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Short Communication: New record of *Linum austriacum* var. *album* from Iran

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Abstract. *Talebi SM, Sheidai M, Noori M. 2016. Short Communication: New record of* Linum austriacum *var.* album *from Iran. Nusantara Bioscience 8: 174-179.* Different infraspecific taxa were described for *Linum austriacum* in various flora all over the world, while no verity/subspecies was recorded for this species in Iran. In the present study, for the first time, a new variety of the species, in the name of *L. austriacum* var. *album* were introduced for Iran. This variety was found in near Famenin city in Hamadan province. Comparison of morphological and phytochemical traits of var. *album* with its two nearest taxa, *Linum austriacum* var. *austriacum* and *L. glaucum*, showed that the main morphological difference of this variety with *L. austriacum* var. *austriacum* was the petal color, however most of morphological characteristics of var. *album* were bigger than var. *austriacum.* In addition, the kinds as well as amount of flavonoids differed between the studied taxa

Keywords: *L. austriacum* var. *album*, flavonoid, morphology, new record.

INTRODUCTION

The genus *Linum* of Linaceae family consists of over 180 species, some of these species are garden ornamentals (for example *Linum perenne* L.) because of their absorbing flowers, in addition some species, such as *L. usitatissimum* L., are used for nutrition, medicine, as source of linseed oil as well as fibers (McDill et al. 2009). *Linum austriacum* L. is one of these species, with deflexed fruiting pedicels and living generally in xeric habitats (Peruzzi 2011). Mohagheghzadeh et al. (2002) stated that *L. austriacum* is an herbaceous medicinal plant containing some important lignans such as aryl naphthalene, lignan and justicidin. These compounds show antifungal, antiprotozoal and also cytotoxic properties (Gertsch et al. 2003).

Linum austriacum is widely distributed all over the word, such as Europe, Russia, Crimea, Caucasus, Iran, Iraq, Afghanistan and Turkey (Yilmaz and Kaynak 2008). Because of the wide distribution, high morphological variations exist in different populations of this species, therefore there were many discussions about infraspecific variations in this species and different taxonomic ranks such as subspecies/variety were definite for it in different flora. For example, Davis (1967) recognized two subspecies, namely subsp. *austriacum* as well as subsp. *glaucescens*, in Flora of Turkey. Ascherson and Graebner (1914) identified three subspecies in this species: subsp. *squamulosum*, subsp. *austriacum* and subsp. *collinum*, but this member of *Linum perenne* group has two subspecies (subsp. *austriacum*, subsp. *collinum*) in Flora of Europe (Ockendon and Walters 1968; Ockendon 19681971). *L. austriacum* L. subsp. *marschallianum* (Juz.) Greuter & Burdet as well as *L. austriacum* subsp. *austriacum* are reported from Flora of Crime (Rescher et al. 2007). *L. austriacum* subsp. *tommasinii* (Rchb.) Greuter & Burdet was recorded from Istria. In addition, *L. austriacum* subsp. *euxinum* (Juz.) Ockendon and subsp. *mauritanicum* (Pommel.) Maire were identified in different flora. While in some flora, especially Flora Iranica (Rechinger 1974) and Flora of Iran (Sharifnia and Assadi 2001), no infraspecific taxon was reported of *L. austriacum*. However, in this paper we introduced one member of the genus *Linum* namely *L. austriacum* var. *album*. This variety was recently found in Iran and therefore is a new record for the country. Furthermore, its morphological and phytochemical characteristics were compared with its two Iranian closet taxa; *L. austriacum* var. *austriacum* and *L. glaucum.*

MATERIALS AND METHODS

In the present study, morphological as well as phytochemical features of three *Linum* taxa, *L. austriacum* var. *austriacum*, *L. austriacum* var. *album* and *L. glaucum*, were investigated. Plant samples were collected from natural populations during spring 2014. Plant specimens were identified on the bases of provided descriptions in valuable references such as Flora Iranica and Flora of Iran. Vouchers are deposited in the herbarium of Shahid Beheshti University, Tehran, Iran.

Morphological studies

Morphometric examinations were performed on one population of each taxon. In total fifteen qualitative and quantitative morphological traits were studied. From each

taxon fourteen to fifteen individuals were selected randomly and for each feature two measurements were taken per each flowering stem. The mean and standard deviations of quantitative features were determined.

Phytochemical investigations

For this study, 200 mg of powdered plant materials (petals) were boiled for 2 min in 5 ml of 70% ethanol. The obtained mixture was cooled and left to extract for 24 h. Then it was filtered and evaporated by rotary evaporation to dryness at 40°C. For 2-Dimensional Paper Chromatography (2-DPC) analyses, extracts taken up in 2 ml of 80% methanol. For flavonoids detection, nearly 20 µl of extracted material was placed on the corner of a Whatman No. 1 chromatography paper quarter sheet as a concentrated spot (10 applications of 2µl). The sample chromatogram was expanded for first direction in BAW (n-Buthanol-HOAc-H2O=4:1:5; V/V; upper layer), for second direction, HOAc (=15% aqueous acetic acid) with rutin (=quercetin 3-O-rutinoside) were used as a standard. At this stage, they were seen in long wave UV light (366 nm) and any dark absorbent as well as fluorescent stains were signed. RF -values in BAW and HOAc were computed.

Methods of identification of the flavonoids

The purified flavonoids were identified by means of UV spectroscopy using shift reagents to check the substitution flavonoids patterns (Mabry et al 1970; Markham 1982) and acid hydrolysis were used for identification the aglycone as well as sugar moieties. Co chromatography with standards was carried out where feasible. Apigenin, luteolin (Sigma), rutin (Merck), chrysin, isorhamnetin, kaempferol, morine, myricetin, narengenin, quercetin, rhamnetin, tricine and itexin (Fluka) were the existing flavonoid standards for measurement.

Acid hydrolysis and identification of flavonoid aglycones

Small quantities of each purified flavonoid (approximately 0.5 mg) were dissolved in 0.5 ml of methanol (80%) in a test tube, then 2 ml of HCl (2M) was added to it. The obtained blend was heated in the water bath at 100° C for thirty min. The dilution was chilled and 2 ml of Ethyl acetate was added. This mixture thoroughly blended with the aqueous layer using a whirly mixer. The upper layer of Ethyl acetate was removed by a pipette and was evaporated to aridity. The obtained materials dissolved in 0.5 ml of methanol and used as stains on thin layer chromatograms (cellulose). The plates of TLC were run in three alongside standards solvents to know the moiety of aglycone (Harborne 1998).

Statistical analysis

The mean and also standard deviation of the studied quantitative morphological traits were calculated. For grouping the studied taxa, data were standardized (mean = 0, variance = 1), then multivariate analyses such as UPGMA (Unweighted Paired Group using Average method) and Principal Coordinate Ordination (PCO) and Principal Coordinate Analysis (PCA) were performed (Podani 2000). Furthermore, one-way ANOVA (analyses of variances) test was used to assess the significant quantitative morphological variations between the studied taxa and also the correlations coefficient of Pearson was used to show significant correlations between quantitative morphological features. The used softwares for statistical analyses were MVSP ver. 2 (1998) and SPSS ver. 9 (1998).

RESULTS AD DISCUSSION

Habitat

In the present study, a natural population of *L. austriacum* var. *album* was recorded for the first time from the only known locality, near Famenin city (Hamadan province) at altitude of 1672, in the spring 2014, in Iran.

Plant description

Herbaceous perennial, stems divergent (Figure 1.A), 38-53 cm, bearing a many-flowered panicle of monochasial cymes (Figure 1.B). Leaves alternate, glaucous, basal linear or linear-oblong, acute, thick, 1-nerved, (5-) 9-15×1.5-4 mm(Figure 1.C), floral 6-12×1-1.5 mm, smooth or minutely scaly under the lens (rarely papillose). Pedicels deflexed or recurved in fruit, 10-25 mm. Flowers heterostylous, sepals prominently 3-5-nerved, 3.5-5×2-3 mm, outer oblong, subobtuse or acute, often mucronate, inner elliptical-orbicular, obtuse(Figure 1.D). Petals 20-24×13-17 mm, white (Figure 1.E). Stigmas oblong-capitate. Capsule 5-6 mm.

Comparison

In this section the morphological and phytochemical traits of the new recorded verity were compared with its two closet taxa. Our studies in different flora showed that two nearest taxa for var. *album* were present in flora of Iran, namely *L. austriacum* var. *austriacum* and *L. glaucum*. In comparison to *L. austriacum* var. *austriacum*, *L. austriacum* var. *album* had bigger morphological characters. In this variety all of the examined features with the exception of floral and basal leaf length were larger than var. *austriacum.* Although the distinct character for recognition of these varieties was the color of petal. The main difference between *L. glaucum* with these varieties was related basal leaf shape. In *L. glaucum* two different shapes of basal leaves were seen, while the mentioned varieties had similar leaves all over the stems. *L. glaucum* samples had longer leaves than *L. austriacum* var. *album*, but this verity had bigger sepals and also petals (Table 1).In addition, ANOVA analysis showed significant variation ($p \leq 0.05$) in the studied features with the exception of basal leaf width and floral leaf length (Table 2). Morphological UPGMA tree and PCA plot were used for clustering individuals of the studied taxa (Figures 2, 3, 4).In these diagrams individuals of *L. glaucum* placed separately, while members of *L. austriacum* var. *austriacum* and *L. austriacum* var. *album* were placed together.

In phytochemical study, the kind and also number of floral flavonoids were examined. The obtained results showed that total flavonoid numbers differed between the

studied taxa. In *L. austriacum* var. *austriacum* 10, *L. austriacum* var. *album* 8 and *L. glaucum* 6 types of flavonoids were recorded. Furthermore, kinds of flavonoid varied between them (Table 3).Some types of flavonoids, such as isorhamnetin, kaempferol and quercetin, were found in all of the studied taxa. These conditions did not hold true for other flavonoids. For example, rhamnetin was only observed in *L. austriacum* var. *austriacum* and chrysin were found in *L. austriacum* var. *album* as well as *L. glaucum.*

L. austriacum var. *austriacum* and *L. glaucum* had myricetin, while this compound wasn't found in *L. austriacum* var. *album.* The studied taxa were separated from each others in UPGMA tree of flavonoid data (Figure 5). In this diagram *L. austriacum* var. *album* and *L. glaucum* clustered together, while *L. austriacum* var. *austriacum* placed separately.

In the combined tree of phytochemical and also morphological data (Figures 6, 7), two varieties of *L. austriacum* grouped together, while *L. glaucum* clustered separately. This condition confirmed similarity of these varieties and proved recent findings about *L. glaucum.*

Discussion

In this paper, a new record of *L. austriacum* var. *album* was introduced for Iran, it was found in the western of it. The very interesting case was the co-existence of two varieties of this species in the same habitat. So, it seems that the sympatric speciation mechanism caused this condition. The most important morphological trait for recognition of these varieties was petal color. In addition, most of quantitative morphological traits differed between two varieties. Although, investigations showed that almost all of morphological characteristics such as stem, leaf and flower characters, seed size and pedicel position show some degrees of phenotypic plasticity (Ockendon 1971), co-existence of two mentioned varieties in the same habitat eliminated this idea. In addition, phytochemical data were very useful characteristics for identification var. *album* from *var. austriacum.* Not only quantities of flavonoid sulphate and also total flavonoid differed between them, but also, flavonoid type varied between these varieties. For instance, myricetin and rhamnetin were absent in var. *album*, while the situation was reversed for chrysin.

Figure 1. Images of some morphological characteristics.

Table 1. Comparison of morphological traits between the studied taxa (all values are in Cm.).

taxa		Stem height	Hetero-phyly	Branch	Basal leaf width	Basal leaf length	Floral leaf width	Floral leaf length	Sepal length	Sepal width	Petal length	Petal width	Flower color
L. austriacum var. *album*	Mean	44.8	Absence	2.93	2.42	5.53	1.40	6.59	0.43	0.27	2.14	1.6	White
	N	15		15	15	15	15	15	15	15	15	15	
	SD	4.99		4.06	0.82	1.33	0.42	1.38	0.52	0.37	1.12	1.02	
L. austriacum var. *austriacum*	Mean	42.5	Absence	0.14	2.00	6.39	1.26	8.48	0.42	0.25	1.78	1.47	Blue
	N	14		14	14	14	14	14	14	14	14	14	
	SD	3.58		0.36	0.19	1.60	.37	2.13	0.46	0.43	0.79	0.77	
L. glaucum	Mean	51.2	Present	8.26	2.10	7.55	0.95	10.00	0.23	0.2	1.8	1.34	Blue
	N	15		15	15	15	15	15	15	15	15	15	
	SD	9.88		3.26	0.57	1.95	0.17	0.00	0.48	0.25	0.48	0.24	

Table 2. ANOVA analyses between morphological features

		Sum of Squares	df	Mean Square	F	Sig.
Stem height	BG	588.499	2	294.249	6.399	.004
	WG	1885.229	41	45.981		
	Total	2473.727	43			
Basal leaf length	BG	126.971	2	63.486	6.394	.004
	WG	407.117	41	9.930		
	Total	534.088	43			
Basal leaf width	BG	1.462	2	.731	2.043	.143
	WG	14.669	41	.358		
	Total	16.132	43			
Floral leaf width	BG	1.569	2	.784	6.762	.003
	WG	4.756	41	.116		
	Total	6.325	43			
Floral leaf length	BG	11.160	2	5.580	2.296	.113
	WG	99.636	41	2.430		
	Total	110.796	43			
Sepal length	BG	37.972	2	18.986	77.145	.000
	WG	10.090	41	.246		
	Total	48.062	43			
Sepal width	BG	24.511	2	12.255	93.442	.000
	WG	5.377	41	.131		
	Total	29.888	43			
Petal length	BG	3270.224	2	1635.112	2.286E3	.000
	WG	29.325	41	.715		
	Total	3299.549	43			
Petal width	BG	1970.846	2	985.423	1.730E3	.000
	WG	23.360	41	.570		
	Total	1994.206	43			

Note: BG = Between Groups, WG = Within Groups

Table 3. Thin Layer Chromatography data of the studied taxa

Compound	*L. austriacum* var. *album*	*L. austriacum* var. *austriacum*	*L. glaucum*
Flavonoid sulphate	4	6	4
Flavon *C* & *C-/O* glycosides	4	4	2
Rhamnetin	-	+	-
Chrysin	+	-	+
Isorhamnetin	+	+	+
Kaempferol	+	+	+
Quercetin	+	+	+
Myricetin	-	+	+
Total flavonoid	8	10	6

Formerly, no infraspecific taxon, neither subspecies nor varieties, was identified for *L. austriacum* for Iran in differences flora such as Flora Iranica (Rechinger 1974) and Flora of Iran. Davis (1967) in Flora of Turkey (the neighboring country of Iran) recognized a subspecies for this species, in the name of *L. austriacum* subsp. *glaucescens*, while, Rechinger in Flora Iranica (1974) not accepted this subspecies and introduced it an independent species; *L. glaucum*. Although our phytochemical data did not confirm the above idea, the morphological characteristics and also combination of morphological and phytochemical characteristics confirmed that *L. glaucum* is the independent separated species.

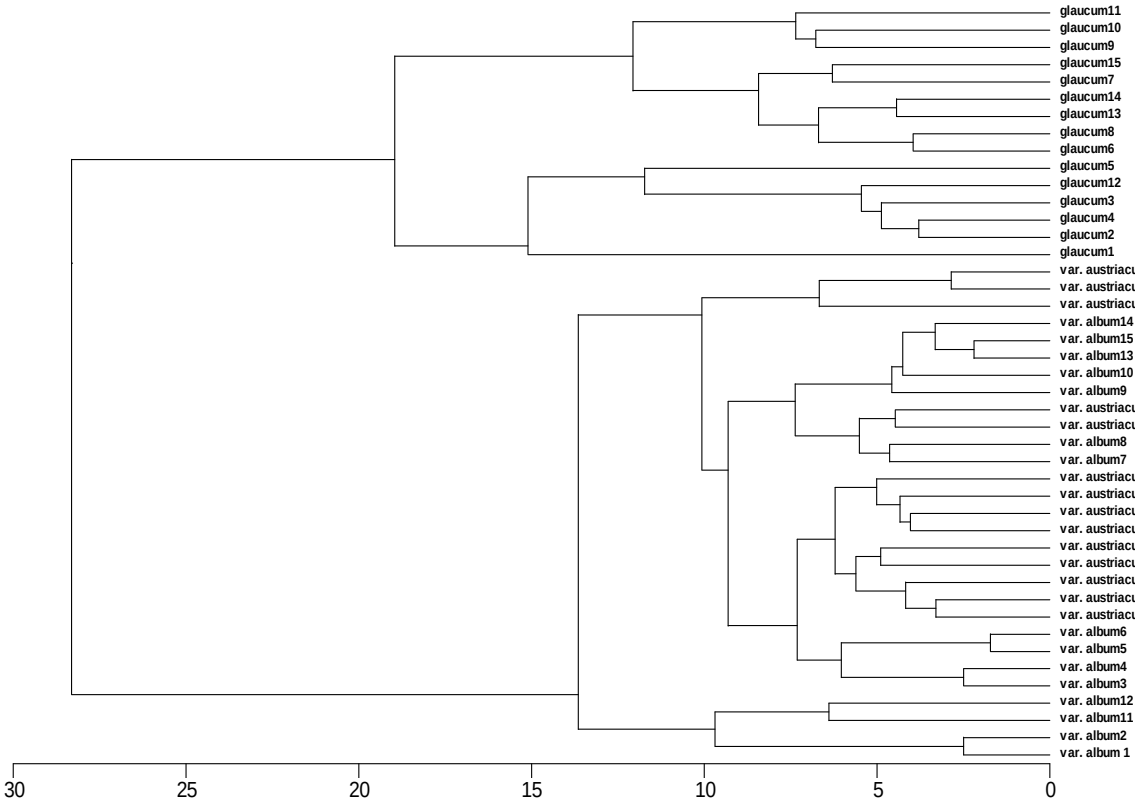

Figure 2. Morphological UPGMA tree of individuals of the studied taxa.

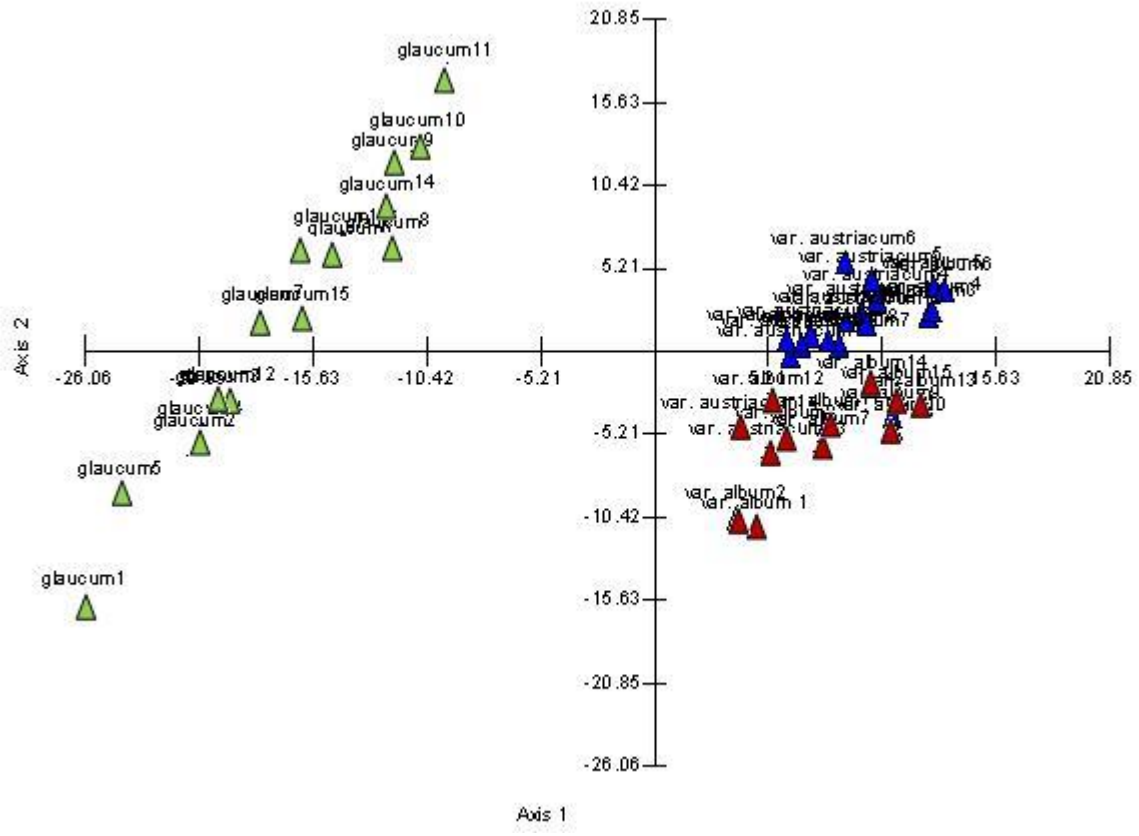

Figure 3. PCA plot of the studied taxa individuals on the bases of morphological traits.

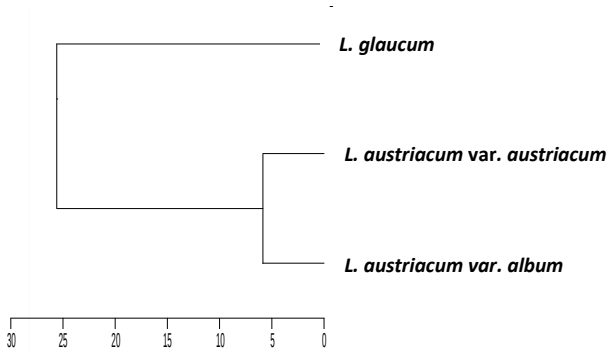

Figure 4. Morphological UPGMA tree of the studied taxa.

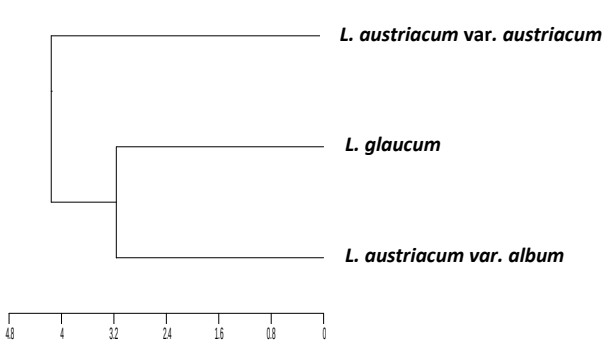

Figure 5. UPGMA tree of studied taxa on the bases of flavonoid data.

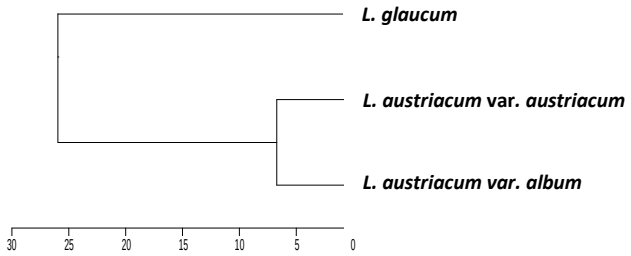

Figure 6. UPGMA tree of studied taxa on the bases of combined data.

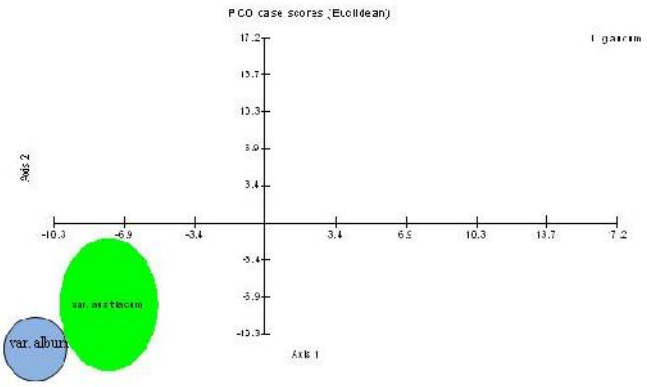

Figure 7. PCO plot of studied taxa on the bases of combined data.

Previous studies (for example, Sheidai et al. 2014; Afshar et al. 2015; Noormohammadi et al. 2015) confirmed that *L. austriacum* is variable specie. These investigations confirmed that high infraspecific morphological, cytological as well as genetic variations were existed between populations. Therefore it seems that classification of this species in Flora Iranica and Flora of Iran is not correct and different populations of this species should be rechecked.

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