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EVALUATION OF GENETIC VARIABILITY *Rindera* USING RAPD MARKERS

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Genetic diversity investigations are critical for understanding how to conserve and manage plant resources in every setting. Six *Rindera* species are reported in Iran. No detailed *Rindera* genetic diversity was investigated using Random Amplified Polymorphic DNA (RAPD) experiments. Six plants from Iran's seven provinces were gathered and studied for our scientific purposes. Seventy plant specimens have been gathered in total. Our objectives were as follows: 1) to determine genetic variability among *Rindera* species, and 2) is there a correlation between the genetic and geographical distance of the species? 3) Populations and taxon genetic structure we revealed that quantitative morphological features varied significantly across plant species. *Rindera* species were classified into two groups using the unweighted pair group approach with arithmetic mean and principal component analysis. The unbiased expected heterozygosity (UHe) of *Rindera Regia* was in the 0.18 range. *Rindera lanata* has important Shannon information (0.30). The lowest value was 0.22 for *Rindera Regia*. In *R. cyclodonta* and *Rindera media,* the observed number of alleles (Na) varied between 0.33 and 0.49. *R. cyclodonta* and *Rindera bungei* had Ne values between 1.034- 1.17, indicating an effective number of alleles. *Rindera* has a comparatively low gene flow (Nm) (0.45). According to the Mantel test, there was a significant correlation (r = 0.33, p=0.0001) between genetic and geographical distances. We identified a substantial level of genetic variation, which demonstrates that the *Rindera* species can adapt to altering environments because genetic diversity is associated with species adaptability. The current findings

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indicated the efficacy of RAPD markers and morphometry approaches for studying genetic variation in *Rindera* species.

Keywords: Gene flow, Random Amplified Polymorphic DNA (RAPD) *Rindera*, isolation, morphometry

INTRODUCTION

The family Boraginaceae s.str consists of approximately 131 genera and 2,500 species, is primarily discovered in arid, cliffy, and sunny settings across Eurasia, the Mediterranean area, and western North America (BINZET and AKCIN, 2009). They seem to be primarily annual, bi-annual, or perennial herbs and shrubs, including a few trees and lianes that are found across the temperate and subtropical globe (RETIEF and VANWYK, 1997) the world's temperate and subtropical areas (RETIEF and VANWYK, 1997) with high dispersion in Iran (WILLIS, 1973). Subfamily Cynoglossoideae Weigend is the largest subfamily having about 900 species and 50 genera. Current molecular research has demonstrated that such subfamily includes many previously reported tribes (CHACÓN *et al*., 2016). The subtribe Cynogolossinae Dumort. (Tribe Cynoglosseae W.D.J.Koch) is completely limited to the Old World, with a diversity hotspot in western Asia and the Mediterranean (CHACÓN *et al*., 2016).

The genus *Rindera* Pallas (1771: 486) comprises about 20–25 species distributed in central-eastern Europe to central Asia (BIGAZZI *et al*., 2006). This taxon is closely related to *Paracaryum* Boissier (1849: 128) and *Mattiastrum* Brand (1915: 150), nested in *Cynoglossum* Linnaeus (1753: 134) s.str. (WEIGEND *et al*., 2013; WEIGEND *et al*., 2016). All species of *Rindera* seem to be perennial to the dry and continental climate of the steppe and semidesertic belts (BIGAZZI *et al*., 2006). *Rindera* is represented by six species in Iran, 4 of which *Rindera albida* (Wettst.) Kusn.; *Rindera bungei* (Boiss.) Gürke; *Rindera regia* Kusn, *rindera media* (Turrill) Riedl are endemic (KHATAMSAZ, 2001). Tubular corollas characterize *Rindera*, stamens usually inserted at the throat of the corolla, with style mostly exserted from the corolla, and usually eglochidiate large mericarpids with a broad, membranous wing (BIGAZZI *et al*., 2006).

Rindera species are commonly called "Yünlü gelin" and are utilized in Anatolian traditional medicine as an anti-inflammatory (ALTUNDAG and OZTURK, 2001). MOSADDEGH *et al*. (2012) suggested that *R. lanata* is utilized to relieve joint pains in Iranian folk medicine. In numerous studies, fruit morphology was utilized as the most significant attribute for a synthetic approach to the systematics of this family, considering both phylogenetic and evolutionary elements (CRONQUIST, 1981; TAKHTAJAN, 1997; SELVI *et al*., 2006). Numerous traits of fruits provide significant taxonomic characteristics for identifying the Boraginaceae tribes, including a straight or incurved nutlet, a specific type of emergence, the location of the attachment scar, and the unique shape of prickles or glochids (AL-SHEHBAZ, 1991; BAILLON, 1888; GURKE, 1893; HILGER, 2014; RIEDL, 1997). Furthermore, these features contribute to the description of genera, species, and subspecies (LANGSTROM and CHASE, 2002; SELVI and BIGAZZI, 2003; SELVI *et al*., 2006).

Genetic diversity studies are usually tapped due to molecular markers. Molecular markers are an excellent method to disentangle phylogenetic associations between species and populations. Among molecular methods or markers, RAPD (Random Amplified Polymorphic DNA) is sensitive to detect variability among individuals of species. The RAPD method is cost-

effective and can work with limited sample quantities. In addition to this, RAPD can amplify and target genomic regions with potential and several markers (ESFANDANI-BOZCHALOYI *et al*., 2017). Taxonomical Systematics studies were conducted in the past to identify the *Rindera* species. According to the best of our knowledge, there is no existing RAPD data on genetic diversity investigations in Iran. We studied seventy samples. Our objectives were as follows: 1) to determine genetic variability among *Rindera* species2) Are there correlations between species and geographical distance? 3) Population and taxon genetic structure4) Are the *Rindera* species exchanging genes?.

MATERIALS AND METHODS

Plant materials

Six *Rindera* species were gathered from various parts of Iran (Table 1). These species have been studied via morphological and molecular methods. Seventy plant samples (5-25 per plant species) were examined for morphometry (Figure 1). The random amplified polymorphic DNA analysis method was limited to 70 examples. We focused on the following species *Rindera albida* (Wettst.) Kusn.; *Rindera bungei* (Boiss.) Gürke; *Rindera lanata* (Lam.) Bunge; *Rindera cyclodonta* Bunge*; Rindera regia* Kusn. and *Rindera media* (Turrill) Riedl. According to previous references, all the species were identified (KHATAMSAZ, 2001; POPOV, 1953; RIEDL, 1967).

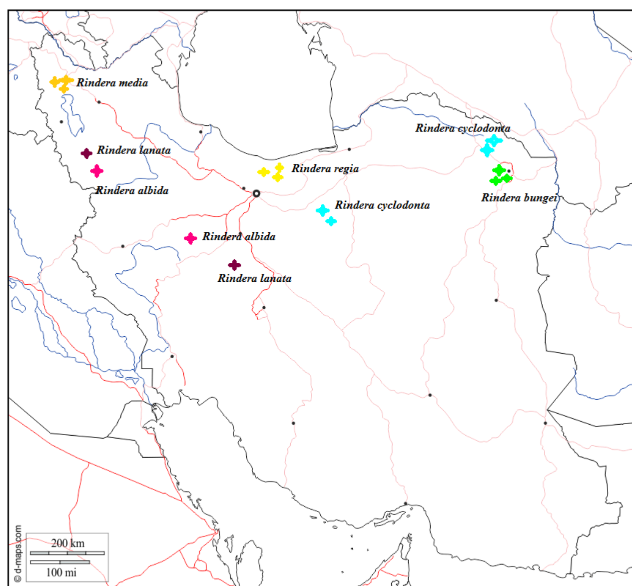

Figure 1. Presence of species in different regions of Iran.

Table 1. List of the investigated taxa including origin of voucher specimens

Taxa	Locality	Latitude	Longitude	Altitude(m)
Rindera albida (Wettst.) Kusn.	Kordestan, Sanandaj Hamedan, 20km s of Nahavand	37° 07' 48 "	49° 54' 04"	165
Rindera bungei (Boiss.) Gürke	Razavi Khorasan, Kashmar, Kuhsorkh District	37° 07' 08"	49°54' 11"	159
Rindera lanata (Lam.) Bunge	Kordestan, Sanandaj Esfahan, ardestan on road to taleghan	38 ˚ 52' 93"	47 ˚25' 92"	1133
Rindera cyclodonta Bunge	Bojnord, Ghorkhod protected area Semnan, 20km NW of shahrud	38˚52' 93"	47 ˚25' 92"	1139
Rindera regia Kusn v	Mazandaran, 40 km Tonekabon to janat abad Mazandaran, Nowshahr	35 ˚50' 36"	51˚ 24' 28"	2383
Rindera media (Turrill) Riedl n	West-Azarbaijan, Urumieh, Silvana	35 ˚42'29"	52 ˚20'51"	2421

Morphometry

In total, four quantitative and three qualitative characters of the flowers and the nutlets were studied; calyx length, calyx width, corolla length, corolla color, faucal appendages, nutlet length, and stamens position (Table 2). Prior to ordination, the data were converted (Mean = 0, variance = 1). Euclidean distance was implemented to cluster and ordinate plant species (PODANI, 2000).

Table 2. Morphological characters and coding of diagnostic nutlet and flower characteristics in studied species. Corolla color: 1: dark maroon to deep pink,2: pale yellow, 3: bluish-purple,

taxon	Nutlet size (mm)	Stamens position	Calyx length (mm)	Calyx width (mm)	Corolla length (mm)	Corolla color	Focal scale
Rindera lanata	23.4-24.2	2	6.5-6.9	3-3.9	9-10.9	1	2
Rindera cyclodonta	14.3-15.2	2	5.2-6.7	3-3.3	7.9-8	1	1
Rindera regia	14.55-16.8	2	4.6-6.5	3-3.9	8.9-8.7	1	2
Rindera albida	14.7-15.3	1	5-6.3	1-1.1	7.4-9	2	2
Rindera bungei	7.5-8	1	3.4-5	1-1.9	4.4-8	3	2
Rindera media	7.8-8.8	1	3.5-7.9	2-2.8	5.4-6	3	2

Random Amplified Polymorphic DNA

We extracted DNA from fresh leaves. Leaves were dried. The extraction of DNA was carried out under the prior procedure (ESFANDANI-BOZCHALOYI *et al.*, 2019). For verifying the

purity of the DNA, its quality was determined using an agarose gel. We employed RAPD primers to amplify the DNA (Operon technology, Alameda, Canada). These primers belonged to OPA, OPB, OPC, OPD sets. We selected those primers (10), which showed clear bands and polymorphism (Table 3). Overall, the polymerase chain reaction contained 25µl volume. This 25 volume had ten mM Tris-HCl buffer, 500 mM KCl; 1.5 mM $MgCl_2$; 0.2 mM of each dNTP; 0.2 µM of a single primer; 20 ng genomic DNA and 3 U of *Taq* DNA polymerase (Bioron, Germany). We observed the following cycles and conditions for the amplification. Five minutes initial denaturation step was carried out at 94°C after this forty cycles of 1 minute at 94°C were observed. Then 1-minute cycle was at 52-57°C followed by two minutes at 72°C. In the end, the final extension step was performed for seven to ten minutes at 72°C. We confirmed the amplification steps while observing amplified products on a gel. Each band size was confirmed according to 100 base pair molecular ladder/standard (Fermentas, Germany).

Data analyses

We employed the Ward techniques and the Unweighted pair group method with arithmetic mean (UPGMA). Furthermore, ordination techniques, including multidimensional scaling and principal coordinate analysis, have been utilized (PODANI, 2000). The morphological difference among species and population was assessed through analysis of variance (ANOVA). PCA analysis (PODANI, 2000) was done to find the variation in plant population morphological traits. Multivariate and all the necessary calculations were done in the PAST software, 2.17 (HAMMER *et al*., 2001). Unbiased expected heterozygosity (UHe) and heterozygosity were assessed in GenAlEx 6.4 software (PEAKALL and SMOUSE, 2006). Neighbor joining (NJ) and networking were studied to fathom genetic distance plant populations (HUSON and BRYANT, 2006; FREELAND *et al*., 2011). The Mantel test was used to determine the relationship between genetic and geographical distances (PODANI, 2000). We were interested in knowing the genetic structure and diversity; we also investigated the genetic difference between populations through AMOVA (Analysis of molecular variance) in GenAlEx 6.4 (PEAKALL and SMOUSE, 2006). Furthermore, gene flow (Nm) was estimated in PopGene ver. 1.32 using genetic statistics (GST) (YEH *et al*., 1999). We also did STRUCTURE analysis to detect an optimum number of groups. For this purpose, the Evanno test was conducted (EVANNO *et al*., 2005).

RESULTS

Morphometry

ANOVA findings revealed significant variations in quantitative morphological features across plant species ($P < 0.01$). Principal component results explained 74% variation. The first component of PCA demonstrated 50% of the total variation. Nutlet morphology and traits such as calyx length calyx width positively correlated with corolla length corolla color (>0.7). The second and third components explained floral characters such as faucal appendages, nutlet length, and stamens position. The principal component analysis (PCA) and unweighted pair group technique with arithmetic mean (UPGMA) plots revealed symmetrical findings (Figure 2). Generally, plant specimens belonging to different species were separated due to differences in morphology. Morphological characters divided *Rindera* species into two groups, as evident in the UPGMA tree (Figure 2).

The first group belonged to *Rindera albida*, *Rindera bungei*, and *Rindera media*. On the other hand, the second group consisted of two sub-groups. *Rindera Regia* formed the first sub-group. *Rindera lanata* and *R. cyclodonta* formed the second sub-group. These groups and sub-groups were formed due to morphological differences among the individuals of *Rindera*. Our PCA results also confirmed the application of morphological characters in separating and clustering the species into separate groups (Figure 3). Identical results were also reported in the UPGMA tree (Figure 2). In *Rindera albida* and *R. bungei* nutlets are 8–14 mm, two-winged; outer wing 3 mm broad, margin undulate, inner 2 mm broad, incurved, margin cristate-dentate, glochids absent, while in *R. lanata, R. cyclodonta* nutlets are 15.8–23 mm, smooth, the wing with smooth or often undulate blue margin, without glochids.

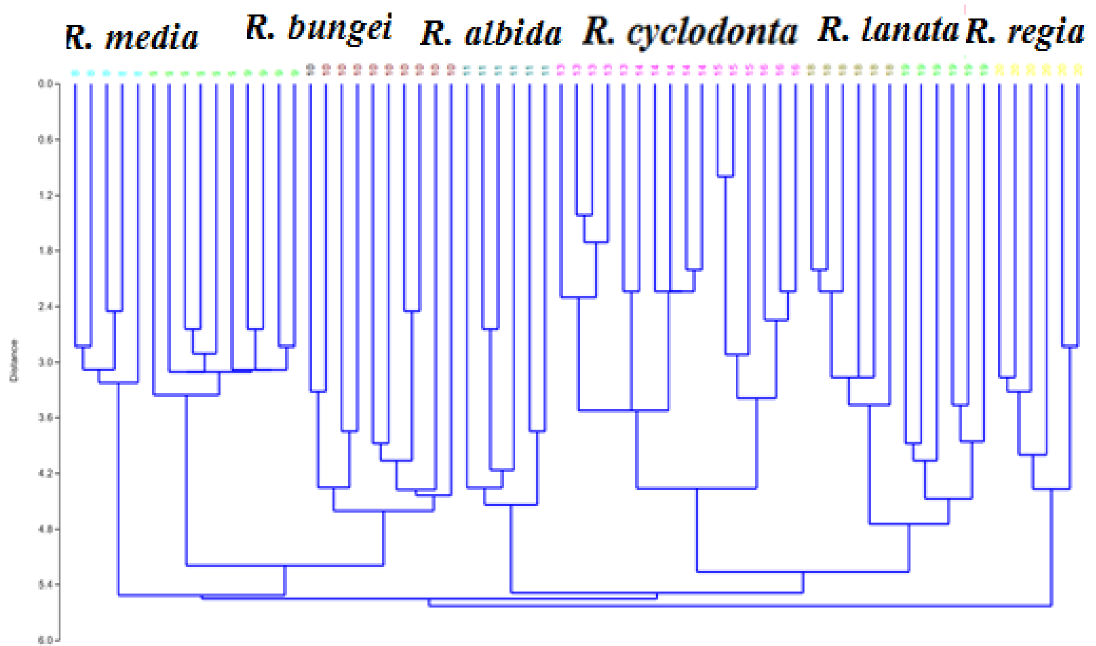

Figure 2. UPGMA clusters of morphological characters revealing species delimitation in *Rindera* species.

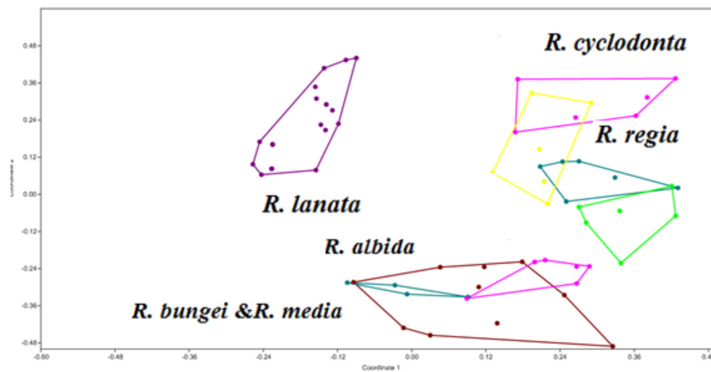

Figure 3. PCA plot morphological characters revealing species delimitation in *Rindera* species.

Species Identification and Genetic Diversity

The primers, i.e., OPC-04 and OPA-05, could amplify plant (*Rindera*) DNA (Figure 4). One hundred and eleven polymorphic bands were generated and amplified. The amplified products varied in power from 100 to 3000 bp. We identified OPA-05's most polymorphic bands. OPD-03 had the lowest polymorphic bands. The average polymorphic bands ranged to 11.5 for each primer. The polymorphic information content (PIC) had values in the range of 0.27 (OPA-05) to 0.66 (OPB- 02). Primers had 0.42 average polymorphic information content values. Marker index (MI) values were 2.88 (OPB-01) to 5.66 (OPD-05), with an average of 3.7 for each primer. Effective multiplex ratio (EMR) values are useful to distinguish genotypes. Our study reported 8.20 (OPD-11) to 11.88 (OPD-02) EMR values. EMR values averaged 8.5 per primer (Table 3). The necessary genetic features calculated of six *Rindera* species are shown (Table 4).

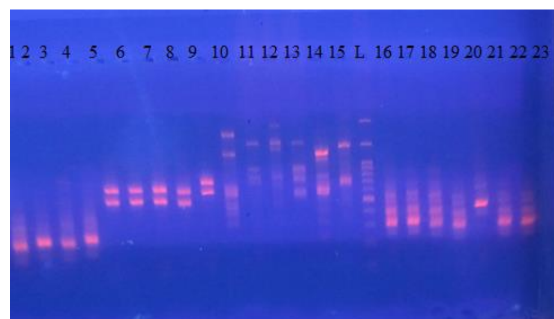

Figure 4. Gel Electrophoresis image of DNA fragments of *Rindera* species. 1,8,15,22: *Rindera albida* ; 2, 9,16,23: *Rindera bungei* ; 3,10, 17, 24: *Rindera lanata*; 4, 11, 18, 25: *R. cyclodonta*; 5, 12, 19, 26: *Rindera regia* and 6-7, 13-14, 20-21,27-28: *Rindera media*. L = Ladder 100 bp. Arrows show polymorphic bands.

Table 3. RAPD primers and other parameters. Note: 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.

Primer name	Primer sequence (5'-3')	TNB	NPB	PPB	PIC	PI	EMR	MI
OPA-05	5′-AGGGGTCTTG-3′	15	14	93.74%	0.27	5.66	8.56	3.67
OPA-06	5′-GGTCCCTGAC-3′	13	12	92.31%	0.54	4.21	8.23	4.55
OPB-01	5′-GTTTCGCTCC-3′	12	12	100.00%	0.47	4.32	9.55	2.88
OPB-02	5′-TGATCCCTGG-3′	11	9	82.89%	0.66	6.56	9.34	3.18
OPC-04	5'-CCGCATCTAC-3'	10	10	100.00%	0.39	4.25	10.11	3.87
OPD-02	5′-GGACCCAACC-3′	11	11	100.00%	0.36	4.86	11.88	3.45
OPD-03	5′-GTCGCCGTCA-3′	9	7	84.99%	0.43	3.51	8.43	3.85
OPD-05	5′ -TGAGCGGACA-3′	9	9	100.00%	0.44	4.34	10.55	5.66
OPD-08	5′-GTGTGCCCCA-3′	11	11	100.00%	0.37	2.18	9.56	3.65
OPD-11	5′-AGCGCCATTG-3′	10	10	100.00%	0.45	4.28	8.20	3.47
Mean		12.1	11.5	90.88%	0.42	3.5	8.5	3.7
Total		115	111					

Table 4. Genetic diversity variables of Rindera. (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 in populations).

taxon	N	Na	Ne	I	He	UHe	%P
Rindera lanata	4.000	0.344	1.042	0.30	0.33	0.30	57.53%
Rindera cyclodonta	5.000	0.336	1.034	0.25	0.25	0.29	51.83%
Rindera regia	6.000	0.458	1.039	0.22	0.11	0.18	30.38%
Rindera albida	6.000	0.448	1.049	0.28	0.18	0.23	49.38%
Rindera bungei	5.000	0.455	1.177	0.277	0.24	0.22	55.05%
Rindera media	8.000	0.499	1.067	0.24	0.19	0.24	49.26%

The unbiased expected heterozygozygosity (UHe) of Rindera regia was in the 0.18 range. *Rindera lanata* showed a 0.33 UHe value heterozygosity had a mean value of 0.25 in overall *Rindera* species. Shannon's information was high (0.30) in *Rindera lanata*. *Rindera regia* showed the lowest value, 0.22. The mean values for Shannon information were 0.24. In *R.*

cyclodonta and *Rindera media*, the observed number of alleles (Na) varied between 0.33 and 0.49. For *R. cyclodonta* and *Rindera bungei,* the effective number of alleles (Ne) ranged between 1.034 and 1.17. Analysis of Molecular Variance (AMOVA) test highlighted genetic differences among *Rindera* species (P = 0.001). According to AMOVA, 65% of genetic diversity exists across species. Relative less variation (35%) was reported within the species. Genetic similarity and dissimilarity assessed through Genetic statistics (GST) showed significant differences i.e., (0.123, P = 0.001) and D_est values (0.245, *p* = 0.001). The neighbor-joining tree also revealed some major groups (Figure not included). The neighbor-joining tree also repeated the same pattern as indicated in Figures 2 and 3. In the current work, molecular findings also coincided with the traditional taxonomical (morphology) approaches for *Rindera* species. In the *Rindera* species, gene flow (Nm) was relatively low (0.45). Genetic identity and phylogenetic distance in the *Rindera* members are mentioned (Table 5). *Rindera bungei* and *Rindera media* were genetically closely related (0.96). *R. cyclodonta* and *Rindera bungei* were dissimilar due to low (0.79) genetic similarity. The mantel tests indicated a link between genetic and geographical distances (r = 0.33, p=0.0001). The Evanno test showed ΔK =6 (Figure 2). Figure 2 shows the genetic details of the *Rindera* species. According to STRUCTURE analysis, *Rindera bungei* and *Rindera albida* were closely related to common alleles (Figure 2). The rest of the *Rindera* species are genetically differentiated due to different allelic structures (Figure 2). The neighbor-joining plot also showed the same result. Limited gene flow results were supported by K-Means and STRUCTURE analyses too. We were unable to detect significant gene flow among the Rindera species. This finding is consistent with the grouping produced by Neighbor-Net (Figure not included) since these populations were located close to each other. As indicated by the STRUCTURE plot relying on the admixture model, these common alleles form a relatively small portion of the genomes in these populations. These findings concur in demonstrating a significant degree of genetic stratification among *Rindera* groups.

Table 5. The Nei genetic similarity (Gs) estimates using RAPD markers.

Rindera lanata	*Rindera cyclodonta*	*Rindera regia*	*Rindera albida*	*Rindera bungei*	*Rindera media*	
1.000						*Rindera lanata*
0.820	1.000					*Rindera cyclodonta*
0.807	0.928	1.000				*Rindera regia*
0.829	0.873	0.860	1.000			*Rindera albida*
0.807	0.794	0.874	0.952	1.000		*Rindera bungei*
0.829	0.826	0.905	0.842	0.966	1.000	*Rindera media*

DISCUSSION

The *Rindera* is a relatively complex taxonomic group, and several morphological characters make it difficult to identify and classify *Rindera* species (AKCIN, 2008). Given the complexity, exploring other methods could complement the traditional taxonomical approach (ERBANO *et al*., 2015). Advent and molecular techniques have enabled plant taxonomists to utilize molecular protocols to study plant groups (ERBANO *et al*., 2015). We examined genetic diversity in *Rindera* by morphological and molecular methods. We mainly used RAPD markers to investigate genetic diversity and genetic affinity in *Rindera*. Our clustering and ordination techniques showed similar patterns. Morphometry results clearly showed the utilization or significance of morphological characters in *Rindera* species. PCA plot results also confirmed the application of morphological characters to separate *Rindera* species. The present study also highlighted those morphological characters such as corolla color, nutlet shape, nutlet length, stamens position, nutlet margin, nutlet disc could delimit the *Rindera* group. The *Rindera* species highlighted morphological differences. We argue that such dissimilarity was due to differences in quantitative and qualitative traits.

In our study, morphology and micro-morphology of flower and nutlet characters in six taxa of *Rindera* species are given in detail for the first time. This research aimed to discover diagnostic characteristics that could be used to identify *Rindera* species in Iran. As stated, morphological characteristics are a valuable aid for species identification (AKCIN, 2008).

According to AKCIN (2008), fruits and seeds are useful characters in identifying *Cynoglossum creticum, C. officinale, C. montanum,* and *C. glochidiatum*. However, due to differences in the seed coat and fruit surface, these species were classified as having two tuberculate and granulate kinds and two subtypes of granulate-punctuate and granulate-tuberculate. The reticulate seed coat type and its particular subtypes have been identified depending on the ornamentation of the seed coats (AKÇIN, 2008). Previous research has examined the micromorphology of seed and fruit in a variety of taxa and underlined their significance in plant taxonomy (OLGUN and BEYAZOGH LU, 1997; COSZKUNCELEBI *et al*., 2000; KHALIK *et al*., 2008). Present findings on morphological differences are in line with the previous studies (COSZKUNCELEBI *et al*., 2000, KHALIK *et al*., 2008). Polymorphic information content (PIC) values are useful to detect genetic diversity. The current study recorded average PIC values of 0.42. This value is sufficient to study genetic diversity in the population (KEMPF *et al*., 2016). High genetic diversity among the *Rindera* population was reported in the present study. The previous scientific data (KURATA *et al*., 2019) supports our current high diversity results. Genetic analysis conducted via analysis of molecular variance and STRUCTURE showed genetic differences among the species.

Interestingly, STRUCTURE results showed the presence of shared alleles in *Rindera* species. Shared alleles are related to self-pollination in *Rindera* (WILLIAMS *et al*., 2000). Bees, flies, and honeybees (LEFEBVRE *et al*., 2019) pollinate some *Rindera* members. Present findings revealed limited gene flow, and it is quite logical to report low gene flow. Similar low gene flow values were recorded using RAPD markers (FISCHER *et al*., 2000). Other probable reasons for limited gene flow are geographical isolation (FISCHER *et al*., 2000) among the *Rindera* species and population. Low or limited gene flow results were according to the Mantel test results. The Mantel test found that genetic and geographical distances were positively correlated. Therefore,

it is concluded that distance isolation and limited genes determine the *Rindera* population's genetic structure. Molecular markers (RAPD) and morphometry analyses were used to investigate genetic variability and population structure in identifying *Rindera* species. All the species had distinct genetic differentiation. Present results highlighted isolation and limited gene flow are the main deterministic factors that shape the *Rindera* population. Furthermore, we observed considerable genetic variation, which indicates that the *Rindera* species can adapt to changes in the environment since genetic diversity is associated with species adaptability.

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ISPITIVANJE GENETIČKE VARIJABILNOSTI *Rindera* KORIŠĆENJEM RAPD MARKERA

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Izvod

Istraživanja genetičke raznovrsnosti su kritična za razumevanje kako sačuvati i upravljati biljnim resursima u svakom okruženju. U Iranu je prijavljeno šest vrsta *Rindera*. Nije istražena detaljna genetska raznolikost *Rindera* korišćenjem eksperimenata nasumične amplifikovane polimorfne DNK (RAPD). Sakupljeno je i proučavano šest biljaka iz sedam iranskih provincija za naše naučne svrhe. Ukupno je prikupljeno sedamdeset biljnih primeraka. Naši ciljevi su bili sledeći: 1) da odredimo genetičku varijabilnost među vrstama *Rindera*, i 2) da li postoji korelacija između genetičke i geografske udaljenosti vrste? 3) Populacije i genetička struktura taksona otkrili su da su kvantitativne morfološke karakteristike značajno varirale među biljnim vrstama. Vrste *Rindera* su klasifikovane u dve grupe korišćenjem pristupa grupe neponderisanih parova sa aritmetičkom sredinom i analizom glavne komponente. Nepristrasna očekivana heterozigotnost (UHe) *Rindera Regia* bila je u opsegu od 0,18. *Rindera lanata* ima važne Šenonove informacije (0,30). Najniža vrednost je bila 0,22 za *Rindera Regia*. U podlozi *R. ciclodonta* i *Rindera*, posmatrani broj alela (Na) varirao je između 0,33 i 0,49. *R. ciclodonta* i *Rindera bungei* su imale Ne vrednosti između 1,034-1,17, što ukazuje na efektivni broj alela. *Rindera* ima relativno nizak protok gena (Nm) (0,45). Prema Mantel testu, postojala je značajna korelacija (r = 0,33, p=0,0001) između genetske i geografske udaljenosti. Identifikovali smo značajan nivo genetičke varijacije, što pokazuje da se vrsta *Rindera* može prilagoditi promenljivom okruženju, jer je genetska raznolikost povezana sa prilagodljivošću vrsta. Sadašnji nalazi su ukazali na efikasnost RAPD markera i morfometrijskih pristupa za proučavanje genetske varijacije kod vrsta *Rindera*.

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