UDC 575.630
https://doi.org/10.2298/GENSR1802533A
Original scientific paper

BIOSYSTEMATIC STUDY AND POPULATION GENETIC ANALYSIS IN *Lamium amplexicaule* L. (LAMIOIDEAE, LAMIACEAE)

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Azimishad F., M. Sheidai, S. Mehdi Talebi, Z. Noormohammadi (2018): *Biosystematic study and population genetic analysis in Lamium amplexicaule L. (Lamioideae, Lamiaceae).*- Genetika, Vol 50, No.2, 533- 548.

L. amplexicaule L. grows in different regions of Iran and forms several populations that can be invasive in some area. Morphological and genetic variation of *L. amplexicaule* in Iran was studied to evaluate the differentiation among three presumed varieties within this species. Morphometric study was accompanied by the analysis of ISSR and chloroplast genome (psbA-trnH). Results of the molecular methods show that *L. amplexicaule* var. *bornmuelleri* Mennema. does not form any genetically distinct group. Genetic study of 103 plant specimens of 13 different populations using ISSR molecular marker revealed a high degree of within-population (67%) and among (33%) populations genetic diversity. AMOVA and Gst analyses produced a significant difference. Nm estimation revealed certain degree of gene flow/shared alleles between these populations, indicating that *L. amplexicaule* is predominantly an out-crossing species in Iran. Mantel test produced significant correlation between genetic distance and geographical distance of the studied populations (r = 0.097, P = 0.04). STRUCTURE plot identified two main gene pools for this species in Iran.

Keywords: chloroplast genome, genetic diversity, gene flow, ISSR, *L. amplexicaule*,

INTRODUCTION

The genus *Lamium* L. (Lamiaceae), contains about 17–30 herbaceous annual and perennial species, depending on circumscription (HARLEY *et al.*, 2004). This genus is distributed from North Africa to Eurasia with highest species richness and diversity in the Irano - Turranean region (MENNEMA, 1989; MABBERLEY, 1997).

Nine *Lamium* species occurs in Iran (JAMZAD, 2012). *Lamium amplexicaule* L. (sect. *Amplexicaule*, subgen. *Lamium*) (MENNEMA, 1989), shows extensive morphological variability in

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plant height, flowers size, as well as shape and size of basal and inflorescent leaves. Three varieties were reported for this species in Iran namely 1 - *L. amplexicaule* var. *amplexicaule*, 2 - *L. amplexicaule* var. *aleppicum* (Boiss. & Hausskin. ex Boiss.) Bornm. and 3 - *L. amplexicaule* var. *bornmuelleri* Mennema (MENNEMA, 1989). *L. amplexicaule* var. *aleppicum* is distinguishable from other varieties morphologically. It has narrower leaves and long nutlets (MENNEMA, 1989).

Identification of *L. amplexicaule* var. *bornmuelleri* is based on some morphological features distinguishing it from other varieties: having small size of plant (stem < 5 -10 cm) and merely cleistogamous flowers (MENNEMA, 1989), but small size of specimens of var. *amplexicaule* correspondence with features of var. *bornmuelleri* and therefore, their separation is difficult (JAMZAD, 2012). *L. amplexicaule* var. *amplexicaule* is an annual herbaceous plant with rose-colored flowers, which produces either or both cleistogamous and open flowers. It is a wild growing plant, widespread in Iran and creates populations on different ecological conditions like hills and mountain grasslands, in cultivated lands, gardens and open waste places. This plant acts invasive anywhere introduced and brings about substantial problems to natural ecosystems and crop-lands (PIMENTEL *et al*., 2000). It reduced the yield of wheat (CONLEY and BRADLEY, 2005) and has allelopathic effect probably due to its non-volatile chemical components or due to biotic factors (JONES *et al*., 2012). However, it contains essential oils and vitamins, and it is a promising weed as a renewable natural resource for forage and pharmaceutical purposes (ABU-ZIADA *et al*., 2014). Aerial parts of this plant are used as medicine by locals in Iran (AKBARZADEH *et al*., 2010; YAZDANPANAH *et al*., 2013).

The aims of this investigation were: 1 - Delineation of the varieties. Specifically, we wanted to test whether *L. amplexicaule* var. *bornmuelleri* is confirmed by molecular data. 2 - Provide information on population genetics of var. *amplexicaule* which is used as medicinal plant. We used both morphological and molecular approaches for these aims.

For molecular study, we used both chloroplast DNA sequences (psbA- trnH) and ISSRs (inter-simple sequence repeats).

The genetic diversity is vital for each species as it influences the adaptability of species in the presence of fluctuating biotic and abiotic factors (VRIJENHOEK, 1994). Data produced by population genetics can be used in developing effective management approaches for endangered and/or invasive species (ELLIS and BURKE, 2007).

Population genetic analysis mainly uses different neutral molecular markers, like AFLP (Amplified fragments length polymorphism), SSRs (Simple sequence repeats), ISSRs (Inter-simple sequence repeats), etc. (FREELAND *et al*., 2011). ISSRs have been successfully used to estimate population genetic diversity and structure in different plant species (SHEIDAI *et al*., 2014a; KOOHDAR *et al*., 2015; MINAEIFAR *et al*., 2016; MOSAFERI *et al*., 2015), and to delimit the species (SAFAEI *et al*., 2016), and identify infra-specific taxonomic forms (SHEIDAI *et al*., 2014b).

MATERIALS AND METHODS

Plant material

In total 114 plants were collected in April to May 2016 for the morphometric study. Each sampled plant was immediately pressed and dried as a herbarium specimen, and tentatively determined (according to original description) as one of the three studied taxa. For the study of ISSR, 10 - 20 samples of three studied group were chosen. 103 accessions from 13 natural localities of *L. amplexicaule* var. *amplexicaule*, were collected for study of genetic structure,

during the field investigation and collections. Young and healthy leaves were sampled randomly and quickly dried with silica gel in the field and stored frozen until DNA extraction.

Details of the studied populations were provided in Table. 1, Fig. 1. Voucher Specimens were deposited in Herbarium of Shahid Beheshti University (HSBU), Tehran, Iran.

Table 1. Populations studied, their locality and ecological features

R	Province	Locality	Alt (m)	Long	Lat	Voucher No.
1	Alborz	Asara, Emam zadeh Ebrahim	2000	36° 05′ 60″	51° 19′ 01″	HSBU100
2	Gilan	Damash	1700	36° 75′ 54″	49° 81′ 07″	HSBU101
3	Ardabil	Khalkhal-asalem road	1500	37° 57′ 36″	48° 61′ 03″	HSBU102
4	East Azarbaijan	Liqvan village	2300	37° 82′ 77″	46° 41′ 49″	HSBU103
5	Razavi Khorasan	Gonabad,south of kakhak	1630	34° 08 ′42″	58° 38′ 12″	HSBU104
6	Kohgiluyeh and buyer Ahmad	Sisakht	2300	30° 86′ 05″	51° 46′ 79″	HSBU105
7	Qazvin	Ziya Abad	1450	35° 99′ 61″	49° 44′ 69″	HSBU106
8	West Azarbaijan	Targavar	1750	37° 27′ 68″	44° 85′ 90″	HSBU107
9	Isfahan	Semirom	2000	31° 19′ 74″	51° 71′ 39″	HSBU108
10	Kurdistan	Kachaleh village	1680	34° 94′ 37″	46° 96′ 18″	HSBU109
11	Fars	Shahr miyan	2700	30° 84′ 40″	52° 06′ 76″	HSBU110
12	Chahar mahaal and Bakhtiari	Dimeh village	2600	32° 47′ 76″	50° 03′ 33″	HSBU111
13	Tehran	Firuzkuh	2400	35° 75′ 39″	52° 75′ 14″	HSBU112
14	Fars	Shahr miyan	2700	30° 84′ 40″	52° 06′ 76″	HSBU113
15	Chahar mahaal and Bakhtiari	Dimeh village	2600	32° 47′ 76″	50° 03′ 33″	HSBU114

Note: Populations 1-13: *L. amplexicaule* var. *amplexicaule*; 14: *L. amplexicaule* var. *aleppicum* and 15: *L. amplexicaule* var. *bornmuelleri*.

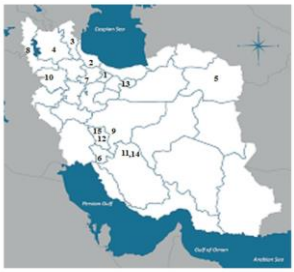

Figure 1. Distribution map of *Lamium amplexicaule* populations.
Note: Population numbers are according to Table 1.

Morphometry

In total 23 morphological characters including 18 quantitative and 5 qualitative characters were studied (Table. 2). Data obtained were standardized (Mean = 0, variance =1) and used to estimate Euclidean distance for clustering and ordination analyses (PODANI, 2000).

Table 2. Morphological characters studied

Morphological characters	
1	Plant height(cm)
2	Stem position
3	Stem ramification
4	Basal leaf shape
5	Floral leaf shape
6	Basal leaf length(mm)
7	Basal leaf width(mm)
8	Basal leaf length/Basal leaf width ratio
9	Basal petiole length(mm)
10	Floral leaf length (mm)
11	Floral leaf width (mm)
12	Floral leaf length /Floral leaf width ratio
13	Flowers cycle
14	Calyx length(mm)
15	Calyx width(mm)
16	Calyx length/Calyx width ratio
17	Calyx teeth length(mm)
18	Calyx teeth length(mm)/ Calyx length(mm) ratio
19	Corolla length(mm)
20	Corolla length(mm) / Calyx length(mm) ratio
21	Seed length(mm)
22	Seed width(mm)
23	Seed length/Seed width ratio

DNA extraction and ISSR assay

Genomic DNA was extracted using CTAB 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)9T, (GT)7CA, (CA)7AC, (GA)9A and (GA)9C commercialized by UBC (the University of British Columbia) were used.

PCR reactions were performed in a 25 ml volume containing 10 mM TriseHCl buffer at PH 8; 50 mM KCl; 1.5 mM MgCl2; 0.2 mM of each dNTP (Bioron, Germany), 0.2 mM 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, followed by 40 cycle of denaturing: 30s at 94°C, 1 min at 57°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).

cp-DNA

We generated five DNA sequences (of three presumptive groups) of plastid DNA region (psbA-trnH). Furthermore, six plastid sequences (two outgroups) were obtained from GenBank (supplementary Table). The intergenic spacer of chloroplast genome psbA-trnH was amplified and sequenced with universal primers following the methodology of SHAW *et al.*, (2005). The psbA-trnH forward primer was (psbA) 5´- GTTATGCATGAACGTAATGCTC -3´ and, the reverse primer was (trnH) 5´ CGCGCATGGTGGATTCACAATCC -3´. The cpDNA spacer were carried out in a total volume of 30 ml PCR mixture contained 10 ml of 2_ PCR buffer, 0.5 mM of each primer, 200 mM of each dNTP, 1Unit of Taq DNA polymerase (Bioron, Ludwigschafen, Germany), and 1 µl of template genomic DNA at 20 ng µl_1. The PCR amplification program was: 2 min at 95°C, followed by 35 cycles of 60s at 94°C, 60s at 52°C, and 75s at 72°C, with a final extension step of 10 min at 72°C.

Data analysis

Morphological analysis

Morphological characters were standardized (Mean = 0, variance =1) and used to establish the Euclidean distance among the pairs of taxa (PODANI, 2000). Different clustering and ordination methods like, UPGMA (Unweighted paired group using average), and Ward (Minimum spherical variance) clustering as well as principal coordinate analysis (PCoA) were used for grouping of the species. To identify the most variable morphological characters, Principal components analysis (PCA), was used (PODANI, 2000; SAFAEI *et al*., 2016).

Genetic diversity and population structure

ISSR bands obtained were scored as present (1) or absent (0) at each locus. Genetic diversity parameters like, Nei's gene diversity (H), Shannon information index (I), number of effective alleles (Ne), and percentage of polymorphism were determined in each population (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).

Neighbor Joining (NJ) clustering method and Neighbor Net method of networking were performed after 100 times bootstrapping/ permutations, for grouping of the plant specimens, (HUSON and BRYANT, 2006; FREELAND *et al*., 2011).

PAST ver. 2.17 (HAMER *et al*., 2012), was used for performing the Mantel test to check correlation between geographical distance and the genetic distance of the studied species (PODANI, 2000). DARwin ver. 5 (2012) and SplitsTree4 V4.13.1 (2013) programs were used for grouping of populations.

Significant genetic difference among the studied populations was determined by AMOVA (Analysis of molecular variance) test (with 1000 permutations) by using GenAlex 6.4 (PEAKALL and SMOUSE, 2006).

Bayesian model-based STRUCTURE analysis (PRITCHARD *et al.*, 2000) and Maximum likelihood-based method of K-Means clustering were used to study the genetic structure of populations (SHEIDAI *et al*., 2014a). For STRUCTURE analysis, data were scored as dominant markers (FALUSH *et al*., 2007). The Evanno test (EVANNO *et al.*, 2005) and K-Means clustering were used to identify optimum k genetic groups (SHEIDAI *et al*., 2014a). Two summary 5 statistics: (1) pseudo-F and (2) Bayesian Information Criterion (BIC) provide the best fit for *k* (MEIRMANS, 2012).

Gene flow was determined by Calculating Nm an estimate of gene flow from Gst by PopGene ver. 1.32 (1997) as: Nm = 0.5(1 -Gst)/Gst.

Chloroplast region analysis

The cp-DNA spacer psbA-trnH was aligned and cured. These were then used to construct Neighbor Joining (NJ), UPGMA, and maximum parsimony (ML) phylogenetic trees as performed in MEGA 5 software (TAMURA *et al*., 2011). Bootstrapping was performed with 100 replications. Networking was done by Splits Tree4 program (HUSON and BRYANT, 2006).

Biogeography

For biogeography analysis we used ISSR data. RASP program (Reconstruction Ancestral State in Phylogenies) (YU *et al*., 2015) was used for biogeography analyses. RASP is a tool for inferring ancestral state using S-DIVA. We used both Binary MCMC and S-Diva methods of RASP for our analyses. Based on ancestral area of population distribution, the route of this species in the country was reconstructed.

RESULTS

Taxonomic delineation of varieties in L. amplexicaule

PCoA plot of both quantitative and qualitative morphological characters separated the studied species in three distinct groups (Fig. 2). var. *amplexicaule* (No.1) and var. *bornmuelleri* (No.3) were placed close to each other in one side of the plot, while var. *aleppicum* (No.2) was placed in other side.

In PCoA plot of ISSR data, plants of var. *aleppicum* (denoted as 2 in Fig. 3) and plants of var. *bornmuelleri* (denoted as 3 in Fig. 3) were intermixed with each other, while plants of var. *amplexicaule* (denoted as 1 in Fig. 3) were almost separated from them.

Maximum likelihood tree of cp-DNA (Fig. 4) produced three separate clades. The out-group taxa formed the first clade, and were separated from the other taxa studied.

The second clade was made by the members of *L. amplexicaule* var. *aleppicum*, while, the third clade was formed by members of *L. amplexicaule* var. *amplexicaule* and *L. amplexicaule* var. *bornmuelleri*. Therefore, *L. amplexicaule* var. *bornmuelleri* is nested within *L. amplexicaule* var. *amplexicaule* and they cannot be distinguished by molecular data used.

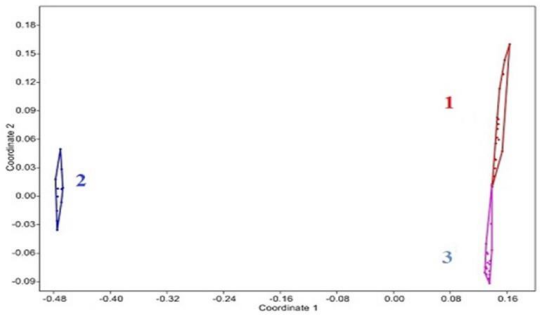

Figure 2. PCoA plot of morphological data of studied populations.
Species abbreviations: Varieties 1-3 are: var. *amplexicaule*, var. *aleppicum*, and var. *bornmuelleri*, respectively.

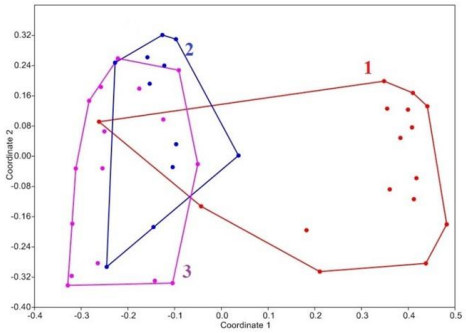

Figure 3. PCoA plot of ISSR data of studied populations
Varieties numbers are according to Figure 2.

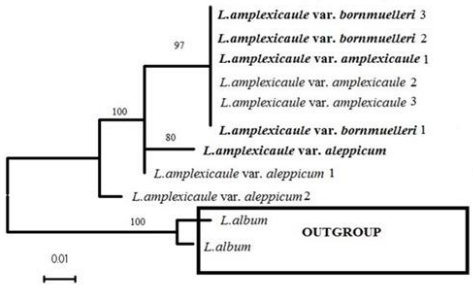

Figure 4. cp-DNA Maximum likelihood tree of *L. amplexicaule*. Specimens which we analyzed are in bold. Bootstrap values ≥ 50% from 100 replicates were indicated above branches.

Genetic diversity and population structure

Genetic diversity parameters of the studied populations are presented in Table. 3. The highest level of genetic polymorphism (47.75%) occurred in population No. 3, while the lowest level (17.12%) occurred in population No. 8 and 10.

Population No. 3 also had the highest values for effective number of alleles (Ne = 1.209) and Shannon information index (I = 0.205).

Table 3. Genetic diversity parameters in the studied populations

Pop	N	Na	Ne	I	He	UHe	%P
Pop1	10	0.721	1.172	o.161	0.104	0.109	36.03%
Pop2	10	0.766	1.160	0.157	0.100	0.105	36.94%
Pop3	10	0.955	1.209	0.205	0.131	0.137	47.75%
Pop4	10	0.739	1.155	0.152	0.096	0.107	36.94%
Pop5	9	0.775	1.135	0.149	0.091	0.096	37.84%
Pop6	8	0.496	1.110	0.102	0.066	0.070	23.42%
Pop7	7	0.604	1.134	0.133	0.084	0.091	29.73%
Pop8	6	0.369	1.097	0.087	0.058	0.063	17.12%
Pop9	6	0.640	1.143	0.141	0.090	0.098	31.53%
Pop10	6	0.328	1.109	0.094	0.063	0.069	17.12%
Pop11	6	0.523	1.102	0.105	0.065	0.071	25.23%
Pop12	9	0.477	1.109	0.102	0.066	0.070	22.52%
Pop13	6	0.541	1.119	0.118	0.075	0.082	26.13%

Abbreviations: N = Number of individuals, Na = No. of alleles, Ne = No. of effective alleles, He = Gene diversity index, UHe = Unbiased gene diversity, %P = Percentage of polymorphism. Population codes are according to Table 1.

AMOVA test revealed significant molecular difference (PhiPT = 0.33, P = 0.001) among the studied populations. It also revealed that 33% of total genetic variability occurred among the studied populations while, 67% occurred within populations. These results indicate a high-level genetic diversity both within and among populations. This conclusion was supported by Gst value obtained after 999 permutations (Gst = 0.33, P = 0.001).

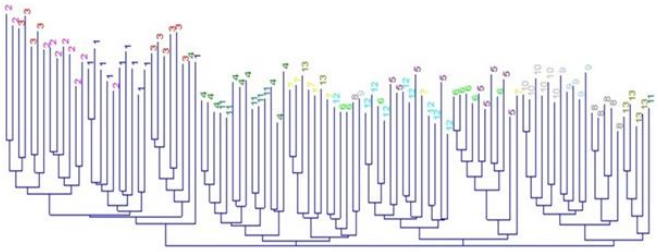

Figure 5. NJ tree of *L. amplexicaule* var. *amplexicaule* (the population code is according to Table 1).

NJ tree of the populations based on ISSR data (Fig. 5) produced two major clusters. Plants of populations 1-3 formed the first cluster, while plants of populations 4-13 comprised the second cluster. Most of the plants in each population were placed close to each other, but some were placed intermixed with the other populations. This result indicated a certain degree of gene flow/shared alleles in the studied populations.

Nei's genetic identity and genetic distance were determined among the studied populations. The highest degree of genetic similarity (0.9801) occurred between population 5 and population 6, followed by population 4 and population 11 (0. 0.98). The lowest degree of genetic similarity occurred between population 2 and 10 (0.9106).

The Nm analysis produced the mean Nm = 1.00, which indicates a good level of gene flow/shared ancestral alleles among var. *amplexicaule* populations, indicating that *L. amplexicaule* is predominantly an out-crossing species in Iran.

The Mantel test produced significant correlation between genetic distance and geographical distance of the studied populations (r = 0.097, P = 0.04). This indicated the occurrence of isolation by distance (IBD) in *L. amplexicaule* var. *amplexicaule* populations. The Evanno test and pseudo-F value of K-Means clustering produced the optimum number of k = 2. Therefore, two genetic groups can be identified in the studied populations. The STRUCTURE plot (Fig. 6) based on k = 2, revealed that populations 1-3 are genetically more alike and form the first gene pool, while populations 4-13 comprise the second gene pool.

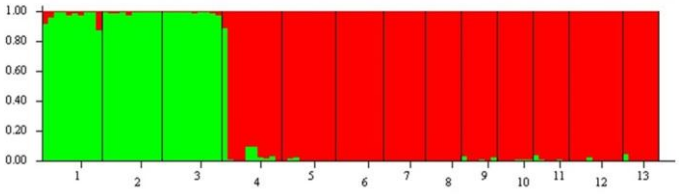

Figure 6. STRUCTURE plot of *L. amplexicaule* var. *amplexicaule* populations (population numbers are according to Table 1).

Biogeography

RASP analysis and reconstruction of the ancestral areas of the studied populations (Fig. 7) revealed that three regions, viz. Ardabil province in North - West of Iran, as well as Alborz and Gilan provinces in North of Iran are the most probable ancestral regions occupied by *L. amplexicaule* var. *amplexicaule*. The second probable ancestral area was Razavi Khorasan in east of Iran and Kohgiluyeh and Buyerahmad in south west of Iran. Based on these findings, the path of geographical distribution of this species is presented in Fig. 7.

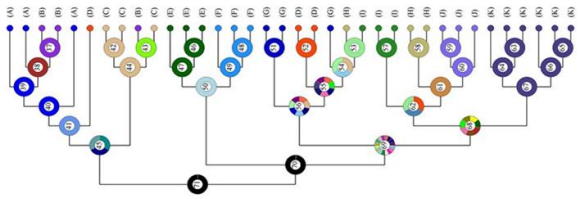

Figure 7. A: RASP results by Bayesian Binary method (MCMC). B: geographical distribution of populations based on ISSR data.

Locality abbreviations: A = Alborz, B = Gilan, C = Ardabil, D = East Azarbaijan, E = Razavi Khorasan, F = Kohgiluyeh and Buyerahmad, G = Qazvin, H = West Azarbaijan, I = Isfahan, J = Kurdistan, K = Fars (outgroup).

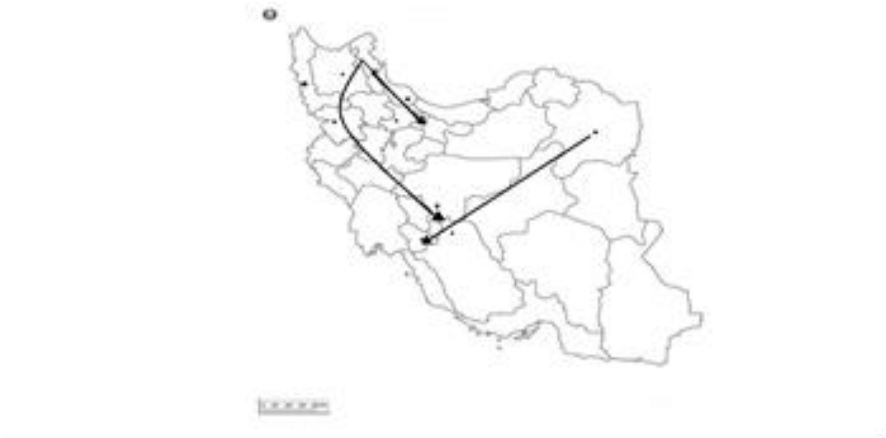

Figure 7. B: geographical distribution of populations based on ISSR data.

DISCUSSION

Taxonomic delineation of varieties

Three varieties were reported for *L. amplexicaule* in Iran (MENNEMA, 1989). This species shows great variability, especially in quantitative morphological characters. These characters have been used to differentiate the three presumed varieties.

Molecular phylogeny investigation of the genus *Lamium* performed by BENDIKSBY *et al*., (2011) showed that *L. amplexicaule* is polyphyletic and its varieties were not placed in a single clade (var. *aleppicum* was placed far from others).

Systematic position of *Lamium aleppicum* Boiss. is controversial. It was originally described as a species, but latter it was considered as a variety under *L. amplexicaule* by BORNMÜLLER (1907) (BENDIKSBY *et al*., 2011). However, BENDIKSBY *et al*. (2011), based on molecular data and support from morphology (narrower leaves, faint grayish-bluish tint of the leaves and long nutlets), proposed that *L. aleppicum* should be resurrected as a separate species. Our morphological results showed that the leaf length/ width ratio ranged in size from 1.8–2.5 in var. *aleppicum*. This range was 0.5-1.0 in the other two presumed varieties. Moreover, var. *aleppicum* had 2.8-3.1 mm long nutlets, while the other varieties had 2.1–2.5 mm long nutlets. Therefore, the present study supports the species rank for *L. aleppicum*.

Our molecular results, based on plastid sequences clearly showed that plants preliminary collected as *L. amplexicaule* var. *bornmuelleri* were placed inter-mixed with *L. amplexicaule* var. *amplexicaule*, and therefore, these two varieties cannot be distinguished based on cp-DNA data.

The genus *Lamium* is evidently the subject of complex evolutionary history caused mainly by intensive hybridization events (BENDIKSBY *et al*., 2011; SALMAKI *et al*., 2015). Therefore, the genus is still in need of a comprehensive molecular phylogenetic study including adequate numbers of taxa and markers (SALMAKI et al., 2015).

Population genetic structure

L. amplexicaule is weedy invasive species, which can aggressively spread through farms, grasslands, and gardens. This species is a cleistogamous plant that produces both closed flowers (obligate self-pollinated) (FRYXELL, 1957), and open flowers (potentially outcrossed) (ORUETA and VIEJO, 1999).

The AMOVA test indicated that 67% of total variation was due to within population genetic variability. This should be related to outcrossing of this plant species. The presence of high within population genetic variability helps the population to cope with local environmental changes (ÇALIŞKAN, 2012; SHEIDAI *et al*., 2013, 2014a). The within-population genetic diversity observed may be due to the ongoing gene flow, too. Gene flow via introducing new genes into the local populations increases their genetic variability (HOU and LOU, 2011; SHEIDAI *et al*., 2014a). The present study revealed high degrees of gene flow among *L. amplexicaule* populations.

The Nm analysis produced the mean Nm = 1, which indicates a good degree of gene flow due to cross-pollination of the studied populations. LORD (1982) showed that the percentage of open flowers produced during the flowering season can vary in response to environmental causes such as photoperiod and temperature, being as low as zero when plants are exposed to short, cold days. He also suggested that such a plastic production of open flowers is an adaptation to long, warm days, i.e., spring season, when pollinators are abundant (LORD, 1982). Similarly, in the short, cold, autumn days, when pollinators are few, the production of closed flowers providing autonomous self-fertilization is favored (LORD, 1982). Such an adaptation assumes that open flowers are able to outcross substantially, thus increasing individual fitness by avoiding the deleterious effects of inbreeding (CHARLESWORTH and WILLIS, 2009; STOJANOVA *et al*., 2013). Our result revealed high levels of within population genetic variability indicating that *L. amplexicaule* is predominantly an out-crossing species in Iran.

The STRUCTURE result revealed that *L. amplexicaule* contains two main gene pools in the country. The first gene pool is comprised of populations that are mainly located in the Northern parts of Iran. The second gene pool showed a higher concentration in western and southwestern parts and Eastern regions of the country. These populations are genetically and morphologically diverged from each other. The local environmental and ecological features, such as local mountains (Alborz Mountains reaching up to 5000 m in height), which separate populaions 1-3 (first gene pool) from other populations, can act as a block against gene flow between these populations.

In conclusion, the present study provide information on taxonomy of *L. amplexicaule* and potential gene pools of this medicinal plant in the country which may be used for further conservation and breeding studies.

ACKNOWLEDGEMENTS

We thank Dr. Fahimeh Koohdar and Miss. Somayeh Ghasemzadeh-Baraki for technical support.

Supplementary Table. Alphabetical list of specimens used in the present study including voucher information and GenBank accession numbers.

Taxon	Voucher information	GenBank accession number
L .album L.	Krawczyk., et al	KF055097
L .album L.	Xiang., et al	JX893256.1
L. amplexicaule var. *aleppicum* (Boiss. & Hausskin. ex Boiss.) Bornm.	Stapf O. 209	JF780118.1
L. amplexicaule var. *aleppicum* (Boiss. & Hausskin. ex Boiss.) Bornm.	Pichler Th, anno s.n.1882	JF780119.1
L. amplexicaule var. *amplexicaule* L.	Elven R. 280241	JF780123.1
L. amplexicaule var. *amplexicaule* L.	Leenhouts P. W. 3568	JF780122.1

Received, July 09th, 2017
Accepted February 15th, 2018

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BIOSYSTEMATICS STUDY AND POPULATION GENETIC ANALYSIS IN *Lamium amplexicaule* L. (LAMIOIDEAE, LAMIACEAE)

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Izvod

L. amplexicaule L. raste u različitim regionima u Iranu i formira nekoliko populacija koje mogu biti invazivne u nekim oblastima. Morfološke i genetske varijacije *L. amplexicaule* u Iranu su proučavane kako bi se procenila diferencijacija između tri taksona. Rezultati su pokazali da *L. amplexicaule* var. *bornmuelleri* Mennema ne formiraju ni jednu genetički različitu grupu. Genetičko proučavanje 103 biljna uzorka 13 različitih populacija korišćenjem ISSR molekularnih markera otkrila je visok stepen genetičke raznovrsnosti unutar populacije (67%) i između njih (33%). AMOVA i Gst analize su proizvele značajnu razliku. Procena Nm pokazala je određeni stepen protoka gena / zajedničkih alela između ovih populacija, što ukazuje na to da je *L. amplexicaule* pretežno netipična vrsta u Iranu. Mantelov test je pokazao značajnu korelaciju između genetičke distance i geografske udaljenosti proučavanih populacija (r = 0,097, P = 0,04). STRUCTURE je identifikovao dva osnovna genska pula za ovu vrstu u Iranu.

Primljeno 09.IX. 2017.
Odobreno15. II. 2018.