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GENETIC DIVERSITY AND POPULATION STRUCTURE ANALYSIS IN *Lonicera* L. (Caprifoliaceae) WITH THE USE OF ISSR MOLECULAR MARKERS

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Species delimitation is essential since species is regarded as the basic unit of analysis in nearly all biological disciplines, such as ecology, biogeography, conservation biology, and macroevolution. The genus *Lonicera*, which includes approximately 200 species, is a major component of the family Caprifoliaceae, comprising a large number of horticultural and economically important shrubs and tree species. This genus is represented in Iran by 9 species. In spite vast distribution of many *Lonicera* species that grow in Iran, there are not any available report on their genetic diversity, mode of divergence and patterns of dispersal. Therefore, data molecular (ISSR markers) with four primer pairs generated 77 polymorphic bands and morphological studies of 70 accessions from 7 species of *Lonicera*, that were collected from different habitats in Iran were performed. The aims of the present study are 1) to find the diagnostic value of ISSR markers in delimitation of *Lonicera* species, 2) to find the genetic structure of these taxa in Iran, and 3) to investigate the species inter-relationship. The present study revealed that combination of morphological and ISSR data can delimit the species. AMOVA and STRUCTURE analysis revealed that the species of *Lonicera* are genetically differentiated but have some degree of shared common alleles.

Key words: Inter-simple sequence repeat, Morphology, Species delimitation, *Lonicera*

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INTRODUCTION

Species delimitation is important in different biological disciplines, like ecology, biogeography, and plant conservation (ESFANDANI-BOZCHALOYI *et al*., 2017a; 2017b) Species delimitation is done by tree-based and non-tree-based approaches (JIA *et al*, 2021). In the first method, species form distinguishing clades (phylogenetic species concept), whereas in non-tree-based method, the species are recognized on the basis of gene flow assessments (biological species concept; BI *et al*, 2021; CHENG *et al*, 2021).

Lonicera L. (Caprifoliaceae) includes more than 200 species (MABBERLEY, 2008) worldwide, with 19 species in the region of Flora Iranica (WENDELBO, 1965). The genus is mainly distributed in temperate to subtropical regions of the northern hemisphere: Europe, Russia, East Asia, and North America (HSU and WANG, 1988; MABBERLEY, 2008). In the flora of Iran, the genus *Lonicera* is represented by nine species (KHATAMSAZ, 1995; GHAHREMANINEJAD and EZAZI, 2009) across the north, northwest and northeast of the country. Some species are medicinal plants (ZENG *et al*., 2017). Dried *Lonicera* flowers and buds are known as Flos Lonicera and have been a recognized herb in the traditional Chinese medicine for more than 1500 years. It has been applied for treatment of arthritis, diabetes mellitus, fever, and viral infections (SHANG *et al*., 2011). The plants are erect shrubs, occasionally small trees. Members of *Lonicera* are characterized by opposite, narrowly elliptic to ovate leaves, white, yellow, reddish, or purple-red corolla with capitate stigma (JUDD *et al*., 2007), and undulate calyx margin. In Flora Iranica, WENDELBO (1965) classified 19 species of the *Lonicera* into two subgenera (*Chamaecerasus* and *Lonicera*) and three sections, named *Coloxylosteum*, Isika and *Coeloxylosteum.* The four studied species belong to subgenus *Chamaecerasus* and sections *Isika* and *Coeloxylosteum*.

Molecular data have been obtained in phylogenetic studies and species divergence researches (KAZEMPOUR OSALOO *et al*., 2003; 2005). These data can also provide supportive and extra criteria for systematic classification of the studied species that have been based only on the morphological characters (CHASE *et al*., 1993). The internal transcribed spacer (ITS) is the region of the 18S-5.8 S-26S nuclear ribosomal cistron (BALDWIN *et al*., 1995). The spacers contain the signals needed to process the rRNA transcript (BALDWIN, 1992; BALDWIN *et al*., 1995) and have often been used for inferring phylogeny at the generic and infrageneric levels in plants (e.g. BALDWIN, 1992; BALDWIN *et al*., 1995; KAZEMPOUR OSALOO *et al*., 2003; 2005). THEIS *et al*. (2008) studied phylogenetics of the *Caprifolieae* and *Lonicera* (*Dipsacales*) on the basis of nuclear and chloroplast DNA sequences. Their analysis indicates monophyly in *Lonicera* and highlights instances of homoplasy in several morphological characters. Molecular phylogenetics of *Lonicera* in Japan has been studied by NAKAJI *et al*. (2015) on the basis of chloroplast DNA sequences. *Lonicera* is well known for its taxonomic complexity resulting from overlapping morphological characters.

In the past four decades, researchers have developed several molecular marker techniques including inter-simple sequence repeat (ISSR) (MA *et al*, 2021; PENG *et al*, 2021; SI *et al*., 2018; ZOU *et al*, 2019). ISSR is rapid, inexpensive, simple, and efficient DNA marker method that does not require prior knowledge of the DNA sequence or large amounts of starting DNA template. ISSR is widely used for germplasm identification, fingerprint construction, and genetic diversity and relationship analyses (ESFANDANI-BOZCHALOYI *et al*., 2017c; 2017d). There

are no attempt to study genetic diversity, ecological adaptation and intra- and inter-specific differentiation along with morphometric studies on *Lonicera* of Iran. Therefore, we performed morphological and molecular study of 7 collected species of *Lonicera.* The project try to answer the following questions: 1) Is there infra- and inter-specific genetic diversity among studied species? 2) Is genetic distance among these species correlated with their geographical distance? 3) What is the genetic structure of populations and taxa? 4) Is there any gene exchange between *Lonicera* species in Iran? Therefore it is important to delimit the identified species for performing further detailed molecular studies.

MATERIAL AND METHODS

Plant materials

In present study, 70 plant samples were collected from 12 geographical populations belong to7 *Lonicera* species. The species studied are *L. bracteolaris* Boiss. & Buhse, *L. hypoleuca* Decne., *L. iberica* M. Bieb., *L. korolkowii* Stapf, *L. floribunda* Boiss. & Buhse, *L. nummulariifolia* Jaub. et Spach and *L. caucasica.* Different references were used for the correct identification of species (Flora Iranica (WENDELBO, 1965)). Details of sampling sites are mentioned (Table 1, Figure 1). Vouchers were deposited at the herbarium of Islamic Azad University, Science and Research Branch, Tehran, Iran (IAUH).

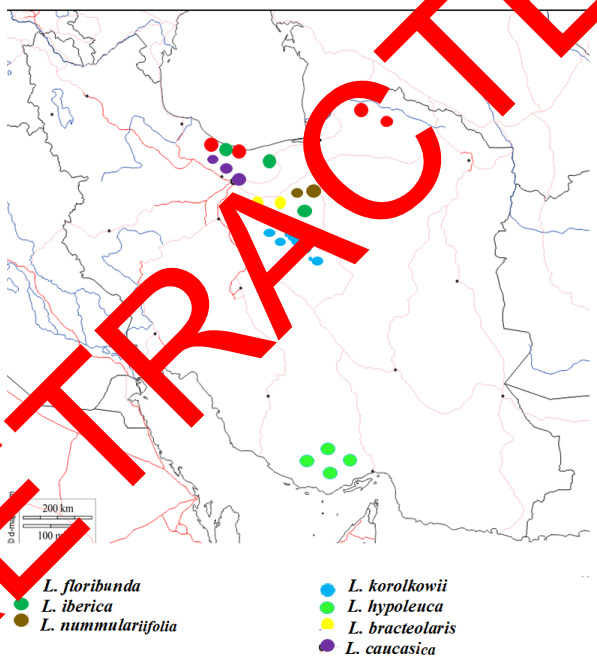

Fig. 1. Distribution map in studied species

Table 1. Lonicera species and populations, their localities and voucher numbers

Sp.	Locality	Latitude	Longitude	Altitude (m)	Voucher no.
1. *Lonicera floribunda* Boiss. & Buhse	Mazandaran, Chalus, Valiabad	38 ° 52'393"	47 ° 25' 92"	1133	IAUH 201677
	Mazandaran, Noshahr, Kajoor	38 ° 52'353"	47 ° 27 92"	1143	IAUH 201678
2. *L. iberica* M. Bieb.	Golestan, Jahan Nama	38 ° 52'373	47 ° 23' 92	1144	IAUH 201679
	Tehran, Firuzkuh Road, Gaduk	38 ° 52'353	47 ° 27' 92"	1143	IAUH 201680
	Mazandaran, Marzanabad, Kelardasht Dalir	38 ° 52'393	47 ° 25' 92"	1137	IAUH 201681
	Semnan, Mehdishahr, Fenisk Jungle	38 ° 51' 51"	47 ° 02' [illegible]8"	[illegible]55	IAUH 201682
3. *L. nummulariifolia* Jaub. et Spach	Semnan, tange parvar	38 ° 52'372	[illegible]7 ° 23' 92	1144	IAUH 201683
4. *L. bracteolaris* Boiss. & Buhse	Semnan, Shahrud, Abr Forest	38 [illegible]52'353	47 ° 27' 92	1143	IAUH 201686
5. *L. caucasica*	Mazandaran, Chalus, Pole Zangoole	3[illegible]'09' 55"	49 ° 55' 49 "	32	IAUH 201689
6. *L. hypoleuca* Decne.	Hormozg[illegible], Ban[illegible]ar Abbas, [illegible]iyahu	370702.32	494432.6	48	IAUH 201690
7. *L. korolkowii* Stapf	Se[illegible]an, M[illegible]dishahr, [illegible]li	38 ° 52'373	47 ° 23' 92"	1144	IAUH 201695

Morphological studies

Five [illegible] sam[illegible]les from each species were used for Morphometry. In total 52 morpho[illegible]g[illegible]al (2[illegible]qual[illegible]tative, 29 quantitative) characters were studied. Data obtained were stand[illegible]rdized (Mean=0, variance = 1) and used to estimate Euclidean distance for clustering and ordin[illegible]ion [illegible]nalys[illegible] (PODANI, 2000).

DNA extrac[illegible]ion and ISSR assay

Fresh leaves were used randomly from 5-10 plants in each of the studied populations. These were dried by silica gel powder. CTAB activated charcoal protocol was used to extract

genomic DNA (ESFANDANI-BOZCHALOYI *et al.*, 2019). The quality of extracted DNA was examined by running on 0.8% agarose gel. 4 ISSR primers; (AGC) 5GT, (AGC) 5GG, UBC 810, (GA) 9T commercialized by UBC (the University of British Columbia) were used. PCR reactions were carried in a 25µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM $MgCl_2$; 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, followed by 40 cycles of 1 min at 94°C; 1 min at 52-57°C and 2 min at 72°C. The reaction was completed by final extension step of 7-10 min at 72°C. The amplification products were observed by running on 1% agarose gel, followed by the ethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany).

Data analyses

Morphological studies

Morphological characters were first standardized (Mean = 0, Variance = 1) and used to establish Euclidean distance among pairs of taxa (PODANI, 2000). For grouping of the plant specimens, The UPGMA (Unweighted paired group using average) and Ward (Minimum spherical characters), as well as ordination methods of MDS (Multidimensional scaling) and PCoA (Principal coordinate analysis), were used (PODANI, 2000). ANOVA (Analysis of variance) were performed to show morphological differences among the populations while, PCA (Principal components analysis) biplot was used to identify the most variable morphological characters among the studied populations (PODANI, 2000). PAST version 2.17 (HAMMER *et al.*, 2012) was used for multivariate statistical analysis of morphological data.

Molecular analyses

ISSR bands obtained were coded as binary characters (presence = 1, absence = 0) and used for genetic diversity analysis. Parameters like Nei's gene diversity (H), Shannon information index (I), the number of effective alleles, and percentage of polymorphism were determined (WEISING *et al.*, 2005; FREELAND *et al.*, 2011). Nei's genetic distance among populations was used for Neighbor Joining (NJ) clustering and Neighbor-Net networking (FREELAND *et al.*, 2011; HUSON and BRYANT, 2006). Mantel test checked the correlation between geographical and genetic distance of the studied populations (PODANI, 2000). These analyses were done by PAST ver. 2.17 (HAMMER *et al.*, 2012), DARwin ver. 5 (2012) and SplitsTree4 V4.13.1 (2013) software. AMOVA (Analysis of molecular variance) test (with 1000 permutations) as implemented in GenAlex 6.4 (PEAKALL and SMOUSE, 2006), and Nei's Gst analysis as implemented in GenoDive ver.2 (2013) (MEIRMANS and VAN TIENDEREN, 2004) were used to show genetic difference of the populations. Moreover, 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). The genetic structure of populations was studied by Bayesian based model STRUCTURE analysis (PRITCHARD *et al.*, 2000), and maximum likelihood-based method of K-Means clustering of GenoDive ver. 2. (2013). For STRUCTURE analysis, data were scored as dominant markers (FALUSH *et al.*, 2007). We used

the admixture ancestry model under the correlated allele frequency model. A Markov chain Monte Carlo simulation was run 20 times for each value of K after a burn-in period of 10^5. The Evanno test was performed on STRUCTURE result to determine proper number of *K* by using delta *K* value (EVANNO *et al*., 2005). In K-Means clustering, two summary statistics, pseudo-F, and Bayesian Information Criterion (BIC) provide the best fit for k (MEIRMANS, 2012).

Gene flow was determined by (i) Calculating Nm an estimate of gene flow from Gst by PopGene ver. 1.32 (1997) as: Nm = 0.5(1 - Gst)/Gst. This approach considers the 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). The presence of shared alleles was determined by drawing the reticulogram network based on the least square method by DARwin ver 5. (2012).

RESULTS

Species delimitation and inter-relationship

Morphometry

ANOVA showed significant differences (P<0.01) in quantitative morphological characters among the species studied. In order to determine the most variable characters among the taxa studied, PCA analysis has been performed. It revealed that the first three factors comprised over 77% of the total variation. In the first PCA axis with 40% of total variation, such characters as shape of petals, shape of sepals, peduncles and pedicels hair, stem hair, petiols hair density have shown the highest correlation (>0.7). Length of bract and pedicel, length and width of the petal, length and width of stem leaves were characters influencing PCA axis 2 and 3 respectively. Different clustering and ordination methods produced similar results therefore, UPGMA clustering and PCA plot of morphological characters are presented here (Figure. 2 and 3). In general, plant samples of each species, were grouped together and formed separate cluster. This result show that morphological characters studied can delimit *Lonicera* species in two different major clusters or groups. In the studied specimens we did not encounter intermediate forms. In general, two major clusters were formed in WARD tree (Figure. 2).

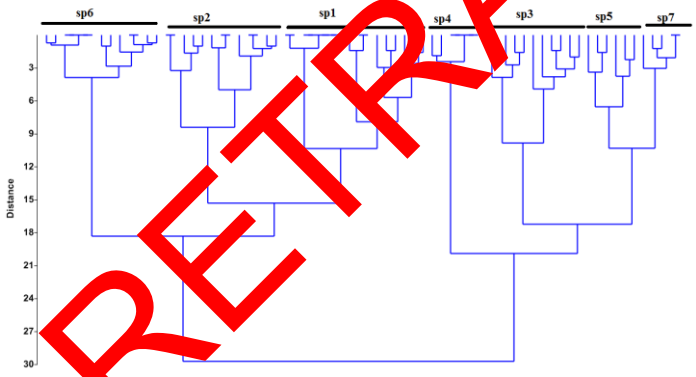

Fig 2. WARD clustering of morphological characters revealing species delimitation in Lonicera species

Populations of *Lonicera floribunda*, *L. iberica* and *L. hypoleuca* were placed in the first major cluster and were placed with great distance from the other species. The second major cluster included two sub-clusters. Plants of *L. nummulariifolia*, *L. caucasica* and *L. korolkowii* comprised the first sub-cluster, while plants of *L. bracteolaris* formed the second sub-cluster.

The PCA plot of morphological characters (Figure. Not included) separated the species into distinct groups with no inter-mixing. This is in agreement with WARD tree presented before. The plants of *L. nummulariifolia* showed more similarity to *L. bracteolaris* and were placed close to each other, Similarly, plants of *L. iberica* and *L. caucasica* were placed close to each other due to morphological similarity and were separated from the other species.

Species delimitation and genetic diversity

All ISSR primers produced polymorphic bands. All 3 primer AGC) 5GT, UBC 810, (GA) 9T produced 60 bands, while the lowest level of bands for (AGC) 5GG with 17 bands. For each ISSR locus were estimated by calculating Na, Ne, I and He and uHe index for each locus ranged from (0–0.09), (0.05–0.07), (0.0–0.2) and (0-0.1) respectively (Table 2).

Genetic diversity parameters determined in the studied species (Table 3) revealed that *Lonicera floribunda* (sp1) had the highest level of genetic polymorphism (65.91%), while the lowest level of genetic polymorphism (10.38%) occurred in *L. nummulariifolia* (sp3). *Lonicera floribunda* also had the highest values for effective number of alleles (Ne = 1.310) and Shannon information index (I =0.293).

AMOVA test showed significant genetic differences (P = 0.001) among studied species. It revealed that 75% of total variation was among species and 25% was within species. Pair-wise FST values showed significant difference among all studied species (Table 4). Moreover, genetic differentiation of these species was demonstrated by significant Nei's GST (0.44, P = 0.02) and D_est values (0.155, P = 0.01).

Table 2. Genetic diversity and differentiation of 4 ISSR Loci

Locus name	Alleles observed	Na	Ne	I	H_e	uH_e
Locus GT	20	0.017	0.052	0.003	0.001	0.001
Locus GA	20	0.000	0.050	0.000	0.000	0.000
Locus GG	17	0.029	0.062	0.005	0.003	0.002
Locus UBC 810	20	0.092	0.071	0.022	0.014	0.015

Na = the total number of alleles, Ne = the effective number of alleles, I = Shanon's information index, H_e = the expected heterozygosity, uH_e = Unbiased Expected Heterozygosity

Non-metric MDS plots of ISSR data (Figure 3) showed higher within species genetic diversity in the species number 1(*Lonicera floribunda*), supporting genetic diversity parameters obtained (Table 3). The MDS plot separated the species into distinct groups. This indicates that ISSR molecular markers can be used in *Lonicera* species delimitation. This is in agreement with AMOVA and genetic diversity parameters presented before. The species are genetically well differentiated from each other. The Nm analysis by Popgene software also produced mean Nm= 0.31, that is considered very low value of gene flow among the studied species.

Table 3. Genetic diversity parameters in the studied Lonicera species.

Pop	N	Na	Ne	I	He	UHe	%P
sp1	12.000	1.247	1.310	0.293	0.284	0.192	65.91%
sp2	8.000	0.419	1.097	0.084	0.056	0.060	16.13%
sp3	6.000	0.258	1.029	0.033	0.011	0.020	10.38%
sp4	12.000	0.925	1.259	0.233	0.155	0.162	44.09%
sp5	11.000	0.774	1.171	0.162	0.104	0.109	36.56%
sp6	14.000	0.344	1.069	0.064	0.041	0.043	13.98%
sp7	14.000	0.570	1.106	0.098	0.064	0.066	21.51%

(N = number of samples, Ne = number of effective alleles, I= Shannon's information index, He = gene diversity, UHe = unbiased gene diversity, P%= percentage of polymorphism, populations).

Mantel test with 5000 permutations showed a significant correlation (r = 0.22, p=0.0002) between genetic distance and geographical distance, so isolation by distance (IBD) occurred among the *Lonicera* species studied.

Nei's genetic identity and the genetic distance determined among the studied species (Table is not included). The results showed that the highest degree of genetic similarity (0.88) occurred between *L. iberica* and *L. hypoleuca*. The lowest degree of genetic similarity occurred between *L. iberica* and *L. korolkowii* (0.65).

NJ tree based on Nei,s genetic distance (Figure. Not included), showed that *L. nummulariifolia* differed genetically from the other studied species, as it stands far from them. This dendrogram showed close genetic affinity between *L. iberica* and *L. hypoleuca* supporting our morphological result presented before (Figure. 2). The other *Lonicera* species were placed closer to each other based on ISSR data, but their genetic affinity is not evident as is in morphology tree.

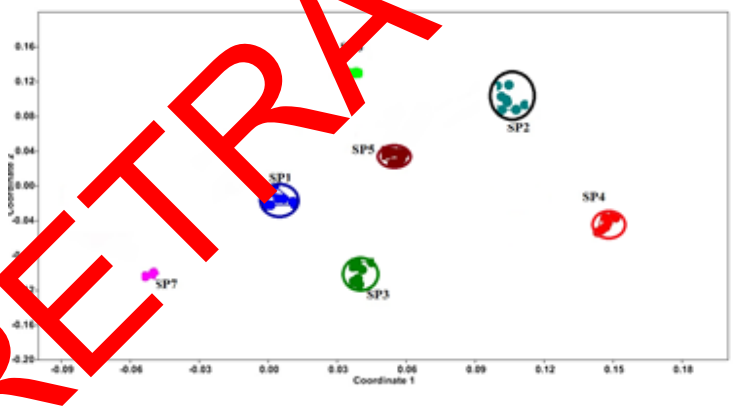

Fig 3. MDS plot of *Lonicera* species based on ISSR data

Table 4. Pair-wise FST values among the studied Lonicera species

	sp1	sp2	sp3	sp4	sp5	sp6	sp7
sp1	-	0.010	0.010	0.010	0.010	0.010	0.010
sp2	0.682	-	0.010	0.010	0.010	0.010	0.010
sp3	0.597	0.743	-	0.020	0.010	0.010	0.010
sp4	0.429	0.513	0.815	-	0.010	0.010	0.010
sp5	0.520	0.500	0.593	0.359	-	0.010	0.010
sp6	0.636	0.699	0.775	0.576	0.598	-	0.010
sp7	0.628	0.568	0.709	0.554	0.631	0.720	-

(Above diagonal = FST value, bellow diagonal =P value)

The species genetic STRUCTURE

We performed STRUCTURE analysis followed by the Evanno test to identify the optimal number of genetic groups. We used the admixture model to illustrate interspecific gene flow or/and ancestrally shared alleles in the species studied.

STRUCTURE analysis followed by Evanno test produced ΔK=7. The STRUCTURE plot (Figure. Not included) produced more detailed information about the genetic structure of the species studied as well as shared ancestral alleles and/or gene flow among *Lonicera* species. This plot revealed that Genetic affinity between *L. korolkowii* and *Lonicera floribunda* (similarly colored, No. 1, 7), as well as *L. iberica* and *L. hypoleuca* (sp No. 2,6) due to shared common alleles. This is in agreement with Neighbor joining dendrogram presented before. The other species are distinct in their allele composition.

The low Nm value (0.31) indicates limited gene flow or ancestrally shared alleles between the species studied and supports genetic stratification as indicated by K-Means and STRUCTURE analyses. Population assignment test also agreed with Nm result and could not identify significant gene flow among members of the studied species. However, reticulogram obtained based on the least square method (Figure is not included), revealed some amount of shared alleles between species 2 and 6 and between 2 and 7 also between 1 and 3. As evidenced by STRUCTURE plot based on admixture model, these shared alleles comprise very limited part of the genomes in species studied and all these results are in agreement in showing a high degree of genetic stratification in species studied.

DISCUSSION

Species delimitation and taxonomic consideration

Lonicera is one of the most important genera of *Caprifoliaceae*. Morphological analyses of the studied *Lonicera* species showed that they are well differentiated from each other both in quantitative measures (the ANOVA test result) and qualitative characters (The PCA plot result). In addition, PCA analysis suggests that characters like pedicel length, bract length, calyx shape, petal shape, length and width of stem-leaf, length and width of petal, peduncle and pedicel hair, hair and petal claw could be used in species groups delimitation.

Four species and 12 populations of the genus *Lonicera* have been studied in terms of pollen and seed micro-morphology and molecular phylogeny **(**AMINI *et al*., 2019**)**. Their results showed that micro-morphological and molecular data provide reliable evidence for

differentiation of some populations from others. Since *Lonicera* systematically is a problem genus, it is necessary to use alternative methods to distinguish its taxa. Statistical evaluation of taxa can be used for taxa delimitation. The present study intends to provide further evidence for taxonomists, so as to help them in separating these seven species. Our morphological results support close affinity between *L. iberica* and *L. hypoleuca*, as well as between *L. korolkowii* and *L. bracteolaris*, and these results are consistent with molecular findings. Our results correspond with the findings of THEIS *et al*. (2008) and NAKAJI *et al*. (2015).

Genetic structure and gene flow

Population genetic study provides valuable information about genetic structure of plants, the stratification versus gene flow among the species populations, genetic divergence of the populations, etc. (ESFANDANI-BOZCHALOYI, 2018a; 2018b; 2018c; 2018d; ZHENG *et al*., 2021; ZHU *et al*, 2021). This information have different applications, and from pure understanding of biology of the species to conservation of endangered species, choosing of proper parents for hybridization and breeding and phylogeography and mechanism of invasion (FREELAND *et al*., 2011; YIN *et al*., 2021).

AMOVA and STRUCTURE analysis revealed that the species of this genus are genetically differentiated but have some degree of shared common alleles. Several trends in pollination mechanism can be observed in *Lonicera* species with gradual transition between them. According to PHILIPP (1985), the methods we used are indirect estimation of gene flow and if it is identified to occur among species may be either due to ancestral shared alleles or ongoing gene flow. The Nm value obtained based on ISSR data revealed very limited amount of gene flow among the studied species that was also supported by STRUCTURE analysis as *Lonicera* species mostly had distinct genetic structure. Reticulation analysis also showed some degree of gene flow for ISSR. We did not observe any intermediate forms in our extensive plant collection, but morphological variability within each species did occur to some extent. Therefore, the low degree of gene flow identified by indirect methods applied may be due to a low degree of gene flow both ancestral shared alleles and ongoing gene.

In the study conducted by CHEN *et al*. (2012), 20 ISSR primers amplified 186 bands with 103 (54.63%) polymorphic bands and 5[illegible] sequence-related amplified polymorphism (SRAP) primer combinations amplified 591 bands with 347 (55.46%) polymorphic bands. Both ISSR and SRAP analyses revealed a middle level of genetic diversity in *Lonicera macranthoides* cultivars. SMOLIK *et al*. (2006) found a level of similarity for 6 populations of *Lonicera periclymenum* ranging from 82.3% to 86.6%, indicating their closely related nature. ISSR amplification was used by SMOLIK *et al*. (2010) to analyze polymorphisms of microsatellite sequences in the honeysuckle genome and to evaluate genetic diversity among 14 Polish and Russian blue honeysuckle accessions. Random amplified polymorphic DNA (RAPD) analysis was used by NAUGŽEMYS *et al*. (2011) to assess the genetic relationships among 51 accessions of blue honeysuckle. The pairwise genetic distance (GDxy) values among studied accessions ranged from 0.024 to 0.479; the mean GDxy was 0.283. Knowledge of the content of secondary metabolites in individual genotypes allows us to choose the best in *Lonicera* breeding programs in order to increase the nutritional value and health benefits.

*N*m is the movement of genes among and within populations and is negatively correlated with their genetic differentiation (GRANT, 1991). It is important for the mobility and evolution of plant populations and can be calculated using the following formula (MCDERMOTT and MCDONALD, 1993): $Nm = 0.5 (1 - GST)/GST$.

WRIGHT (1951) reported that *N*m values greater than 1 indicate that gene flow is occurring between populations. In the present study, *N*m among varietal populations of *Lonicera* was 0.31, indicating a low level of *N*m and a high degree of genetic differentiation. *N*m among bud-type varieties populations was even lower, at 0.110. To conclude, the present study revealed the use of ISSR molecular markers along with morphological characters in *Lonicera* species delimitation. Some degrees of interspecific genetic admixture occur in *Lonicera*, but the studied species are strongly differentiated during the speciation process and invasion in new habitats. Genetic drift, strong inbreeding, and local adaptation are effective evolutionary forces operating in *Lonicera* species and population divergence and adaptation.

Plant species delimitation is of central importance in phylogenetic systematics, evolution, biogeography and biodiversity. It is significant to infer patterns and mechanisms of speciation and hybridization, the evolutionary process by which new biological species arise and gene flow between closely related phylogenetic species can occur (DUMINIL and DI MICHELE, 2009). Isolation by distance, local adaptation and gene flow are different mechanisms responsible for species differentiation and genetic diversity (FREELAND *et al.* 2011).

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GENETIČKI DIVERZITET I ANALIZA STRUKTURE POPULACIJE KOD *Lonicera* L. (Caprifoliaceae) POMOĆU ISSR MOLEKULARNIH MARKERA

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Izvod

Razgraničenje vrsta je bitno jer se vrste smatraju osnovnom jedinicom analize u gotovo svim biološkim disciplinama, kao što su ekologija, biogeografija, biologija očuvanja i makroevolucija. Rod *Lonicera*, koji obuhvata približno 200 vrsta, glavna je komponenta porodice *Caprifoliaceae*, koja obuhvata veliki broj hortikulturnih i ekonomski važnih grmova i vrsta drveća. Ovaj rod je u Iranu zastupljen sa 9 vrsta. Uprkos velikoj rasprostranjenosti mnogih vrsta *Lonicera* koje rastu u Iranu, nema dostupnih izveštaja o njihovoj genetskoj raznolikosti, načinu divergencije i obrascima širenja. Zbog toga su molekularni podaci (ISSR markeri) sa četiri para prajmera generisali 77 polimorfnih traka. Izvršena su i morfološka ispitivanja 30 pristupa iz 7 vrsta *Lonicera*, koji su prikupljeni sa različitih staništa u Iranu. Ciljevi ove studije su: 1) pronaći dijagnostičku vrednost ISSR markera u razgraničenju vrsta *Lonicera*, 2) pronaći genetsku strukturu ovih taksona u Iranu, i 3) istražiti povezanost između vrsta. AMOVA i STRUCTURE analiza otkrile su da su vrste *Lonicera* genetski diferencirane, ali imaju određeni stepen zajedničkih alela.

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