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Bayesian and Multivariate Analyses of combined molecular and morphological data in *Linum austriacum* (Linaceae) populations: Evidence for infraspecific taxonomic groups

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Abstract. *Afshar F, Sheidai M, Talebi SM, Keshavarzi M. 2015. Bayesian and Multivariate Analyses of combined molecular and morphological data in* Linum austriacum *(Linaceae) populations: Evidence for infraspecific taxonomic groups. Biodiversitas 16: 179-187.* Plant specimens of *Linum austriacum* (Linaceae) were collected from 16 geographical populations of nine provinces in Iran and used for morphological and molecular (ISSR) analyses. Different multivariate and Bayesian methods were used to study interpopulations differences. Analysis of variance test and Principal coordinate analysis plots indicated morphological difference of the populations. Mantel test revealed positive significant correlation between morphological and geographical distance of these populations. Pearson' coefficient of correlation showed significant correlations between basal leaf length, width and length/width ratio with latitude and altitude of the studied populations. Bayesian analysis of combined molecular and morphological features revealed divergence of the studied populations and consensus tree showed separation of 6 populations in different clusters. Canonical Variate Analysis plot of these populations showed that Sang Sefid and Salmas populations differed greatly from the other populations. New ecotypes are suggested for these populations.

Keywords: Infraspecific variation, morphology, Iran, ISSR, population.

INTRODUCTION

The presence of intraspecific variation in organisms is a source of natural variation and is a way of response of organisms to their environment. Intraspecefic variation brings about biodiversity and is thought as the main origin and storage of speciation (Christine and Monica 1999; Hufford and Mazer 2003).

Many plant species have wide geographical distribution and face a wide range of climatic and edaphic conditions. Individuals of these species are able to give appropriate response to a tremendous variety of different conditions. These responses or adaptations change the descents of these individuals of a species, genotypically, phenotypically and physiologically and led to infraspecific diversity. Therefore, descents of these individuals tend to appear as new ecotypes, ecophenes, chemotypes, cytotypes and even subspecies (Christine and Monica 1999).

Habitat heterogeneity, combined with natural selection, often results in multiple, genetically distinct ecotypes within a single species. In addition, the populations of a given species facing different environmental conditions may undergo genetic changes to adapt to their local conditions (Linhart and Grant 1996; Hufford and Mazer 2003).

Infraspecific variation, difference between individuals or populations of a given species, is responsible for a relation of functionally relevant niche space occupation in biological communities (Albert et al. 2010; Fridley and Grime 2010; Violle et al. 2010). It is confirmed that plants occupy different space to forbear competitive interactions by means of variations in morphological and physiological characteristics. In addition, same plant species elude competition with each other and variegate their biological strategies by means of feature variation.

Linum L. genus (Linaceae) contains about 180 species that are source of fiber (Smeder and Liljedahl 1996), seed oils (Diederichsen and Raney 2006), and fodder (Bhathena et al. 2002). Some species are of medicinal value and contain Omega-3 fatty acids and potential anti-cancer compounds (Rogers 1982) as well as Lignans (Schmidt et al. 2010). *Linum* species grow in temperate and subtropical regions of the world (Rogers 1982; Muir and Westcott 2003). *Linum austriacum* L. is an herbaceous medicinal plant containing important lignans such as arylnaphthalene lignan and justicidin (Mohagheghzadeh et al. 2002), with antifungal, antiprotozoal, cytotoxic and piscicidal properties (Gertsch et al. 2003).

Although extensive biosystematics and phylogenetic studies have been carried out in the genus *Linum* (see for example, Velasco and Goffman 2000; Everaert et al. 2001; Sharifnia and Albouyeh 2002; Hemmati 2007; Rogers 2008; Schmidt et al. 2010; Soto-Cerda et al. 2011, Talebi et al. 2012a,b), little is known about the infraspecific diversity and taxonomic forms of wild *Linum* species (Sheidai et al. 2014b).

The neutral molecular data have been extensively used to study the speciation process (see for example, Cassel-Lundhagen et al. 2009; Pampoulie et al. 2011), the genetic diversity (Sheidai et al. 2012, 2013), the populations' genetic structure (Sheidai et al. 2012**,** 2014), and the genetic drift (Heather and Freeland 2011). In particular, ISSR (Inter simple Sequence Repeats) markers have been applied in genetic characterization and taxonomy studies on cultivated flax as well as on wild flax species.

Recently we reported inter-population genetic diversity in *L. austriacum* L. (Sheidai et al. 2014). Furthermore, we also encountered morphological variability among these geographical populations. The aim of present study was to illustrate if genetic variability of populations is associated with morphological variability and if combined effects of these variation lead to the formation of infra-specific taxonomic forms.

Therefore, in the present study randomly collected plants of *L. austriacum* from 16 geographical populations were studied from morphological and molecular (ISSR) points view. Bayesian and multivariate analyses were performed on combined data set obtained to identify potential infraspecific forms. The genetic data employed in this current manuscript has been entirely taken from the former Sheidai et al. (2014).

MATERIALS AND METHODS

Plant materials

Plant specimens of *L. austriacum* (Linaceae) were collected from 16 geographical populations of nine provinces in Iran (Figure 1) and used for morphological and molecular (ISSR) analyses. In order to acquire data of the studied populations, four plant samples were selected of each population.

Samples were identified based on the descriptions provided in accessible references such as Flora Iranica (Rechinger 1974) and Flora of Iran (Sharifnia and Assadi 2001). The voucher specimens were deposited in the herbarium of Shahid Beheshti University (HSBU), Iran (Table 1). Detail of DNA extraction and molecular study is provided in our previous report (Sheidai et al. 2014). In short, genomic DNA was extracted using CTAB activated charcoal protocol (Križman et al. 2006). Ten ISSR primers; (AGC) $_5$GT, (CA) $_7$GT, (AGC) $_5$GG, UBC810, (CA) $_7$AT, (GA) $_9$C, UBC807, UBC811, (GA) $_9$A and (GT) $_7$CA commercialized by UBC (the University of British Columbia) were used. PCR reactions were performed in a 25µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM $MgCl_2$; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of a single primer; 20 ng genomic DNA and 3 U of *Taq* DNA polymerase (Bioron, Germany). The amplifications, reactions were performed in Techne thermocycler (Germany) with the following program: 5 minutes initial denaturation step 94°C, 30 S at 94°C; 1 minutes at 50°C and 1 minute at 72°C. The reaction was completed by final extension step of 7 minutes at 72°C.

Figure 1. Distribution map of the studied populations in north-west Iran.

Table 1. Locality and herbarium voucher number of the studied populations.

Habitat address	Collector	Herbarium No.
Zanjan, Abhar, 1785 m	Talebi	HSBU2011133
Kurdistan, Sanandaj, Abidar Mountain, 1645 m	Talebi	HSBU2011112
East Azerbaijan, Ahar, 1593 m	Talebi	HSBU2011134
Guilan, Darestan Forest, 544 m	Talebi	HSBU2011126
Hamedan, Famenin, 1761m	Talebi	HSBU2011103
East Azerbaijan, Kalibar, 1460 m	Talebi	HSBU2011135
Kermanshah, Kangavar,1564 m	Talebi	HSBU2011116
Markazi, Nobaran, 1654 m	Talebi	HSBU2011102
Qazvin 1, Highway, 1476 m	Talebi	HSBU2011124
Qazvin 2, Cargo Terminal, 1205 m	Talebi	HSBU2011122
Hamedan, Razan,1898 m	Talebi	HSBU2011118
Guilan, Rudbar, 228 m	Talebi	HSBU2011125
Zanjan, Saeen Qaleh, 1805 m	Talebi	HSBU2011131
West Azerbaijan, Salmas, 1700 m	Talebi	HSBU2011138
Markazi, Arak, Sang Sefid, 2084 m	Talebi	HSBU2011151
East Azerbaijan, Tabriz, 1552 m	Talebi	HSBU2011136

Data analyses

In total thirty two, nine qualitative and twenty three quantitative, morphological characteristics were studied. Four plants were measured or scored of each population and for each characteristic one measurement was taken per each flowering stem. The used terminologies for qualitative morphological traits were on the basis of descriptive terminology provided by Stearn (1983). Qualitative characters were: petal color, basal leaf shape, floral leaf shape, apex, margin and base shape of floral and basal leaf blade. While, basal leaf width, basal leaf length, stem height, branch number, basal leaf length/width ratio, floral leaf width, floral leaf length, floral leaf length/width ratio,

calyx length, calyx width, calyx length/width ratio, sepal length, sepal width, sepal length/width ratio, capsule length, capsule width, capsule length/width ratio, stem diameter, leaf diameter, pedicle length, corolla length, corolla width and corolla length/width ratio were quantitative features.

Mean as well as standard deviation of the quantitative traits were determined. The analysis of variance (ANOVA) test was performed to show significant morphological difference between the studied populations. Principal coordinate analysis (PCoA) and Correspondence Analysis (PCA) were performed to group the plants specimens based on morphological characters. Morphological data were standardized (mean = 0, variance = 1) for these analyses (Podani 2000). Non-metric Multidimensional Scaling (MDS) and Canonical Variate Analysis (CVA), were used to illustrate populations' morphological distinctness.

For combined morphological and molecular analyses, the characters were coded as binary and multistate characters. Grouping of the populations was done by two methods. First we carried out structure analysis (Pritchard et al. 2000). For this analysis, data were scored as dominant markers (Falush et al. 2007). Second, we performed K-Means clustering as done in GenoDive ver. 2. (2013).

In order to identify the populations that are genetically and morphologically differentiated from the others, a consensus tree was constructed from morphological and genetic obtained trees. The Mantel test was performed to check correlation between geographical, morphological and genetic distances of the populations (Podani 2000). PAST ver. 2.17 (Hamer et al. 2012), DARwin ver. 5 (2012) programs were used for these analyses.

RESULTS AND DISCUSSION

Morphological analysis

In present study thirty two quantitative and qualitative morphological features of the both vegetative as well reproductive organs were investigated (Table 2). Among the studied qualitative traits, the shapes of blade apex, margin and also base of basal and floral leaves were stable among the studied sample, and were in the shapes of acute, entire and cuneate respectively, furthermore the petal color was invariable inter-populations and presented as blue. While other characteristics such as floral and basal leaf shape varied between populations and were recorded in the shapes of linear, lanceolate or linear-lanceolate.

In addition, quantitative traits differed between populations and the performed ANOVA test for these characters showed significant difference ($p = 0.05$) for all the studied traits with the exception of basal and floral leaf length/width ratio, calyx length/width ratio, capsule length as well as capsule length/width ratio among the studied populations (Table 3). Moreover, PCoA plots of all morphological characters separated these populations from each other (Figure2). These results indicated that the studied populations differed significantly in their morphological characters.

PCA analysis of morphological features revealed that the first three PCA components comprised about 73% of total variability of the studied populations. In the first PCA axis with about 35% of total variation, morphological traits like sepal length and basal leaf diameter possessed the highest correlation ($r > 0.90$) while in the second PCA axis, characters like stem diameter, sepal length/width ratio, and pedicel length, possessed the highest correlation ($r > 0.80$). Therefore, these morphological characters were the most variable morphological characteristics among the studied populations. The obtained data showed that characteristics such as floral leaf width as well as corolla width had lowest correlation, this mean that the mentioned traits were the most stable morphological features between the studied samples.

PCA biplot (Figure 3) revealed that morphological characters of sepal length and basal leaf diameter separated Famenin population from the other populations, while morphological characters like stem diameter, sepal length/width ratio, and pedicel length separated Sang Sefid population.

Pearson's coefficient of correlation determined between morphological characters and geographic traits of the studied populations (longitude, latitude and altitude) produced significant positive correlations between basal leaf length and latitude, and between basal leaf width and basal leaf length/width ratio with altitude.

The Mantel test performed between morphological distance and geographical distance of the studied populations produced significant correlation ($P = 0.01$, Figure 4), indicating that with an increase in geographical distance of these populations they showed a higher magnitude of morphological difference.

Combined molecular and morphological results

A combined data matrix of 44×91 was formed from ISSR and morphological data and used for further analyses. MDS plot of combined data is presented in Figure 5. Almost complete separation of the studied population indicated genetic and morphological distinctness of these populations. STRUCTURE plot of the combined data that is based on Bayesian method is presented in Figure 6. Although it showed some degree of similarity between Abidar and Darestan populations (populations numbers 2 and 4, respectively), and Rudbar and Salmas populations (populations numbers 12 and 14, respectively), complete difference was observed among the other studied populations. This indicates populations' divergence in both genetic and morphological features.

Delta K results of Evanno test that is based on STRUCTURE analysis is presented in Figure 7. It produced K value of 13 as the optimum K number and indicated that the studied populations were placed in 13 different groups. Therefore *L. austriacum* populations are highly stratified with regard to their morphological and genetic features.

Table 2. The most important morphological traits of the studied populations (all values were in cm).

Population		Stem high	Branch no.	Basal leaf shape	Basal leaf length	Basal leaf width	Floral leaf shape	Floral leaf length	Floral leaf width	Calyx length	Calyx width	Pedicle length	Sepal length	Sepal width	Corolla length	Corolla width
Abhar	Mean	43.87	3.00	Linear	1.52	.10	Lanceolate	.82	.09	.38	.37	.10	.12	.16	0.74	0.74
	N	4	4		4	4		4	4	4	4	4	4	4	4	4
	SD	8.74	.81		.25	.01		.14	.015	.02	.04	.005	.05	.02	4.98	4.99
Abidar	Mean	50.05	2.50	Lanceolate	1.90	.11	Linear	.97	.10	.38	.34	.08	.10	.19	1.05	.80
	N	4	4		4	4		4	4	4	4	4	4	4	4	4
	SD	3.24	.57		.21	.01		.12	.00	.04	.05	.01	.01	.01	.05	.08
Ahar	Mean	57.25	6.75	Linear	1.50	.197	Linear	1.05	.10	.40	.40	.12	.10	.23	1.47	1.20
	N	4	4		4	4		4	4	4	4	4	4	4	4	4
	SD	2.25	1.25		.32	.00		.10	.00	.01	.00	.05	.01	.04	.35	.08
Darestan	Mean	33.50	2.25	Linear-	1.22	.10	Lanceolate	.85	.07	.40	.38	.08	.16	.20	1.32	.98
	N	4	4	Lanceolate	4	4		4	4	4	4	4	4	4	4	4
	SD	6.28	.50		.17	.00	Lanceolate	.17	.02	.00	.02	.01	.03	.00	.17	.19
Famenin	Mean	38.30	4.50	Lanceolate	1.73	.30		1.05	.14	.37	.40	.10	.15	.25	1.22	1.10
	N	4	4		4	4		4	4	4	4	4	4	4	4	4
	SD	6.27	.57		.67	.08		.129	.04	.06	.00	.00	.05	.05	.22	.20
Kalibar	Mean	54.25	10.25	Linear	1.45	.16	Lanceolate	.67	.08	.31	.30	.05	.16	.18	1.20	.82
	N	4	4		4	4		4	4	4	4	4	4	4	4	4
	SD	8.14	3.30		.36	.03		.09	.01	.03	.00	.01	.04	.00	.14	.09
Kangavar	Mean	54.75	6.00	Lanceolate	1.55	.17	Lanceolate	.87	.08	.40	.39	.10	.10	.21	1.41	.96
	N	4	4		4	4		4	4	4	4	4	4	4	4	4
	SD	4.34	2.44		.19	.04		.09	.02	.00	.00	.00	.00	.02	.46	.30
Nobaran	Mean	31.87	12.75	Linear	1.02	.07	Lanceolate	.80	.08	.32	.37	.06	.10	.18	1.20	.90
	N	4	4		4	4		4	4	4	4	4	4	4	4	4
	SD	8.29	7.41		.20	.04		.20	.01	.05	.05	.02	.00	.04	.18	.15
Qazvin1	Mean	44.72	9.99	Linear	1.12	.12	Lanceolate	.72	.09	.40	.40	.06	.20	.22	1.53	1.17
	N	4	4		4	4		4	4	4	4	4	4	4	4	4
	SD	6.28	.00		.26	.02		.09	.02	.00	.00	.01	.08	.05	.19	.27
Qazvin2	Mean	56.00	3.25	Linear-	2.10	.20	Linear	1.00	.12	.35	.33	.09	.12	.19	1.35	.82
	N	4	4	Lanceolate	4	4		4	4	4	4	4	4	4	4	4
	SD	8.75	1.25		.11	.04		.08	.02	.05	.04	.01	.05	.01	.129	.20
Razan	Mean	62.12	7.00	Lanceolate	1.87	.23	Linear	.90	.10	.38	.37	.10	.10	.23	1.60	1.10
	N	4	4		4	4		4	4	4	4	4	4	4	4	4
	SD	4.40	3.55		.34	.04		.29	.00	.03	.05	.00	.00	.02	.34	.24
Rudbar	Mean	53.62	7.00	Linear	1.15	.14	Lanceolate	.82	.09	.43	.42	.10	.20	.26	1.25	1.05
	N	4	4		4	4		4	4	4	4	4	4	4	4	4
	SD	3.22	1.63		.12	.04		.09	.01	.04	.05	.00	.00	.02	.12	.09
Saeen	Mean	49.37	4.50	Lanceolate	1.67	.18	Lanceolate	1.12	.12	.46	.49	.10	.13	.20	2.15	1.15
Qaleh	N	4	4		4	4		4	4	4	4	4	4	4	4	4
	SD	5.02	1.29		.30	.08		.35	.04	.04	.02	.00	.04	.00	.12	.05
Salmas	Mean	58.00	17.50	Linear	1.67	.21	Linear	.75	.09	.40	.38	.09	.13	.20	1.40	1.05
	N	4	4		4	4		4	4	4	4	4	4	4	4	4
	SD	9.09	4.50		.35	.06		.12	.02	.00	.02	.01	.04	.00	.35	.19
Sang	Mean	62.25	6.75	Lanceolate	1.27	.15	Linear	.72	.08	.30	.35	.05	.15	.23	1.09	.91
Sefid	N	4	4		4	4		4	4	4	4	4	4	4	4	4
	SD	7.07	1.50		.22	.05		.15	.00	.08	.05	.00	.05	.04	.08	.09
Tabriz	Mean	63.62	5.00	Linear	1.30	.12	Lanceolate	.90	.10	.38	.47	.27	.42	.26	1.10	1.05
	N	4	4		4	4		4	4	4	4	4	4	4	4	4
	SD	4.71	.81		.29	.02		.08	.05	.04	.12	.20	.04	.11	.00	.00

Finally, in order to identify those populations that are differentiated in both morphological and genetic tree, a consensus tree was obtained which is presented in Figure 8. It revealed that 6 populations, Sang Sefid, Salmas, Darestan, Qazvin 1, Abhar, and Razan were separated from the others and were differentiated in both morphological and NJ trees. Therefore, although all studied populations differed in their morphological and genetic content as indicated by STRUCTURE plot and MDS plot of combined data, only these 6 populations were placed in a similar position on the constructed tree in both analyses and formed a distinct cluster.

Detailed analysis of morphological characters in these 6 populations revealed that Sang Sefid population possessed highest value of stem diameter, pedicel length, sepal length and sepal length/width ratio. Similarly, Salmas population contained the largest basal leaf length and largest floral leaf length. It had the shortest calyx leaf length, shortest corolla

width, shortest sepal length, and the lowest corolla length/width ratio.

These two populations with maximum number of morphological characters which were significantly different from the other studied populations.

Qazvin 1 population had the shortest calyx width and the lowest pedicel length, while Abhar population had the highest floral leaf length, the shortest sepal length and the lowest pedicel length. Razan population had the longest calyx length. This suggestion is supported by CVA plot of combined data in these 6 populations (Figure 9). These Figures showed that Sang Sefid and Salmas populations were placed far from the other 4 populations and took position in different corners of this plot. The other 4 populations were placed close to each other. Therefore Sang Sefid and Salmas populations are much more differentiated from the other studied populations. These populations may be considered as different ecotypes.

Table 3. ANOVA test of quantitative morphological features of the studied populations

Characteristics		Sum of Squares	df	Mean Square	F	Sig.
Stem height	BG	5923.677	15	394.912	9.706	.000
	WG	1953.062	48	40.689		
	Total	7876.740	63			
Basal leaf length	BG	5.754	15	.384	4.094	.000
	WG	4.497	48	.094		
	Total	10.251	63			
Basal leaf width	BG	.205	15	.014	6.357	.000
	WG	.103	48	.002		
	Total	.308	63			
Floral leaf length	BG	1.050	15	.070	2.589	.006
	WG	1.299	48	.027		
	Total	2.349	63			
Floral leaf width	BG	.021	15	.001	2.154	.023
	WG	.031	48	.001		
	Total	.053	63			
Calyx length	BG	.103	15	.007	4.194	.000
	WG	.079	48	.002		
	Total	.182	63			
Calyx width	BG	.139	15	.009	4.168	.000
	WG	.106	48	.002		
	Total	.245	63			
Pedicle length	BG	.156	15	.010	3.587	.000
	WG	.139	48	.003		
	Total	.295	63			
Sepal length	BG	.364	15	.024	14.042	.000
	WG	.083	48	.002		
	Total	.447	63			
Sepal width	BG	.056	15	.004	2.220	.019
	WG	.080	48	.002		
	Total	.136	63			
Capsule length	BG	.127	12	.011	1.967	.061
	WG	.183	34	.005		
	Total	.310	46			
Capsule width	BG	.203	12	.017	2.569	.015
	WG	.224	34	.007		
	Total	.427	46			

Note: BG = Between Groups, WT = Within Groups

Discussion

Ellison et al. (2004), stated that geographic variations in morphological characters of plants is a function of changes in phenotypic characters in response to local ecological conditions, variations in genetic structure as well as evolution between populations, and the biogeographic history of an individual species.

Although some qualitative morphological features such as petal color as well as leaf apex, margin and base fixed among the studied populations, but floral and basal leaf shape varied between the studied populations, so different shapes of leaves were recorded. Different studies showed that morphological characteristics such as leaf shapes are constrained genetically, but they also can be affected greatly by the local environment in which they develop (Thompson 1991; Schlichting and Pigliucci 1998).

In addition, most of quantitative morphological traits varied between populations and ANOVA test as well as PCA analysis of morphological traits confirmed these conditions. Some of these variations had either taxonomical or ecological (adaptive) importance. For example, foliar features were used in identification keys of this genus in different references such as Flora Iranica (Rechinger 1974) and Flora of Iran (Sharifnia and Assadi 2001). Furthermore, PCA biplot revealed that some quantitative feature had diagnostic value and were useful in identification of populations.

The obtained results of Mantel test confirmed that, in the studied *L. austriacum* populations, morphological distance (difference) of these populations was not correlated to their genetic distance, but morphological distance was positively correlated with their geographical distance. It showed that morphological changes of these populations were not merely under influence of genetic difference and other factors along with genetic variation affect morphological differences.

Moreover, some of the morphological characters like basal leaf length, basal leaf width and basal leaf length/width ratio were correlated to latitude and altitude. Because results of this study showed that populations that grow in higher latitude possess larger basal leaf length, while populations that grow in eastern parts of the country have larger basal leaf width, and bigger basal leaf length/width ratio.

Since the ecological and environmental conditions varied between population's habitats, it might be possible that floristical composition of neighboring plants of *L. austriacum* samples, ecological factors, pollinator species as well as nature of dominant species differed between populations, these conditions were seen in *Linum album* populations (Talebi et al. 2014a). In order to adaptation to these conditions, different morphological traits of *L. austriacum* adapted to ecological factors of habitat, therefore morphological variations occurred between populations. Infraspecific studies on various species of this genus or other genera such as *Linum album* (Talebi et al. 2014 a), *Linum glaucum* (Talebi et al. 2015) as well as *Stachys inflata* (Talebi et al. 2014b) confirmed these findings.

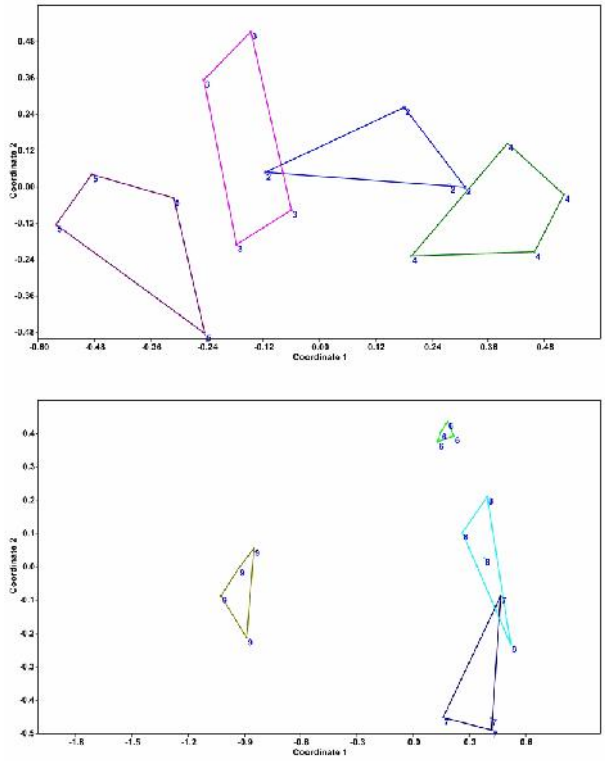

Figure 2. Representative PCoA plots of morphological data showing distinctness of the studied populations. Note: 1. Abhar, 2. Abidar, 3. Ahar, 4. Darestan, 5. Famenin, 6. Kalibar, 7. Kangavar, 8. Nobaran, 9. Qazvin 1, 10. Qazvin 2, 11. Razan, 12. Rudbar, 13. Saeen Qaleh, 14. Salmas, 15. Sang Sefid, 16. Tabriz.

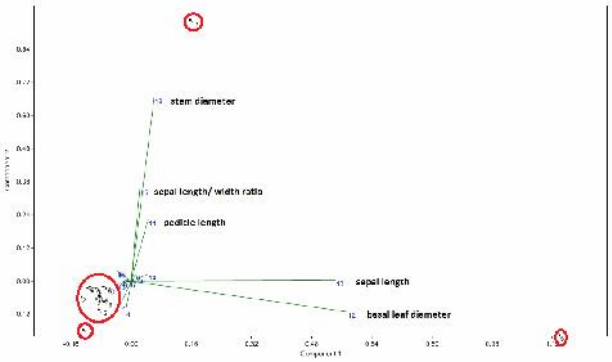

Figure 3. PCA biplot of populations based on the morphological characters. Note: 1. Abhar, 2. Abidar, 3. Ahar, 4. Darestan, 5. Famenin, 6. Kalibar, 7. Kangavar, 8. Nobaran, 9. Qazvin 1, 10. Qazvin 2, 11. Razan, 12. Rudbar, 13. Saeen Qaleh, 14. Salmas, 15. Sang Sefid, 16. Tabriz.

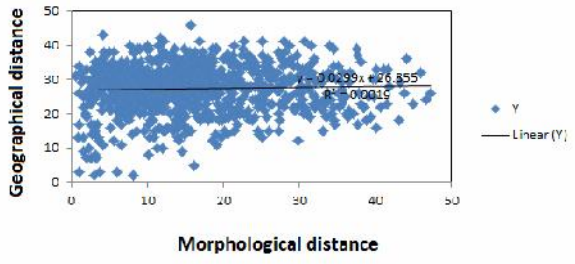

Figure 4. Mantel test result between morphological and geographical distance of the studied populations.

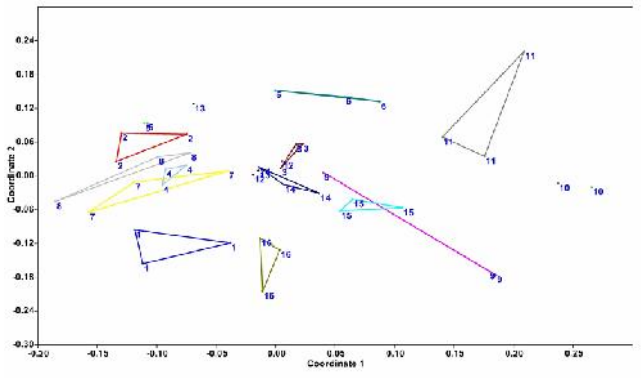

Figure 5. MDS plot of *L. austriacum* populations based on combined morphological and molecular data. Note: 1. Abhar, 2. Abidar, 3. Ahar, 4. Darestan, 5. Famenin, 6. Kalibar, 7. Kangavar, 8. Nobaran, 9. Qazvin 1, 10. Qazvin 2, 11. Razan, 12. Rudbar, 13. Saeen Qaleh, 14. Salmas, 15. Sang Sefid, 16. Tabriz.

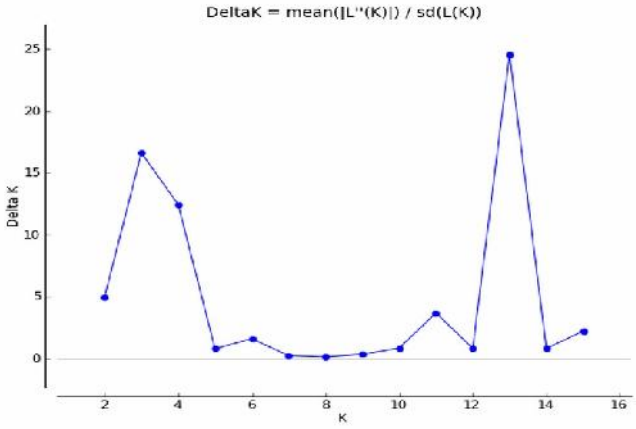

Figure 7. Delta K results of Evanno test based on the combined data.

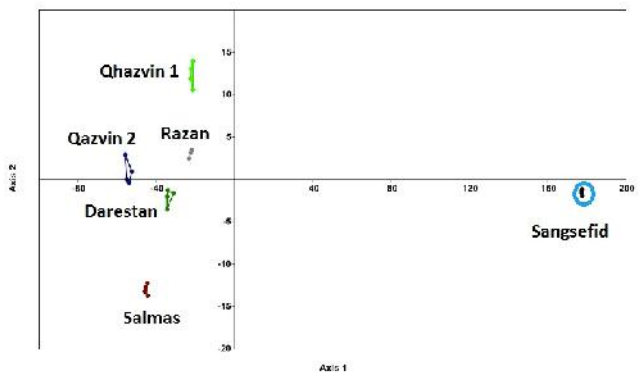

Figure 9. CVA plot of reduced data.

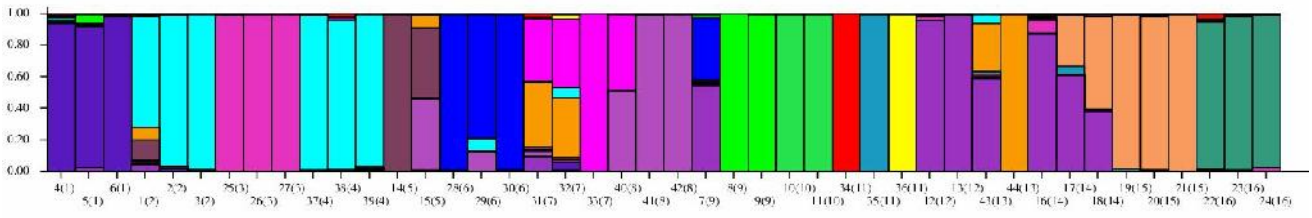

Figure 6. STRUCTURE plot of the combined data. Note: 1. Abhar, 2. Abidar, 3. Ahar, 4. Darestan, 5. Famenin, 6. Kalibar, 7. Kangavar, 8. Nobaran, 9. Qazvin 1, 10. Qazvin 2, 11. Razan, 12. Rudbar, 13. Saeen Qaleh, 14. Salmas, 15. Sang Sefid, 16. Tabriz.

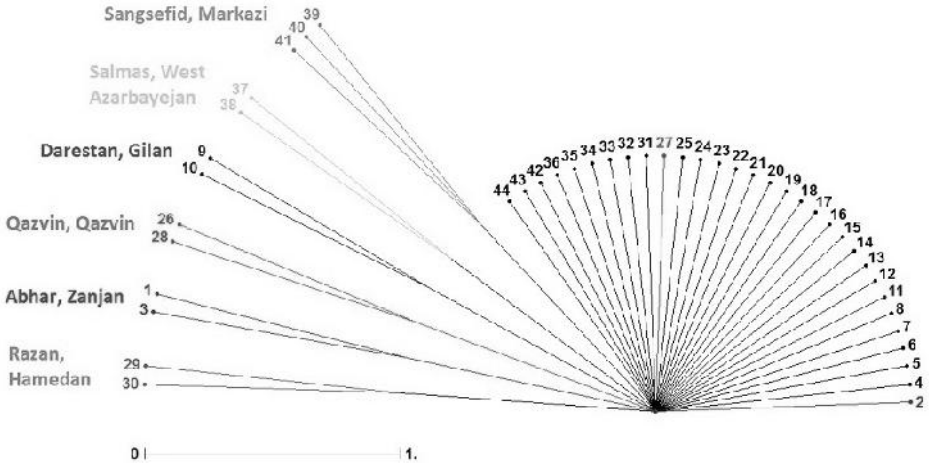

Figure 8. Consensus tree of molecular and morphological characters.

Different studies (for example, Bradshaw 1965; Travis 1994; Schmitt et al. 1999) showed that the phenotypic responses to various environments may also consist of highly special physiological, developmental as well as reproductive adaptive that multiply plant function in those environments. Sultan (1995), believed that capability for particular functionally appropriate environmental answer is called *adaptive plasticity,* as differentiated from the inevitable effects of resource restricts and other suboptimal environments on phenotypic expression. Both adaptive and inevitable natures of developmental plasticity are basic to ecological development, because they influence the success of organisms in their natural contexts. However, functionally adaptive plasticity is of particular interest because it allows individual genotypes to successfully grow and reproduce in many different habitats. Consequently, such plasticity can play a major pert in both the organism's ecological distribution and also its evolutionary diversifications (Sultan 2003).

In our earlier study (Sheidai et al. 2014 a), we reported a significant positive correlation between genetic distance and geographical distance of these populations. However, Mantel test did not show significant correlation between morphological and genetic distance of the studied populations.

Linum austriacum is a widespread species which its different populations occur in different regions of the word, and also this species grow naturally in different parts of Iran in wide ranges of environmental conditions. Sample collecting by authors as well as herbarium vouchers mentioned in different flora such as Flora Iranica (Rechinger 1974) as well as Flora of Iran (Sharifnia and Assadi 2001) confirmed this condition and showed that this species finds in various phytogeographical regions. There are several reasons for such distribution; one of the most important factors for this ability is plasticity in genomic structure of this species. Various studies (Such as Baker 1974; Oliva et al. 1993) confirmed that taxa consisting of adaptively plastic genotypes may inhabit a wide range of ecological conditions; many widespread generalist species may upon examination show this property. Williams et al. (1995), suggested that the adaptive plasticity may also contribute specifically to species invasiveness by allowing rapid colonization of diverse new habitats without the need to undergo local selection. As Sultan and Spencer (2002) believed, plasticity of individual may influence evolutionary diversification models at the population level by precluding selective divergence in environmentally distinct habitats.

Losos and Glor (2003), believed that morphological variation and geographical separation among populations are important and considered as the prerequisite to the formation of new taxonomic ranks such as subspecies and species. It is suggested that phylogeographic analysis can be used to illuminate the interplay of climate, geographical history, and evolutionary dynamics in generating new taxa (Avise et al. 1987; Arbogast and Kenagy 2001). Similarly, the Bayesian and consensus tree obtained in the present investigation produced the results that suggested the presence of potential subspecies and ecotypes in *L. austriacum*. Moreover, population studies on the pattern of variation in many plant species have revealed the existence of localized populations each adapted to the particular environmental conditions of their habitat (Christine and Monica 1999).

"The term ecotype is defined as: distinct genotypes (or populations) within a species resulting from adaptation to local environmental conditions; capable of interbreeding with other ecotypes or epitypes of the same species (Hufford, and Mazer 2003)". This definition suits morphological and genetic discontinuities observed in 4 populations of *L. austriacum* species.

Ockendon (1968), studied morphological diversity in several geographical populations of the *L. perenne* group in Europe. He reported that most of these characters vary continuously and no sharp differences existed among populations. However, significant difference was observed in some of the quantitative characters and therefore, different geographical populations were considered to be ecotypes within each subspecies. In a similar investigation, Nicholls (1986), carried out multivariate analysis of morphological characters in populations of *L. tenuifolium* and recognized sub-specific taxa in this species. The same argument holds true for Sheidai et al. (2014b), who based on molecular and morphological difference of the studied population in *Linum album*, considered them as ecotypes within this species.

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