

Intraspecific morphological and genome size variations in *Linum glaucum* in Iran

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Abstract. Talebi SM, Sheidai M, Atri M, Sharifnia F, Noormohammadi Z. 2015. Intraspecific morphological and genome size variations in *Linum glaucum* in Iran. *Biodiversitas* 16: 69-78. There are many discussions about taxonomic position of *Linum glaucum* in different Flora. In this study, the morphological traits associated with the nuclear genome size were used for identification of intraspecific variations in the nine populations of *L. glaucum*. Twenty three qualitative and quantitative morphological characteristics were investigated. The Analysis of variance tests showed significant difference for some morphological features. The Canonical Correspondence Analysis of habitat ecological factors showed that each of the habitats had prominent characteristics, and also Pearson's coefficient of correlation confirmed the significant correlations between the morphological features in relation to ecological factors. In the morphological Unweighted Paired Group using Average method tree, populations were separated from each other, so that population No. 4 was separated from others. In the flow cytometry investigations, variation of about 1.19 times was presented between the maximum and minimum averages of the genome sizes in the populations. Minimum amount of genome size was found in populations No. 4. Significant correlations occurred between the nuclear genome size with habitat elevation as well as some of the quantitative morphological features. The mentioned variations caused to difference between the populations and lead to creation of ecotype and ecophene in the studied populations.

Key words: Ecology, genome size, morphology, population.

INTRODUCTION

Biodiversity is mostly investigated at the species level as well as higher taxonomic ranks. However, the basis for biodiversity and organic evolution is the genetic difference within species (Ramel 1998). Linhart and Grant (1996) suggested that Comparable developmental variation presents between different populations of a species (intraspecific), which likely resounds verifications to different natural environments and it is the source of plant species differentiation. Therefore, humans have used these intraspecific variations for the domestication and genetic improvement of many of plant species (Diamond 2002).

Linum glaucum Boiss. & Nöe belongs to section *Linum* of the genus *Linum* L. This species is an Irano-Turanian element which naturally spread in the slopes of the Zagros Mountains in the different provinces of Iran such as Kurdistan, Kermanshah, Zanjan, Hamadan, Lorestan as well as Markazi. In addition this species are found in the neighboring countries as Iraq and Turkey (Rechinger 1974; Mobayen 1996; Sharifnia and Assadi 2001).

There are many discussions about taxonomic position of *L. glaucum*. This taxon was described for the first time as *L. alpinum* Jacq, var. *glaucescens* Boiss. in *Flora Orientalis* (Boissier 1867), then Stapf recognized it as *L.*

strile (Rechinger 1974). In 1967, Davis named it as *L. austriacum* L. subsp. *glaucescens*, following Rechinger (1974) recognized this taxon as a synonym of *L. glaucum*. Therefore there are three different synonyms for *L. glaucum* in different Flora such as *Flora Iranica* (Rechinger 1974) and *Flora of Iran* (Sharifnia and Assadi 2001).

Although *L. glaucum* is morphologically very similar to *L. austriacum*, the other member of this section, but in different taxonomical treatments such as palynology (Talebi et al. 2012a), seed micromorphology (Talebi et al. 2012b), anatomy (Rashnou-Taei 2014) and also molecular investigation (ISSR) (Talebi 2013) these species placed separately. The obtained results of these studies showed that *L. glaucum* and *L. austriacum* are separate species.

In addition to morphological characteristics, variation between populations can be investigated with using 2C-value or nuclear genome size. One of the most important features of all living organisms is nuclear DNA amount. Swift (1950) definite C-value as DNA content in the unreplicated haploid nucleus. Nuclear DNA amount is positively related to the number of cellular traits such as: time of mitotic cell cycle, nuclear DNA synthesis rate, the size of cell and its nuclear, through to the whole plant traits such as minimum generation time and geographic distribution (Bennett 1972, 1987; Kidd et al. 1987). Studies

confirmed that infraspecific variation in nuclear genome size amount is most significant for plant taxonomy as an indicator of taxonomic heterogeneity (Murray 2005).

Because, the lacks of previously infraspecific study for *L. glaucum* in Iran as well as the world, we have used for the first time in the present work morphological characteristics as well as nuclear genome size for identification and determining kind and level of infraspecific variations between the nine populations of this species in Iran.

MATERIAL AND METHODS

Plant material

Nine populations of *L. glaucum* were randomly collected from western regions of Iran during spring 2011 (Table 1). Plant 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). From each population four samples were selected randomly and then examined for their nuclear DNA content and morphological features.

Morphometry

Twenty three quantitative and qualitative morphological characteristics from the both of vegetative and reproductive organs of this species were examined. The studied morphological characteristics include: stem lengths and its branch number, stem diameter, shape, length and width of basal and floral leaves, the shape of apex, margin and base of basal and floral leaf blade, basal leaf diameter, calyx dimensions as well as corolla color and dimensions. Four replications were used for quantitative characters measurements and the used terminologies for qualitative morphological traits were on the basis of descriptive terminology provided by Stearn (1983).

Flow cytometry

In order to preparation of nuclei suspensions, small amounts of mature fresh leaf tissue together with an equal weight of mature leaf tissue of the standard reference were used. In this study *Allium cepa* L. was used as standard references, which has a 2C DNA value of 33.5 pg (Greilhuber and Ebert 1994).

For preparation of the nuclear suspension two-stepped method was employed. Plant materials were chopped with a sharp scalpel with adding 400 µl nuclei isolation buffer in the plastic Petri dish at room temperature. For staining the extracted nuclei, 1600 µl DNA fluorochrome or 4', 6-Diamidino-2-phenylindole (DAPI) added and the suspensions were filtered through a 50 µm nylon mesh into a labeled sample tube. Then the suspensions of stained nuclei were analyzed with a Partec PI Flow Cytometer (Partec Germany). In the obtained histograms, some flow cytometric statistics such as: coefficient of variation (CV), mode, mean and the number of counted cells were investigated. The obtained nuclear DNA amounts were

measured in picograms (pg) and the nuclei status described in terms of 'C' value (Doležel et al. 2007). Both of the C-value as well as the genome size can be expressed either in DNA picograms ($=10^{-9}$ g) or mega base pairs (1 pg = 978 Mbp). The nuclear DNA amount of the studied samples calculated on the basis of the values of the G₁/G₂ peak means (Doležel and Bartoš 2005).

$$\frac{\text{Sample 2C peak mean position}}{\text{Standard 2C peak mean position}} \times \text{Standard 2C DNA amount} = \text{Sample 2C DNA (pg) amount}$$

Ecological factors

In order to compare the effect of different environmental factors on the nuclear genome size and morphological characteristics of *L. glaucum* populations, five factors were examined for each population's habitat, such as: longitude (E°), latitude (N°), altitude (in meters), average of annual minimum and maximum temperature (in C°). Longitude, latitude and altitude were calculated with Garmin GPS map76CSx, and the averages annual minimum and maximum temperature for each population were taken from the web site of the meteorological organization of Iran.

Statistical analysis

The mean and standard deviation of the studied morphological characteristics were determined. In order to group the studied populations on the basis of morphological features, data were standardized (mean = 0, variance = 1) and used for multivariate analyses including Unweighted Paired Group using Average method (UPGMA) as well as Principal Coordinate Analysis (PCoA) (Podani 2000). Analysis of variance (ANOVA) test was employed to assess the significant quantitative morphological characteristics difference among the studied populations, and Pearson's coefficient of correlation was used to determine significant correlations of nuclear genome size and quantitative morphological traits in relation to ecological factors, as well as, longitude, latitude, altitude, average of annual minimum and maximum temperature, so as to show possible relationship between populations. The multivariate method, Canonical Correspondence Analysis (CCA), was used to elucidate the relationship between the studied populations and their environmental factors. NTSYS ver. 2 (1998) and SPSS ver. 9 (1998) softwares were used for statistical analyses.

RESULTS AND DISCUSSION

Morphometry

In present study, in order to compare the effect of different environmental factors on the phenotype of *L. glaucum* populations, twenty three traits of the both vegetative and reproductive organs were selected and examined between nine natural populations. The mean and standard deviations of the studied characteristics are presented in Table 2. Qualitative characteristics such as: the shape of blade and also its margin, apex and basis of the

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

No. Population	Habitat address	Collector	Herbarium nos.
1	Kurdistan, road of Sanandaj to Hasan Abad, 1684 m.	Talebi	HSBU2011353
2	Kurdistan, Sanandaj, Darbandeh village, 1559 m.	Talebi	HSBU2011354
3	Kurdistan, Sanandaj, Kani Moshkan village, 1678 m.	Talebi	HSBU2011355
4	Kurdistan, Sanandaj, Kilaneh village, 1471 m.	Talebi	HSBU2011356
5	Kurdistan, Sanandaj, Abidar Mountain, 1585 m.	Talebi	HSBU2011358
6	Kurdistan, road of Saqqez to Baneh, 1546 m.	Talebi	HSBU2011360
7	Kurdistan, 25 km Baneh to Saqqez, 1623 m.	Talebi	HSBU2011361
8	Kurdistan, Saqqez, 1570 m.	Talebi	HSBU2011362
9	Kurdistan, road of Saqqez to Divandareh, 1617 m.	Talebi	HSBU2011363

Table 2. Selected morphological traits of the studied population of *L. glaucum* (all values are in cm, details of each population are given in Table 1).

Populations	Stem length	Stem ramification	Basal leaf length	Basal leaf width	Basal leaf shape	Floral leaf length	Floral leaf width	Floral leaf shape	Calyx width	Calyx length	Pedicle length	Sepal length	Sepal width	Petal length	Petal width
1 Mean	49.12	10.50	1.77	0.19	Linear-	0.87	0.09	Linear-	0.37	0.45	0.10	0.20	0.10	1.55	1.20
N	4	4	4	4	Lanceolate	4	4	Lanceolate	4	4	4	4	4	4	4
SD	9.54	2.64	0.10	0.10		0.15	0.02		0.05	0.06	0.00	0.00	0.00	0.33	0.14
2 Mean	60.37	8.75	1.96	0.25	Linear-	0.87	0.11	Linear-	0.35	0.45	0.09	0.18	0.10	1.72	1.25
N	4	4	4	4	Lanceolate	4	4	Lanceolate	4	4	4	4	4	4	4
SD	6.75	1.25	0.49	0.04		0.09	0.02		0.05	0.05	0.00	0.02	0.00	0.22	0.05
3 Mean	55.37	10.25	1.67	0.21	Linear	1.07	0.12	Linear-	0.38	0.40	0.10	0.23	0.10	1.55	1.22
N	4	4	4	4		4	4	Lanceolate	4	4	4	4	4	4	4
SD	6.79	4.78	0.38	0.06		0.12	0.05		0.02	0.00	0.00	.04	0.00	0.38	0.17
4 Mean	40.25	8.00	1.27	0.15	Linear	1.04	0.31	Lanceolate	0.37	0.47	0.10	0.27	0.10	2.32	1.60
N	4	4	4	4		4	4		4	4	4	4	4	4	4
SD	5.17	3.65	0.17	0.00		0.13	0.45		0.05	0.05	0.00	0.05	0.01	0.37	0.31
5 Mean	49.75	6.50	1.50	0.22	Lanceolate	0.80	0.09	Linear-	0.33	0.42	0.07	0.22	0.12	1.65	1.27
N	4	4	4	4		4	4	Lanceolate	4	4	4	4	4	4	4
SD	6.65	1.73	0.11	0.05		0.18	0.00		0.04	0.05	0.02	0.02	0.05	0.51	0.15
6 Mean	47.75	16.75	1.57	0.35	Linear-	1.05	0.16	Lanceolate	0.37	0.42	0.10	0.22	0.10	1.62	1.35
N	4	4	4	4	Lanceolate	4	4		4	4	4	4	4	4	4
SD	8.46	3.09	0.26	0.05		0.23	0.04		0.05	0.05	0.00	0.05	0.00	0.15	0.17
7 Mean	53.87	15.00	1.87	0.18	Linear-	1.37	.17	Lanceolate	0.45	0.47	0.10	0.22	0.12	1.97	1.82
N	4	4	4	4	Lanceolate	4	4		4	4	4	4	4	4	4
SD	7.37	6.78	0.26	0.04		0.12	0.05		0.05	0.05	0.00	0.02	0.05	0.17	0.17
8 Mean	55.25	8.75	1.47	0.15	Linear	1.12	.10	Linear	.38	.45	.10	.25	.13	1.82	1.35
N	4	4	4	4		4	4		4	4	4	4	4	4	4
SD	4.85	1.70	.37	0.03		0.25	0.00		0.06	0.05	0.00	0.05	0.04	0.22	0.23
9 Mean	59.00	4.50	1.60	0.23	Lanceolate	1.02	0.24	Lanceolate	0.36	0.52	0.09	0.23	0.10	2.00	1.65
N	4	4	4	4		4	4		4	4	4	4	4	4	4
SD	8.36	0.57	0.14	0.02		0.12	0.24		0.04	0.05	0.02	0.04	0.00	0.08	0.12

both of basal and floral leaf as well as petal color were examined between the populations. Basal and floral leaf shape varied between populations and presented in the shape of linear, lanceolate or linear-lanceolate. In spite of variations in blades shape in the basal and floral leaf, blade apex, margin and base shapes were stable between populations and were in the shape of acute, entire and cuneate respectively, furthermore the petal colors were stable inter-populations and presented as blue.

The performed ANOVA test on the quantitative morphological traits between the studied populations showed significant difference ($p < 0.05$) in characteristics

such as: stem height and diameter, branch number, basal leaf width and thickness, floral leaf length, petal length and width (Table 3).

Highest and smallest stems lengths being recorded in populations No. 2 (62.16 cm) and No. 4 (40.25 cm), respectively. Stem branch number was between 5 (population No. 9) to 17 (population No. 6). Biggest basal leaf (1.57×0.35 cm) occurred in population No. 6, while population No. 4 had smallest basal leaf (1.27×0.15 cm). Widest floral leaf (0.31 cm) registered in population No. 4 and narrowest (0.1 cm) belonged to population No. 1. Biggest calyx (0.45×0.45 cm) recorded in population No. 7,

Table 3. Results on the ANOVA analysis to assess for differences in the quantitative morphological traits in *L. glaucum* populations.

Characters		Sum of Squares	df	Mean Square	F	Significant
Stem height	Between Groups	1252.889	8	156.611	2.977	.016
	Within Groups	1420.250	27	52.602		
	Total	2673.139	35			
Stem ramification	Between Groups	481.556	8	60.194	5.079	.001
	Within Groups	320.000	27	11.852		
	Total	801.556	35			
Basal leaf length	Between Groups	1.457	8	.182	2.082	.074
	Within Groups	2.362	27	.087		
	Total	3.819	35			
Basal leaf width	Between Groups	.118	8	.015	8.910	.000
	Within Groups	.045	27	.002		
	Total	.163	35			
Floral leaf length	Between Groups	.926	8	.116	4.184	.002
	Within Groups	.747	27	.028		
	Total	1.674	35			
Floral leaf width	Between Groups	.184	8	.023	.748	.649
	Within Groups	.832	27	.031		
	Total	1.016	35			
Calyx width	Between Groups	.032	8	.004	1.554	.186
	Within Groups	.070	27	.003		
	Total	.102	35			
Calyx length	Between Groups	.042	8	.005	2.040	.079
	Within Groups	.070	27	.003		
	Total	.112	35			
Pedicle	Between Groups	.002	8	.000	1.294	.288
	Within Groups	.005	27	.000		
	Total	.007	35			
Sepal length	Between Groups	.021	8	.003	1.552	.186
	Within Groups	.046	27	.002		
	Total	.066	35			
Sepal width	Between Groups	.005	8	.001	.838	.578
	Within Groups	.022	27	.001		
	Total	.028	35			
Petal length	Between Groups	2.122	8	.265	2.879	.019
	Within Groups	2.488	27	.092		
	Total	4.610	35			
Petal width	Between Groups	1.581	8	.198	5.783	.000
	Within Groups	.922	27	.034		
	Total	2.503	35			
Leaf thickness	Between Groups	.062	8	.008	3.111	.013
	Within Groups	.067	27	.002		
	Total	.130	35			
Stem diameter	Between Groups	.137	8	.017	4.974	.001
	Within Groups	.093	27	.003		
	Total	.230	35			

while smallest calyx (0.35×0.4 cm) was seen in population No. 2. Largest (0.27 cm) and smallest sepal (0.1 cm) were found in populations No. 4 and 6, respectively. Biggest and smallest corolla (2.32×1.6 cm) were registered in populations No. 4 and 1, respectively. Thickest basal leaf (0.2 mm) and stem (0.33 cm) were found in populations No. 2 and 3, respectively. Slimmest basal leaf (0.1 mm) and stem (0.13 cm) occurred in populations No. 7 and 4, respectively.

Five geographic and climatic factors of population's habitat were examined. CCA analysis showed that these factors varied between habitat, and each of them had a prominent environmental feature. For example, populations No. 6 and 7 placed at coldest environments or population

No. 5 rested in eastern regions (Figure 1). CCA biplot of morphological traits and environmental factors showed that the mentioned parameters had strong effect on the plant phenotype. For example, branch number and also basal leaf shape were varied along annual minimum temperature and also variations in floral leaf length, petal width and floral leaf width were parallel with northern distribution of populations; furthermore, annual maximum temperature had strong effect on floral leaf shape (Figure 2).

In addition, Pearson's coefficient of correlations were determined between the quantitative morphological features in relation to environmental factors of habitat, including longitude, latitude, altitude and average of annual minimum

and maximum temperature, showed significant negative/positive correlations. For example, floral leaf length had a significant negative correlation ($p < 0.05$, $r = -0.65$) with maximum temperature of year, but this feature had significant positive correlations ($p < 0.05$) with minimum temperature of year ($r = -0.65$) and northern distribution ($r = 0.69$) of populations. A significant negative correlation ($p < 0.001$, $r = -0.94$) occurred between calyx width with annual maximum temperature, while this trait had a significant positive correlation ($p < 0.05$, $r = 0.72$) with northern distribution of populations.

The studied populations were separated in the UPGMA tree of morphological features (Figure 3). In the mentioned diagram two main branches were seen, one of these had one population, namely population No. 4, which arranged separately far from others. The rest of populations placed in another branch. This branch had two sub branches; populations No. 7 and 6 became detached from others and placed in one sub branch. As seen in the box and whisker plots (Figure 4), each of the studied populations had distinct morphological trait (s).

Genome size

Nuclear DNA amounts of the studied populations were calculated with flow cytometer apparatus (Table 4). In the analyzed samples, most of the leaf nuclei of the both *L. glaucum* and standard references were at the G₁ phase of the cell division and thus represented the 2C DNA amount. The average of nuclear genome size differed between populations. Maximum average of genome size was recorded in population No. 5 with about 2.06 pg, while the

minimum average value (1.73 pg) being registered in population No. 4, showing variation of about 1.19 times.

A significant negative correlation ($p < 0.05$, $r = -0.73$) occurred between genome size with habitat altitude. This means that, *L. glaucum* populations that grow in higher altitude possess smaller nuclear DNA amounts, while the populations found in lower had larger nuclear DNA amounts. Not only significant correlation occurred between nuclear genome sizes in relation to environmental factors of habitat, but also morphological traits of the studied populations had significant correlations with 2c-value amount of plants. For example, nuclear genome size had significant negative correlations ($p < 0.05$) with floral leaf width ($r = -0.67$) and also with petal length ($r = -0.72$). A significant positive correlation ($p < 0.05$, $r = 0.67$) found between nuclear DNA amount with stem height.

Table 4. Nuclear genome size of the studied populations (details of each population are given in Table 1).

Population nos.	2C DNA amount (picograms)	Genome size (mega base pairs)
1	1.99	1946.22
2	2.05	2004.9
3	1.99	1946.22
4	1.73	1691.94
5	2.06	2014.68
6	1.96	1916.88
7	1.94	1897.32
8	2.02	1975.56
9	2.01	1965.78

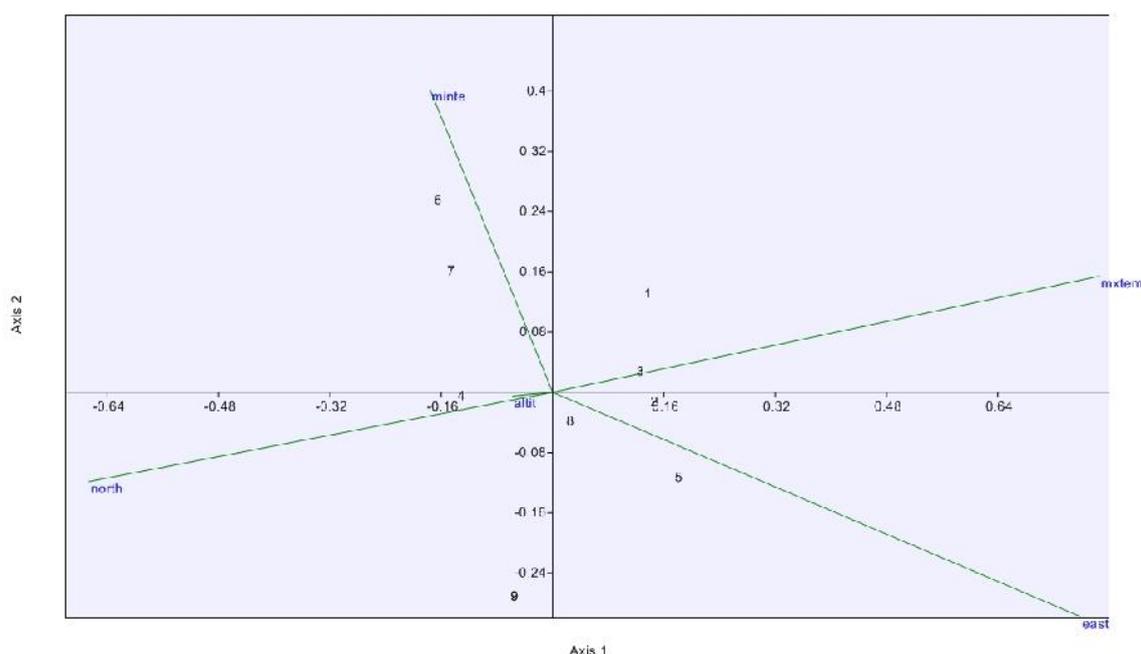


Figure 1. CCA plot of the ecological factors of *L. glaucum* populations. Abbreviations, minte: average of annual minimum temperature, maxtem: average of annual maximum temperature, north: latitude, altit: altitude, east: Longitude (details of each population are given in Table 1).

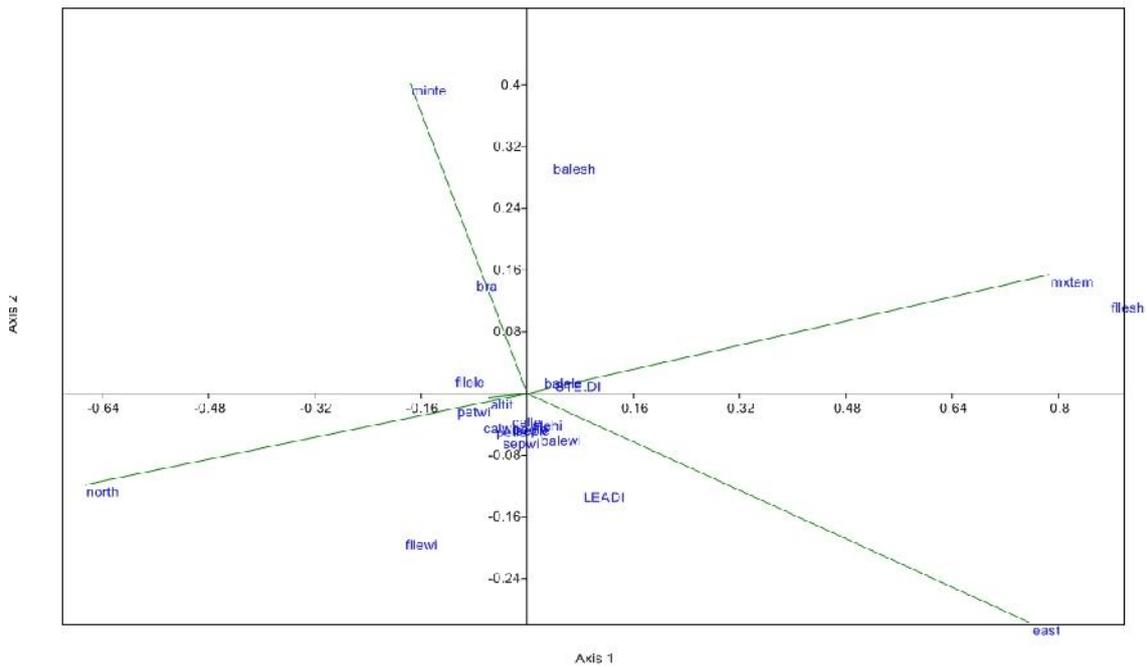


Figure 2. CCA biplot of morphological traits with ecological factors of the studied populations. Abbreviations, minte: average of annual minimum temperature, maxtem: average of annual maximum temperature, north: latitude, altit: altitude, east: Longitude, balesh: basal leaf shape, flesh: floral leaf shape, filewi: floral leaf width, fillele: floral leaf length, leadl: basal leaf dimentions, petwi: petal width, balewi: basal leaf width, bra: branch number (details of each population are given in Table 1).

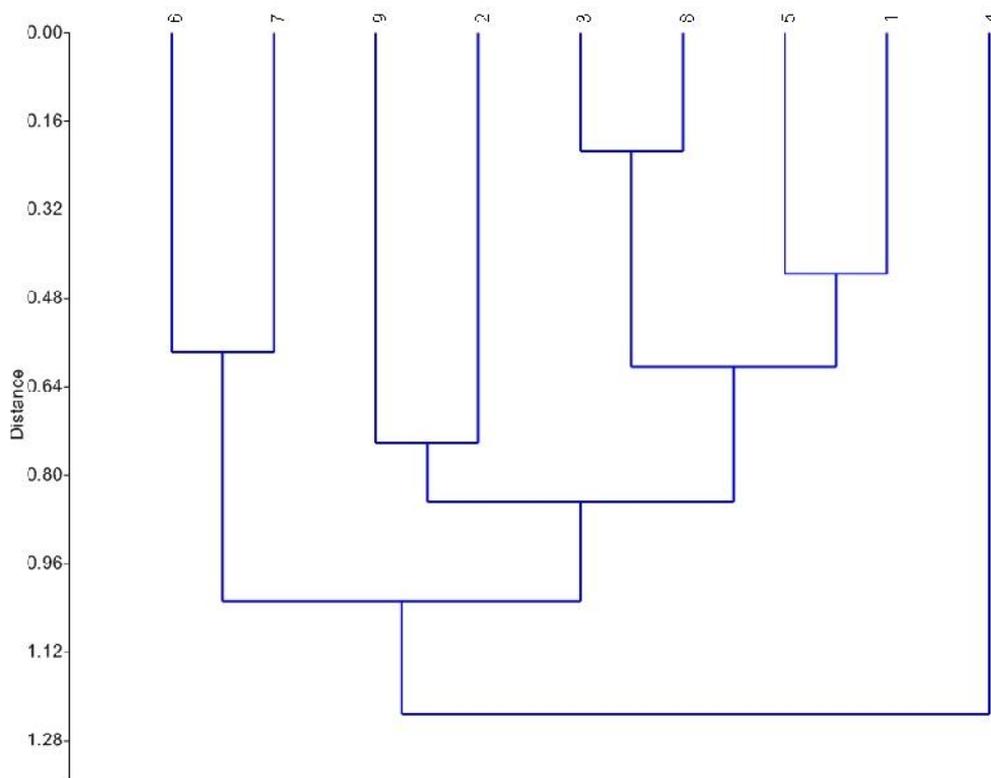


Figure 3. Morphological UPGMA tree of the *L. glaucum* populations (details of each population are given in Table 1).

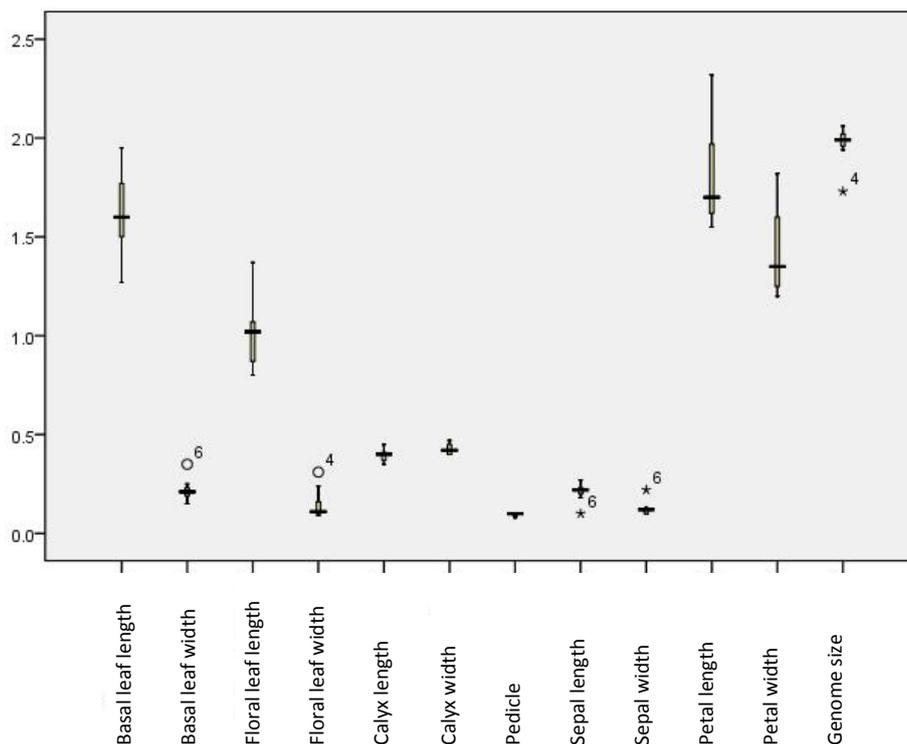


Figure 4. Box and whisker plot of some important characteristics of the studied populations (details of each population are given in Table 1).

Mixoploidy

In mixoploidy condition, the plant body consisting of diploid and polyploid cells. Genome size of all cells undergoes cyclic changes. In the resting (G_0 phase) and pre-replicative stages of cells (G_1 phase), each cell has $2C$ of nuclear DNA amount, but synthesis of new DNA occurs during S phase of cell cycle, resulting in doubled ($4C$) nuclear DNA content. In the post-replicative period of cell growth (G_2 phase), the DNA content is maintained at $4C$ level. In population No. 1, in addition to peak of G_1 phase nuclei ($2C= 1.99$ pg), the peak of G_2 phase nuclei were seen and represented the $4C$ ($DNA4C = 4.11$ pg). So mixoploids can be identified in flow cytometry histograms by the presence of two peaks of *Linum* in the histogram (Figure 5).

Discussion

Alonso-Blanco et al. (2005) suggested that plant diversity has fascinated man all over history, primarily due to the great difference that presents in nature for morphological and other developmental features. According to Cronk (2001), the effects of fitness of such naturally occurring variation exist in the different populations of a species (intraspecific) have driven plant macroevolution by means of natural selection, this developmental diversity being the principle of plant classification and phylogeny.

In the present study, in addition to morphological characters, nuclear genome size was used for identification of intraspecific variations in the *L. glaucum* populations. Not only quantitative morphological traits differed between populations and ANOVA test showed significant difference for some of them, but also qualitative features such as floral as well as basal leaf shape varied among the studied samples. In the UPGMA tree of morphological features, populations were detached, not only population No. 4 placed in separated branch but also populations No. 6 and 7 separated from the rest. This subject confirmed high morphological variations between the populations. The individuals of the mentioned populations had distinct morphological characteristics, for example population No.4 had smallest stem, basal leaf and sepal and also biggest corolla with widest floral leaf in comparison to other populations. Therefore, these traits differentiated these populations from others. Different morphological studies on the populations of this species confirmed the obtained results of the present study. For example morphological characters of long-styled and short-styled plant populations

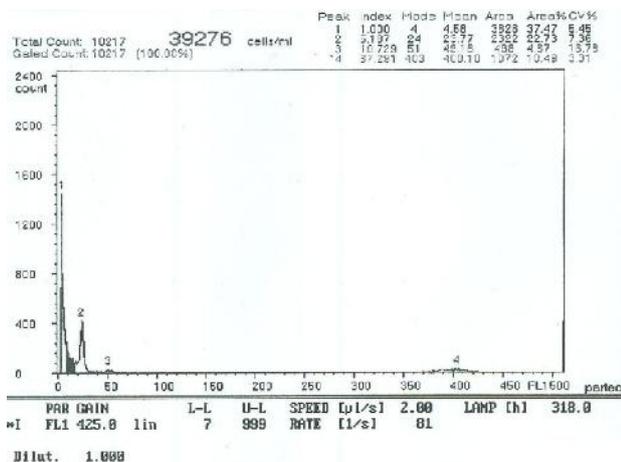


Figure 5. Flow cytometry histogram of mixoploidy in *L. glaucum* population (peak 2: *L. glaucum* $2c$ value amount, peak 3: *L. glaucum* $4c$ value amount and peak 4: standard reference).

in *L. glaucum* were investigated by Talebi et al. (2012c). In their study, T-test analysis of morphological characters between long-styled and short-styled plant populations, showed significant difference ($p < 0.05$) for some characters such as basal leaf length, calyx width and length. Understanding the sources of morphological variation in organisms such as plants is central to the understanding of natural variation and the responses of organisms to their environment. Morphological traits variability expression can show differentiation of plant populations in a particular habitat (Schlichting 1986).

The observed variations between the studied populations can be caused by two main factors: it may be related to ecological factors of the population's habitat or variations in genetic structures of populations. Most of the existing relationships between the morphological features with environmental factors were significant; this subject showed that variation in phenotypical characteristics is predictable and had significant correlations with environmental variations. Referring again to the mentioned above populations, it can see that both of the populations No. 4 and 6 were found in habitats which had salient characteristic (s), for example population No.4 found in the highest altitude (1741 m), conversely population No. 6 was seen in the lowest elevation habitat (1546 m), with variation about 200 m. These conditions were true about other ecological factors of habitats such as annual minimum and maximum temperatures and also geographical latitude and altitude. Furthermore, significant positive or negative correlations found between environmental factors with morphological characteristics of populations individuals. For example, increase in habitat temperature shortens the length of floral leaf or northern populations have more elongated floral leaf. Different studies on the other species, either of the genus *Linum* or other genus/ families confirmed this condition. For example, Morphological characteristics of twenty one populations of *Linum album* Kotschy ex Boiss. were survived (Talebi et al. 2014a). The results of the study have shown that the different populations of *L. album* were adapted to their habitat and the ranges of phenotype plasticity were high between populations which led to creation of ecomorphs (Talebi et al. 2014a). Furthermore, Talebi et al. (2014b) investigated morphological traits of different populations of *Stachys inflata* Benth. in Iran. The results of this study showed that different populations of same species that grow under different ecological conditions, altered their morphological features for adaptation with their habitat condition.

The relationships between nuclear DNA mounts and phenotypic features have been discussed in different studies at different levels, for example effect of genome size on cell size and stomatal density in angiosperms (Beaulieu et al. 2008), effect of genome size on phenotypic traits at the cellular level i.e. guard cell length and epidermal cell area (Knight and Beaulieu 2008); relationship between genome size and production of above-ground biomass, seedling establishment success as well as seed weight and seed dormancy (Munzbergova 2009), but few studies have studied how and whether nuclear genome

size can alter phenotypic features at the population scale (but see Lavergne et al. 2010).

The obtained results showed that, in addition to morphological features, nuclear genome size showed variations of about 1.19 times between populations. These variations are widespread phenomenon in this genus. ANOVA test revealed a significant variation in genome size among the Iranian populations of *Linum austriacum* L. (Sheidai et al. 2014a) as well as *Linum album* (Sheidai et al. 2014b). Some of *Linum* species are known as a good example of plastic genome in plant (Evans et al. 1966; Evans 1968a, b; Durrant and Jones 1971; Joarder et al. 1975), for example different cultivars of *L. sativum* Hasselq. had a 10 % difference in genome size. Some degree of chromosomal variation within populations such as duplications and deletions, spontaneous aneuploidy or polyploidy, heterochromatic segments, B-chromosomes and sex chromosomes will naturally cause some inter-individual DNA content variation (Greilhuber 1998).

Results of this study showed that nuclear genome size had strong effect on morphological characteristics of the studied populations, and significant positive/negative correlations recorded between morphological characteristics with $2c$ -value amounts. Furthermore, population's arrangements in the morphological UPGMA tree were coinciding with $2c$ -value amounts of the populations. Population No. 4 which separated of others had smallest amount of nuclear genome size. This condition was true about populations No. 6 and 7. These populations placed together and approximately had equal nuclear genome size. Quantitative differences in nuclear DNA contents within a species result in changes in cell cycle and cell size in many plant species such as *Zea mays* L. (McMurphy and Rayburn 1992), *Festuca arundinacea* Schreb. (Minelli et al. 1996) and *Poa annua* L. (Mowforth and Grime 1989). These changes at the cellular level have multitudinous effects on various phenotypic traits through allometric effects of the changes in cell dimension (Cavalier-Smith 1985). DNA is effected growth and development of each organism in two ways, directly through its informational and gene content, and indirectly by the physical-mechanical effects of nuclear DNA mass. Although the amount of nuclear DNA does not involve expression and regulation of single genes, it has great effects on cellular metabolic processes (Cavalier-Smith 1985).

The term 'nucleotype' was used to describe those conditions of the nucleus, which affect the phenotype (Bennett 1972). The infraspecific changes in genome size, being therefore subject to natural selection is naturally related with those leading to the divergence and evolution of species. Therefore, a thorough appraisal of such changes is required for the application of genome size data to demarcate infrageneric taxa and in microsystematics.

In the studied populations, habitat ecological factors influenced nuclear genome size, as significant negative correlations occurred between genome sizes with habitat altitude. Different studies confirmed this condition for other *Linum* species. For example, various genotrophs of *L. usitatissimum* L. created by a process of environmentally

induced changes in genomes structure (Cullis et al. 1999). The mentioned heritable alternations are due to specific rearrangements at prominent positions of the genome, furthermore Cullis et al. (1999) stated that some changes in genome structure such as highly repetitive, middle-repetitive, as well as low-copy-number sequences are involved in the polymorphisms detected, and sequence alterations of specific subsets of 5SrDNA have been distinct, in addition different varieties of *L. usitatissimum* which grew in different environmental conditions for example: high nitrogen concentrations or high temperatures increased 10% in nuclear DNA amounts (Evans 1966).

Price et al. (1981) studied DNA contents of 222 plant samples of *Microseris douglasii* (DC.) Sch. Bip. representing geographically, ecologically and morphologically diverse populations in California which showed a 14% variation between population means, with those having higher and lower DNA amounts restricted to mesic and xeric sites, respectively. Similarly, Ceccarelli et al. (1992, 1993) recorded a 32.3 % difference covering 35 natural populations of hexaploid *Festuca arundinacea* was positively correlated with mean temperature during the year, with the coldest month at stations and with generation time, while it was negatively correlated with latitude and germination power of seeds and growth rate. A negative significant correlation was occurred between C-value and altitude in *Dactylis glomerata* L. The finding results showed that plants growing at lower altitudes have larger C-values than those at higher altitudes, which suggest that there was selection for smaller C-values with increasing altitude (Reeves et al. 1998).

The results of our study demonstrated the difference in nuclear DNA content between different populations of *L. glaucum*, and suggested that this variation play important roles in determining phenotypic differences between the populations in this species. Based on all the examined data, it can be concluded that populations No. 4, 6 and 7 detached of others and had unique morphological characteristics as well as nuclear DNA amount, which lead to difference of the mentioned populations from the others. Therefore high inter-population variations obviously seen between the studied populations of *L. glaucum*. Clausen et al. (1948) suggested that individuals of a given species typically varied in morphological features. Although some of the mentioned variations may be random, but ecological theory posits that many of these variations may represent adaptive matching of phenotypes to a variable environmental condition. As Sultan (1995) believed this matching can occur either through natural selection process that create genetically-differentiated ecotypes, or through phenotypic plasticity, in which various phenotypical characters are produced from the same genotypes in different ecological condition.

The term ecotype is definite as a preferable set of populations within a species, resulting from conformity to positional environmental situations, able of interbreeding with other ecotypes of the same species (Hufford and Mazer 2003). In ecotype, populations are adapted to local conditions, at a variety of spatial and temporal scales. Based on above definition, at least one ecotype was

distinguished between the studied populations. Population No. 4 had distinct morphological characteristics with least nuclear DNA amount, which with these traits adapted to its habitat, so this population could be definite as ecotype. Creation of ecotype is very important because this type of variation had adaptive value for each species and increase biodiversity in occurring ecosystem. Matching ecotypes to local conditions increases restoration success. A practical value of recognizing ecotypic variation in grasses is in allowing recognition of the most suitable ecotypes for conservation, restoration, renovation, landscaping, and bioremediation (Gibson 2009).

Other interpopulation variation was phenotypic plasticity (accommodation ecophene) which found between the studied populations, for instance populations No. 7 and 6 that morphologically separated from others, because no appreciable difference registered in nuclear DNA amount between these in comparison with other populations. The ability of plant to alter its morphology in response to changes in environments can be regarded as phenotypic plasticity or accommodation ecophene (Bradshaw 1965; Schlichting 1986). As Thompson (1991) suggested, the resistor of population to a variable environment is usually achieved by plastic phenotypic answer because it has been agreed that plastic reactions are required for species for the possession of damaged habitats.

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