VERSITA

Biologia **69**/1: 32—39, 2014
Section Botany
DOI: 10.2478/s11756-013-0281-4

Infra-specific genetic and morphological diversity in *Linum album* (Linaceae)

Masoud Sheidai[1]*, Somayeh Ziaee[1], Fatemeh Farahani[1], Seyed-Mehdi Talebi[2], Zahra Noormohammadi[3] & Yeganeh Hasheminejad-Ahangarani Farahani[1]

[1]*Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran; e-mail: msheidai@yahoo.com*
[2]*Department of Biology, Faculty of Sciences, Arak University, Arak, Iran*
[3]*Department of Biology, Science and Research Branch, Islamic Azad University, Teheran, Iran*

Abstract: The genus *Linum* L. (Lineacea) has over 15 species, subspecies or ecotypes in Iran. These species show extensive geographical distribution and form many local populations throughout the country. *Linum album* is herbaceous medicinal plant containing important lignans such as podophyllotoxin (PTOX) and 6-methoxy podophyllotoxin (MPTOX), which have antiviral and anticancer properties. Studying the genetic and morphological diversity of different geographical populations produces detailed knowledge about population divergence and identification of the infra-species taxa if at all they are present. Moreover, the populations that differ in their genetic content and structure may also differ in their chemical and medicinal properties. The present study considers morphological and genetic diversity analyses of 20 *L. album* geographical populations by using nuclear ISSR markers, genome size, and cytogenetic characteristics. These populations differed significantly in many of their quantitative morphological characters and in some of their qualitative features. They also differed significantly in their molecular characteristics and genome size. Details of morphological and molecular variations are reported and discussed.

Key words: genetic variation; ISSR; morphological diversity; wild flax

Introduction

Linum is the largest genus of Linaceae, with about 180 species. These species mainly grow in temperate and subtropical regions of the world (Rogers 1982; Muir & Westcott 2003). *Linum* species have been used as a source of fiber (*L. usitatissimum*), seed oils, fodder and ornamentals. Flax seed oils contain omega-3 fatty acids and anti-cancer compounds (Rogers 1982), while lignans have been identified in flowering aerial parts of these species (Schmidt et al. 2010).

Linum album is an herbaceous medicinal plant containing important lignans such as podophyllotoxin (PTOX) and 6-methoxy podophyllotoxin (MPTOX), which show antiviral and anticancer properties (Seidel et al. 2002). It has been suggested that the roots of *L. album* could be used for mass production of important lignans (Chashmi et al. 2011).

In recent years extensive research has been done to conserve and explore germplasm of crop wild relatives (Maxted et al. 2008). Crop wild relatives are species closely related to crops, including their progenitors, which may contain beneficial traits such as pest or disease resistance and yield improvement (Dwivedi et al. 2008). Fu et al. (2010) state that challenges still exist in conservation and utilization of wild plants, as well as in the species delimitation, and understanding of their genetics and species crossability.

Due to economic importance of flax plant, about 48,000 *Linum* germplasm accessions are maintained in national and international gene banks, including ca. 900 accessions representing 53 species of flax wild relatives (Diederichsen 2007). There have been extensive taxonomic, biosystematic and phylogenetic studies in the genus *Linum* (e.g., Ockendon 1968; Ockendon & Walters 1968; Seetharam 1972; Rogers 1982; Nicholls 1986; Velasco & Goffman 2000; Everaert et al. 2001; Sharifnia & Albouyeh 2002; Wiesnerová & Wiesner 2004; Fu 2006; Hemmati 2007; Rogers 2008; McDill 2009; Fu et al. 2010; Schmidt et al. 2010; Soto-Cerda et al. 2011). Moreover, the genetic diversity analysis has been carried out using ISSR specific diversity in wild relatives of cultivated flax including pale flax (*L. bienne* Mill.) (Uysal et al. 2010). It remains unclear if the populations' genetic divergence leads to the formation of new infra-specific taxonomic forms. Thus, the present study was aimed to investigate molecular, morphological, cytological and genome size characteristics of 20 *L. album* geographical populations in Iran.

* Corresponding author

Table 1. *Linum album* populations and their voucher number.

Populations	Habitat	Voucher No
1	Markazi, Saveh, Ghargh-Abad, 1,464m	2011101
2	Markazi, Saveh, Nobaran, 1,654m	2011102
3	Kurdistan, Sanandaj, Abidar mountain 1,645m	2011112
4	Kordestan, Sanandaj, 1,476m	2011113
5	Kurdistan, Sanandaj to Kamyaran, 1,329m	2011114
6	Hamedan to Tehran, 50km to Avaj, 1,898m	2011118
7	Markazi, Zrandiyeh, Vardeh, 1,566m	2011119
8	Markazi, Zrandiyeh, Noshveh, 2,121m	2011120
9	Markazi, Zrandiyeh, Peyghambar, 1,997m	2011121
10	Qazvin, 1,408m	2011123
11	50 km Abhar to Zanjan, 1,805m	2011131
12	Markazi, Tafresh, 2,237m	2011145
13	Markazi, Saveh, Sankak, 2,237m	2011146
14	Kordestan, Sanandaj, Karehsi, 1,585m	2011157
15	Kordestan, 140km Sanandaj to Saghez,1,620m	2011159
16	Markazi, Saveh, Chamran, 1,783m	2011182
17	Tehran, Fasham, 1,900m	2011187
18	Tehran, Sohanak, 1,900m	2011191
19	Kordestan, Baneh, 1,600m	2011188
20	Hamedan, Malayer, 1,818m	2011152

Material and methods

Plant materials

Extensive field visit and collection was undertaken during 2010-2013 throughout the country and several geographical populations were identified for different wild flax species including *Linum album* Ky. ex Boiss. We collected fresh plant materials from 20 *L. album* populations (Table 1). For morphological studies, 5 randomly collected plants were studied in detail, while for the molecular study fresh leaves were collected from 10 randomly selected plants. These leaves were mixed together and used for DNA extraction. Plant specimens were identified based on descriptions provided in Flora Iranica (Rechinger1974) and Flora of Iran (Sharifnia & Assadi 2001). The voucher specimens were deposited in herbarium of Shahid Beheshti University (HSBU).

Morphometry

Morphological characters studied were: stem height and diameter, branch number, basal leaf shape, the floral leaf shape, size of the width and length of basal and floral leaves, length/width ratio of leaves, the shape of leaf apex, the size of calyx width and length, calyx width /length ratio, the size of sepal width, size of corolla width and length, the corolla width/length ratio, the size of petal width and length, petal width/length ratio, the style length, the length of anther and stamens' filament. The mean value of quantitative characters was measured in at least 10 samples for each population and different forms of qualitative characters were noted any time encountered. The analysis of variance (ANOVA) was performed for quantitative morphological characters to indicate significant difference among populations. UPGMA (Unweighted Paired Group using Average mean) clustering and principal coordinate analysis (PCoA) were performed to group the plants specimens based on morphological characters. Standardized (mean = 0, variance = 1) data were used to determine Manhatan distance among populations followed by clustering.

Cytological study

Suitable flower buds for meiotic analysis could be obtained in four populations of *L. album*. These were fixed and used for cytological investigation by squash method according to our previous report (Sheidai et al. 2012). Meiotic features including polyploidy level, chiasma frequency and distribution as well as chromosome pairing and segregation were determined in plants collected.

Gnome size determination by flow cytometery

The nuclei suspensions were prepared from small amount of mature fresh leaf tissue together with an equal weight of mature leaf tissue of the standard reference. The standard reference used for *L. album* was *Rosa wichurana* which has a 2C DNA value of 1.13 pg (Yokoya et al. 2000). The amount of nuclear DNA of each sample was calculated based on the values of the G1/G2 peak means (Doležel & Bartoš 2005) as follows: Sample 2C peak mean position/Standard 2C peak mean position X Standard 2C DNA amount.

ISSR assay DNA extraction

For molecular studies, fresh leaves were collected randomly in each population and dried in silica gel powder. Genomic DNA was extracted using CTAB activated charcoal protocol (Križman et al. 2006). The extraction procedure was based on activated charcoal and polyvenyl pyrrolidone (PVP) for binding of polyphenolics during extraction and on mild extraction and precipitation conditions, promoting high-molecular weight DNA isolation without interfering contaminants. Quality of extracted DNA was examined by running on 0.8% agarose gel.

Ten ISSR primers; (AGC)5GT, (CA)7GT, (AGC)5GG, UBC 810, (CA)7AT, (GA)9C, UBC 807, UBC 811, (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). Amplifications reactions were performed in Techne thermocycler (Germany) with the following program: 5 min initial denaturation step 94 ℃, 30 s at 94 ℃; 1 min at 50 ℃ and 1 min at 72 ℃. The reaction was completed by final extension step of 7 min at 72 ℃. Amplification products were visualized by running on 2% agarose gel, following ethidium

bromide staining. Fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany).

Data analyses

ISSR bands obtained were treated as binary characters and coded accordingly (presence = 1, absence = 0). Dice as well as Nei's genetic distance (Weising 2005; Freeland et al. 2011), was determined among populations and used for grouping them by unweighted paired group method with arithmetic average (UPGMA) and Neighbor Joining (NJ) clustering methods after 100 times bootstrapping (Freeland et al. 2011). Similarly ordination plot based on principal coordinate analysis (PCoA), and multidimentional scaling (MDS) (Podani 2000) were performed using PAST ver. 2.17 (Hamer et al. 2012) and DARwin ver. 5 (2012). Genetic diversity parameters were determined in each population. These parameters were percentage of allelic polymorphism, allele diversity (Weising 2005), Nei's gene diversity (H), Shannon information index (I) (Weising 2005; Freeland et al. 2011), the number of effective alleles and percentage of polymorphism. AMOVA (Analysis of molecular variance) test (with 1000 permutations) as performed in GenAlex 6.4 (Peakall & Smouse 2006), was used to show molecular difference among the populations. Mantel test (Podani 2000) was performed to study association between molecular distance and geographical distance of the populations.

Results and discussion

Morphometry

Detailed comparison of morphological characters among populations studied showed that, the highest value of stem length (36.4 cm) occurred in populations 19 (Kordestan, Baneh) and 10 (Qazvin), while the lowest value of the same (21.4 cm) occurred in population 13 (Saveh, Sankak). The largest floral leaf size (25.2 mm) occurred in population 19 (Kordestan, Baneh), while the shortest floral leaf size (11.4 mm) was observed in population 17 (Tehran, Fasham). The longest calyx size (12 mm) occurred in population 8 (Markazi, Zrandiyeh, Noshveh) while, the shortest size (8.6 mm) occurred in populations 19 (Kordestan, Baneh), and 3 (Kordestan, Sanandaj, Abidar mountain).

The widest calyx size (3.7 mm) was observed in population 20 (Hamedan, Malayer), but the narrowest calyx size (2.2 mm) occurred in population 10 (Qazvin). Similarly, the highest value of calyx length/width ratio (4.56) occurred in population 10 (Qazvin), while the smallest value of this ratio (2.7) was observed in population 19 (Kordestan, Baneh). The highest (12 mm) and the lowest (8.6 mm) values of sepal length occurred in populations 8, 19 and 3 respectively. The narrowest size of sepal (1.04 mm) occurred in population 10, but the widest sepal size (2.8 mm) occurred in population 20. The longest corolla size (31.6 mm) was observed in population 15 (Kordestan, Sanandaj to Saghez), while, the smallest corolla size (20.5 mm) occurred population17 and 19. Similarly, the lowest (1.16) and highest (2.43) corolla length/width ratio occurred in populations 20 and 8 respectively.

ANOVA test showed significant difference among populations for: The branch number, width size of the basal leaves, width and length size of the floral leaves, as well as for the width and length of calyx and corolla ($p < 0.05$).

Qualitative morphological characters showed variation among populations. For example, in case of basal leaf; some of the populations had linear to spear-shaped leaves, while few populations had only spear-shaped leaves. Moreover, some other populations had reverse spear-shaped leaves, oval-shaped or merely linear-shaped leaves.

Similarly, most of the populations had smear-shaped inflorescence leaves, while some others had linear-shaped, reverse spear-shaped or merely oval-shaped inflorescence leaves. Significant positive correlation was observed between some of the morphological characters, for example, between the width and length of inflorescence leaves and the width of basal leaves ($p < 0.05$). However, significant negative correlation occurred between calyx length and corolla length. Environmental and ecological characteristics of the populations studied differed greatly. For example, altitude of the plants populations studied varied from 2,180 m to 1,329 m, with most of the populations growing above 1,700 m. These plant populations showed mostly southern and northern distribution (rarely western) and pH of soil varied from 6.12 to 7.77. Significant negative correlation was observed between the stem height and slope of the plants habitat ($r = -0.50$, $p < 0.05$), and also between the number of stem branches and eastern distribution of plants ($r = -0.49$, $p < 0.05$). Similarly, the width and length of basal leaves was negatively correlated to the soil pH ($r = -0.47$, $p < 0.05$).

Significant positive correlation occurred between the number of stem branches and soil pH. The corolla length was also positively correlated to the northern distribution of plants and the mean annual temperature ($p < 0.05$).

UPGMA tree and PCoA plot produced similar results; only UPGMA tree is discussed here. The populations studied were placed in 3 major clusters (Fig. 1). Populations 1 (Saveh) and 17 (Fasham) showed morphological similarity and were placed close to each other in the first major cluster. This was followed by populations 14 (Karehsi, Sanandaj) and 3 (Abidar mountain, Sanandaj), both of them occurring in Kordestan province.

The populations 2, 18 and 13 (Nobaran Saveh, Sohanak and Sankak Saveh, respectively) comprised the second sub-cluster of this major cluster, which were followed by populations 5 (Sanandaj to Kamyaran) and 11 (Abhar to Zanjan).

The second major cluster was comprised of 2 sub-clusters. The populations 7 (Vardeh, Zarandiyeh), 12 (Tafresh), 10 (Qazvin) and 8 (Noshveh, Zrandiyeh) formed the first sub-cluster, in which, populations 7 and 12 showed more similarity and joined each other. The other 2 populations joined them with some distance. The populations 9, 15, 16, 6 and 20 formed the sec-

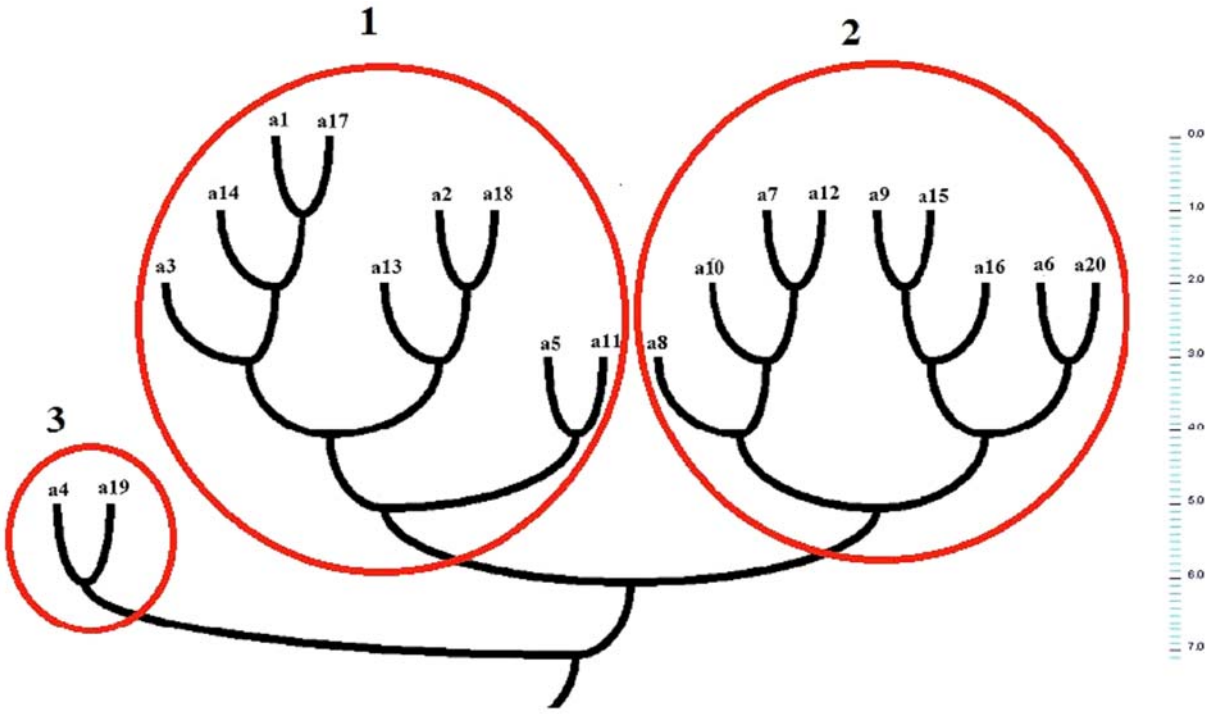

Fig. 1. UPGMA dendrogram of morphological characters showing 3 major clusters. (Numbers a1–a20 are the populations, number according to the Table 1).

Table 2. Meiotic characteristics in *L. album* populations.

Population	N	TOX	IX	TX	ROD	RB	UNI	IV
Tafresh	15	16.36	1.09	15.27	12.36	1.82	1.27	0.09
Tehran	15	20.38	1.17	19.21	9.07	5.07	0.9	0.17
Sankak	15	17.06	0.56	16.5	13.06	1.93	0	0
Salehabad	15	17	0.28	16.71	12.86	1.71	0.28	0.14

TOX, total chiasmata; IX, intercalary chiasmata; TX, terminal chiasmata; ROD, rod bivalents; RB, ring bivalents; UNI, univalents, and IV, quadrivalents.

ond sub-cluster, in which, populations 9 (Peyghambar, Zrandiyeh) and 15 (Sanandaj to Saghez) showed more similarity and were joined each other. The same was true for the populations 6 (Hamedan to Tehran) and 20 (Malayer, Hamedan). The populations 4 (Sanandaj) and 19 (Baneh), both from Kordestan province formed the third major cluster. All these results indicate large morphological diversity among 20 *L. album* populations studied.

Cytology

Results of meiotic study are presented in Table 2 and Fig. 2. Four populations studied showed $n = 15$ ($2n = 2x = 30$) chromosome number supporting Gill (1987) report. The mean value for total chiasmata varied from 16.36 in Tafresh population to 20.38 in Tehran population. These populations had the lowest and highest values of terminal chiasmata too (15.27 and 19.21 respectively).

The lowest value of intercalary chiasma (0.28) occurred in Saleh-abad while, the highest value of the same parameter occurred in Tehran population (1.17). Although these populations are diploid and mostly formed bivalents (Table 1), they formed few univalent and quadrivalents in metaphase I cells (Fig. 2). Quadrivalent formation in diploid plants is due to the occurrence of heterozygote translocations, which may have adaptive value (Sheidai et al. 2012).

Most of the meiotic cells showed normal chromosomes segregation during anaphase and telophase stages. However, laggard chromosomes and micronuclei were formed in some cases (Fig. 2). ANOVA test showed significant difference ($p < 0.05$) for chiasma frequency and chromosome pairing among populations studied. This suggests a change in genetic control of chromosome pairing during population diversification.

Genome size analysis

The mean genome size ranged from 3.51 pg in population 1 (Saveh, Ghargh-abad) to 6.80 pg in population 4 (Kordestan, Sanandaj) (Fig. 3). ANOVA test showed significant difference ($p < 0.05$) in genome size among the populations studied. This indicates a significant change in DNA content during population diversification.

It is interesting to mention that; population 1 with

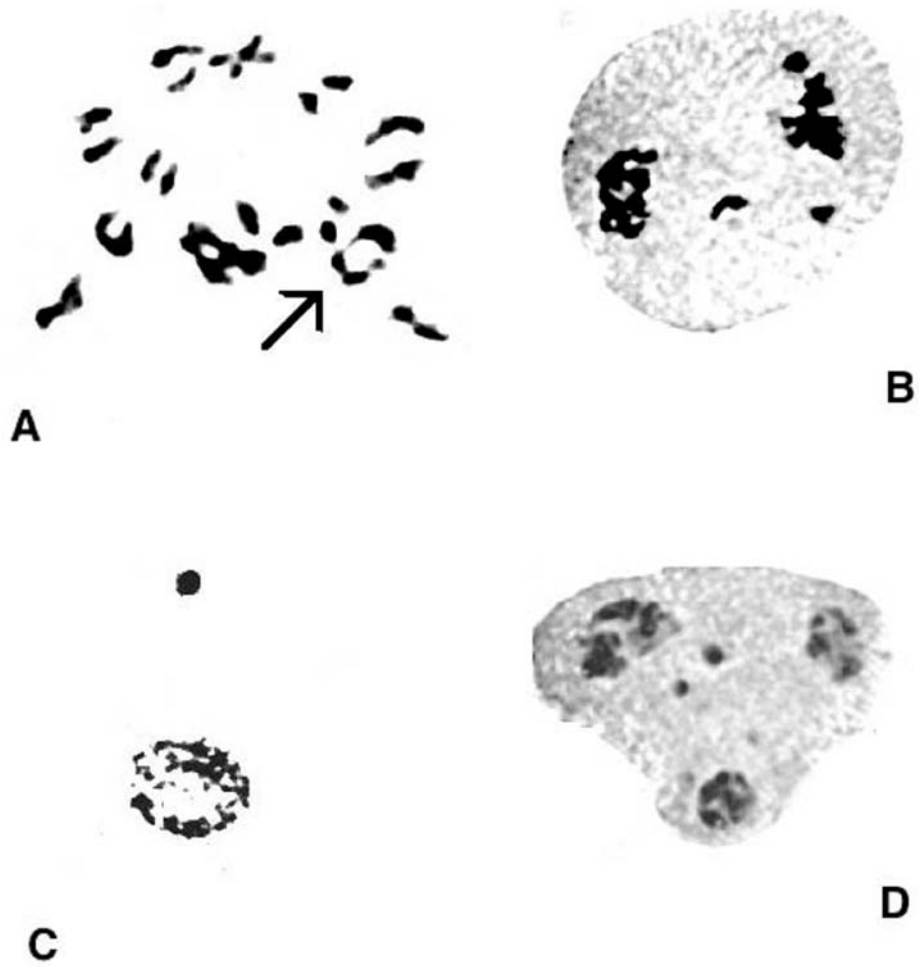

Fig. 2. Representative meiotic cells in *L. album* populations. A – quadrivalent (arrow); B – laggard chromosome; C & D – micronucleus formation.

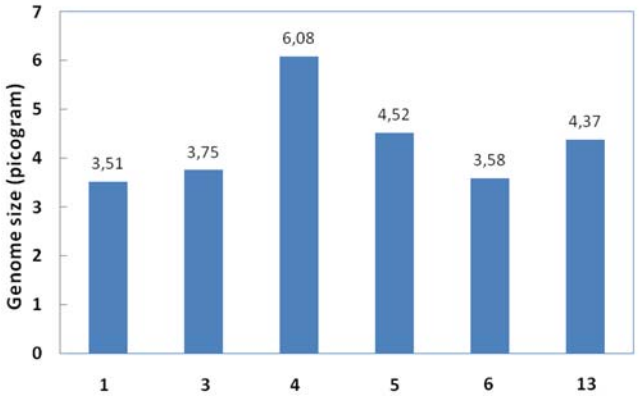

Fig. 3. Genome size (picogram) in *Linum album* populations. The populations' number are according to the Table 1.

Table 3. Genetic diversity parameters in genetic population groups recognized.

Pop	N	Ne	I	He	%P
Pop1	8.000	1.233	0.214	0.139	46.43
Pop2	6.000	1.262	0.246	0.159	51.43
Pop3	2.000	1.131	0.112	0.077	18.57
Pop4	2.000	1.096	0.082	0.056	13.57
Pop5	2.000	1.081	0.069	0.047	11.43
Total	4.000	1.161	0.145	0.096	28.29

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)), He = Expected Heterozygosity = 2*p*q, UHe = Unbiased Expected Heterozygosity = (2N/(2N – 1))*He, Where for Diploid Binary data and assuming Hardy-Weinberg Equilibrium, q = (1 – Band Freq.)$^{0.5}$ and p = 1 – q.

the lowest gnome size was placed in the first major cluster of morphological tree, while the population 4 with the highest genome size was placed in the third major cluster. Other populations viz. 3, 5, 6 and 13 were placed in clusters 1 and 2. We found no correlation between the genome size and morphological characters studied, but significant negative correlation occurred between the genome size and soil pH and EC ($r = 0.90$ and $r = 0.5$, $p < 0.05$, respectively). Therefore, populations with a higher genome size value grow in soil with less pH.

Genetic diversity analysis

All 10 SSR primers produced 140 bands in 20 populations studied. Therefore, a data matrix of 140 X 20 was used for genetic distance determination and clustering. Genetic diversity parameters were determined both based on 5 geographical provinces from which the

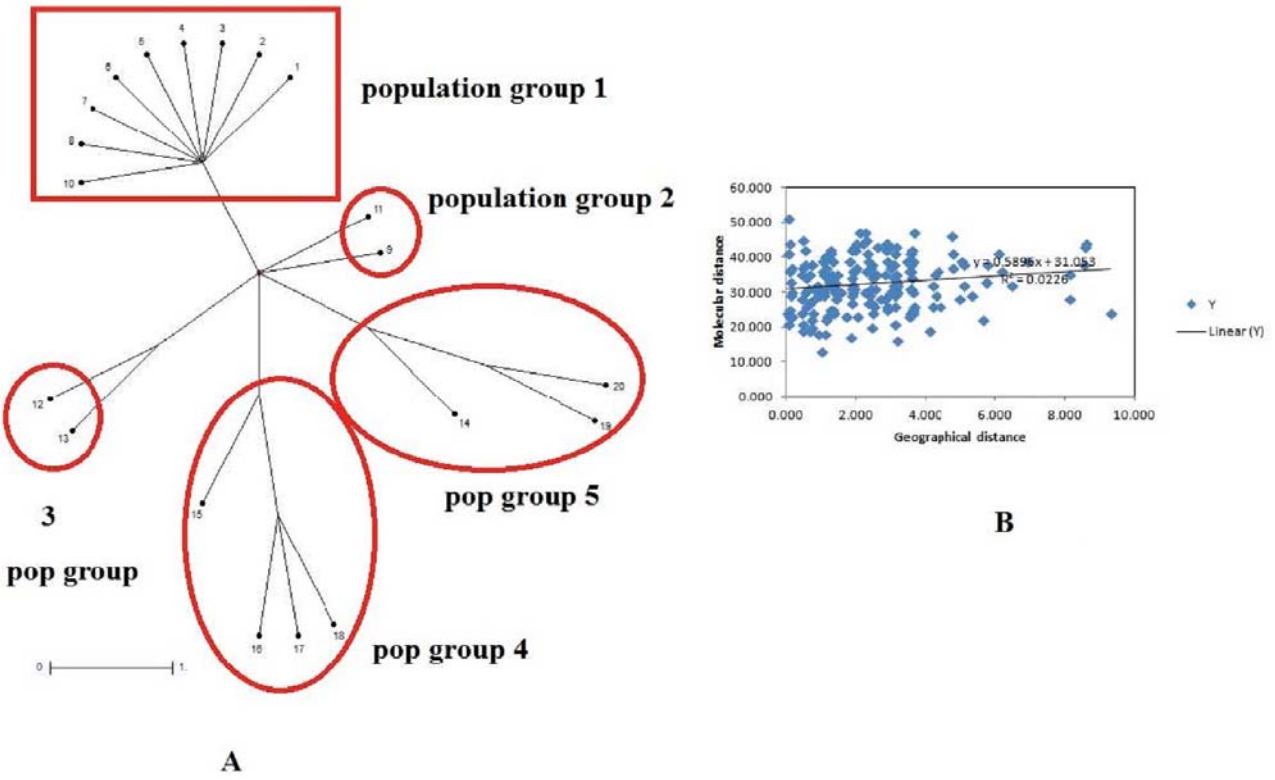

Fig. 4. NJ tree of ISSR data in *Linum album* populations (A) and Mantel test plot between genetic and geographical distance of the population (B).

plant specimens were collected and also between genetic population groups identified by NJ tree. Among the provinces studied, the highest value of effective alleles (1.262) occurred in Kordestan province (pop 2), while the lowest value of the same parameter occurred in province 4 (Ghazvin + Zanjan) and 5 (Tehran) (1.09 and 1.08 respectively). Similarly the highest values for Shanon index, expected heterozygosity and polymorphism percentage occurred in Kordestan province (0.246, 0.159 and 51.43% respectively).

AMOVA test followed by pair-wise comparison showed significant difference among the provinces studied ($p < 0.01$). It showed that 26% of total variation is due to among provinces and 74% due to within populations in each province.

NJ and UPGMA clustering showed genetic differences among the populations in each province as they were separated from each other and were placed close to populations from the other provinces. This result supported the high value of within population variance revealed by AMOVA test as indicated before.

The consensus tree obtained from NJ and UPGMA analyses is presented in Fig. 4; A. Populations were grouped in 5 distinct clusters and were designated population groups 1–5 accordingly. Population group 1 contains populations 1–8 and 10 (Table 1), population group 2 includes population 9 and 11, while population numbers 12 and 13 comprised the population group 3. Similarly, the populations 15-18 formed the population group 4, while populations 14, 19 and 20 comprised the population group 5. As stated before, these population groups contain populations from different provinces.

The highest value for the number of different alleles and the number of effective alleles occurred in population group 1 (populations 1–8 and 10) (1.19 & 1.24 respectively), while the lowest value of the same parameters occurred in population group 3 (populations12 and 13) (0.50 & 1.06 respectively). The highest values for Shanon index and expected heterozygosity (0.23 & 0.15) also occurred in the same population groups. AMOVA test showed significant molecular difference among the population groups ($p = 0.01$). It revealed that 21% of total genetic diversity is due to among population difference, while 79% is due to within populations' genetic difference. These data showed high genetic diversity among *L. album* populations studied.

The Mantel test showed almost positive significant correlation ($p < 0.08$, Fig. 4, B) between genetic distance and geographical distance among the populations. This indicates the genetic difference of *L. album* populations increased with increased geographical distance.

All these findings indicate genetic and morphological divergence of geographical populations in *L. album*. Change in genetic structure of plant populations may also affect their chemical contents and medicinal properties, there fore we may encounter some change in quantity or kind of chemical compounds as well as medicinal properties of these diversified populations. Our future plan is to screen the most divergent populations with regards to lignans and omega-3 contents.

Taxonomic consideration (infra-specific forms)
Populations 19 and 20 which were separated from the other populations in both morphological and molecular analyses, showed distinct morphological characters. For example, the population 19 (Kordestan, Baneh) had the highest value of stem length, the largest floral leaf size, the smallest corolla size, the shortest calyx size, the smallest calyx length/width ratio and the lowest size, the widest sepal size, the smallest corolla length/width ratio, the shortest petal size and the largest style size. Ockendon (1971) 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 27 morphological characters in several populations of *L. tenuifolium* and found that, these characters varied within this species. He interpreted the results within the context of reproductive biology, chromosome number and ecological response of populations and confirmed recognized sub-specific taxa in this species.

In the present study we also encountered intermixed position of some of the plant specimens from one population with plant specimens from another population in UPGMA tree (not presented here). This was mainly due to continuity of quantitative morphological characters studied. However, populations 19 and 20 not only differed significantly from other populations in these morphological characters (as stated before), they also differed in their molecular characteristics. Therefore, we consider them as potential new subspecies within *Linum album*, which will be introduced in following publications in which we shall provide their detailed description.

References

Chashmi N.A., Sharifi M., Yousefzadi M., Behmanesh M. & Palazon J. 2011. The production of cytotoxic lignans by hairy root cultures of *Linum album*. World Academy of Science, Engineering Tech. **56:** 401–402.

Diederichsen A. 2007. Ex situ collection of cultivated flax (*Linum usitatissimum* L.) and other species of the genus *Linum* L. Genet. Resour. Crop Evol. **54:** 661–678.

Doležel J. & Bartoš J. 2005. Plant DNA flow cytometry and estimation of nuclear genome size. Ann.Bot. **95:** 99–110.

Dwivedi S.L., Hari D., Upadhyaya H., Stalker T., Blair M.W., Bertioli D.J., Nielen S. & Ortiz R. 2008. Enhancing crop gene pools with beneficial traits using wild relatives. Plant Breed. Rev. **30:** 179–230. Everaert I., de Riek J., de Loose M., van Waes J. & van Bockstaele E. 2001. Most similar variety grouping for distinctness evaluation of flax and linseed (*Linum usitatissimum* L.) varieties by means of AFLP and morphological data. Plant Variet. Seeds **14:** 69–87.

Freeland J.R., Kirk H. & Peterson S.D. 2011. Molecular Ecology, 2nd ed., Wiley-Blackwell, UK, 449 pp.

Fu Y.B. 2006. Redundancy and distinctness in flax germplasm as revealed by RAPD dissimilarity. Plant Genet. Resour. **4:** 117–124.

Fu Y.B. & Allaby R.G. 2010. Phylogenetic network of *Linum* species as revealed by non-coding chloroplast DNA sequences. Genet. Resour. Crop Evol. **57:** 667–677.

Fu Y.B., Diederichsen A. & Allaby R. 2012. Locus-specific view of flax domestication history. Ecol. Evol. **2:** 139–152.

Gill K.S. 1987. Linseed. Indian Council of Agricultural Research, New Delhi, India

Hamer Ř., Harper D.A.T. & Ryan P.D. 2012. PAST: Paleontological Statistics software package for education and data analysis. Paleontol. Electronica **4:** 9.

Hemmati Sh. 2007. Biosynthesis of lignans in plant species of the section *Linum*: pinoresinol- lariciresinol reductase and justicidin B 7-hydroxylase. Ph. D. Thesis, Faculty of Mathematics and Natural Sciences, Heinrich-Heine University, Düsseldorf, Germany.

Križman M., Jakse J., Baricevic D., Javornik B. & Prošek M. 2006. Robust CTAB-activated charcoal protocol for plant DNA extraction. Acta Agri. Slov. **87:** 427–433.

Maxted N., Ford-Lloyd B.V., Kell S.P., Iriondo J.M., Dulloo M.E. & Turok J. 2008. Crop Wild Relative Conservation and Use, CABI Publishing Series, Oxfordshire 720 pp.

McDill J.R. 2009. Molecular phylogenetic studies in the Linaceae and *Linum*, with implications for their systematics and historical biogeography. Doct. dissert., University of Texas at Austin. Available electronically from http: //hdl.handle.net/2152/ETD-UT-2009-12-657.

Muir A.D. & Westcott N.D. (eds) 2003. Flax: The genus *Linum*. Taylor and Francis Inc., London, UK and NY, USA, 307 pages.

Nicholls M.S. 1986. Variation and evolution in *Linum tenuifolium* (Linaceae). Plant Syst. Evol. **153:** 243–258.

Ockendon D.J. 1968. Biosystematic studies in *Linum perenne* group. New Phytol. **67:** 787–813.

Ockendon D.J. & Walters S.M. 1968. *Linum*. In: Tutin T.G., Heywood V. H., Burges N.A., Moore D.M., Valentine D. H., Walters S.M. & Webb D.A. (eds), Flora Europaea, Cambridge University Press, Cambridge, vol. 2, pp. 206–211.

Pritchard J.K., Stephens M. & Donnelly P. 2000. Inference of population structure using multilocus genotype Data. Genetics **155:** 945–959.

Podani J. 2000. Introduction to the Exploration of Multivariate Data, Backhuyes, Leide Ltd.

Rogers C.M. 1982. The Systematics of *Linum* sect. *Linopsis* (Linaceae). Pl. Syst. Evol. **140:** 225–234.

Rogers C.M. 2008. A revision of the genus *Linum* in southern Africa. Nordic J. Bot. **1:** 711–722.

Schmidt T.J., Hemmati S., Klaes M., Konuklugil B., Mohagheghzadeh A., Ionkova I., Fuss E. & Wilhelm Alfermann A. 2010. Lignans in flowering aerial parts of *Linum* species. Chemodiversity in the light of systematics and phylogeny. Phytochem. **71:** 1714–28. doi: 10.1016/j.phytochem.2010.06.015.

Seetharam A. 1972. Interspecific hybridization in *Linum*. Euphytica **21:** 489–495.

Seidel V., Windhövel J., Eaton G., Alfermann A.W., Arroo R.R., Medarde M., Petersen M. & Woolley J.G. 2002. Biosynthesis of podophyllotoxin in *Linum album* cell cultures. Planta **215:** 1031–1039.

Semenova O.Y., Khotyuleva L.V. & Zelenin A.V. 2003. Genome comparisons with chromosomal and molecular markers for three closely related flax species and their hybrids. Russian J. Genet. **39:** 414–421. Sharifnia F. & Albouyeh R.M. 2002. Anatomical studies in relation to taxonomy of Persian *Linum* species. Pakistan J. Biol. Sci. **5:** 1240–1245.

Sharifnia F. & Assadi M. 2001. Flora of Iran, No. 34: Linaceae. Research Inst. Forests and Rangelands, Ministry of Jahad-e-Sazandegi, Iran (in Farsi).

Soto-Cerda B.J., Saavedra H.U., Navarro C.N. & Ortega P.M. 2011. Characterization of novel genic SSR markers in *Linum usitatissimum* (L.) and their transferability across eleven *Linum* species. Plant Biotech. **14:** 0717–3458.

Uysal H., Fu Y.B., Kurt O., Peterson G.W., Diederichsen A. & Kusters P. 2010. Genetic diversity of cultivated flax (*Linum usitatissimum* L.) and its wild progenitor pale flax (*Linum bienne* Mill.) as revealed by ISSR markers. Genetic Crop Evol. **57:** 1109–1119.

Velasco L. & Goffman F.D. 2000. Tocopherol, plastochromanol and fatty acid patterns in the genus *Linum*. Plant Syst. Evol. **221:** 77–88.

Vromans J. 2006. Molecular genetic studies in flax (*Linum usitatissimum* L.). PhD thesis, Wageningen University, The Netherlands.

Weising K., Nybom H., Wolff K. & Kahl G. 2005. DNA Fingerprinting in Plants. Principles, Methods, and Applications (2nd ed.), Taylor & Francis, 444 pp.

Wiesnerová D. & Wiesner I. 2004. ISSR-based clustering of cultivated flax germplasm is statistically correlated to thousand seed mass. Mol. Biotech. **26:** 207–214.

Wilson I.J. & Balding D.J. 1998. Genealogical inference from microsatellite data. Genetics **150:** 499–510.

Yokoya K., Roberts A.V., Mottley J., Lewis R. & Brandham P.E. 2000. Nuclear DNA amounts in Roses. Ann. Bot. **85:** 557–561.

Received April 26, 2013
Accepted July 19, 2013