Genetic diversity, population structure and morphological variability in the *Lallemantia royleana* (*Lamiaceae*) from Iran

Fahimeh Koohdar[1], Masoud Sheidai[1], Seyed Mehdi Talebi[2], Zahra Noormohammadi[3] & Somayyeh Ghasemzadeh-Baraki[1]

[1] Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran, e-mail: f_koohdar@yahoo.com (corresponding author), msheidai@yahoo.com

[2] Biology Department, Arak University, Arak, Iran, e-mail: seyedmehdi_seyedmehdi_talebi@yahoo.com

[3] Biology Department, Islamic Azad University. Sciences and Research Branch, Tehran, Iran, e-mail: bahar.gh127@googlemail.com

Received: January 29, 2015 ▷ Accepted: February 15, 2016

Abstract. Genetic variability and population structure were studied in 11 geographical populations of *Lallemantia royleana* Benth. (*Lamiaceae*). DNA was extracted from 102 plant specimens and used for ISSR assay. Genetic diversity parameters were determined in these populations. AMOVA and Gst analyses revealed the presence of extensive genetic variability within populations and significant molecular difference among the studied populations. Mantel test showed positive significant correlation between genetic distance and geographical distance of the populations. STRUCTURE and K-means clustering revealed populations genetic stratification. Networking showed some degree of gene exchange among the studied populations. UPGMA dendrogram of the populations based on morphological characters was in agreement with the NJ tree of molecular data. These results indicated that geographical populations of *Lallemantia royleana* are well differentiated both in genetic content and morphological characteristics. This information may be of use for future conservation and breeding of this medicinally important plant species.

Key words: gene flow, genetic admixture, *Lallemantia royleana*, medicinal plant

Introduction

The genus *Lallemantia* L. (*Lamiaceae*) is included in *Nepetinae* and contains herbaceous annual or biennial plants. It is characterized by simple leaves; a thyrsoid, spike-like or oblong, often interrupted inflorescence; ovate to rotund or occasionally linear, aristate-toothed bracteoles; and oblong, trigonous, smooth and mucilaginous nutlets (Harley & al. 2004). The *Lallemantia* species can be used for a variety of purposes, including for nutrition and healing. For example, *Lallemantia iberica* Fisch. & C.A. Mey. is cultivated in Iran and southern parts of the former USSR as an oil-seed plant (Rivera-Nunez & Obonde-Gastro, 1992).

Lallemantia is represented by five species that are distributed in Afghanistan, China, India, Kazakhstan, Kyrgyzstan, Pakistan, Iran, Russia, Tajikistan, Turkmenistan, Uzbekistan, SW Asia, and Europe. *L. royleana* Benth. is an annual herb, commonly known as Lady's Mantle. This medicinally important plant is originally native to tropical Asia, Afghanistan, Turkestan, India, and Pakistan. Traditionally, it is a very common practice of local people to use these plants to cure infectious diseases. Seeds contain carbohydrates, fiber, oil, protein, and tannins (Razavi & Karazhiyan 2009; Razavi & Moghaddam 2011). They displayed a significant anti-bacterial effect and can be used as a good remedy for skin diseases and gastrointesti-

nal problems caused by human pathogenic bacterial strains (Mahmood & al. 2013). *L. royleana* grows in different regions of Iran and comprises many local geographical populations.

The advent of molecular markers has resulted in an improved ability to track evolution through a better understanding of genetic variation within and among populations and added phylogenetic perspectives. These molecular markers produce valuable data for plant biology in general and also for specific purposes, such as species and populations, divergence, genetic drift and migration, genetic fingerprinting, etc. (Heather & al. 2011). Molecular markers have been used extensively in genetic diversity analysis, as well as in populations genetic structure (Sheidai & al. 2012, 2013). We have used ISSR molecular markers in the present study as they have proved to be informative about the genetic diversity and population structure studies (e.g. Sheidai & al. 2012, 2013; Azizi & al. 2014).

Due to medicinal and economic importance of *L. royleana*, it is frequently used by local people and therefore in the long term is subjected to elimination. The goal of the present study was to reveal genetic variability both within and among *L. royleana* local populations, to reveal population genetic structure, and study the gene flow among geographical populations. Similarly, we have investigated morphological diversity of the studied populations and whether these morphological variations accompany genetic diversity in divergent populations.

Material and methods

Plant material

Eleven geographical populations have been identified. Each population contained 40–50 plants. Altogether, 102 plants were randomly collected from these populations and used for molecular and morphological studies. Details of localities are provided in Table 1, Fig. 1. Voucher specimens are deposited in the Herbarium of Shahid Beheshti University (HSBU). Fresh leaves were collected and used for DNA extraction and molecular study.

DNA extraction and ISSR assay

Fresh leaves were collected randomly from 10 plants in each of the studied populations and dried in silica gel powder. Genomic DNA was extracted using CTAB activated charcoal protocol (Sheidai & al. 2013). The quality of extracted DNA was examined by running on 0.8 % agarose gel. Ten ISSR primers – (AGC)5GT, (CA)7GT, (AGC)5GG, UBC810, (CA)7AT, (GA)9C, UBC807, UBC811, (GA)9T, 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 amplification reactions were performed in Techne thermocycler (Germany) with the following program: 10 min initial denaturation step 94 °C, 30 S at 94 °C; 1 min at 57 °C and 1 min at 72 °C. The reaction was completed by final extension step of 7 min at 72 °C.

The amplification products were visualized by running on 2 % agarose gel, followed by ethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany).

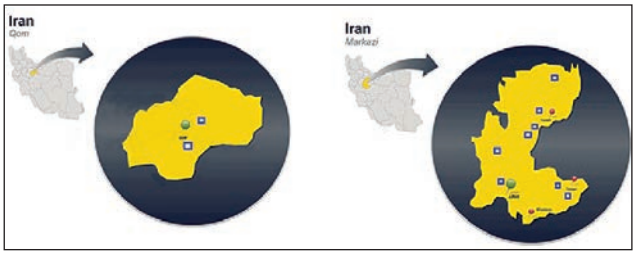

Fig. 1. Distribution map of *Lallemantia royleana* populations.

Table 1. Populations, their locality and ecological features.

	Province	Locality	Altitude (m)	Longitude	Latitude
1	Markazi	Delijan, near Fojoor	1523	33.55	50.37
2	Markazi	Saveh-Salafchegan	1440	34.34	50.25
3	Markazi	Zarandiueh, Roodeshoor village	1267	35.30	50.29
4	Markazi	Saveh, Samavak village	1248	35.01	50.06
5	Markazi	Mahallat, Abegarm	1725	33 .59	50.33
6	Markazi	Tehran-Saveh	1442	35.12	50.24
7	Qom	Tehran-Qom	1080	35.13	51.07
8	Markazi	Delijan, Nimvar village	1574	33.53	50.31
9	Markazi	Mahallat, opposite the University	1680	33.54	50.29
10	Qom	Delijan to Salafchegan	1364	34.21	50.30
11	Markazi	Arak, Hoseinabad	1777	34.04	49.47

Data analyses

Morphological studies

The studied morphological characters were: 1 – qualitative characters: habitat, type of stem, stem color, shape of basal leaf, base of basal leaf, tip of basal leaf, shape of stem leaf, position of inflorescence leaves relative to the inflorescence whorls, nutlet color. 2 – quantitative characters: plant height, length of basal leaf, width of basal leaf, length of petiole, length of stem leaf, width of stem leaf, length of petiole in stem leaf, length of bracteole, width of bracteole, size of bract arista, number of veins in calyx, size of tooth in calyx, length of calyx, width of calyx, length of corolla, width of corolla, length of nutlet, width of nutlet, length of areole, length of style, length of stamen, length of middle lobe in corolla, width of middle lobe in corolla.

An analysis of variance (ANOVA) test was performed to show the significant morphological difference between the studied populations. For grouping the plant specimens, UPGMA (unweighted paired group with arithmetic average) and CVA (Canonical Variates Analysis) were used. Morphological data were standardized (mean = 0, variance = 1) for these analyses (Podani 2000). A principal components analysis (PCA) was performed to identify the most variable morphological characters among the studied populations.

Molecular analyses

The obtained ISSR bands were coded as binary characters (presence = 1, absence = 0). Genetic diversity parameters were determined for dominant molecular markers in each population. These parameters were: percentage of allelic polymorphism, allele diversity, Nei's gene diversity (H), genetic diversity due to populations (Hs), Shannon information index (I), number of effective alleles, and percentage of polymorphism (Weising 2005). Nei's genetic distance was determined among the studied populations and used for clustering. For grouping the plant specimens, Neighbor Joining (NJ) clustering methods as well as Neighbor-Net method of networking were performed after 100 times bootstrapping (Huson & Bryant 2006).

The Mantel test was performed to check correlation between geographical distance and genetic distance of the studied populations (Podani 2000; Weising 2005). PAST ver. 2.17 (Hamer & al. 2012), DARwin ver. 5 (2012) and SplitsTree4 V4.13.1 (2013) programs were used for these analyses.

The significant genetic difference among the studied populations and provinces were determined by: 1 – AMOVA (analysis of molecular variance) test (with 1000 permutations) for dominant molecular markers as implemented in GenAlex 6.4 (Peakall & Smouse 2006); 2 – Nei's Gst analysis of dominant markers as implemented in GenoDive ver.2 (2013) (Meirmans & Van Tienderen 2004). Furthermore, the populations' genetic differentiation was studied by G'ST est = standardized measure of genetic differentiation (Hedrick 2005), and D_est = Jost measure of differentiation (Jost 2008).

In order to overcome potential problems caused by the dominance of ISSR markers, a Bayesian program, Hickory (ver. 1.0) (Holsinger & al. 2003), was used to estimate the parameters related to genetic structure (Theta B value). Three runs were conducted with default sampling of parameters (burn-in = 50 000, sample = 250 000, thin = 50) to ensure consistency of results (Tero & al. 2003).

The genetic structure of geographical populations and provinces was studied by two methods. First, we carried out a structure analysis (Pritchard & al. 2000) for dominant markers (Falush & al. 2007). Second, we performed K-means clustering as in GenoDive ver. 2. (2013).

Model-based clustering, as performed by STRUCTURE software ver. 2.3 (Pritchard & al. 2000), was carried out to group the studied populations on the basis of genetic affinity. That program was also used to reveal the genetic admixture of studied populations. For this analysis, an admixture ancestry model under the correlated allele frequency model was used. The Markov chain Monte Carlo simulation was run 20 times for each value of K (2–11) for 20 iterations after a burn-in period of 10^5. All other parameters were set at their default values. Data were scored as dominant markers and analysis followed the method suggested by Falush & al. (2007). STRUCTURE Harvester web site (Earl & von Holdt 2012) was used to visualize the STRUCTURE results and also to perform Evanno method to identify the proper number of K (Evanno & al. 2005). The most likely number of clusters (K) was chosen by comparing log probabilities of data [Pr (X|K)] for each value of K (Pritchard & al. 2000), as well as by calculating an ad hoc statistic ΔK based on the rate of change in the log probability of data between successive K values, as described by Evanno & al. (2005). In K-means clustering, the optimal clustering is the one

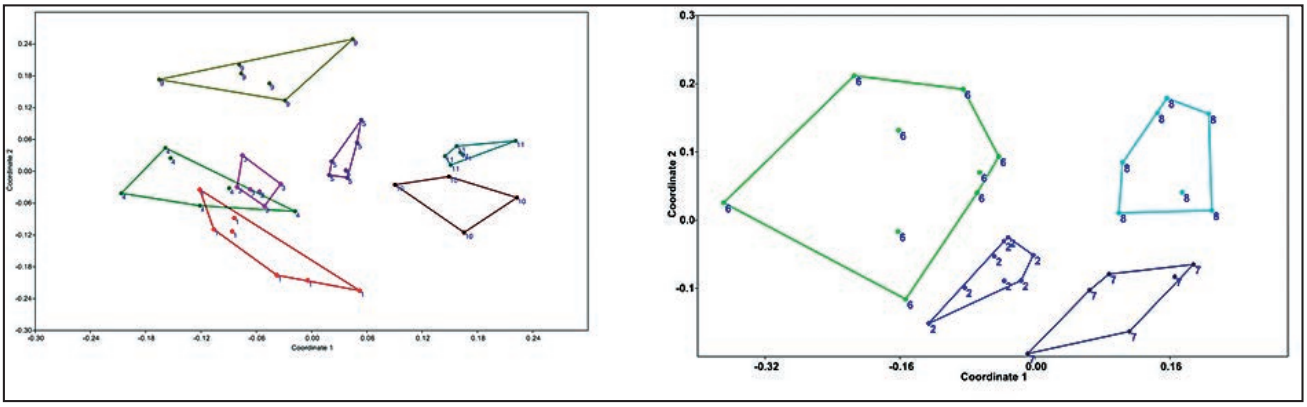

Fig. 2. MDS plots of genetic data, showing populations, genetic differentiation. Populations 1–11 are: Delijan, near Fojoor, Saveh-Salafchegan, Roodeshoor village, Samavak village, Abegarm, Tehran-Saveh, Tehran-Qom, Nimvar village, Mahallat, opposite university, Delijan to Salafchegan, and Arak, respectively.

with the smallest amount of variation within clusters. This is calculated by using the within-clusters sum of squares. Minimization of the within-groups sum of squares that is used in K-means clustering, in the context of a hierarchical AMOVA, is equivalent to minimizing the among-populations-within-groups sum of squares, SSDAP/WG (Meirmans 2012).

We used two summary statistics to present K-means clustering, 1 – pseudo-F (Calinski & Harabasz 1974); and 2 – Bayesian Information Criterion (BIC) (Schwarz 1978). The clustering with the highest value for pseudo-F is regarded as providing the best fit, while clustering with the lowest value for BIC is regarded as providing the best fit (Meirmans 2012). Similarly, non-metric multidimentional scaling (MDS) (Podani 2000) was performed to study genetic distinctiveness of the provinces. The occurrence of gene flow among populations was checked out by different methods. First, we performed indirect Nm analysis of POPGENE ver. 2 for ISSR loci studied according to the following formulae: Nm = estimate of gene flow from Gst , Nm = 0.5(1 – Gst)/Gst. Then we used reticulation (Legendre & Makarenkov 2002) and NeighborNet analyses (Huson & Bryant 2006).

Recently, Frichot & al. (2013) have introduced a statistical model called latent factor mixed models (LFMM) that tests correlations between environmental and genetic variation, while estimating the effects of hidden factors that represent background residual levels of population structure. We used this method to check if ISSR markers show correlation with environmental features of the studied populations. The analysis was done by LFMM program Version: 1.2 (2013).

Results

Populations' genetic diversity

We obtained high number of reproducible bands from almost all used ISSR primers and finally a data matrix of 102 × 59 was formed for further analysis. A DCA plot revealed (Fig. 2.) scattered distribution of the studied ISSR loci, which indicated that these loci are not linked and are suitable for population genetic structure analysis.

Genetic diversity parameters determined in 11 geographical populations of *Lallemantia royleana* are presented in Table 2. The highest value of percentage polymorphism (55.17 %) was observed in Delijan, Nimvar village (population No. 8). This population al-

Table 2. Genetic diversity parameters in the studied populations.

	Population	Ne	I	h	P%	Structure Fst	Hs in AMOVA
1	Delijan, near Fojoor	1.321	0.272	0.185	48.28	0.343	0.223
2	Saveh-Salafchegan	1.194	0.177	0.116	36.21	0.4895	0.147
3	Zarandiueh, Roodeshoor village	1.162	0.149	0.097	31.03	0.2492	0.129
4	Saveh, Samavak village	1.236	0.221	0.143	46.55	0.5184	0.205
5	Mahallat, Abegarm	1.212	0.17	0.117	29.31	0.3199	0.13
6	Tehran-Saveh	1.240	0.232	0.149	51.72	0.4923	0.216
7	Tehran-Qom	1.290	0.258	0.171	50.00	0.5005	0.236
8	Delijan, Nimvar village	1.285	0.267	0.174	55.17	0.3466	0.236
9	Mahallat, opposite the University	1.211	0.196	0.128	39.66	0.4331	0.190
10	Delijan to Salafchegan	1.172	0.165	0.107	32.76	0.3141	0.184
11	Arak, Hoseinabad	1.193	0.169	0.113	32.76	0.4924	0.149

so had the highest values of the Shanon' information index (0.267) and genetic diversity due to populations (Hst), as estimated in AMOVA (0.267) (this will be discussed subsequently).

Roodeshoor village population (Population 3) had the lowest values for percentage polymorphism (31.03), Shanon' information index (0.149), and Hs (0.129).

Gst analysis revealed that the total genetic diversity obtained was 2.045, with the mean value of 0.185. Genetic diversity due to each population (Hs) ranged from 0.129 in Population 3, to 0.236 in Populations 7 and 8.

AMOVA test (Table 3) revealed the presence of a significant molecular difference among the studied populations (PhiPT = 0.37, P = 0.01). It also revealed that 37% of the total genetic variability occurred among the studied populations, while 63% occurred within these populations. The AMOVA result was supported by Gst analysis and Hickory test. The Gst value obtained among the populations after 999 permutations was 0.354 (p = 0.001), while Hickory test produced a theta-II value of 0.50. This value is also very high and significant. Populations' differentiation parameters determined among the studied populations produced high values for Hedrick' standardized fixation index after 999 permutation (G'st = 0.443, P = 0.001) and Jost' differentiation index (D-est = 0.138, P = 0.001). These results indicate that the geographical populations of *Lallemantia royleana* are genetically differentiated from each other.

The significant difference value obtained by AMOVA and Gst tests may be due to genetic difference between only two populations. Therefore, we performed pair-wise Fst and Gst analyses. Both tests produced a significant difference (p = 0.01) for all pair-wise population'comparisons.

Table 3. AMOVA test of the studied populations.

Source	df	SS	MS	Est. Var.	%
Among Pops	10	259.428	25.943	3.188	37
Within Pops	60	326.375	5.440	5.440	63
Total	70	585.803	—	8.628	100
Stat	Value	P(rand > = data)			
PhiPT	0.370	0.010			

* Best clustering, according to Calinski & Harabasz' pseudo-F: k = 2.
Best clustering, according to Bayesian Information Criterion: k = 4.

Genetic affinity of the populations

Nei' genetic identity versus genetic distance of the studied populations showed closer genetic similarity (0.925) between the populations of Delijan and Saveh-Salafchegan (Populations 1 and 2), and between Roodeshoor village and Saveh populations (Populations 3 and 4) (0.920), respectively.

Similarly, the lowest value for genetic similarity (0.75) occurred between Nimvar village and Delijan-Salafchegan populations (Populations 8 and 10), followed by Nimvar village and Arak populations (Populations 8 and 11–0.76).

The grouping of the plant populations obtained by NJ tree based on Nei' genetic distance is presented in Fig. 3. It produced two major clusters. Populations 1–6 (Delijan, Saveh-Salafchegan, Roodeshoor village, Saveh, Mahallat and Tehran-Saveh, respectively) comprised the first major cluster. In this cluster, Populations 1 and 2 (Delijan and Saveh-Salafchegan populations) showed higher genetic similarity and were joined to each other. The same holds true for Populations 3 and 4 (Roodeshoor village and Saveh populations).

Populations 7–11 (Tehran-Qom, Nimvar village, Mahallat, Delijan to Salafchegan and Arak popula-

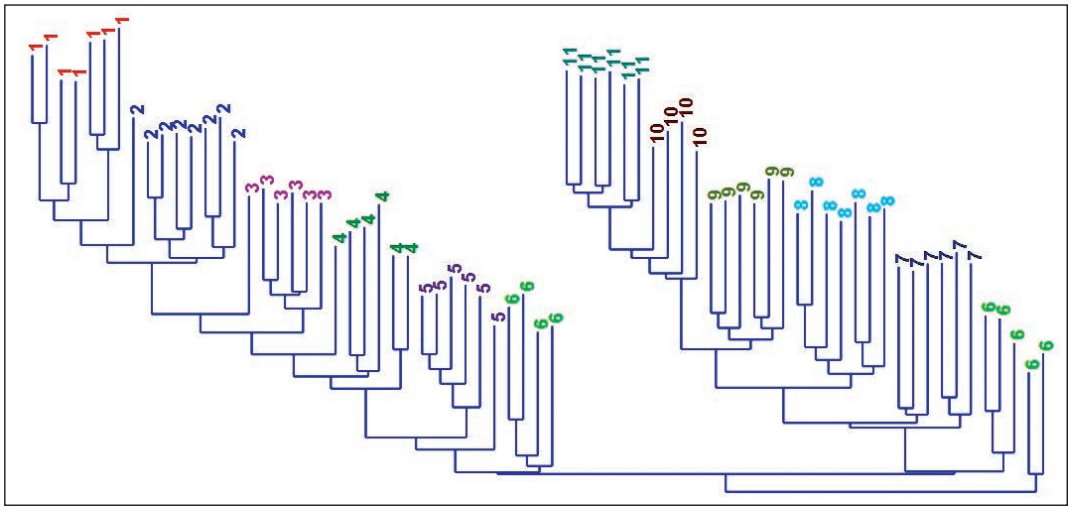

Fig. 3. NJ tree of the studied populations based on ISSR data. (Populations 1–11 are according to Table 1).

tions, respectively) formed the second major cluster. In this cluster, Populations 10 and 11 showed higher genetic affinity.

A Mantel test for the populations genetic distance and their geographical distance (determined from pair-wise difference in populations coordinates by GeneAlex program) produced a significant positive correlation ($p = 0.05$). This result indicates that with the increase in geographical distance among *Lallemantia royleana* populations, a lower amount of gene exchange occurred between them and we encounter the populations' isolation by distance (IBD) phenomenon. Pearson' coefficient of correlation determined between Hs values (genetic diversity due to populations) and ecological parameters produced a significant positive correlation between Hs and longitude ($r = 0.98$, $P < 0.01$). This indicates that plant populations located according to the species longitudinal distribution had a higher degree of genetic diversity. Similar analysis performed between the populations' Fst values estimated by STRUCTIRE analysis and ecological parameters produced a significant positive correlation between Fst values and latitude ($r = 0.87$, $P = 0.05$). Therefore, plant populations located according to the species latitudinal distribution are genetically more differentiated. However, the studied populations did not show any correlation between altitude with either Hs or Fst value.

Populations genetic structure

Ggenetic structure of the *Lallemantia royleana* populations was studied by two methods: 1 – K-means clustering, and 2 – Bayesian based model STRUCTURE analysis for dominant molecular markers (Falush & al. 2007).

K-means clustering results are presented in Table 4. The results showed that the best clustering (optimum number of genetic groups= k), according to Calinski & Harabasz' pseudo-F, was k = 2 (the highest value of pseudo-F = 8.361). The optimum number of k, according to the Bayesian Information Criterion, was 4 (the lowest value of BIC = 451.061).

Table 4. K-means clustering results.

K	SSD (T)	SSD (AC)	SSD (WC)	r-squared	pseudo-F	BIC	Rho
2*	585.803	63.312	522.491	0.108	8.361	452.887	0.172
3	585.803	105.799	480.004	0.181	7.494	451.127	0.217
4&	585.803	134.193	451.61	0.229	6.636	451.061	0.245
5	585.803	159.782	426.021	0.273	6.188	451.182	0.274

Evanno test performed on STRUCTURE analysis by STRUCTURE HARVESTER produced the best number of k = 3. This genetic grouping is in agreement with NJ tree results presented before. Both analyses revealed that the *Lallemantia royleana* populations are genetically differentiated and contain two to three gene pools in the country.

Gene flow among populations

The presence of isolation by distance among the *Lallemantia royleana* populations and also the presence of a significant molecular difference among the studied populations raise the question whether these populations are completely isolated and do not exchange genes at all. To answer this question, we have compared the results of STRUCTURE, NeighborNet and reticulation analyses.

In the STRUCTURE analysis we have used an admixture model to investigate a possible genetic admixture among the studied populations. A detailed analysis of allelic combinations based on 102 plant specimens from 11 geographical populations is presented in Fig. 4. It shows certain degree of genetic admixture among the studied populations. It is particularly true for Populations 4–8. Occurrence of differently colored segments in these populations indicates genetic admixture. The gene flow observed may be due to ancestral shared alleles, or ongoing gene flow that cannot be seen at present.

The NeighborNet diagram (Fig. 5) also reveals a certian degree of gene flow between Populations 1 and 2 (Delijan, near Fojoor and Saveh-Salafchegan, respectively) and between Populations 6 and 7 (Tehran-Saveh and Tehran-Qom, respectively), 5 and 7 (Mahallat and Tehran-Qom, respectively), 6 and 10 (Tehran-Saveh and Delijan to Salafchegan, respectively), and 10 and 11 (Delijan to Salafchegan and Arak, respectively). Therefore, despite genetic differentiation and divergence of the studied *Lallemantia royleana* populations, they are not totally isolated and still some amount of gene flow occurrs between them (in the past or at present). The mean Nm value of 0.50 for ISSR loci revealed that the amount of gene flow is not high among the studied populations (Nm >1 indicates high gene flow).

Frichot & al. (2013) have proposed new algorithms based on the population genetics, ecological modeling, and statistical learning techniques for screening genomes for signatures of local adaptation. This is implemented by the LFMM computer program.

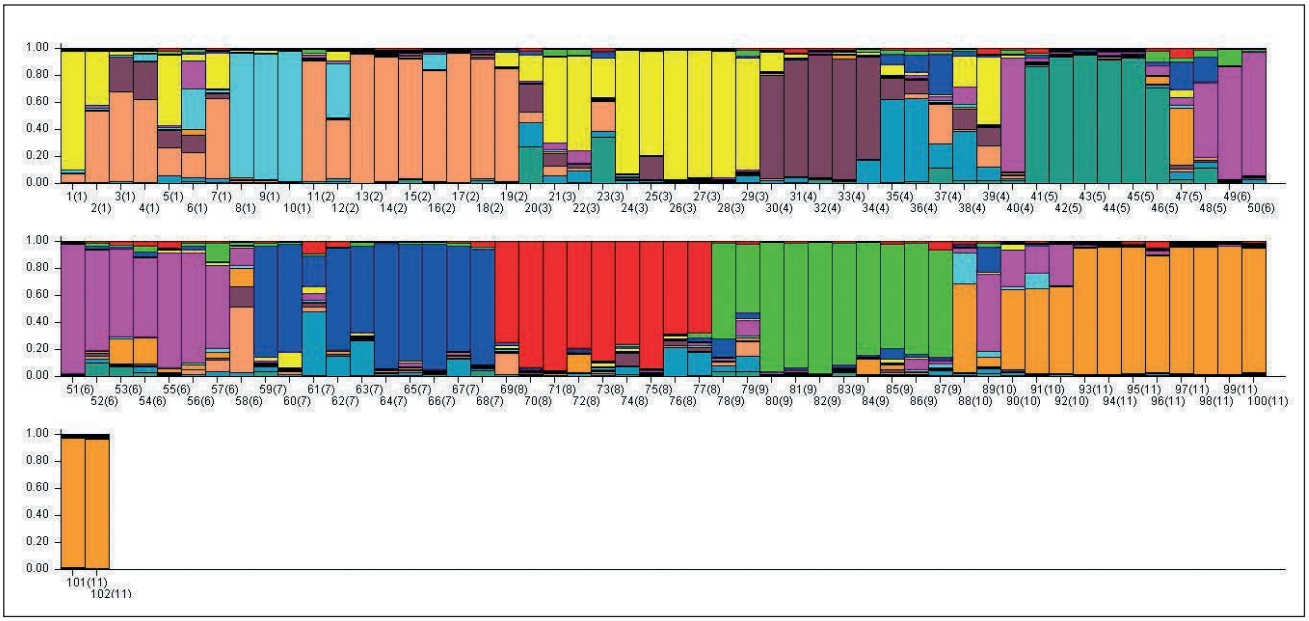

Fig. 4. STRUCTURE plot of ISSR data based on k = 3. (Populations 1–11 are according to Table 1).

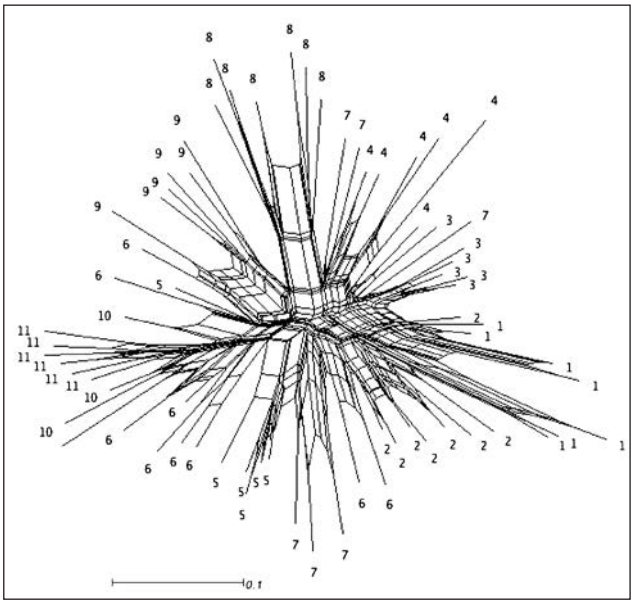

Fig. 5. NeighborNet diagram of ISSR data. (Populations 1–11 are according to Table 1).

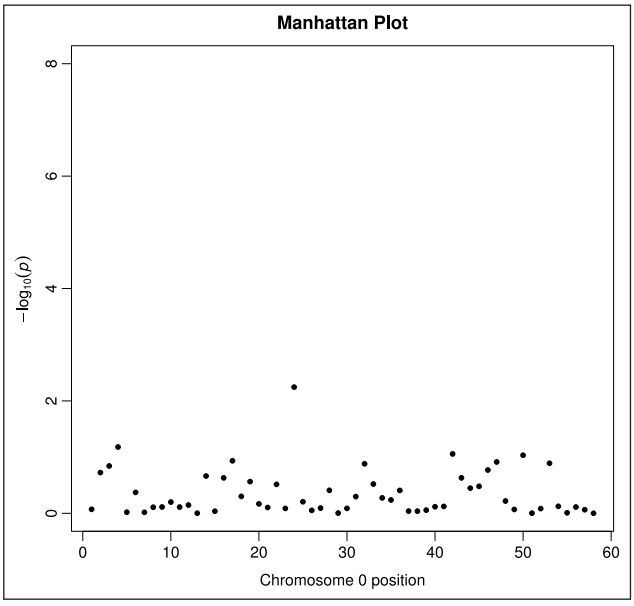

Fig. 6. Manhattan plot of LFMM analysis in ISSR loci.

LFMM analysis showed that ISSR loci 40 (Nm = 3.69), 42 (Nm = 0.25) and 24 (Nm = 0.62) had -log10 (p-value) of 1–0.05-2.10 and were significantly correlated with the studied environmental parameters (P = 0.05). These results are summarized in a Manhattan plot which is presented in Fig. 6. As it is evident, one of these loci had high Nm value, while the other two had low to medium Nm value. Therefore, the adaptive nature of these loci is not related to their migration nature.

Morphological variability

An ANOVA test performed for quantitative morphological characters showed a significant difference (P = 0.01) among the studied populations. Moreover, the CVA plots of the studied populations based on all morphological characters (quantitative and qualitative) separated these populations from each other (Fig. 7). This provides a discriminant analysis for two or more groups. A scatter plot of specimens along the first two canonical axes produces maximal and sec-

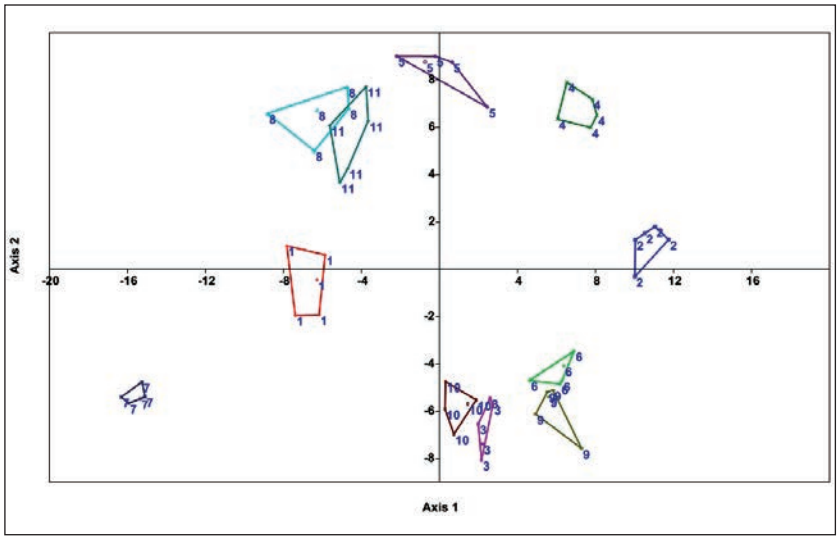

Fig. 7. A CVA plot of the studied populations based on morphological characters. Populations 1–11are: Delijan, near Fojoor, Saveh-Salafchegan, Roodeshoor village, Samavak village, Abegarm, Tehran-Saveh, Tehran-Qom, Nimvar village, Mahallat, opposite the University, Delijan to Salafchegan, and Arak, respectively.

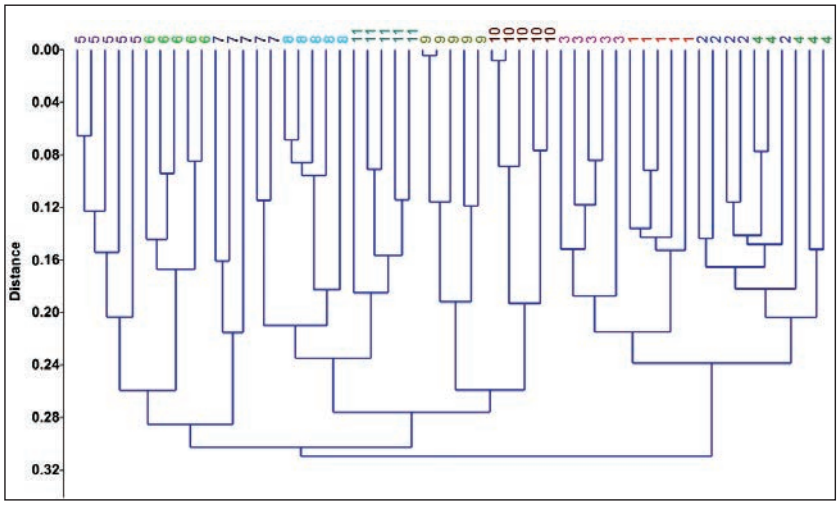

Fig. 8. A UPGMA dendrogram of populations based on morphological characters. Populations 1 – 11 are: Delijan, near Fojoor, Saveh-Salafchegan, Roodeshoor village, Samavak village, Abegarm, Tehran-Saveh, Tehran-Qom, Nimvar village, Mahallat, opposite the University, Delijan to Salafchegan, and Arak, respectively.

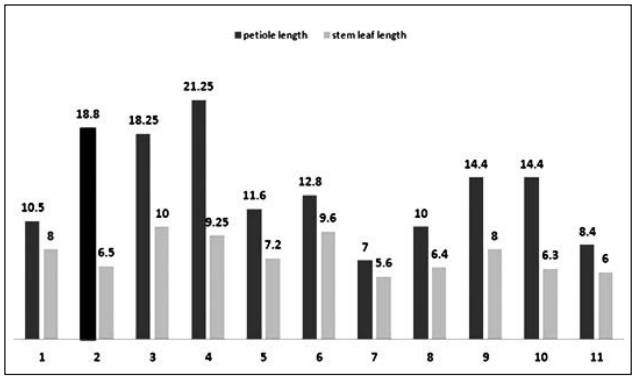

Fig. 9. Variation in petiole size and stem leaf length among the studied populations.

ond to maximal separation between all groups. The axes are linear combinations of the original variables as in PCA, and eigenvalues indicate an amount of variation explained by these axes. Therefore, these populations differed significantly in both genetic and morphological features.

Taxonomic consideration (infraspecific forms)

A UPGMA clustering of morphological characters is presented in Fig. 8. The general grouping obtained is in agreement with the genetic grouping of NJ tree presented before. In both analyses, Populations 1–4 (Delijan, Saveh-Salafchegan, Roodeshoor village, and Samavak village, respectively), and 5–7 (Abegarm, Tehran-Saveh, and Tehran-Qom, respectively), as well as 8, 10 and 11 (Nimvar village, Delijan-Salafchegan, and Arak populations, respectively) were placed close to each other. This grouping was supported by the PCA plot too.

A PCA analysis of the morphological characters revealed that the first two PCA components comprised about 70 % of the total variation. Two morphological characters of petiole length and stem leaf length had the highest correlation (>0.70) with the first PCA axis and, therefore, are the most variable morphological characters among the *Lallemantia royleana* populations. A comparison of these two morphological characters among the studied populations is presented in Fig. 9. Populations 2, 3 and 4 (Saveh-Salafchegan, Roodeshoor village, and Saveh, respectively) had the greatest number of these morphological characters that differed significantly from the other populations. No qualitative character was identified by PCA. This result indicates that these populations are uniform in their qualitative morphological characters and all belong to a single species. This result, in turn, supports their correct identification.

Discussion

Genetic diversity is an important criterion for plant populations in the process of facing the environmental changes and adapting to them. Occurrence of high genetic diversity within population has been reported in different plant species and outcrossing nature of these species has been suggested to be the reason for that (Sheidai & al. 2013). The same may hold true for the *Lallemantia royleana* populations.

Among-population differentiation in phenotypic traits and allelic variation is expected to occur as a consequence of isolation, drift, founder effects, and local selection (Jolivet & Bernasconi 2007).

A Mantel test revealed that with the increase in geographical distance among the *Lallemantia royleana* populations, a lower amount of gene exchange occurred between them. In a similar study, Egelund & al. (2012) reported a reduced gene flow among the populations of Tea-Leaved Willow (*Salix phylicifolia*, *Salicaceae*) and considered genetic drift and inbreeding as possible causes of such poor gene flow. Therefore, we may suggest that a combination of genetic drift, limited gene flow and local adaptation may have played a role in the genetic divergence of *Lallemantia royleana* populations. However, as it was mentioned earlier, ISSR markers only cover some parts of the genome. Definitely, a more detailed genetic analysis with the use of other molecular data must be performed to get a more precise result.

According to Knaus (2008), "If we take the species to be the unit of distinction, the infra-taxa (the subspecies, the variety and the ecotype) are consequently non-distinct. The process in which a group of organisms diverge from being one cohesive group to becoming two or more distinct groups is the process of speciation". Stebbins (1993) also contributed the idea that "species are systems of populations, which resemble each other, yet contain genetically different ecotypes that could be arranged in a continuous series. These allopatric infra-specific categories are usually recognized as infra-taxa".

Ockendon (1971) studied morphological diversity in several geographical populations of the *Linum perenne* group in Europe. He reported that most of these characters vary continuously and no sharp differences exist among the populations. However, a significant difference was observed in some of the quantitative characters. Moreover, a PCA plot showed that the different populations were separated from each other. Therefore, different geographical populations were considered to be ecotypes within each subspecies.

In the present study, the *Lallemantia royleana* populations differed significantly in their genetic and morphological features. We have obtained almost similar groupings of the studied populations in both molecular and morphological tree and ordination. Therefore, we may consider them as different ecotypes or varieties of *Lallemantia royleana*. This holds particularly true for the Populations 2, 3 and 4 (Saveh-Salafchegan, Roodeshoor village, and Saveh, respectively), which had significantly larger-sized petiole length and stem leaf length, as compared to the other studied populations. These populations were separated from the others in the PCA plot too.

References

Azizi, N., Sheidai, M., Mozafarian, V. & Noormohammadi, Z. 2014. Genetic, cytogenetic and morphological diversity in *Helicrysum leucocephalum* (*Asteraceae*) populations**.** – Biologia, **69**: 566-573.

Calinski, R.B. & Harabasz, J. 1974. A dendrite method for cluster analysis. – Commun. State., **3**: 1-27.

Egelund, B., Pertoldi, C. & Barfod, A.S. 2012. Isolation and reduced gene flow among Faroese populations of tea-leaved willow (*Salix phylicifolia, Salicaceae*). – New Zealand J. Bot., **12**: 9-15.

Earl, D.A. & von Holdt, B.M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. – Conserv. Genet. Resour., **4**: 359-361.

Evanno, G., Regnaut, S. & Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. – Molec. Ecol., **14**: 2611-2620.

Falush, D., Stephens, M. & Pritchard, J.K. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. – Molec. Ecol. Notes, **7**: 574-578.

Frichot, E., Schoville, S.D., Bouchard, G. & Francois, O. 2013. Testing for associations between loci and environmental gradients using latent factor mixed models. – Molec. Biol. Evol., **30**:1687-1699.

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

Harley, R.M., Atkins, S., Budantsev, A.L., Cantino, P.D., Conn, B.J., Grayer, R., Harley, M.M., De Kok, R., Krestovskaja, T., Morales, R., Paton, A.J., Ryding, O. & Upson, T. 2004. *Lamiaceae*. – In: **Kadereit J.W. (Ed)**. The Families and Genera of Vascular Plants. Vol. VII, *Lamiales*. Berlin, Springer, 167-282.

Heather, K. & Freeland, J.R. 2011. Applications and implications of neutral versus non-neutral markers in molecular ecology. – Int. J. Molec. Sci., **12**: 3966-3988.

Holsinger, K.E. & Lewis, P.O. 2003. Hickory: a package for analysis of population genetic data V1.0. Available at http://www.eeb.uconn.edu; accessed 2003.

Huson, D.H. & Bryant, D. 2006. Application of phylogenetic networks in evolutionary studies. – Molec. Biol. Evol., **23**: 254-267. (Software available from http:// www. splitstree.org.)

Jolivet, C. & Bernasconi, G. 2007. Molecular and quantitative genetic differentiation in European populations of *Silene latifolia* (*Caryophyllaceae*). – J. Hum. Genet., **177**: 1239-1247.

Jost, L. 2008. GST and its relatives do not measure differentiation. – Molec. Ecol., **17**: 4015-4026.

Legendre, P. & Makarenkov, V. 2002. Reconstruction of biogeographic and evolutionary networks using reticulograms. – Syst. Biol., **51**: 199-216.

Knaus, B.J. 2008. A Fistful of *Astragalus*: Phenotypic and genotypic basis of the most taxon-rich species in the North American flora. Ph.D. Thesis, Oregon State University, USA.

Mahmood, S., Hayat, M.Q., Sadiq, A., Ishtiaq, Sh., Malik, S. & Ashra, M. 2013. Antibacterial activity of *Lallemantia royleana* (Benth.) indigenous to Pakistan. – Afr. J. Microbiol. Res., **7**: 4006-4009.

Meirmans, P.G. 2012. AMOVA-based clustering of population genetic data. – J. Heredity, **103**: 744-750.

Meirmans, P.G. & Van Tienderen, P.H. 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. – Molec. Ecol. Notes, **4**: 792-794.

Ockendon, D.J. 1971. Taxonomy of the *Linum perenne* group in Europe. – Watsonia, **8**: 205-235.

Peakall, R. & Smouse, P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. – Molec. Ecol. Notes, **6**: 288-295.

Podani, J. 2000. Introduction to the Exploration of Multivariate Data [English translation], Leide, Netherlands: Backhuyes,

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

Razavi, S.M.A. & Karazhiyan, H. 2009. Flow properties and thixotropy of selected hydrocolloids, experimental and modeling studies. – Food Hydrocolloids, **23**: 908-912.

Razavi, S.M.A. & Moghaddam, T.M. 2011. Influence of different substitution levels of *Lallemantia royleana* seed gum on textural characteristics of selected hydrocolloids. – Electron. J. Environ. Agric. Food Chem., **10**: 2826-2837.

Rivera-Nunez, D., Obon, D.E. & Gastro, C. 1992. The ethnobotany of *Lamiaceae* of old world. – In: **Harley, R.M. & Reynolds, T. (eds)**, Advances in *Lamiaceae* Science, 455-473. Royal Botanical Gardens, Kew.

Schwarz, G. 1978. Estimating the dimension of a model. – Ann. Statist., **6**: 461-464.

Stebbins, G.L. 1993. Concepts of species and genera in the flora of North America. – In: Flora of North America, Editorial Committee, ed., vol. 1, cap. 11. New York & Oxford Univ. Press.

Sheidai, M., Seif, E., Nouroozi, M. & Noormohammadi, Z. 2012. Cytogenetic and molecular diversity of *Cirsium arvense* (*Asteraceae*) populations in Iran. – J. Japan. Bot., **87**: 193-205.

Sheidai, M., Zanganeh, S., Haji-Ramezanali, R., Nouroozi, M., Noormohammadi, Z. & Ghsemzadeh-Baraki, S. 2013. Genetic diversity and population structure in four *Cirsium* (*Asteraceae*) species. – Biologia, **68**: 384-397.

Tero, N., Aspi, J., Siikamaki, P., Jakalaniemi, A. & Tuomi J. 2003. Genetic structure and gene flow in a metapopulation of an endangered plant species *Silene tatarica*. – Molec. Ecol., **12**: 2073-2085.

Weising, K., Nybom, H., Wolff, K. & Kahl, G. 2005. DNA Fingerprinting in Plants. Principles, Methods, and Applications. (2nd ed.), Boca Rayton, Fl., USA: CRC Press, pp. 472.