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Genetic variations and interspesific relationships in *Lonicera* L. (Caprifoliaceae), using SCoT molecular markers

Fengzhen Chen[1], Dongmei Li[2,*], Mohsen Farshadfar[3]

[1] *Peony Research Institute, Heze University, Heze , Shandong 274000, China*
[2] *College of Horticulture Science and Engineering, Shandong Agricultural University, Taian, Shandong 271018, China*
[3] *Department of Agriculture, Payame Noor University (PNU), Tehran, Iran*
*Corresponding author: E-mail:majidkhayatnezhad126@gmail.com; spfood3200@163.com

Abstract. *Lonicera* L. (Caprifoliaceae) includes more than 200 species worldwide. The genus is mainly distributed in temperate to subtropical regions of the northern hemisphere: Europe, Russia, East Asia and North America. Some species are medicinal plants. 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. Due to the importance of these plant species, we performed a combination of morphological and molecular data for this species. For this study, we used 85 randomly collected plants from six species in 6 provinces. Amplification of genomic DNA using 10 primers produced 103 bands, of which 95 were polymorphic (90.98%). The obtained high average PIC and MI values revealed high capacity of SCoT primers to detect polymorphic loci among *Lonicera* species. The genetic similarities of 6 collections were estimated from 0.67 to 0.90. According to the SCoT markers analysis, *L. hypoleuca* and *L. iberica* had the lowest similarity and the species of *L. korolkowii* and *L. nummularifolia* had the highest similarity. The aims of present study are: 1) can SCoT markers identify *Lonicera* species, 2) what is the genetic structure of these taxa in Iran, and 3) to investigate the species inter-relationship? The present study revealed that SCoT markers can identify the species.

Keywords: gene flow, genetic admixture, *Lonicera,* Network, population structure.

INTRODUCTION

Genetic diversity is a basic component of biodiversity and its conservation is essential for long term survival of any species in changing environments (Mills and Schwartz 2005, Tomasello *et al.* 2015). This is very important in fragmented populations because are more vulnerable due to the loss of allelic richness and increased population differentiation by genetic drift (decreases heterozygosity and eventual fixation of alleles) and inbreeding

depression (increases homozygosity within populations; Frankham 2005). Among different populations, genetic diversity is non randomly distributed and is affected by various factors such as geographic variations, breeding systems, dispersal mechanisms, life span, etc (Khatamsaz 1995; Ghahremaninejad and Ezazi 2009).Change in environmental conditions often leads to variation in genetic diversity levels among different populations and populations with low variability are generally considered less adapted under adverse circumstances (Falk and Holsinger 1991, Olivieri *et al.* 2016). Most of the authors agree that genetic diversity is necessary to preserve the long-term evolutionary potential of a species (Falk and Holsinger 1991). In the last decade, experimental and field investigations have demonstrated that habitat fragmentation and population decline reduce the effective population size. In the same way, most geneticists consider population size as an important factor for maintaining genetic variation (Turchetto *et al.* 2016).

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 (Li *et al.* 2015). It has been applied for treatment of arthritis, diabetes mellitus, fever, and viral infections (Shang *et al.* 2011; Li *et al.* 2015). The plants are erect shrubs, occasionally small trees. Members of *Lonicera* are characterized by opposite, narrowly elliptic to obovate 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, namely *Isoxylosteum*, 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. According to the results, circumscription of the higher taxonomic groups for the Japanese species of *Lonicera* proposed by Hara in 1983 is fundamentally acceptable. *Lonicera* is well known for its taxonomic complexity resulting from overlapping morphological characters.

With the progress in plant molecular biology, numerous molecular marker techniques have been developed and used widely in evaluating genetic diversity, population structure and phylogenetic relationships. In recent years, advances in genomic tools provide a wide range of new marker techniques such as, functional and gene targeted markers as well as develop many novel DNA based marker systems (Wu *et al.* 2013). Start codon targeted (SCoT) polymorphism is one of the novel, simple and reliable gene-targeted marker systems. This molecular marker offers a simple DNA-based marker alternative and reproducible technique which is based on the short conserved region in the plant genes surrounding the ATG (Collard and Mackill 2009) translation start codon (Collard and Mackill 2009). This technique involves a polymerase chain reaction (PCR) based DNA marker with many advantages such as low-cost, high polymorphism and extensive genetic information (Collard and Mackill 2009, Luo *et al.* 2011, Wu *et al.* 2013).

The present investigation has been carried out to evaluate the genetic diversity and relationships among *Lonicera* species using new gene-targeted molecular markers, i.e. SCoT. This is the first study on the use of SCoT markers in *Lonicera* genus; Therefore, we performed molecular study of 85 specimens of 6 *Lonicera* species. We try to answer the following questions: 1) Is there infra and interspecific 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?

MATERIALS AND METHODS

Plant materials

A total of 85 individuals were sampled representing six geographical populations belong six *Lonicera* species (sp1= *Lonicera caucasica*; sp2= *Lonicera iberica* M. Bieb.; sp3= *Lonicera nummulariifolia* Jaub. et Spach; sp4= *Lonicera bracteolaris* Boiss. & Buhse; sp5= *Lonicera korolkowii* Stapf; sp 6= *Lonicera hypoleuca* Decne.) in East Azerbaijan, Guilan, Mazandaran, Tehran, Khorasan and Hormozgan Provinces of Iran during July-Agust 2017-2019. For morphometric and SCoT analysis we used 85 plant accessions (nine to eighteen samples from each populations) belonging to six different species with different eco-geographic characteristics were sampled and stored in -20 till further use. Voucher specimens are deposited in Herbarium of Azad Islamic University (HAIU). More information about geographical distribution of accessions are in Table. 1.

Morphological studies

Nine to eighteen samples samples from each species were used for Morphometry. In total 17 morphological (9 qualitative, 8 quantitative) characters were studied. Data obtained were standardized (Mean= 0, variance = 1) and used to estimate Euclidean distance for clustering and ordination analyses (Podani 2000). Morphological characters studied are: corolla shape, bract shape, seed color, seed shape, bract color, leaf surface, calyx shape, basal leaf shape, pedicel length, calyx length, bract length, corolla length, basal leaf length, basal leaf width, corolla color, stem leaf length and stem leaf width.

DNA extraction and SCoT assay

Fresh leaves were used randomly from nine to eighteen plants in each of the studied populations. These were dried by silica gel powder. CTAB activated charcoal protocol was used to extract genomic DNA (Doyle and Doyle 1987). The quality of extracted DNA was examined by running on 0.8% agarose gel. A total of 25 SCoT primers developed by Collard and Mackill (2009), 10 primers with clear, enlarged, and rich polymorphism bands were chosen (Table 2). 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) ordination methods were used (Podani 2000). ANOVA (Analysis of variance) were performed to show morphological difference 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 analyses of morphological data.

Table 1. Voucher details of *Lonicera* species and relative genera examined in this study from Iran.

Sp.	Locality	Sample size	Latitude	Longitude	Altitude (m)	Voucher no.
L. caucasica	Mazandaran, Chalus	18	34°52'393"	46°25'92"	1133	HIAU 201677
L. iberica M. Bieb.	East Azerbaijan, Kaleybar, Road side	16	38°52'373"	47°23'92"	1144	HIAU 201683
L. nummulariifolia Jaub. et Spach	Tehran, Alamut	14	33°52'353"	48°27'92"	1330	HIAU 201686
L. bracteolaris Boiss. & Buhse	Guilan, Gole rodbar, Road sid	9	34°09'55"	47°55'49"	1600	HIAU 201689
L. korolkowii Stapf	Khorasan, Bojnurd	15	320702.32	504432.6	2300	HIAU 201690
L. hypoleuca Decne.	Hormozgan, Bandar Abbas, Siyahu	13	38°52'373"	47°23'92"	1144	HIAU 201695

Table 2. SCoT primers used for this study and the extent of polymorphism.

Primer name	Primer sequence (5'-3')	TNB	NPB	PPB	PIC	PI	EMR	MI
SCoT-1	CAACAATGGCTACCACCA	15	14	93.74%	0.47	5.66	17.56	5.67
SCoT-3	CAACAATGGCTACCACCG	13	12	92.31%	0.54	3.21	15.60	5.55
SCoT-6	CAACAATGGCTACCACGC	7	7	100.00%	0.47	4.32	9.55	3.45
SCoT-11	AAGCAATGGCTACCACCA	11	9	82.89%	0.43	5.56	6.34	5.11
SCoT-14	ACGACATGGCGACCACGC	10	10	100.00%	0.56	4.86	9.55	3.22
SCoT-15	ACGACATGGCGACCGCGA	9	8	84.99%	0.41	4.91	7.43	4.85
SCoT-16	CCATGGCTACCACCGGCC	8	8	100.00%	0.44	4.34	11.55	6.44
SCoT-17	CATGGCTACCACCGGCCC	16	16	100.00%	0.67	5.88	8.56	3.65
SCoT-18	ACCATGGCTACCACCGCG	13	13	100.00%	0.55	6.23	8.23	6.47
SCoT-19	GCAACAATGGCTACCACC	10	10	100.00%	0.59	6.25	9.7	5.87
Mean		10	9	90.98%	0.56	5	9.5	5.9
Total		103	95					

Abbreviations: TNB = the number of total bands, NPB = the number of polymorphic bands, PPB (%) = the percentage of polymorphic bands, PI = polymorphism index, EMR = effective multiplex ratio, MI = marker index, PIC, polymorphism information content for each of CBDP primers.

Molecular analyses

SCoT bands obtained were coded as binary characters (presence = 1, absence = 0) and used for genetic diversity analysis. Discriminatory ability of the used primers was evaluated by means of two parameters, polymorphism information content (PIC) and marker index (MI) to characterize the capacity of each primer to detect polymorphic loci among the genotypes (Powell *et al.* 1996). MI is calculated for each primer as MI = PIC × EMR, where EMR is the product of the number of polymorphic loci per primer (*n*) and the fraction of polymorphic fragments (β) (Heikrujam *et al.* 2015). The number of polymorphic bands (NPB) and the effective multiplex ratio (EMR) were calculated for each primer. Parameter like Nei's gene diversity (H), Shannon information index (I), number of effective alleles, and percentage of polymorphism (P% =number of polymorphic loci/number of total loci) were determined (Weising *et al*, 2005, Freeland *et al.* 2011). Shannon's index was calculated by the formula: H' = -Σpiln pi. Rp is defined per primer as: Rp = ∑ Ib, were "Ib" is the band informativeness, that takes the values of 1-(2x [0.5-p]), being "p" the proportion of each genotype containing the band. The percentage of polymorphic loci, the mean loci by accession and by population, UHe, H' and PCA were calculated by GenAlEx 6.4 software (Peakall & Smouse 2006). Nei's genetic distance among populations was used for Neighbor Joining (NJ) clustering and Neighbor-Net networking (Huson & Bryant 2006, Freeland *et al.* 2011). Mantel test checked the correlation between geographical and genetic distances of the studied populations (Podani 2000). These analyses were done by PAST ver. 2.17 (Hammer *et al.* 2012), DARwin ver. 5 (2012) 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). To assess the population structure of the *Lonicera* accessions, a heuristic method based on Bayesian clustering algorithms were utilized. The clustering method based on the Bayesian-model implemented in the software program STRUCTURE (Pritchard *et al.* 2000; Falush *et al.* 2007) was used on the same data set to better detect population substructures. This clustering method is based on an algorithm that assigns genotypes to homogeneous groups, given a number of clusters (K) and assuming Hardy-Weinberg and linkage equilibrium within clusters, the software estimates allele frequencies in each cluster and population memberships for every individual (Pritchard *et al.* 2000). The number of potential subpopulations varied from two to ten, and their contribution to the genotypes of the accessions was calculated based on 50,000 iteration burn-ins and 100,000 iteration sampling periods. The most probable number (K) of subpopulations was identified following 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 (Nm) which were calculated using POPGENE (version 1.31) program (Yeh *et al.,* 1999). Gene flow was estimated indirectly using the formula: Nm = 0.25(1 - FST)/FST. In order to test for a correlation between pairwise genetic distances (FST) and geographical distances (in km) between populations, a Mantel test was performed using Tools for Population Genetic Analysis (TFPGA; Miller, 1997) (computing 999 permutations). This approach considers equal amount of gene flow among all populations. (ii) Population assignment test based on maximum likelihood as performed 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 identification 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 65% of the total variation. In the first PCA axis with 47% of total variation, such characters as seed shape, calyx shape, calyx length, bract length and basal leaf shape have shown the highest correlation (>0.7), seed color, leaf surface, corolla length and basal leaf length, were characters influencing PCA axis 2 and 3 respectively. Different clustering and ordination methods produced similar results therefore, PCA plot of morphological characters are presented here (Fig. 1). In general, plant samples of each species were grouped together and formed separate groups. This result show that both quantitative and qualitative morphological characters separated the studied species into distinct groups. In the studied specimens we did not encounter intermediate forms.

Species identification and genetic diversity

Ten SCoT primers were screened to study genetic relationships among *Lonicera* species; all the primers produced reproducible polymorphic bands in all 6 *Lonicera* species. An image of the SCoT amplification generated by SCoT-14 and SCoT-6 primer is shown in Figure 2. A total of 95 amplified polymorphic bands were generated across 6 *Lonicera* species. The size of the amplified fragments ranged from 100 to 2000 bp. The highest and lowest number of polymorphic bands were 16 for SCoT-17 and 7 for SCoT-6, on an average of 9 polymorphic bands per primer. The PIC of the 10 SCoT primers ranged from 0.41 (SCoT-15) to 0.67 (SCoT-17) with an average of 0.56 per primer. MI of the primers ranged from 3.22 (SCoT-14) to 6.47 (SCoT-18) with an average of 5.9 per primer. EMR of the SCoT primers ranged from

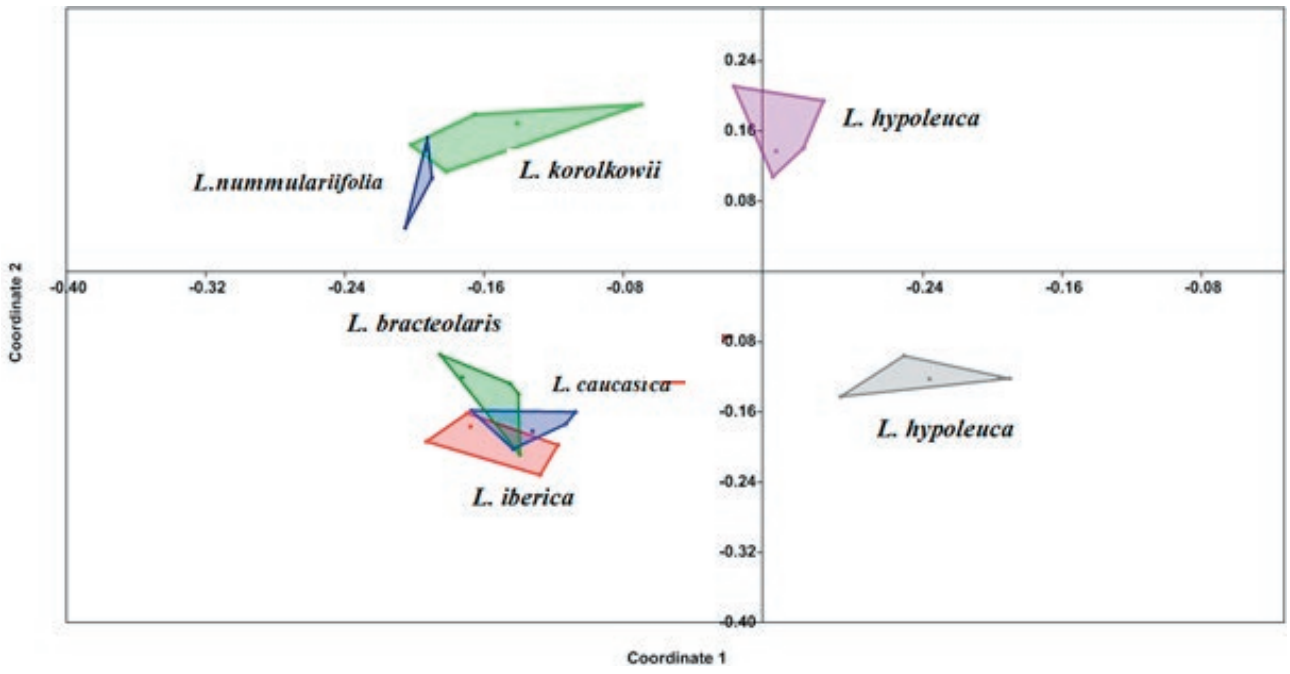

Figure 1. PCA plots of morphological characters revealing species delimitation in the *Lonicera* species.

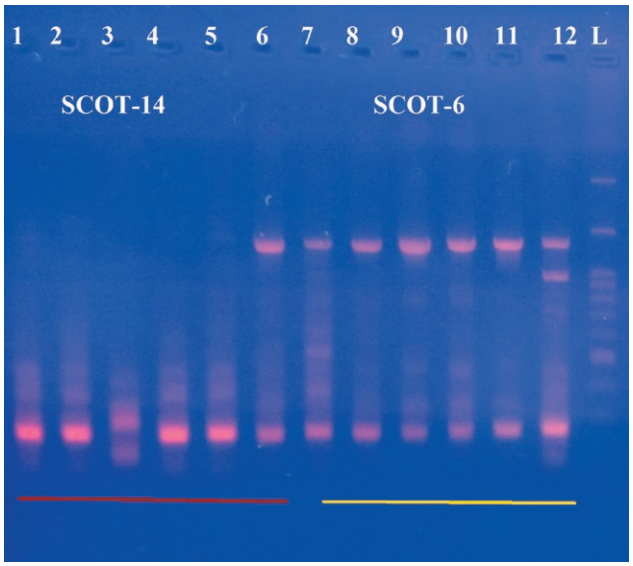

Figure 2. Electrophoresis gel of studied ecotypes from DNA fragments produced by SCoT-14, SCoT-6; sp1= *L. caucasica*; sp2= *L. iberica* M. Bieb.; sp3= *L. nummulariifolia* Jaub. et Spach; sp4= *L. bracteolaris* Boiss. & Buhse; sp5= *L. korolkowii* Stapf; sp 6= *L. hypoleuca* Decne; L = Ladder 100 bp, Arrows are representative of polymorphic bands

6.34 (SCoT-11) to 17.56 (SCoT-1) with an average of 9.5 per primer (Table 2). The primers with the high EMR values were considered to be more informative in distinguishing the genotypes.

The genetic parameters were calculated for all the 6 *Lonicera* species amplified with SCoT primers (Table 3). Unbiased expected heterozygosity (*H*) ranged from 0.13 (*L. caucasica*) to 0.33 (*L. hypoleuca*), with a mean of 0.21. A similar pattern was observed for Shannon's information index (*I*), with the highest value of 0.34 observed in *L. hypoleuca* and the lowest value of 0.18 observed in *L. caucasica* with a mean of 0.28. The observed number of alleles (*N*a) ranged from 0.201 in *L. bracteolaris* to 0.892 in *L. caucasica*. The effective number of alleles (*Ne*) ranged from 1.00 (*L. bracteolaris*) to 1.138 (*L. caucasica*).

AMOVA test showed significant genetic difference (P = 0.01) among studied species. It revealed that 53% of total variation was among species and 47% was within species (Table 4) Moreover, genetic differentiation of these species was demonstrated by significant Nei's GST (0.66, P = 0.01) and D_est values (0.222, P = 0.01). These results revealed a higher distribution of genetic diversity among *Lonicera* species compared to within species. Two major clusters were formed in UPGMA tree (Fig. 3). The first major cluster (A) contained two sub-clusters: *L. nummulariifolia* and *L. korolkowii* are separated from the other studied species and join the others with a great distance and comprised the first sub-cluster. The second sub-cluster was formed by *L. caucasica*; *L. iberica* and *L. bracteolaris*. The second major cluster also contained only 1 species of *L. hypoleuca*. In general, relationships obtained from SCoT data agrees well with species relationship obtained from morphological. This is in agree-

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

SP	N	Na	Ne	I	He	UHe	%P
L. caucasica	18.000	0.892	1.138	0.18	0.141	0.13	28.63%
L. iberica	16.000	0.244	1.032	0.26	0.23	0.18	55.53%
L. nummulariifolia	14.000	0.314	1.044	0.26	0.18	0.23	39.38%
L. bracteolaris	9.000	0.201	1.00	0.33	0.17	0.18	52.23%
L. korolkowii	15.000	0.341	1.058	0.24	0.27	0.20	33.75%
L. hypoleuca	13.000	0.567	1.062	0.34	0.324	0.333	64.73%

Abbreviations: N = number of samples, Na= number of different alleles; Ne = number of effective alleles, I= Shannon's information index, He = genetic diversity, UHe = unbiased gene diversity, P%= percentage of polymorphism, populations).

Table 4. Analysis of molecular variance (AMOVA) of the studied species.

Source	df	SS	MS	Est. Var.	%	*ΦPT*
Among Pops	20	1991.364	70.789	12.154	53%	
Within Pops	177	774.443	8.905	2.888	47%	53%
Total	197	2555.807		14.060	100%	

Abbreviations: df = degree of freedom, SS = sum of squared observations, MS = mean of squared observations, EV = estimated variance, *ΦPT* = proportion of the total genetic variance among individuals within an accession, (P < 0.001).

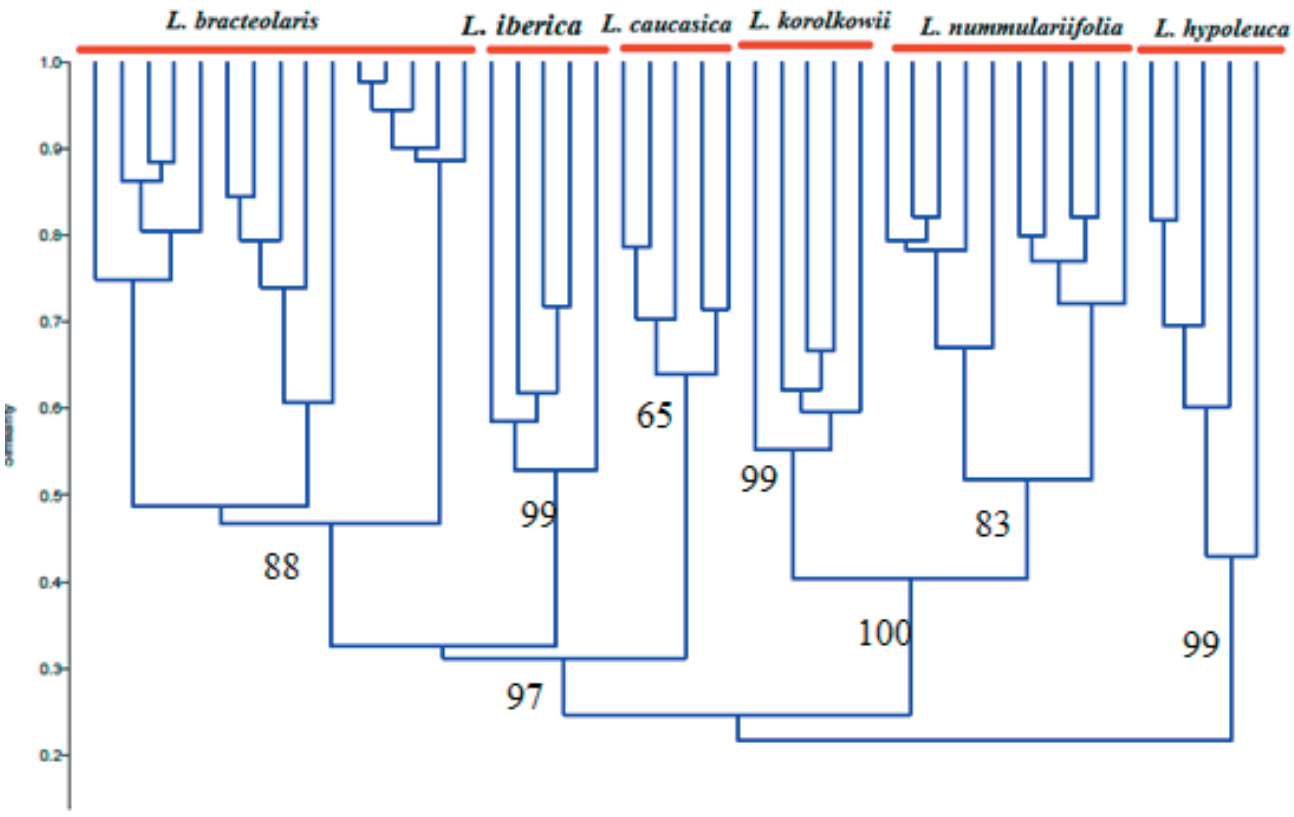

Figure 3. UPGMA tree of SCoT data revealing species delimitation in the *Lonicera* species. Branch support values are given as bootstrap (BP) value above branches.

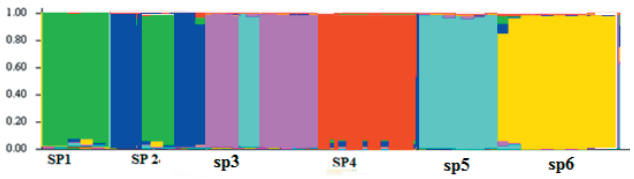

Figure 4. STRUCTURE plot of *Lonicera* species based on SCoT data.

ment with AMOVA and genetic diversity parameters presented before. The species are genetically well differentiated from each other. These results indicate that SCoT molecular markers can be used in *Lonicera* species taxonomy. The Nm analysis by Popgene software also produced mean Nm= 0.186, that is considered very low value of gene flow among the studied species.

Mantel test with 5000 permutations showed a significant correlation (r = 0.177, 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 not included). The results showed that the highest degree of genetic similarity (0.90) occurred between *L. korolkowii* and *L. nummulariifolia*. The lowest degree of genetic similarity occurred between *L. hypoleuca* and *L. iberica* (0.67). The low Nm value (0.186) indicates limited gene flow or ancestrally shared alleles between the species studied and indicating high genetic differentiation among and within *Lonicera* species.

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 =6 (Table 5). The STRUCTURE plot (Figure. 4) 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. cauca-*

TABLE 5 . K-Means clustering result of SCOT data.

K	SSD(T)	SSD(AC)	SSD(WC)	pseudo-F	BIC
1	66.133	0	0	0	192.432
2	66.133	35.707	30.426	16.038	168.916
3	66.133	28.688	37.445	16.089	174.449
4	66.133	35.707	30.426	16.038	168.916
5&	66.133	40.09	26.043	15.394	165.722
6*	66.133	20.586	45.547	19.435	179.457

sica and *L. iberica* (similarly colored, No. 1, 2), as well as *L. nummulariifolia* and *L. korolkowii* (sp No. 3,5) due to shared common alleles. This is in agreement with UPGMA dendrogram presented before. The other species are distinct in their allele composition.

DISCUSSION

knowledge of the genetic variability and diversity within and among different populations is crucial for their conservation and management (e.g. Mills and Schwartz 2005; Khayatnezhad and Gholamin 2021; Guo *et al.* 2021; Ren *et al.* 2021). In the present study we used morphological and molecular (SCoT) data to evaluate species relationship in *Lonicera*. 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 bract length, stipule length, bract shape, calyx shape, petal shape, length and width of stem-leaf, length and width of petal 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 six species.

Genetic structure and gene flow

PIC and MI characteristics of a primer help in determining its effectiveness in genetic diversity analysis. Sivaprakash *et al.* (2004) suggested that the ability of a marker technique to resolve genetic diversity may be more directly related to the degree of polymorphism. Generally, PIC value between zero to 0.25 imply a very low genetic diversity among genotypes, between 0.25 to 0.50 shows a mid-level of genetic diversity and value ≥0.50 suggests a high level of genetic diversity (Tams *et al.* 2005; Hou *et al.* 2021; Huang *et al.* 2021; Khayatnezhad and Gholamin 2020b). In this research, the SCoT primers' PIC values ranged from 0.43 to 0.67, with a mean value of 0.56, which indicated a mid-ability of SCoT primers in determining genetic diversity among the *Lonicera* species.

In the study conducted by Chen *et al.* (2012), 20 ISSR primers amplified 186 bands with 103 (54.63%) polymorphic bands and 58 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 0.054 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.

In conclusion, the results of this study showed that to evaluate the genetic diversity of the *Lonicera* genus, the primers derived from SCoT were more effective than the other molecular markers. Also, *Lonicera* ecotypes/species were clearly separated from each other in the dendrogram and MDS, indicating the higher efficiency of SCoT technique in *Lonicera* species identification.

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