studied leaf as well as stem anatomical traits of 12 species of *Linum* in Iran and obtained valuable data, our present study had three main differences with the previous work. We used infraspecific taxa, such as different varieties and subspecies in our study and conducted statistical analysis. We discussed anatomical traits at species, subspecies or even variety levels, which was not done in the previous study.

Twenty one characteristics of leaf were examined. Box

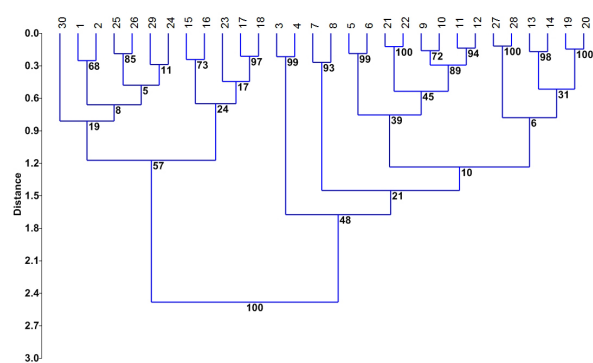


Fig. 4. Relative UPGMA tree of the studied taxa on the basis of anatomical traits. Abbreviations: 1 & 2: *L. mucronatum* subsp. *orientale*, 3 & 4: *L. austriacum*, 5 & 6: *L. bienne*, 7 & 8: *L. nervosum* var. *bungei*, 9 & 10: *L. strictum* var. *strictum*, 11 & 12: *L. strictum* var. *spicatum*, 13 & 14: *L. nervosum* var. *nervosum*, 15 & 16: *L. album*, 17 & 18: *L. mucronatum* subsp. *armenum*, 19 & 20: *L. usitatissimum* var. *usitatissimum*, 21 & 22: *L. glaucum*, 23 & 24: *L. mucronatum* subsp. *mucronatum*, 25 & 26: *L. mucronatum* subsp. *assyriacum*, 27 & 28: *L. corymbulosum*.

and whisker, and PCA ordinations showed that some of these traits had taxonomic importance. Our results showed that leaf epidermal characters (e.g. presence or absence of trichomes on the epidermal surfaces and cell dimensions) had taxonomic value in the genus *Linum*. Pilose indumentum was only seen on leaf surfaces of *L. strictum* var. *strictum*, *L. strictum* var. *spicatum* and *L. mucronatum* subsp. *mucronatum*. In addition, two varieties of *L. strictum* and *L. nervosum* var. *nervosum* had smaller leaf epidermal cells. Two varieties of *L. nervosum* are morphologically very similar (Sharifnia, Assadi 2001), but using leaf epidermal cell dimensions these varieties could easily be distinguished from each other.

Various studies have confirmed that foliar epidermis is one of the most noteworthy taxonomic traits from a biosystematics point of view, and taxonomical treatments of a number of families are made on the basis of this character (e.g. Bhatia 1984; Stace 1984; Jones 1986; Baranova 1972). In addition, features of foliar epidermis have been used previously to settle some taxonomic problems or contribute to increasing taxonomic data in genera such as *Apios* Fabr. and *Colchianthus* Benth. (Ren et al. 2007) and *Eugenia* L. of Myrtaceae (Van Wyk et al. 1982) or even in higher taxonomical level such as family, for example, in Ericaceae (Watson 1964).

Continuity and/ or discontinuity of traits are important feature that can be used to show relationships and differences between plant taxa within a genus or between families. For example, Wilkins and Sabanci (1990) showed that the length and width of epidermal cells are useful aids in distinguishing varieties with similar flowering dates in perennial rye grass. Furthermore, leaf epidermal characteristics are associated with ecological benefits for plants. Among the studied taxa, two varieties of *L. strictum*

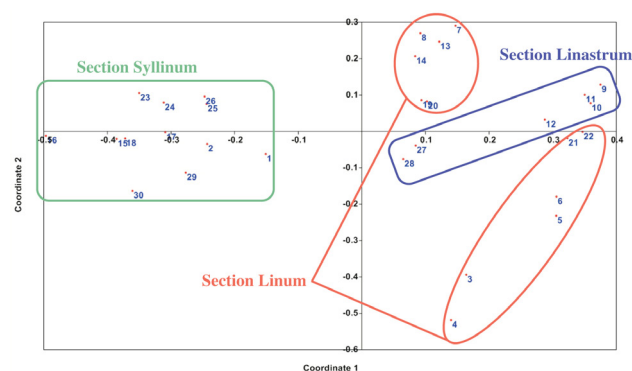


Fig. 5. Representative PCoA plot of anatomical data showing distinctness of the studied taxa. Abbreviations: 1 & 2: *L. mucronatum* subsp. *orientale*, 3 & 4: *L. austriacum*, 5 & 6: *L. bienne*, 7 & 8: *L. nervosum* var. *bungei*, 9 & 10: *L. strictum* var. *strictum*, 11 & 12: *L. strictum* var. *spicatum*, 13 & 14: *L. nervosum* var. *nervosum*, 15 & 16: *L. album*, 17 & 18: *L. mucronatum* subsp. *armenum*, 19 & 20: *L. usitatissimum* var. *usitatissimum*, 21 & 22: *L. glaucum*, 23 & 24: *L. mucronatum* subsp. *mucronatum*, 25 & 26: *L. mucronatum* subsp. *assyriacum*, 27 & 28: *L. corymbulosum*.

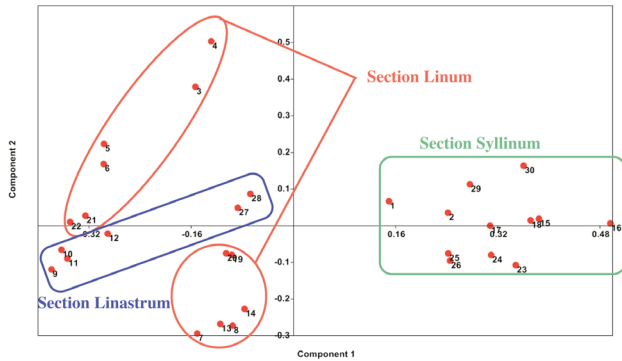


Fig. 6. Representative PCA plot of the studied taxa based on the anatomical features. Abbreviations: 1 & 2: *L. mucronatum* subsp. *orientale*, 3 & 4: *L. austriacum*, 5 & 6: *L. bienne*, 7 & 8: *L. nervosum* var. *bungei*, 9 & 10: *L. strictum* var. *strictum*, 11 & 12: *L. strictum* var. *spicatum*, 13 & 14: *L. nervosum* var. *nervosum*, 15 & 16: *L. album*, 17 & 18: *L. mucronatum* subsp. *armenum*, 19 & 20: *L. usitatissimum* var. *usitatissimum*, 21 & 22: *L. glaucum*, 23 & 24: *L. mucronatum* subsp. *mucronatum*, 25 & 26: *L. mucronatum* subsp. *assyriacum*, 27 & 28: *L. corymbulosum*.

grow in a xerophytic habitat, where epidermal trichomes are vital. Previous studies (for example see, Esau 1977; van der Merwe et al. 1994; Stenglein et al. 2005) indicated that some leaf anatomical characters as epidermal cells together with their waxy cuticles of varying thicknesses, trichomes as well as other indumentums serve a crucial role in regulating water loss as well as protecting leaf tissues from excessive sunlight and other suboptimal abiotic and biotic factors.

The obtained results suggested that, in addition to leaf epidermal features, other leaf traits have taxonomical importance and are useful in identification of species that are morphologically very similar. For example there has been much discussion about the taxonomic position of *L. glaucum* and various synonyms have been given for this species in different floras such as *L. austriacum* subsp. *glaucescens* (Boiss.) P.H. Davis in Flora of Turkey (Sharifnia, Assadi 2001). This species very similar to *L. austriacum* and the basic difference between these species is related to their basal leaf shape. Our results showed that dimensions and layer number of palisade and parenchyma cells in midrib region, phloem and xylem length/width ratio were distinguishing traits in identification of *L. glaucum* from *L. austriacum*. Leaf anatomical characters have been successfully applied in plant research (Kumar et al. 2012; Vasic, Dubak 2012). In addition, leaf features can be used in conjunction with supervised pattern recognition techniques for taxonomic classification, referring to techniques in which a priori knowledge about the category membership of samples is used (Roggo et al. 2003; Chen et al. 2009).

Twenty four anatomical characteristics of stem were examined in the studied taxa. Some of these features had

taxonomic value. Continuity or discontinuity of fiber cell groups, absence or presence of the pith cells as well as trichomes and also stem shape in cross-section view were important stem anatomical traits in classification of these taxa. For example, among the *L. mucronatum* subspecies, one taxon (subsp. *assyriacum*) had contiguous fiber cell groups, while an other subspecies had disconnected ones. In contrast to other subspecies, pith cells were absent in subsp. *armenum*. Stem anatomy features had taxonomic value in delimitation of related taxa. In some genera, for example *Genista* L. (Sanchez-Anta, Navarro-Andres 1985), species can be distinguished from others by variability of the presence and distribution of ribs. In addition to the variations in ribs, other stem characters, such as different arrangements of sclerenchyma, assimilatory tissue, and cortical vascular bundles, have taxonomic importance for distinguishing *Cytisus*, *Genista*, *Ulex* and related genera (Metcalf, Chalk 1950).

In the present study, the clustering of the studied taxa in the UPGMA tree, PCA and PCoA plots did not confirm species classification in the traditional sections, such as in the Flora Iranica (Rechinger 1974) and Flora of Iran (Sharifnia, Assadi 2001), with the exception of section *Syllinum*. Phylogenetic analyses based on chloroplast (*ndhF*, *trnL-F*, *trnK* 3' intron) and nuclear internal transcribed spacer showed that the section *Syllinum* is evidently monophyletic, which is supported also by morphological data (McDill et al. 2009). Moreover, the results of Sharifnia and Albouyeh (2002) showed that members of the section *Syllinum* had similar stem and leaf anatomical features, supporting their membership together under a separate section. However, members of the sections *Linastrum* were placed into different groups. Members of the section *Linum* formed three groups. McDill et al. (2009) stated that section *Linastrum* (= section *Linopsis*) is not monophyletic group and that it is either polyphyletic or paraphyletic. Furthermore in this section, stem anatomical characteristics showed some differences among the studied species (Sharifnia, Albouyeh 2002).

In section *Linum*, *L. austriacum* and *L. nervosum* var. *bungei* were dissimilar from other taxa, and thus the two varieties of *L. nervosum* did not cluster together. In addition, other taxa of this section did not cluster together and they formed separate sub-groups. There has been much discussion about diversity within the section *Linum*. Variations in anatomical traits suggested that the section *Linum* is a heterogeneous group (Sharifnia, Albouyeh 2002). Molecular investigations with phylogenetic markers confirmed that section *Linum* is paraphyletic (McDill et al. 2009). Moreover, taxonomical traits such as anatomy of seed coat as well as leaf ultrastructure have supported this idea (Moroz, Tsymbalyuk 2005; Optasyuk 2006; Svetlova, Yakovleva 2006). Talebi et al. (2012a), investigated palynological features of fifteen taxa of this genus, for which cluster analysis by UPGMA and PCA plots did

not show species classification in traditional sections of the Flora of Iran (Sharifnia, Assadi 2001) or Flora Iranica (Rechinger 1974). In their study, as shown by our results, *L. austriacum* is dissimilar from other members of the section, while *L. nervosum* var. *nervosum* and *L. usitatissimum* var. *usitatissimum* are similar. This was also confirmed by seed micromorphological study authenticated this matter (Talebi et al. 2012b).

To resolve the above problem Ockendon and Walters (1968) suggested to place the *Linum* species into groups, e.g. the *Linum* perenne group, as a preliminary classification until a revision of the genus was completed. The *L. perenne* group consists of *L. leonii*, *L. austriacum* and five subspecies of *L. perenne*. The study of Ockendon (1971) confirmed that, in addition to chromosome number, other taxonomical traits such as heterostyly, ecology, breeding system and pollen characters are more reliable than the quantitative morphological characters. Although Ockendon's (1968) investigations showed that the *L. perenne* group can be easily distinguished from other *Linum* species by use of morphological features, molecular study indicated that classification among the *L. perenne* group is still complicated (Vromans 2006).

Neither *L. perenne* nor *L. austriacum* form a specific group, even though *L. austriacum* is considered a member of *L. perenne* group (Diederichsen, 2007) and they have the same haploid karyotype number $x = 9$ (Nagao 1941; Gill 1987). Some taxonomists (e.g. Yuzepchuk 1949; Egorova 1996) divided section *Linum* into two independent sections: *Linum* containing *L. usitatissimum*, *L. bienne* ($2n = 30$) and *L. grandiflorum* ($2n = 16$) and the section *Adenolinum* comprising *L. austriacum*, *L. perenne* and *L. leonii* ($2n = 18$).

Conclusions

The results of this study suggested that stem and leaf anatomical features are useful for infrageneric classification of *Linum*. Most of the morphological characters used in the taxonomy of the genus *Linum* have proved to be taxonomically unreliable, because they vary almost continuously within this genus and show considerable phenotypic plasticity. Furthermore, heterostyly, which is widely present in the genus *Linum*, affects different features of plant such as nuclear genome size, morphology and palynological characters. For these reasons, other taxonomical traits, such as anatomy, need to be used for taxonomical treatments.

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