

DISTYLY IN *LINUM ALBUM* L. (LINACEAE): GENETIC AND MORPHOLOGICAL DIFFERENCES AND POPULATION'S GENETIC STRUCTURE

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ABSTRACT: *Linum album* L. is a medicinal species that show distyly. The present study was aimed to investigate morphological and genetic differences between long- styled and short-styled plant and if these differences vary with population's genetic structure. For these reason, nine populations of *L. album* were collected from different regions of Iran. The obtained results showed that the long-styled and short-styled plants differed significantly in their morphological as well as genetic content (ISSR loci). Genetic analysis revealed that the genetic rearrangements in allelic forms and combination were associated with these plant forms differentiation. *L. album* populations varied in their magnitude of morphological and genetic differentiation between the two morphs.

KEY WORDS: genetic structure, heterostyly, *Linum album*, morphology.



Introduction

Heterostylous species have two (distyly) or three (tristyly) contrasting flower types. The plants with long styles flowers are known as pin while, plants that have short stamens called thrum (Jhala 2010). This phenomenon is a unique form of sexual polymorphism and herkogamy in flowers. In a heterostylous species, two or three morphological types of flowers, (named morphs), present in the population. On each individual plant, all flowers are in the same morph. The flower morphs differ in the lengths of the pistil and stamens, and these features are not continuous. This morphological character is genetically linked to genes responsible for a unique system of self-incompatibility, termed heteromorphic self-incompatibility, that is, the pollen from a flower on one morph cannot fertilize another flower of the same morph (Armbruster et al. 2006).

Ferrero et al. (2009) stated that discrete sexual polymorphisms - in the other words dioecy and heterostyly - have been used as models for the evolution of reproductive strategies and in particular, sexual systems. To date, heterostyly is known to occur in at least 28 families of flowering plants.

Linum album Ky. ex Boiss. is a heterostylous species which belongs to section *Syllinum* of the genus *Linum* (Rechinger 1974). It is an herbaceous medicinal plant species (Seidel et al. 2002) that naturally grows throughout the country and form several local populations (Sharifnia and Assadi 2001).

In the present study we do not want to find out genetic causes of heterostyly but we are interested to address: (1) - If *Linum album* differ in their morphological features and genetic structure in two different morphs, (2) - Do the two plant morphs in general (considering all populations) differ in their genetic content, and (3) - Do the two plant

morphs differ in accordance with genetic structure of populations.

Materials and methods

Plant materials

In present study, nine different populations of *L. album* were collected from different regions of Iran during spring 2012 (Table 1). Plant specimens were identified based on descriptions provided in Flora of Iran (Sharifnia and Assadi 2001; Rechinger 1974). For morphological and also molecular investigations, from each population 10 randomly collected plants (five plants per each morph) were studied. In molecular study, collected leaves of these plants were mixed together and used for DNA extraction. The voucher specimens were deposited in herbarium of Shahid Beheshti University (HSBU).

Morphological study

Twenty eight, nine qualitative and nineteen quantitative, morphological characters were studied. These traits were : the stem height, the basal as well as floral leaves shapes, the width, length and length/width ratio of the basal and floral leaves, the shape of leaves apex, margin and base, the size of calyx width, length and length/width ratio, the length, width and length/width ratio of sepal, the size of corolla width, length and length/width ratio, petal, color, width and length as well as length/width ratio, the length of style and the length of anther and also filament. The mean value of quantitative characters was measured in each population and different forms of qualitative characters were noted any time encountered.

DNA extraction and ISSR assay

For molecular study, we used ISSR markers since our own previous studies as well as those of other workers showed that these markers, in

spite their limitations, are powerful molecular tools to differentiate the species populations and reveal the genetic structure of populations (see for example Heather and Freeland 2011; Sheidai et al. 2012, 2013).

For molecular studies, fresh leaves were collected randomly from 10 randomly selected plants in each population - five plants per each form. Nuclear DNA was extracted using CTAB activated charcoal protocol (Križman et al. 2006). The extraction procedure was on the base of activated charcoal and Polyvenyl Pyrrolidone (PVP) for binding of polyphenolics during extraction. The mild extraction and precipitation conditions, promoted the high-molecular weight DNA isolation without interfering contaminants. The quality of extracted DNA was examined by running on 0.8% agarose gel.

In total, ten ISSR primers namely (AGC)₅GT, (CA)₇GT, (AGC)₅GG, UBC810, (CA)₇AT, (GA)₉C, UBC807, UBC811, (GA)₉A and (GT)₇CA commercialized by UBC (the University of British Columbia) were used. PCR reactions were performed in a 25 µl volume containing 10 mM Tris- HCl buffer at pH 8; 50 mM KCl; 1.5 mM MgCl₂; 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. The amplification products were visualized by running on 2% agarose gel, followed by the ethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany).

Data analyses

The analysis of variance (ANOVA) test was performed for quantitative morphological traits to indicate significant difference between plant morphs and among the studied populations. Principal coordinate analysis (PCoA), Principal Correspondence Analysis (PCA) as well as multidimensional scaling (MDS) were performed to group the plants specimens based on the standardized (mean = 0, variance = 1) morphological characters. The multivariate method, Canonical Correspondence Analysis (CCA), was used to elucidate the relationship between the studied populations and their environmental factors.

Since in previous studies the impact of longitude and latitude as well as elevation was identified on *Linum* species and populations' divergence and genetic variability (Talebi et al. 2012), we performed CCA (Canonical Correspondence Analysis) using these ecological features of populations to investigate their background effect on plant morphs.

ISSR bands obtained were treated as binary characters and coded accordingly (presence = 1, absence = 0). Genetic diversity parameters were determined in each population. These parameters were percentage of allelic polymorphism, allele diversity (Weising et al. 2005), Nei's gene diversity (H), Shannon information index (I), polymorphic information content (PIC), the number of effective alleles and percentage of polymorphism (Weising et al. 2005; Freeland et al. 2011).

Dice as well as Nei's genetic distance (Freeland et al. 2011; Weising et al. 2005), were determined among the studied populations and used for clustering. Unweighted paired group method with arithmetic average (UPGMA) and Neighbor Joining (NJ) clustering were performed after

100 times bootstrapping (Freeland et al. 2011). Similarly ordination plot based on principal co-ordinate analysis (PCoA), and multidimensional scaling (MDS) (Podani 2000) were performed by using PAST ver. 2.17 (Hamer et al. 2012) and DARwin ver. 5 (2012). The Mantel test was performed to check correlation between geographical distance and the genetic distance of the studied subspecies and populations (Podani 2000).

In order to investigate significant genetic difference among populations (provinces) different methods were used: 1 - AMOVA (Analysis of molecular variance) test (with 1000 permutations) as performed in GenAlex 6.4 (Peakall and Smouse 2006), and 2 - Nei's G_{st} analysis of GenoDive ver.2 (2013) which was originally written by Meirmans and Van Tienderen (2004). Moreover, new parameters of genetic differentiation such as G'_{ST} est = standardized measure of genetic differentiation ((G_{st} est / (n-1+H_s est)) / ((n-1). (1-H_s est))) (Hedrick 2005), and D_{est} = Jost measure of differentiation (Jost 2008) were determined. Moreover, in order to overcome potential problems caused by the dominance of ISSR markers, a Bayesian program, Hickory (ver. 1.0) (Holsinger et al. 2003), was used to estimate parameters related to genetic structure (Theta B value). Three runs were conducted with default sampling parameters (burn-in = 50,000, sample= 250,000, thin = 50) to ensure consistency of results (Tero et al. 2003).

Population's stratification was checked by two methods. First we carried out structure analysis (Pritchard et al. 2000). For this, data were scored as dominant markers and analysis followed the method suggested by Falush et al. (2007). Second, we performed K-Means clustering as done in GenoDive ver. 2. (Meirmans 2012).

We used two summary statistics to

present K-Means clustering, 1 - pseudo-F (Caliński and Harabasz 1974) and 2 - Bayesian Information Criterion (BIC, Schwarz 1978). The clustering with the highest value for pseudo -F and lowest value for BIC is regarded to provide the best fit.

Results

In the present study, morphological as well as molecular data were used for identification of infraspecific variations between long - styled as well as short - styled individuals in *L. album* populations. This species had widespread distributions in Iran and naturally were found in the different regions of the country. *L. album* as many *Linum* species have heterostylous members, which long-styled and also short- styled individuals were found separately in each population.

Morphometry

In order to determine the presence or absence of morphological differences between long-styled and short-styled individuals in each population, twenty eight morphological traits of the both reproductive as well as vegetative organs were investigated. Among the nine studied qualitative features, some characteristics such as blade apex, margin and base shapes of floral and also basal leaves were stable between different morphs as well as among the different populations and were in the shape of acute, entire and cuneate respectively, furthermore the petal colors were stable between heterostylous individuals and presented as white.

Although basal as well as floral leaves shapes were fixed between long-styled and short-styled plant in each populations, while their shapes varied among the studied populations. Floral leaves were present as lanceolate as rarely ob-lanceolate or ovate. Often, the shape of basal leaf was linear-

lanceolate, but in some populations, they were seen as lanceolate, ob-lanceolate, ovate or linear.

The ANOVA test showed significant morphological difference between the plant morphs among the studied populations. The PCA, PCoA and MDS plots of morphological characters produced similar results. Therefore, only the PCA plot is presented and discussed here (Fig. 1). This figure showed that in some of the populations, the long-styled and the short-styled plants were placed close to each other and were almost separated from the other studied populations. But in Kharaghan (coded D in Fig. 1), Qazvin (coded F), Chamran (coded H) and Fasham (coded I) populations, the long-styled and the short-styled plants were placed far from each other. These results showed morphological differences of the two plant morphs in some *L. album* populations.

The CCA plot obtained after removing the plant specimens in Kharaghan (coded D in Fig. 2), Qazvin (coded F), Chamran (coded H) and Fasham (coded I) populations, showed that *L. album* populations varied in the magnitude of morphological divergence between the two plant morphs.

Genetic diversity analysis

The ISSR primers produced polymorphic bands in the both long-styled and the short-styled plants in *L. album* populations. Zarandiyeh population had the highest amount of genetic polymorphism (20.33%), while Fasham population showed the lowest amount of genetic polymorphism (13.01). Similarly, these two populations had the highest and the lowest values of the effective No. of alleles, Shannon information index and Polymorphic Information Content (PIC) (Table 2).

The AMOVA test produced the Fst value (0.351), which showed significant ($p=0.01$) genetic difference among the studied

populations. The same was true for G'st (Nei) = 0.39 ($p = 0.001$) and D_est = 0.10 ($p = 0.001$). The Hickory test also produced Theta B value of 0.29, which is a high value. All of these results indicated that 9 studied geographical distylous plant populations of *L. album* differ genetically from each other. The populations Kharaghan and Fasham had the lowest Hst value = 0.130 (Hst/Ht = 0.09), while population Zarandiyeh had the highest Hst value = 0.203 (Hst/Ht = 0.16).

The AMOVA test between pooled genetic data of the long-styled plants in all studied populations versus all short-styled plant specimens, revealed great genetic difference between the two plant forms (Fst = 0.15, $p = 0.01$). The PCoA plot of ISSR data obtained for the studied populations (Fig. 3) also separated these populations in different corners of the plot. Both plant forms in each species were placed close to each other with some distance. This means that, the kind of genetic differentiation in the two plant forms is unique in each population. For example, plant forms of the populations Chamran and Fasham were placed close to each other (plant forms No. 15-18). Plant forms of the population Peyghambar differed from the other studied populations and were placed alone in the corner of the PCoA plot (plant forms 13 and 14).

The second PCoA plot was obtained for the two plant forms (Fig. 4). This plot also showed almost complete separation of the two plant morphs. The long-styled plants had slightly higher Hst value (0.264) compared to that of the short-styled plants (Hst = 0.225). This result is also supported by PCoA plot of these two plant forms, as the long-styled plants showed greater genetic diversity among each other (they were placed with greater distance from each other in the plot).

The K-Means clustering (Table 3) showed that the best clustering of populations according to the pseudo-F value was $k = 2$, while the Bayesian Information Criterion index produced $k = 3$. These values are in agreement with PCoA results, which separated these populations into 2-3 major groups, and also showed some degree of genetic difference among the 9 studied populations.

The STRUCTURE plot (Fig. 5), showed the presence of 3-4 allelic combinations (differently colored segments) in the studied populations. It revealed the occurrence of extensive genetic recombination and admixture among these populations. The analysis showed difference in the allelic composition of the long-styled plants and the short-styled plants in each population. For example, the two plant forms in the population No. 1, (plants 1 and 2) contained similar allelic combinations (similarly colored segments) but differed in the frequency of these allelic forms (difference in the proportion of the segments with similar colors). This argument holds true for the population No. Sanandaj (plants 3 and 4), population Avaj (plants 5 and 6) and population Chamran (plants 15 and 16).

The STRUCTURE plot also showed that the long-styled and the short-styled plants of a single population differed in their genetic content. For example, in the population Zarandiyeh (plants 9 and 10), the long-styled plant (plant 9) had an orange colored segment (particular allelic form) which was absent in the short-styled plant (plant 10). The same holds true for the population Fasham in which the short-styled plant (plant 18) had allelic form (orange colored segment), which was absent in the long-styled plant. Therefore, this analysis not only showed genetic differences of the studied populations but also showed genetic divergence of the long-styled plants from the short-styled plants.

The Mantel test showed significant positive correlation between the populations' genetic distance and their geographical distance ($p = 0.01$).

Discussion

Our finding confirmed high morphological differences between two plant morphs in these populations. Different studies showed that in heterostylous species some morphological as well as micromorphological features such as the number and size of pollen grains, stamens shape, shape, color and surface papillae of stigma differed in pin and thrum plants (Richards and Barrett 1992; Talebi et al. 2012). These conditions hold true for some species in this genus. For example, in the species *Linum glaucum*, *L. austriacum* (Talebi et al. 2012), *L. mucronatum* subspecies (Farahani 2013), *L. perenne*, *L. grandiflorum* and *L. alpinum* (Dulberger 1981) morphological and also micromorphological characteristics varied between long-styled and short-styled plants. Similarly, in heterostylous species of *Linum* qualitative as well as quantitative palynological features varied between the pin and thrum plants (Dulberger 1981; Talebi et al. 2012, 2014a).

Similar study performed in the plant morphs of *Plumbago auriculata* (Plumbaginaceae) (Ferrero et al. 2009), identified two well-differentiated morphs after morphometric analysis. One morph presents the stigmas above the anthers (L-morph) and the other, stigmas below them (S-morph). Significant differences were found in corolla measurements between the two morphs, with a larger corolla in the S-morph. These two morphs not only differed significantly in the style length as expected, They differed significantly in the stamen height between morphs, with anthers in the S-morph placed

higher (23.3 ± 0.10 mm) than the L ones (20.0 ± 0.00 mm).

In addition to morphological traits, all of the obtained results indicated that the studied populations of *L. album* differ genetically from each other. There were many reasons for these variations which were discussed completely in our previous work (Sheidai et al. 2014), and in this study, only genetic variations between different morphs, long-styled versus short-styled, were investigated.

The obtained data revealed great genetic difference between the two plant forms. Similar study in other *Linum* taxa, for example different subspecies of *L. mucronatum* produced resembling results (Farahani 2013), furthermore, nuclear genome size varied between thrum and pin plant in *L. austriacum* as well as *L. glaucum* (Talebi et al. 2012).

According to Kovalenko et al. (1980), the expression of traits connected to the heterostyly is genetically controlled; however the structure of some flower elements could be modified by environmental conditions (Ciania 1989). Environmental conditions affect populations' genetic structure too, therefore we performed genetic analysis of these populations and the plant morphs to get a better picture of genetic variability of these morphs in the background of population structure.

Hodgins and Barrett (2007), investigated cpDNA sequence and nuclear microsatellite variation among populations of the wild daffodil *Narcissus triandrus* to examine the role of historical vs. contemporary forces in shaping population structure, morphological differentiation and sexual-system evolution. This heterostylous species is composed of two allopatric and populations with either stylar trimorphism or

dimorphism. Dimorphic populations only occur in var. *triandrus* that are mainly restricted to the northwestern portion of the species range, and uniformly lack the mid-styled morph (M-morph). Chloroplast DNA (cpDNA) sequence variation revealed strong geographical structuring and evidence for a fragmentation event associated with differentiation of the two varieties. There were no differences in genetic diversity or population structure between dimorphic and trimorphic populations.

Our findings showed genetic and morphological divergence between the short-styled and the long-styled plants in *Linum album*. The two plant forms differed in both morphological and genetic content in different geographical populations. The change in genetic structure of plant populations may be due to genetic drift, adaptation, etc., which in turn seems has effect on plant morphs in the studied *Linum* species.

Heterostylous species usually possess a sporophytic diallelic incompatibility system, which prevents self- and intra-morph fertilization (Mather and de Winton 1941). Heteromorphic incompatibility system reduces the conflict that can occur in sexually monomorphic animal-pollinated species achieving efficient cross-pollination, but simultaneously avoiding self-interference between female and male sexual organs (Barrett 2002). These mechanisms, by promoting the outcrossing, would have an important influence on diversification and speciation of angiosperms, because effective genetic mechanism preventing inbreeding allowed flowering plants to adapt to different environmental conditions and to accelerate the pace of evolution (Wolko and Slomski 2012). Furthermore, Barrett (1989), stated that the variations in floral morphology and incompatibility system have important

influences on the mating systems of populations and can initiate trait divergence and also speciation. The previous studies (for example, Sheidai et al. 2014; Talebi et al. 2014b) showed high intraspecific variations in morphological and genetic structure between different populations of *L. album*, in addition the results of Talebi et al (2012), study showed that the nuclear genome size as well as qualitative and quantitative palynological features differed between long-styled and short-styled plants in this species. A more complete interpretation of the adaptive significance of heterostyly recognizes different functional roles for the morphological and physiological components of the polymorphism in promoting fitness through male and female function, respectively (Barrett and Shore 2008).

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Table 1. *Linum album* populations, their locality and ecological features.

No.	Name	Locality	Altitude (m)	Longitude	Latitude	Voucher No.
1	Ghargh Abad	Markazi, Saveh, Ghargh Abad, 1464 m, long-style.	1464	35° 07'	49° 44'	2011101 HSBU
2	Ghargh Abad	Markazi, Saveh, Ghargh Abad, 1464 m, short-style.	1464	35° 07'	49° 44'	2011201 HSBU
3	Sanandaj	Kordestan, Sanandaj, 1476 m, long-style.	1476	35° 16'	47° 00'	2011113 HSBU
4	Sanandaj	Kordestan, Sanandaj, 1476 m, short-style.	1476	35° 16'	47° 00'	2011213 HSBU
5	Avaj	Hamedan to Tehran, 50km Avaj, 1898 m, long-style.	1898	35° 24'	49° 01'	2011118 HSBU
6	Avaj	Hamedan to Tehran, 50km Avaj, 1898 m, short-style.	1898	35° 24'	49° 01'	2011218 HSBU
7	Kharaghan	Markazi, Saveh, Kharaghan, 1717m, long-style.	1566	35° 15'	50° 17'	2011168 HSBU
8	Kharaghan	Markazi, Saveh, Kharaghan, 1717m, short-style.	1566	35° 15'	50° 17'	2011268 HSBU
9	Zarandiyeh	Markazi, Zarandiyeh, 2121m, long-style.	2121	35° 11'	50° 10'	2011120 HSBU
10	Zarandiyeh	Markazi, Zarandiyeh, 2121m, short-style.	2121	35° 11'	50° 10'	2011220 HSBU
11	Qazvin	Qazvin, 1408m, long-style.	1408	36° 19'	49° 47'	2011123 HSBU
12	Qazvin	Qazvin, 1408m, short-style.	1408	36° 19'	49° 47'	2011223 HSBU
13	Peyghambar	Markazi, Zarandiyeh, Peyghambar, 1997 m, long-style.	1997	35° 13'	50° 11'	2011121 HSBU
14	Peyghambar	Markazi, Zarandiyeh, Peyghambar, 1997 m, short-style.	1997	35° 13'	50° 11'	2011221 HSBU
15	Chamran	Markazi, Saveh, Chamran, 1783 m, long-style.	783	35° 11'	49° 54'	2011182 HSBU
16	Chamran	Markazi, Saveh, Chamran, 1783 m, short-style.	1783	35° 11'	49° 54'	2011282 HSBU
17	Fasham	Tehran, Fasham, 2100 m, long-style.	2132	33° 65'	55° 00'	2011301 HSBU
18	Fasham	Tehran, Fasham, 2100 m, short-style.	2132	33° 65'	55° 00'	2011401 HSBU

Table 2. Genetic diversity parameters in *Linum album* populations.

Population	Na	Ne	I	PIC	UHe	%P
Ghargh Abad	0.772	1.121	0.103	0.071	0.094	17.07%
Sanandaj	0.634	1.098	0.084	0.057	0.076	13.82%
Avaj	0.683	1.109	0.093	0.064	0.085	15.45%
Kharaghan	0.707	1.092	0.079	0.054	0.072	13.01%
Zarandiyeh	0.764	1.144	0.123	0.084	0.112	20.33%
Qazvin	0.724	1.126	0.108	0.074	0.099	17.89%
Peyghambar	0.764	1.126	0.108	0.074	0.099	17.89%
Chamran	0.829	1.103	0.088	0.061	0.081	14.63%
Fasham	0.870	1.092	0.079	0.054	0.072	13.01%
Total	0.750	1.112	0.096	0.066	0.088	15.90%

Abbreviations: Na = No. of Different Alleles, Ne = No. of Effective Alleles = $1 / (p^2 + q^2)$, I = Shannon's Information Index = $-1 * (p * \ln(p) + q * \ln(q))$, PIC = Polymorphic Information Content = $2 * p * q$, UHe = Unbiased Expected Heterozygosity = $(2N / (2N - 1)) * He$, and % p = percentage of polymorphism.

Table 3. K-Means clustering of *Linum album* populations.

k	SSD(T)	SSD(AC)	SSD(WC)	r-squared	pseudo-F
2*	251.000	56.857	194.143	0.227	4.686
3&	251.000	86.636	164.364	0.345	3.953
4	251.000	108.233	142.767	0.431	3.538

* Best clustering according to Calinski and Harabasz' pseudo-F: k = 2 & Best clustering according to Bayesian Information Criterion: k = 3.

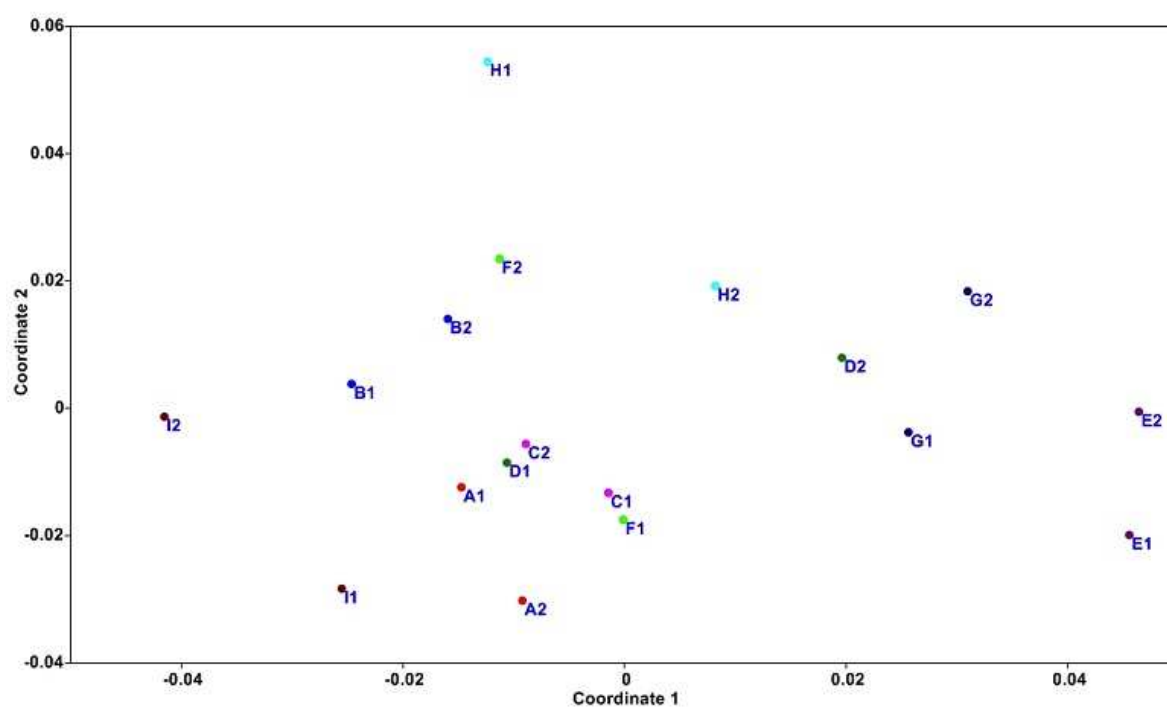


Fig. 1. PCoA of morphological characters in *Linum album* based on distyly.

Populations abbreviations: A = Saveh, B = Sanandaj, C = Avaj, D = Kharaghan, E = Zarandiyeh, F = Qazvin, G = Peyghambar, H = Chamran, and I = Fasham (1 and 2 are the short-styled and the long-styled plants respectively).

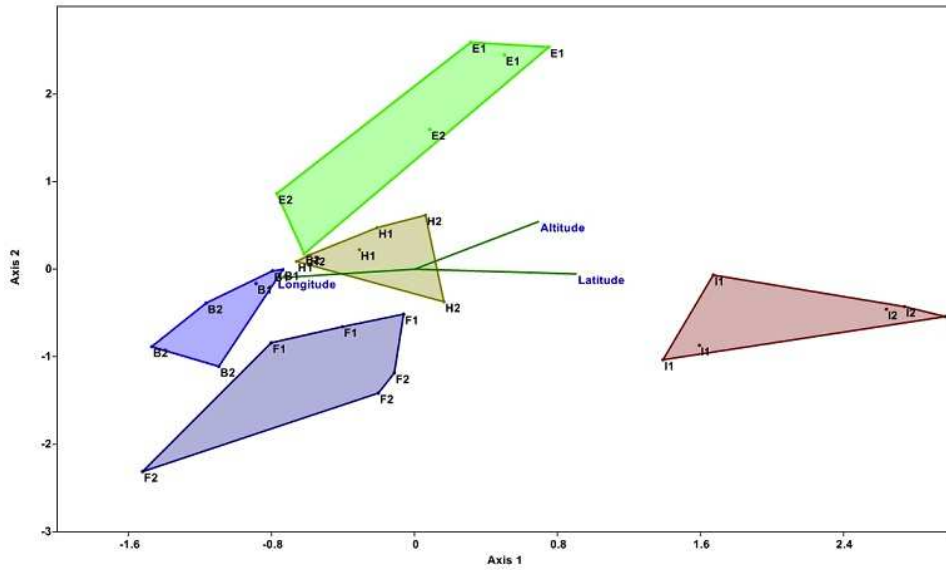


Fig. 2. CCA plot of morphological characters in *Linum album* populations based on distyly. Populations' abbreviations: B = Sanandaj, E = Zarandiyeh, F = Qazvin, H = Chamran, and I = Fasham. (1 and 2 are the short-styled and the long-styled plants respectively).

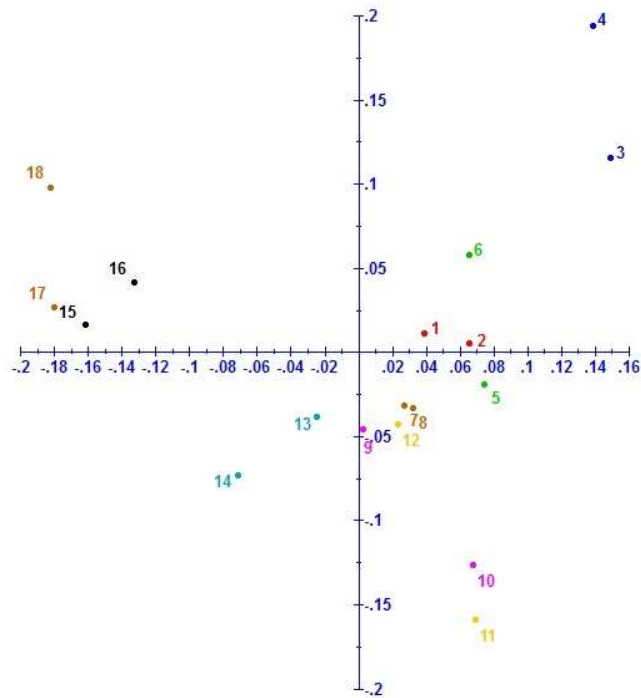


Fig. 3. PCoA plot of ISSR data in *Linum album* populations based on distyly. Populations' code are: 1 & 2 = Saveh, 3 & 4 = Sanandaj, 5 & 6 = Avaj, 7 & 8 = Kharaghan, 9 & 10 = Zarandiyeh, 11 & 12 = Qazvin, 13 & 14 = Peyghambar, 15 & 16 = Chamran and 17 & 18 = Fasham (the first number is the short-styled plant and second number is the long-styled plant in each population).

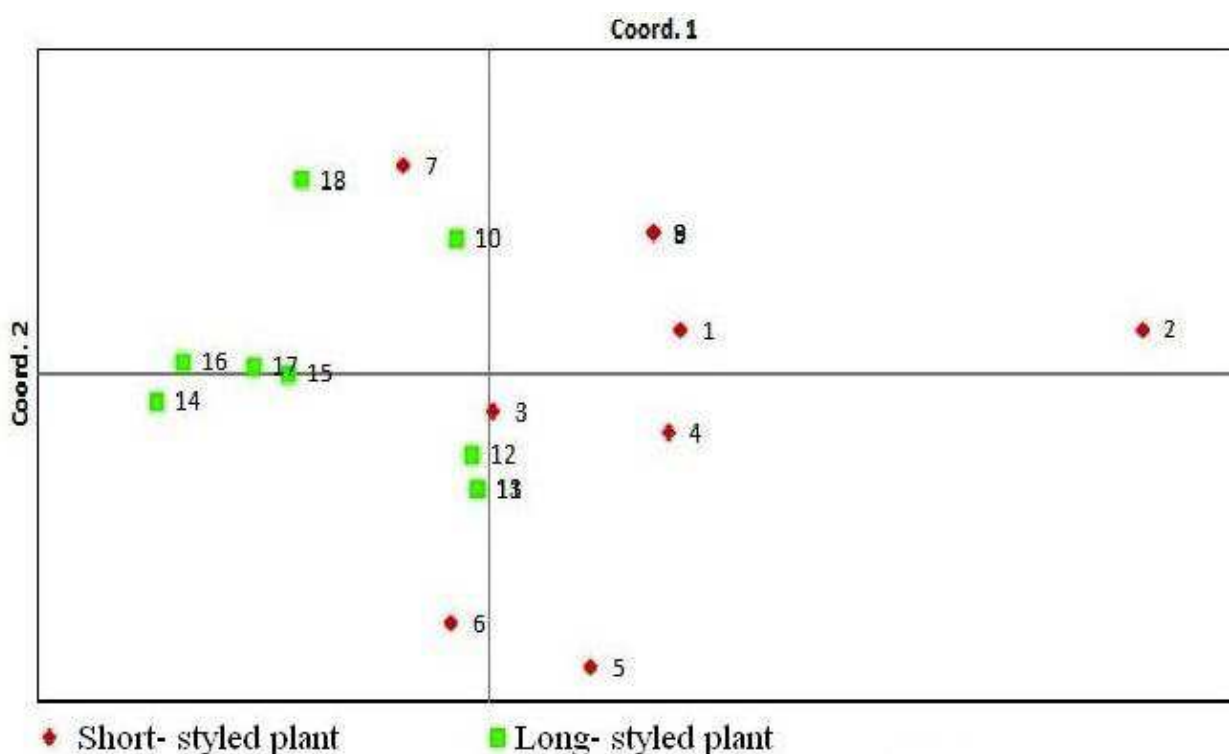


Fig. 4. PCoA plot of distyly in *Linum album* populations based on ISSR data.

Pop1 = short-styled plants, pop2 = long-styled plants. Populations' codes are: 1 & 2 = Saveh, 3 & 4 = Sanandaj, 5 & 6 = Avaj, 7 & 8 = Kharaghan, 9 & 10 = Zarandiyeh, 11 & 12 = Qazvin, 13 & 14 = Peyghambar, 15 & 16 = Chamran and 17 & 18 = Fasham (the first number is the short-styled plant and second number is the long-styled plant in each population).

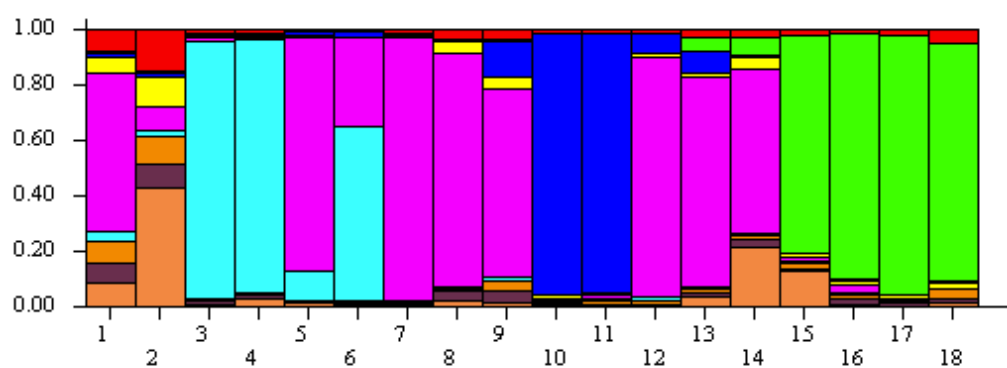


Fig. 5. Structure plot based on $k = 9$ in *Linum album* populations based on distyly.

Populations are: 1 & 2 = Saveh, 3 & 4 = Sanandaj, 5 & 6 = Avaj, and 7 & 8 = Kharaghan, 9 & 10 = Zarandiyeh, 11 & 12 = Qazvin, 13 & 14 = Peyghambar, 15 & 16 = Chamran, 17 & 18 = Fasham (The first number in each population is the short-styled plant and the second number is the long-styled plant).