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Short Communication: Population genetic structure in medicinal plant *Lallemantia iberica* (Lamiaceae)

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Abstract. *Koohdar F, Sheidai M, Talebi SM, Noormohammadi Z. 2015. Population genetic structure in medicinal plant* Lallemantia iberica *(Lamiaceae). Biodiversitas 16: 139-144. Lallemantia iberica* (Bieb.) Fischer and C.A. Meyer (sin. *Dracocephalum ibericum*M. Bieb.) also named Dragon's head" is an annual plant cultivated for its seeds that contain about 30% -38% drying oil (siccative oil). Its seed oil is used in foods, dye and varnish industry. *L. iberica* seeds have traditional uses as reconstitute, stimulant, diuretic and expectorant. *L. iberica* in an important medicinal plant in our country and grows in various regions with different environmental conditions. At present no investigation has been reported about population genetic structure of this valuable plant species in Iran. Therefore, we carried out population genetic analysis of 11 populations of *L. iberica* by using ISSR molecular markers for the first time. Genetic diversity analysis revealed high within population genetic variability. AMOVA test produced significant genetic difference among the studied populations. Mantel test revealed significant correlation between genetic distance and geographical distance of the populations. STRUCTURE analysis and K-Means clustering revealed population genetic fragmentation and the presence of three gene pools for this species. The assignment test revealed the occurrence of limited gene flow among the populations. The results suggested that genetic divergence, limited gene flow, genetic drift and local adaptation have played role in diversification of *L. iberica*.

Key words: Gene flow, population fragmentation, IBD, *Lallemantia iberica*.

INTRODUCTION

Lallemantia iberica (Bieb.) Fischer and C.A. Meyer (syn. *Dracocephalum ibericum* M. Bieb.) also named Dragon's head" is a crop cultivated from the prehistoric times in Southwestern Asia and Southeastern Europe. It is an annual plant and has been cultivated for its seeds that contain about 30% -38% drying oil (siccative oil). The iodic index of its seeds oil is between 163 and 203. The oil is used in foods, but especially in dye and varnish industry (Shafiee et al. 2009; Ion et al. 2011).

Lallemantia iberica seeds have traditional uses as reconstitute, stimulant, diuretic and expectorant. It is considered as a linseed substitute in a number of applications including: wood preservative, ingredient of oil-based paints, furniture polishes, printing inks, soap making, and manufacture of linoleum (Samadi et al. 2007; Shafiee et al. 2009). The plant also has high ornamental value and it is used in arid landscaping and urban horticulture at various places in Turkey (Ozdemir et al 2014).

Population genetics analyses can produce important data on the levels of genetic variation, the partitioning of variability within/between populations, gene flow, inbreeding, selfing versus outcrossing rates, effective population size and population bottleneck. These analyses may be of help in developing effective management strategies for endangered and/or invasive species (Ellis and Burke 2007). *Lallemantia iberica* in an important medicinal plant in our country and grows in various regions with different environmental conditions (Ozdemir et al 2014). This plant forms several local populations. At present no investigation has been reported about population genetic structure of this valuable plant species in Iran. Studying genetic variability and gene flow versus genetic fragmentation of local populations can provide valuable information for conservation of this medicinal plant in the country. Therefore, we carried out population genetic analysis in 11 populations of *L. iberica* for the first time in Iran. Different molecular markers have been used in population genetic studies. We used ISSR (Inter-simple sequence repeats) to study genetic diversity of populations in *L. iberica*, since these markers are reproducible, cheap, easy to work and are known to be efficient in population genetic diversity studies (Sheidai et al. 2012, 2013, 2014).

Materials and Methods

Plant material

Ninety plant specimens were collected from 11 populations of *Lallemantia iberica*. Details of the studied populations are provided in Table 1, Figure 1. Voucher specimens are deposited in Herbarium of Shahid Beheshti University (HSBU), Tehran, Iran.

Table 1. Populations studied, their locality and ecological features.

Province		Locality	Alt. (m)	Long.	Lat.
Zanjan	1.	50 km from Qazvin to Zanjan	1839	3619	4905
Zanjan	2.	23 km from Qazvin to Zanjan	1785	3634	4843
West Azerbaijan	3.	Takab	1729	3627	4703
West Azerbaijan	4.	2km from Takab to Shahindej	1751	3628	4701
West Azerbaijan	5.	65 km from Takab to Shahindej	2047	3630	4658
West Azerbaijan	6.	Sero	1680	3743	4449
Markazi	7.	Sangak	1929	3511	4948
Qazvin	8.	Khereqan	1980	3519	4955
Kermanshah	9.	Paveh	1533	3502	4621
Mazandran	10.	Chalus	1605	3616	5114
Qazvin	11.	Abyek	1263	3602	5031

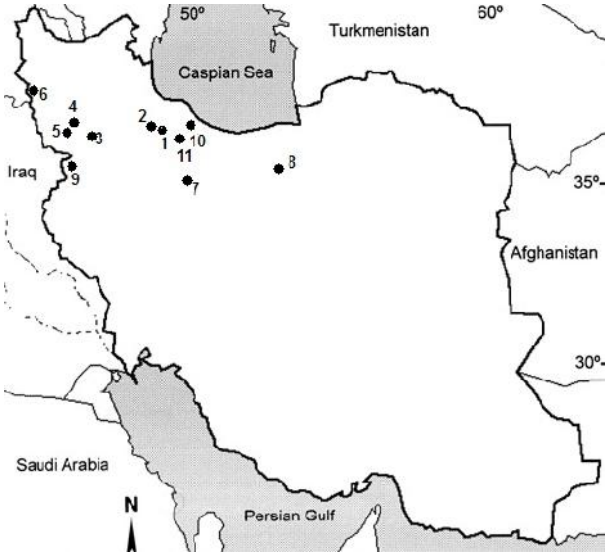

Figure 1. Distribution map of *Lallemantia iberica* populations. Note: Population numbers are according to Table 1.

DNA extraction and ISSR assay

Fresh leaves were collected randomly in each of the studied populations and dried in silica gel powder. Genomic DNA was extracted using CTAB with activated charcoal protocol (Sheidai et 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)9A and (GT)7CA commercialized by UBC (the University of British Columbia) were used. PCR reactions were performed in a 25µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM 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 min initial denaturation step 94°C, 30 S at 94°C; 1 min at 50°C and 1min 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 the ethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany). ISSR bands obtained were coded as binary characters (presence = 1, absence = 0). The following genetic diversity parameters were determined in each population: percentage of allelic polymorphism, allele diversity (Weising et al. 2005), Nei's gene diversity (H), Shannon information index (I), number of effective alleles, and percentage of polymorphism (Freeland et al. 2011).

Data analysis

Genetic diversity and population structure. ISSR bands obtained were scored as binary characters. Genetic diversity parameters were determined in each population. These parameters were Nei's gene diversity (H), Shannon information index (I), number of effective alleles, and percentage of polymorphism (Weising et al. 2005; Freeland et al. 2011). Nei's genetic distance was determined among the studied populations and used for clustering (Weising et al. 2005; Freeland et al. 2011). For grouping of the plant specimens, Neighbor Joining (NJ) clustering method, Neighbor Net method of networking as well as principal coordinate analysis (PCoA) were performed after 100 times bootstrapping/ permutations (Freeland et al. 2011; Huson and Bryant 2006). The Mantel test was performed to check correlation between geographical distance and the genetic distance of the studied species (Podani 2000). PAST ver. 2.17 (Hamer et al. 2012), DARwin ver. 5 (2012) and SplitsTree4 V4.13.1 (2013) programs were used for these analyses.

Significant genetic difference among the studied populations and provinces were determined by: 1- AMOVA (Analysis of molecular variance) test (with 1000 permutations) by using GenAlex 6.4 (Peakall and Smouse 2006), and 2- Nei's Gst analysis of GenoDive ver.2 (2013) (Meirmans and Van Tienderen 2004). The population genetic differentiation was studied by G'st_est = standardized measure of genetic differentiation (Hedrick 2006), 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 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).

The genetic structure of populations was studied by two different approaches. First by using Bayesian based model STRUCTURE analysis (Pritchard et al. 2000), and second by maximum likelihood-based method of K-Means clustering. For STRUCTURE analysis, data were scored as dominant markers (Falush et al. 2007). The Evanno test was performed on STRUCTURE result to determine proper

number of *K* by using delta *K* value (Evanno et al. 2005). We performed K-Means clustering as done in GenoDive ver. 2. (2013). Here, the optimal clustering is the one with the smallest amount of variation within clusters. This is calculated by using the within-clusters sum of squares. The minimization of the within-groups sum of squares that is used in K-Means clustering is, in the context of a hierarchical AMOVA, equivalent to minimizing the among-populations-within-groups sum of squares, SSDAP/WG (Meirmans 2012). Two summary statistics, 1- pseudo-F (Calinski and Harabasz 1974), and 2- Bayesian Information Criterion (BIC) (Schwarz 1978, provide the best fit for k (Meirmans 2012).

Gene flow. Gene flow was determined by two different approaches. (i) Calculating Nm an estimate of gene flow from Gst by PopGen ver. 1.32 (1997) as: Nm = 0.5(1 - Gst)/Gst. This approach considers equal amount of gene flow among all populations. (ii) Population assignment test based on maximum likelihood as performed in Genodive ver. in GenoDive ver. 2. (2013).

Results and discussion

Genetic diversity

Genetic diversity parameters determined in the studied populations are presented in Table 2. The highest values for gene diversity and percentage of polymorphism occurred in population 6 (0.162 and 63.89, respectively). The lowest values for these parameters were observed in population 10 (0.048 and 12.5, respectively).

Population genetic structure

AMOVA test produced significant genetic difference (PhiPT = 0.49, P = 0.010) among the studied populations. It also revealed that, 59% of total genetic variability was due to within population diversity and 41% was due to among population genetic differentiation. Pairwise AMOVA produced significant difference among the studied populations. Hickory test also produced high Theta B value (0.4) supporting AMOVA. Gst (0.40, P = 0.001), Hedrick, standardized fixation index (G'st = 0.47, P = 0.001) and Jost' differentiation index (D-est = 0.13, P = 0.001), revealed that the studied populations are genetically differentiated.

Neighbor Joining (NJ) tree and PCoA plot produced similar results. Therefore, only PCoA plot is presented and discussed (Figure 2). The studied population was placed in separate group which was in agreement with AMOVA result. It also revealed a higher degree of within population genetic variability in population 6 (as plants in this populations were more scattered than the other populations) which is in agreement with genetic diversity parameters presented before. PCoA plot revealed higher genetic affinity between populations 1, 2 and 3, and between 4, 5 and 6, as well as between 9, 10 and 11.

Neighbor-Net diagram (Figure 3), supported grouping obtained by PCoA and also revealed the presence of three distinct genetic groups. The populations 1-3 formed the first genetic group, populations 4-6 and populations 7-11 comprised second and third genetic groups respectively. This result was supported by Evanno test based and K-Means clustering that produced the best k = 3 and 2 respectively. Therefore, we have population genetic fragmentation in *L. iberica*.

STRUCTURE plot (Figure 4) based on k = 3, identified three distinct genetic groups (gene pools) for of *L. iberica* populations. The first gene pool (populations 1-3) is distributed from Ghazvin to Zanjan and to Takab (West Azerbayejan). The second gene pool (populations 4-6) is distributed from Takab to Shahindej and Orumiyeh, while the third gene pool (populations 7-11) is distributed mainly in West and North-West of the country.

Gene flow

The STRUCTURE plot that was based on admixture model, revealed some degree of genetic admixture in the studied populations. For example, populations 1-3 had some alleles from populations 4-6 and vice versa. These shared alleles might be the possible reason for close genetic affinity of these populations as revealed by PCoA plot presented before. Populations 7-11 also had some degree of shared alleles with the other studied populations.

The population assignment test also revealed some degree of gene flow or ancestral shared alleles among the studied populations. This was true particularly for populations 1 and 6, 1 and 2, 2 and 3, 3 and 4, 4 and 6, 7 and 8, 6 and 9, 6 and 10, as well as 6 and 11. Gene flow determined by Nm, produced mean value = 0.60, that is not considered to be high. Therefore, all these results revealed restricted gene flow and strong population differentiation among *L. iberica* populations. The Mantel test produced significant correlation between genetic distance and geographical distance of the studied populations (r = 0.36, P = 0.01, Figure 5). This indicated the occurrence of isolation by distance (IBD) in *L. iberica* populations.

LFMM analysis revealed that 20 out of 72 ISSR loci had >1.3 -log 10 value (P <0.05) and may be considered as adaptive loci. Some of these loci had low Nm value, for example ISSR loci 2, 7, 37, 38, 41, 43, 57, 59. 63, and 71 had Nm <1. However, some loci like ISSR loci 14, 18, 22, 26, 34, 47, 51, 65, 66, had nm >1. Therefore, both ISSR loci that were shared by different populations and loci with lower admixture value were used by *L. iberica* plants to adapt to their environment.

Discussion

The extensive use of natural resources to meet the needs of expanding human populations, deforestation and habitat fragmentation could lead to reductions in the rate of gene flow among populations. This in turn increases the genetic differentiation among populations and genetic structuring and reductions in genetic variation within populations due to genetic drift (Setsuko et al. 2007; Hou and Lou 2011). However, there are cases in which fragmentation did not result in reduced genetic diversity (Prober et al. 1990), due to various reasons, like population size and the time scale of fragmentation. Templeton (1991) stated that "Heterozygosity is not always beneficial, nor does inbreeding always have adverse effects. In some circumstances, a population may be so well adapted to its local circumstances. STRUCTURE analysis and K-Means

Table 2. Genetic diversity parameters in the studied populations of *Lallemantia iberica*

Pop	N	Na	Ne	I	He	UHe	%P
Pop1	10.000	0.931	1.235	0.217	0.142	0.149	44.44%
Pop2	10.000	1.208	1.292	0.267	0.174	0.183	56.94%
Pop3	10.000	1.083	1.211	0.211	0.133	0.140	50.00%
Pop4	10.000	0.944	1.224	0.204	0.133	0.140	43.06%
Pop5	10.000	0.875	1.200	0.179	0.119	0.125	34.72%
Pop6	10.000	1.306	1.249	0.260	0.162	0.170	63.89%
Pop7	10.000	0.639	1.180	0.152	0.102	0.108	29.17%
Pop8	10.000	0.583	1.204	0.162	0.112	0.118	27.78%
Pop9	3.000	0.472	1.105	0.101	0.066	0.079	19.44%
Pop10	3.000	0.417	1.083	0.071	0.048	0.058	12.50%
Pop11	4.000	0.486	1.134	0.119	0.079	0.090	22.22%

Note: N = Number of populations, Na = No. of different alleles, Ne = No. of effective alleles, I = Shannon's Information Index, He = Gene diversity, UHe = Unbiased gene diversity, and %P = Percentage of polymorphism.

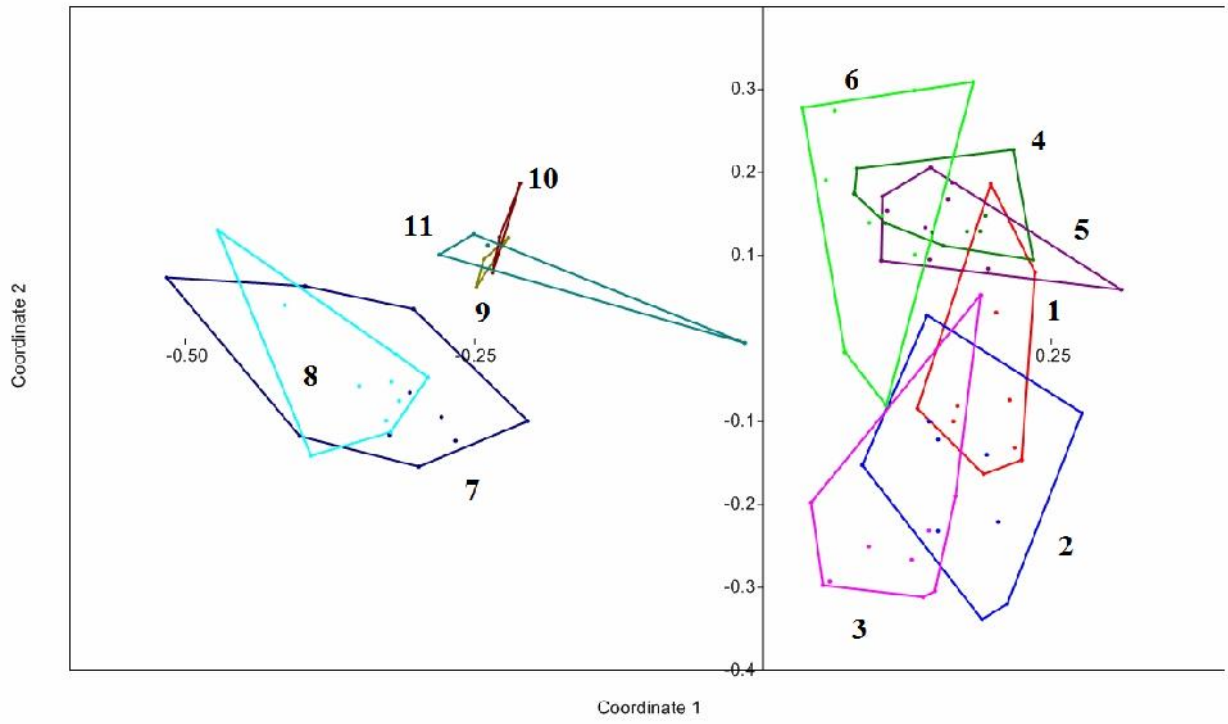

Figure 2. PCoA plot of *Lallemantia iberica* populations based on ISSR data. Note: Population numbers are according to Table 1.

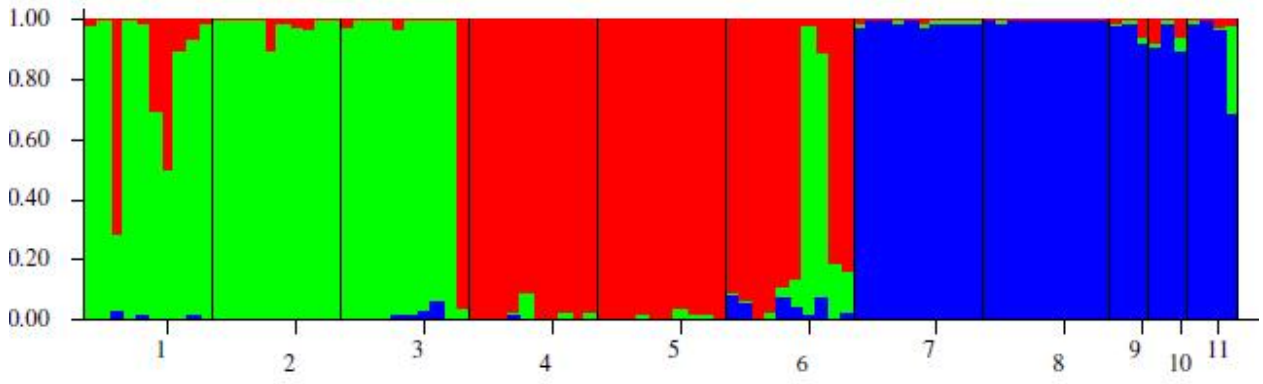

Figure 4. STRUCTURE plot based on k = 3

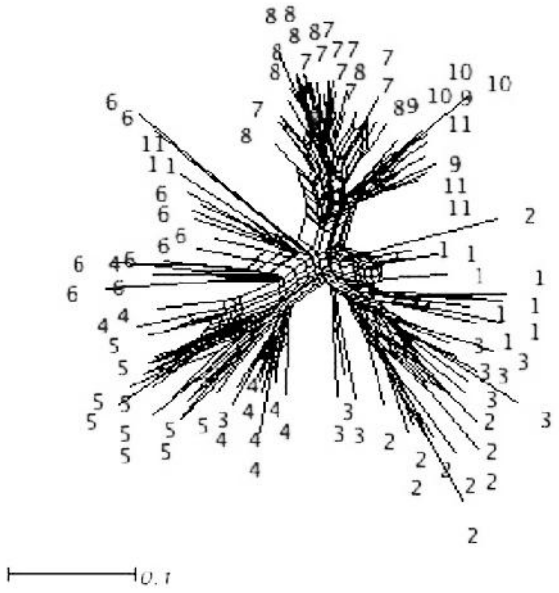

Figure 3. Neighbor-Net diagram of *Lallemantia iberica* populations based on ISSR data. Note: Population numbers are according to Table 1.

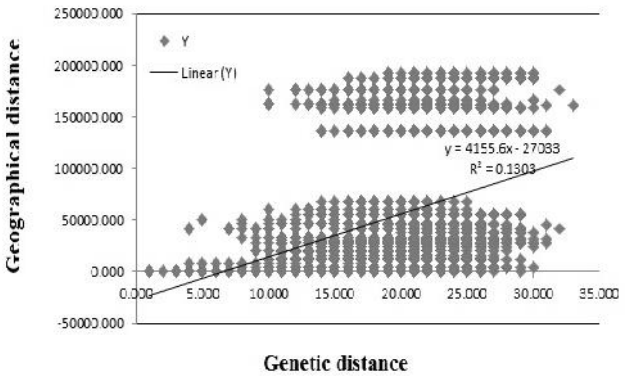

Figure 5. The Mantel test indicated significant correlation between genetic distance and geographical distance

clustering revealed population fragmentation in *L. iberica*. However, population assignment and Nm estimation showed some degree of gene flow among the studied populations. These results show that populations achive some new combination of alleles through limitted gene flow. A metapopulation is an assemblage of local populations that usually are small and are linked by loose relationships, i.e., there is some gene flow among them. In such cases, gene flow among local populations could mitigate losses of genetic variation caused by genetic drift in local populations and thus save them from extinction via so-called "genetic rescue" (Richards 2000).

Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment (Çalişkan 2012; Sheidai et al. 2013, 2014). High value of within population genetic diversity was observed in the studied populations (for instance, high percentage of polymorphism in each population). Moreover, AMOVA test revealed that 59% of total genetic variability was due to within population diversity and 41% was due to among population genetic differentiation. This is possibly related to outcrossing nature of *L. iberica*.

Mantel test revealed a pattern of isolation-by distance across the distribution range of the studied *L. iberica* populations. This pattern suggested that the dispersal of these populations might be constrained by distance and gene flow is most likely to occur between neighboring populations. As a result, more closely situated populations tend to be more genetically similar to one another (Slatkin 1993; Hutchison and Templeton 1999; Medrano and Herrera 2008). In fact population assignment test revealed that limited gene flow occurred mostly between neighboring populations, populations 1-3, 4-6, and 7-11.

LFMM analysis revealed that some of the genetic loci were adaptive nature and possibly used by local populations to adapt to their environment. Therefore, combination of genetic divergence, limited gene flow and local adaptation have played role in diversification of *L. iberica*. In conclusion the present study may provide some useful information about population genetic structure and genetic variability of *L. iberica* that may be used in conservation of these important species.

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