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Original scientific article

EVALUATION OF GENETIC DIVERSITY IN *Geranium* (Geraniaceae) USING RAPD MARKER

Juan YIN[1*], Majid KHAYATNEZHAD[2], Abdul SHAKOOR[3,4]

[1]Forestry College, Xinyang College of Agriculture and Forestry, Xinyang, Henan, 464000, China
[2]Department of Environmental Sciences and Engineering, Ardabil Branch, Islamic Azad University, Ardabil, Iran
[3]College of Environment and Planning, Henan University, Kaifeng, 475004, Henan, China
[4]Key Laboratory of Geospatial Technology for the Middle and Lower Yellow River Regions, Ministry of Education, Kaifeng 475004, Henan, China

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Genetic diversity studies are essential to understand the conservation and management of plant resources in any environment. No detailed Random Amplified Polymorphic DNA (RAPD) studies were conducted to study Geranium genetic diversity. Therefore, we collected and analyzed thirteen species from nine provinces. Overall, one hundred and twenty-five plant specimens were collected. Our aims were 1) to assess genetic diversity among *Geranium* species 2) is there a correlation between species genetic and geographical distance? 3) Genetic structure of populations and taxa. We showed significant differences in quantitative morphological characters in plant species. Unweighted pair group method with arithmetic mean and multidimensional scaling divided *Geranium* species into two groups. *G. sylvaticum* depicted unbiased expected heterozygosity (UHe) in the range of 0.11. Shannon information was high (0.38) in *G. columbinum*. *G. sylvaticum* showed the lowest value, 0.14. The observed number of alleles (*N*a) ranged from 0.25 to 0.55 in *G. persicum* and *G. tuberosum*. The effective number of alleles (*Ne*) was in the range of 1.020-1.430 for *G. tuberosum* and *G. collinum*. Gene flow (Nm) was relatively low (0.33) in *Geranium*. The Mantel test showed correlation (r = 0.27, p=0.0002) between genetic and geographical distances. We reported high genetic

Corresponding author: Juan Yin, Forestry College, Xinyang College of Agriculture and Forestry, Xinyang, Henan, China. E-mail: yinjuan20210313@163.com, ldongmei870@gmail.com

diversity, which clearly shows the *Geranium* species can adapt to changing environments since high genetic diversity is linked to species adaptability. Present results highlighted the utility of RAPD markers and morphometry methods to investigate genetic diversity in *Geranium* species.

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

INTRODUCTION

Genetic diversity is a vital feature that helps plant species survive in an ever-changing environment, and it sheds light on understanding the phylogenetic affinity among the species (Erbano et al. 2015). Quite a significant number of genetic resources and materials programs of plant species have been carried out to preserve the plant species worldwide. Scientific data indicate that genetic diversity plays a pivotal role in conservation programs (GOMEZ *et al*., 2005).

To better understand genetic diversity, the biologist study population size. Population size is considered one of the central factors to understand the variability in a gene (ELLEGREN and GALTIER, 2016; TURCHETTO *et al.*, 2016). Genetic variation and diversity are essential for species to survive because individuals are separated due to genetic or geographical barriers, often resulting in scattered populations. Since these individuals have limited gene flow, there is a greater chance of a decline in population size (FRANKHAM, 2005). Given the significance of genetic diversity in conservation strategies, it is of utmost importance to disentangle genetic diversity in plant species, particularly threatened and rare species (Cires *et al*., 2013).

Around 430 species of *Geranium* L. are reported in the world (AEDO *et al*., 1998). Morphological and systematic studies have been conducted in the past to decipher its classification (AEDO, 1996). The current classification system divides *Geranium* into three subgenera (YEO, 2008). Among them, subgenera Geranium has 370 species (AEDO and ESTRELLA, 2006). The *Geranium* section has a wide range of distribution; on the contrary, *G.* sect. *Dissecta* occurs in the Eurasian, Mediterranean, and Himalaya regions. *Tuberosa* (Boiss.) has a high occurrence record in Western Europe, Central Asia, Northwest Africa. Vegetative characters were implemented in the past to divide *Tuberosa* into subsections *Tuberosa (Boiss.) Yeo and Mediterranea* R. Knuth (YEO, 2008). *G.* subsect. *Tuberosa* has the center of diversity in Turkey and Iran (AEDO and ESTRELLA, 2006; AEDO *et al*., 2007; ESFANDANI-BOZCHALOYI *et al*., 2018a; 2018b; 2018c; 2018d). Different numbers of species have been reported in Iran about the Geranium genus. For instance, twenty-two annual species are described in Iran (SCHONBECK-TEMESY, 1970), but other scientists reported twenty-five species in Iran (ONSORI *et al.*, 2010). Key traits to classify this genus are leaves and fruit morphology. Fruit morphology and characters are vital to identifying the Geranium species (SALIMI MOGHADAM *et al*., 2015).

Genetic diversity studies are usually tapped due to molecular markers. Molecular markers are an excellent method to disentangle phylogenetic association between species and population. Among molecular methods or markers, RAPD (Random Amplified Polymorphic DNA) are sensitive to detect variability among individuals of species. 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 Geranium species. According to the best of our knowledge, there is no existing RAPD data on genetic diversity investigations in Iran. We studied one hundred and twenty-five samples. Our aims were 1) to assess genetic diversity among Geranium species 2) is there a correlation between species and geographical distance? 3) Genetic structure of populations and taxa 4) Are the Geranium species able to exchange genes?

MATERIALS AND METHODS

Plant materials

Thirteen *Geranium* species were collected from different regions of Iran (Table 1).

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

Species	Locations	Latitude	Longitude	Altitude (m)
1. *G. dissectum*	Esfahan, Ghameshlou, Sanjab	37° 07' 48 "	49° 54' 04"	165
	Lorestan, Oshtorankuh, above Tihun village	37° 07' 08"	49°54' 11"	159
2. *G. collinum*	East Azerbaijan kaleybar cheshme ali akbar	38 ˚ 52' 93"	47 ˚25' 92"	1133
	East Azerbaijan kaleybar, Shojabad	38˚52' 93"	47 ˚25' 92"	1139
3. *G. rotundifolium*	Tehran, Tuchal	35 ˚50' 36"	51˚ 24' 28"	2383
4. *G. columbinum*	Ardabil, Khalkhal	35 ˚42'29"	52 ˚20'51"	2421
5. *G. sylvaticum*	East Azerbaijan kaleybar cheshme ali akbar	38 ˚52'39"	47 ˚25' 92"	1133
6. *G. pratense*	East Azerbaijan kaleybar, Shojabad	38 ˚52'39"	47 ˚25' 92"	1137
7. *G. platypetalum*	Hamedan, Nahavand	38 ˚ 52'39"	47 ˚23' 92"	1144
8. *G. gracile*	Mazandaran, Tonekabon-jannat rudbar	36 ˚48'47"	50 ˚5368"	1600
9. *G. ibericum*	Mazandaran, Haraz road, Emam Zad-e-Hashem	36˚ 38 ˈ 05 "	51˚ 29 ˈ 05 "	1250
10. *G. kotschyi*	Alborz, Karaj- Qazvin	35 ˚49'23"	51 ˚ 00'04"	1365
11. *G. tuberosum*	Kermanshah, Islamabad	38˚52'39"	47 ˚25' 92"	1133
12. *G. stepporum*	Esfahan, Fereydun shahr	35˚50'03"	51 ˚ 24'28"	2383
13. *G. persicum*	Tehran, Damavand	35 ˚43'15"	52 ˚ 04'12"	1975

These species were studied via morphological and molecular methods. One hundred and twenty-five plant samples (10-25 per plant species) were examined for morphometry purposes (Figure 1). The random amplified polymorphic DNA analysis method was limited to 102 samples. We focused on the following species *G. dissectum* L. (sec. *Dissecta*); *G. persicum* Schönb.-Tem., *G. tuberosum* L., *G. kotschyi* Boiss., *G. stepporum* P.H.Davis (sec. *Tuberosa* subsect. *Tuberosa* (Boiss.) Yeo); *G. platypetalum* Fisch. & C. A. Mey., *G. gracile* Ledeb. ex Nordm., *G. ibericum* Cav. (sec. *Tuberosa* subsect. *Mediterranea* R. Knuth). *G. columbinum* L., *G. rotundifolium* L., *G. collinum* Stephan ex Willd, *G. sylvaticum* L., *G. pratense* (sec.

Geranium). According to previous references, all the species were identified (SCHONBECK-TEMESY, 1970; ZOHARY, 1972; AEDO *et al*., 1998; JANIGHORBAN, 2009).

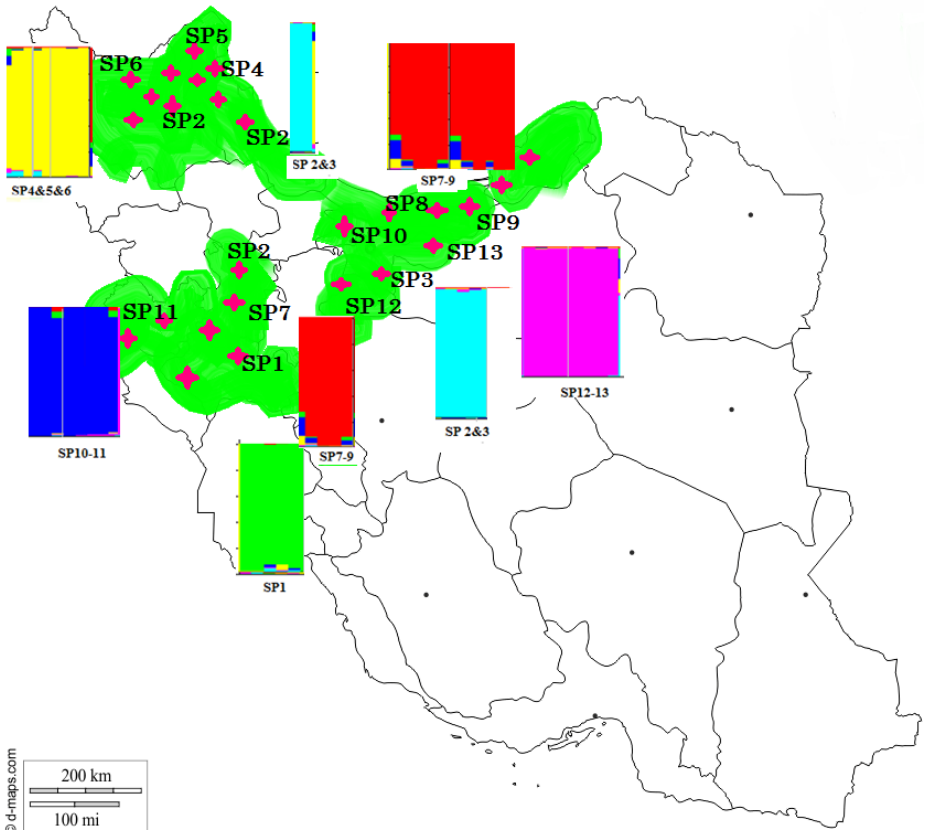

Figure 1. Presence of species in different regions of Iran. sp1= *G. dissectum;* sp2= *G. collinum;* sp3= *G. rotundifolium*; sp4= *G. columbinum;* sp5= *G. sylvaticum*; sp 6= *G. pratense*; sp7= *G. platypetalum*; sp8= *G. gracile;* sp9= *G. ibericum*; sp10: *G. kotschyi* ; sp11: *G. tuberosum;* sp12= *G. stepporum*; sp13= *G. persicum.*

Morphometry

We studied 21 qualitative and 19 quantitative morphological characters (Table 2). Data were transformed (Mean= 0, variance = 1) prior to ordination. Euclidean distance was implemented to cluster and ordinate plant species (PODANI 2000).

Table 2. Quantitative and Qualitative morphological characters of Geranium species.

No	Characters
1	Plant height (mm)
2	Length of stem leaves petiole (mm)
3	Length of stem leaves (mm)
4	Width of stem leaves (mm)
5	Length of stem leaves / Width of stem leaves (mm)
6	Width of stem leaves/ Length of stem leaves (mm)
7	Number of segment stem leaves (mm)
8	Length of basal leaves petiole (mm)
9	Length of basal leaves (mm)
10	Width of basal leaves (mm)
11	Length of basal leaves / Width of basal leaves (mm)
12	Width of basal leaves / Length of basal leaves (mm)
13	Number of segment basal leaves
14	Calyx length (mm)
15	Calyx width (mm)
16	Calyx length/ Calyx width (mm)
17	Petal length (mm)
18	Petal width (mm)
19	Petal length / Petal width (mm)
20	Type root
21	Vegetation-forms
22	State of stem strength
23	State of stem branches
24	Leave shape
25	Phyllotaxy
26	Leaf tips
27	Shape of segments basal leaves
28	Stamen filament color
29	Stigma hair
30	Mericarp shape
31	Mericarp surface
32	Mericarp hair
33	Mericarp Rostrum hair
34	Sepale hair
35	Sepale hair density
36	Peduncle and pedicel hair
37	Anthers colour
38	Stem hair
39	Stem hair density
40	Leaf hair

Random Amplified Polymorphic DNA

We extracted DNA from fresh leaves. Leaves were dried. DNA extraction was carried out according to the previous protocol (ESFANDANI-BOZCHALOYI *et al.*, 2019). DNA quality was checked on an agarose gel to confirm the purity. We amplified the DNA with the aid of RAPD primers (Operon technology, Alameda, Canada). These primers belonged to OPA, OPB, OPC, OPD sets. We selected those primers (10) which could show 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 these 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).

Table 3. RAPD primers and other parameters.

Primer name	Primer sequence (5'-3')	TNB	NPB	PPB	PIC	PI	EMR	MI
OPA-05	5′-AGGGGTCTTG-3′	13	13	100.00%	0.36	4.86	12.55	3.45
OPA-06	5′-GGTCCCTGAC-3′	9	7	84.99%	0.43	3.51	8.43	3.85
OPB-01	5′-GTTTCGCTCC-3′	9	9	100.00%	0.44	4.34	10.55	4.44
OPB-02	5′-TGATCCCTGG-3′	11	11	100.00%	0.37	2.18	9.56	3.65
OPC-04	5'-CCGCATCTAC-3'	10	10	100.00%	0.45	4.28	7.20	3.47
OPD-02	5′-GGACCCAACC-3′	14	13	93.74%	0.37	5.66	8.56	3.67
OPD-03	5′-GTCGCCGTCA-3′	13	12	92.31%	0.54	4.21	8.23	4.55
OPD-05	5′ -TGAGCGGACA-3′	12	12	100.00%	0.47	4.32	9.55	2.18
OPD-08	5′-GTGTGCCCCA-3′	11	9	82.89%	0.43	6.56	9.34	3.18
OPD-11	5′-AGCGCCATTG-3′	10	10	100.00%	0.39	4.25	10.11	3.87
Mean		11.3	10.5	92.68%	0.49	4.5	9.2	3.4
Total		114	109					

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

Data analyses

We used an Unweighted pair group method with arithmetic mean (UPGMA) and Ward methods. Ordination methods such as multidimensional scaling and principal coordinate analysis were also performed (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). To assess genetic diversity, we encoded RAPD bands as present and absent. Numbers 1 and 0 were used to show the presence and absence of bands. It is essential to know the polymorphism information content and marker index (MI) of primers because these parameters serve to observe polymorphic loci in genotypes (ISMAIL *et al*., 2019). Marker index was calculated according to the previous protocol (HEIKRUJAM *et al.*, 2015). Other parameters such as the number of polymorphic bands (NPB) and effective multiplex ratio (EMR) were assessed. Gene diversity associated characteristics of plant samples were calculated. These characteristics include Nei's gene diversity (H), Shannon information index (I), number of effective alleles (Ne), and percentage of polymorphism (P% =number of polymorphic loci/number of total loci) (SHEN *et al*., 2017). 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 carried out to find the correlation between genetic and geographical distances (PODANI, 2000). As 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 through Genetic statistics ($G_{ST)}$ in PopGene ver. 1.32 (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

Significant ANOVA results ($P < 0.01$) showed differences in quantitative morphological characters in plant species. Principal component results explained 65% variation. First component of PCA demonstrated 47% of the total variation. Leaf morphology and traits such as petiole and bract hair positively correlated with mericarp, pedicel, peduncle, and stem hairs (>0.7). The second and third components explained floral characters such as inflorescence, bract and peduncle, sepal hairs, and petals widths. Unweighted pair group method with arithmetic mean (UPGMA) and multidimensional scaling (MDS) plots showed symmetrical results (Figure 2, Figure 3). Generally, plant specimens belonging to different sections were separated from each other due to differences in morphology. Morphological characters divided *Geranium* species into two groups, as evident in the UPGMA tree (Figure 2). Populations belonging to *G. dissectum* (sect. *Dissecta*) were in the first group. On the other hand, the second group consisted of two sub-groups. *G. persicum*, *G. tuberosum*, *G. kotschyi*, *G. stepporum* (sect. *Tuberosa* subsect. *Tuberosa*) and *G. platypetalum*, *G. gracile* and *G. ibericum* (sect. *Tuberosa* subsect. *Mediterranea*) formed the first sub-group. *G. rotundifolium*, *G. collinum*, *G. sylvaticum*, *G. pratense*, *G. columbinum* (sect. *Geranium*) formed the second sub-group. These groups and sub-groups were formed due to morphological differences among the individuals of *Geranium*. Our MDS results also confirmed the application of morphological characters in separating and clustering the species in separate groups (Figure 3). Identical results were also reported in the UPGMA tree (Figure 2).

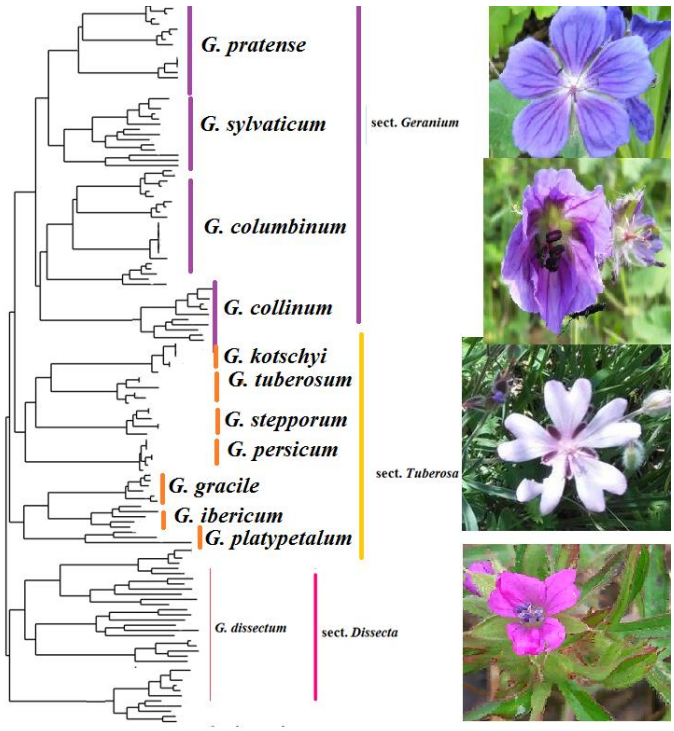

Figure 2. UPGMA clusters of subg. *Geranium*.

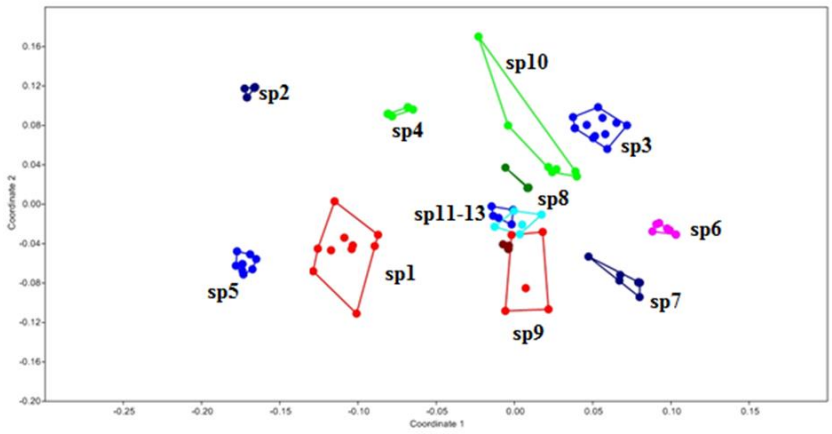

Figure 3. Multidimensional scaling plots. Species codes/numericals are explained (refer to Figure 1 and Table 1 captions).

The primers, i.e., OPD-03 and OPB-01, could amplify plant (*Geranium*) DNA (Figure 4). One hundred and seven polymorphic bands were generated and amplified. Amplified products ranged from 200 to 3000 bp. We recorded the highest polymorphic bands for OPD-02. OPA-06 had the lowest polymorphic bands. The average polymorphic bands ranged to 10.5 for each primer. The polymorphic information content (PIC) had values in the range of 0.36 (OPA-05) to 0.54 (OPD 03). Primers had 0.49 average polymorphic information content values.

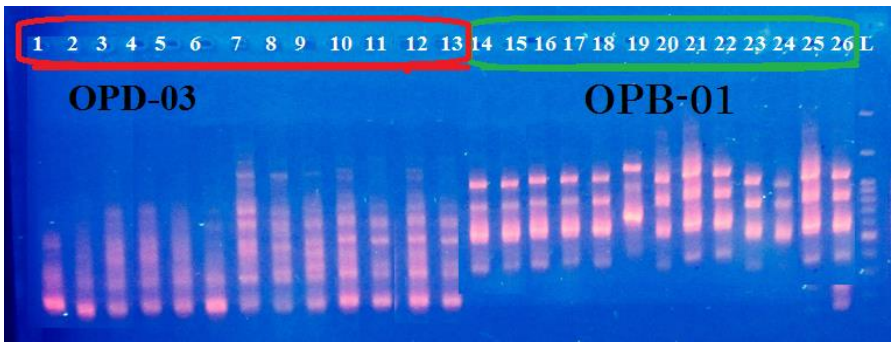

Figure 4. Gel Electrophoresis image of DNA fragments of Geranium species. *G. dissectum;* 2&15= *G. collinum;* 3&16= *G. rotundifolium*; 4&17= *G. columbinum;* 5&18= *G. sylvaticum*; 6&19= *G. pratense*; 7&20= *G. platypetalum*; 8&21= *G. gracile;* 9&22= *G. ibericum*; 10&23: *G. kotschyi* ; 11&24: *G. tuberosum;* 12&25= *G. stepporum*; 13&26= *G. persicum;* L = Ladder 100 bp. Arrows show polymorphic bands.

Marker index (MI) values were 2.18 (OPD-05) to 4.55 (OPC-03), with an average of 3.4 per primer. Effective multiplex ratio (EMR) values are useful to distinguish genotypes. In our study, we reported 7.20 (OPC-04) to 12.55 (OPA-05) EMR values. EMR values averaged 9.2 per primer (Table 3). All the necessary genetic features calculated of thirteen *Geranium* species are shown (Table 4). *G. sylvaticum* depicted unbiased expected heterozygosity (UHe) in the range of 0.11. *G. columbinum* showed a 0.29 UHe value heterozygosity had a mean value of 0.18 in overall *Geranium* species. Shannon information was high (0.38) in *G. columbinum*. *G. sylvaticum* showed the lowest value, 0.14. Mean values for Shannon information was 0.26. The observed number of alleles (*N*a) ranged from 0.25 to 0.55 in *G. persicum* and *G. tuberosum*. The effective number of alleles (Ne) was in the range of 1.020-1.430 for *G. tuberosum* and *G. collinum.*

Analysis of Molecular Variance (AMOVA) test highlighted genetic differences among *Geranium* species (P = 0.01). AMOVA showed that 73% of genetic variation was among the species. Relative less variation (27%) was reported within the species. Genetic similarity and dissimilarity assessed through Genetic statistics (GST) showed significant differences i.e., (0.29, P = 0.001) and D_est values (0.137, p = 0.01). The neighbor-joining tree also revealed two major groups (Figure 5). The neighbor-joining tree also repeated the same pattern as indicated in figures 2 and 3. In current work, molecular findings also coincided with the traditional taxonomical (morphology) approaches for *Geranium* species.

Table 4. Genetic diversity variables of Geranium. (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).

Pop	N	Na	Ne	I	He	UHe	%P
sp1	12.000	0.287	1.233	0.271	0.184	0.192	51.91%
sp2	5.000	0.358	1.430	0.18	0.20	0.29	43.50%
sp3	6.000	0.299	1.029	0.231	0.18	0.23	44.38%
sp4	5.000	0.462	1.095	0.388	0.29	0.22	62.05%
sp5	8.000	0.399	1.167	0.14	0.11	0.113	32.88%
sp6	5.000	0.336	1.034	0.23	0.25	0.19	51.83%
sp7	4.000	0.344	1.042	0.20	0.23	0.20	57.53%
sp8	6.000	0.458	1.039	0.28	0.18	0.23	49.38%
sp9	5.000	0.455	1.077	0.277	0.24	0.22	55.05%
sp10	8.000	0.499	1.067	0.24	0.13	0.14	49.26%
sp11	6.000	0.555	1.020	0.22	0.25	0.28	43.53%
sp12	10.000	0.431	1.088	0.33	0.22	0.13	57.53%
sp13	3.000	0.255	1.021	0.25	0.18	0.12	42.15%

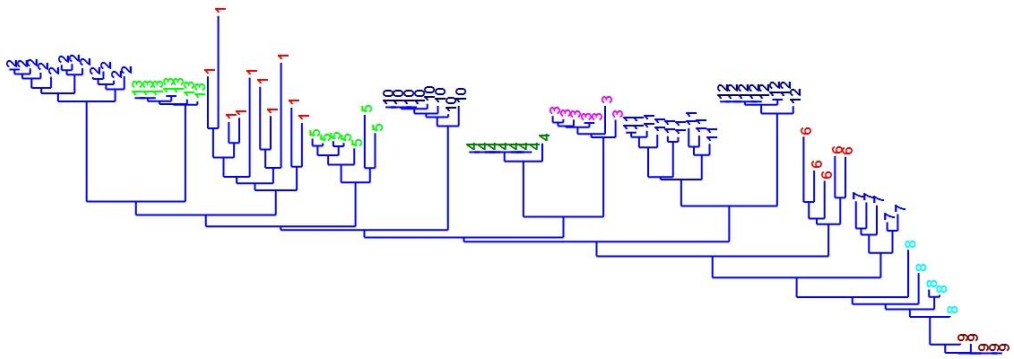

Figure 5. Neigbor-Joingin tree produced while using RAPD data. sp1= *G. dissectum;* sp2= *G. collinum;* sp3= *G. rotundifolium*; sp4= *G. columbinum;* sp5= *G. sylvaticum*; sp 6= *G. pratense*; sp7= *G. platypetalum*; sp8= *G. gracile;* sp9= *G. ibericum*; sp10: *G. kotschyi;* sp11: *G. tuberosum;* sp12= *G. stepporum*; sp13= *G. persicum.*

Gene flow (Nm) was relatively low (0.33) in *Geranium*. Genetic identity and phylogenetic distance in the *Geranium* members are mentioned (Table 5). *G. collinum* and *G. rotundifolium* (sect. *Geranium*). were genetically closely related (0.92) to each other. *G. dissectum* and *G. sylvaticum* were dissimilar due to low (0.71) genetic similarity. The mantel test showed correlation (r = 0.27, p=0.0002) between genetic and geographical distances.

The Evanno test showed ΔK =6 (Figure 6). Figure 6, showed the genetic details of the *Geranium* species. According to STRUCTURE analysis, *G. collinum* and *G. rotundifolium* were closely related to common alleles (Figure 6). The rest of the *Geranium* species are genetically differentiated due to different allelic structures (Figure 6). The neighbor-joining plot also showed the same result. Limited gene flow results were supported by K-Means and STRUCTURE analyses too. We could not identify substantial gene flow among the *Geranium* species. Nonetheless, we were able to construct a consensus tree that agreed with our molecular (RAPD) and morphological findings (Figure 7). The *Geranium* populations showed divergence due to genetic and morphological characters.

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

Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10	Pop11	Pop12	Pop13	
1.000													Pop1
0.800	1.000												Pop2
0.807	0.928	1.000											Pop3
0.829	0.873	0.860	1.000										Pop4
0.719	0.896	0.874	0.862	1.000									Pop5
0.739	0.858	0.844	0.828	0.884	1.000								Pop6
0.771	0.846	0.800	0.796	0.881	0.794	1.000							Pop7
0.759	0.818	0.807	0.794	0.874	0.752	0.862	1.000						Pop8
0.727	0.821	0.829	0.826	0.905	0.742	0.745	0.775	1.000					Pop9
0.759	0.914	0.720	0.745	0.812	0.832	0.825	0.858	0.885	1.000				Pop10
0.743	0.838	0.739	0.738	0.787	0.768	0.773	0.798	0.854	0.842	1.000			Pop11
0.782	0.891	0.771	0.794	0.852	0.797	0.804	0.807	0.789	0.797	0.861	1.000		Pop12
0.829	0.826	0.905	0.742	0.745	0.775	0.846	0.812	0.832	0.825	0.858	0.885	1.000	Pop13

sp1= G. dissectum; sp2= G. collinum; sp3= G. rotundifolium; sp4= G. columbinum; sp5= G. sylvaticum; sp 6= G. pratense; sp7= G. platypetalum; sp8= G. gracile; sp9= G. ibericum; sp10: G. kotschyi ; sp11: G. tuberosum; sp12= G. stepporum; sp13= G. persicum.

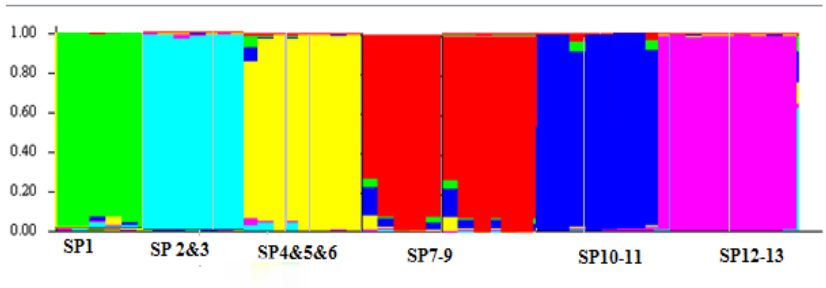

Figure 6. STRUCTURE plot of *Geranium* species

sp1= *G. dissectum;* sp2= *G. collinum;* sp3= *G. rotundifolium*; sp4= *G. columbinum;* sp5= *G. sylvaticum*; sp 6= *G. pratense*; sp7= *G. platypetalum*; sp8= *G. gracile;* sp9= *G. ibericum*; sp10: *G. kotschyi;* sp11: *G. tuberosum;* sp12= *G. stepporum*; sp13= *G. persicum*.

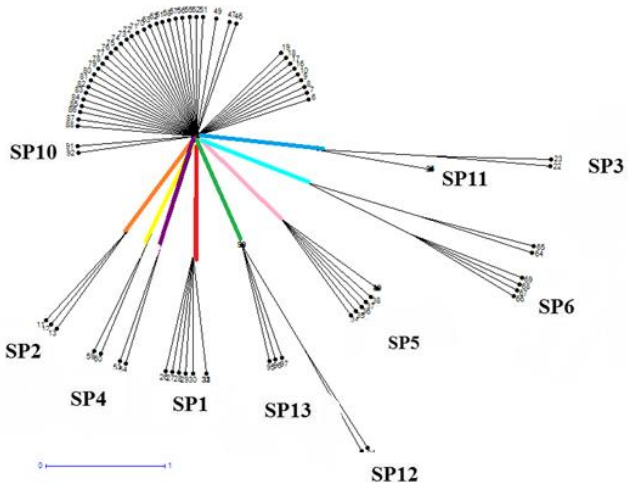

Figure 7. Morphological and molecular consensus tree sp1= *G. dissectum;* sp2= *G. collinum;* sp3= *G. rotundifolium*; sp4= *G. columbinum;* sp5= *G. sylvaticum*; sp 6= *G. pratense*; sp7= *G. platypetalum*; sp8= *G. gracile;* sp9= *G. ibericum*; sp10: *G. kotschyi* ; sp11: *G. tuberosum;* sp12= *G. stepporum*; sp13= *G. persicum*.

DISCUSSION

The *Geranium* is a relatively complex taxonomic group, and several morphological characters make it difficult to identify and classify *Geranium* species (WONDIMU *et al*., 2017). Given the complexity, it is necessary to explore other methods that could complement the traditional taxonomical approach (ERBANO *et al*., 2015). Advent and developments in molecular

techniques have enabled plant taxonomists to utilize molecular protocols to study plant groups (ERBANO *et al*., 2015). We examined genetic diversity in *Geranium* by morphological and molecular methods. We mainly used RAPD markers to investigate genetic diversity and genetic affinity in *Geranium*. Our clustering and ordination techniques showed similar patterns. Morphometry results clearly showed the utilization or significance of morphological characters in *Geranium* species. MDS results also confirmed the application of morphological characters to separate *Geranium* species. The present study also highlighted that morphological characters such as length, bract length, and stipule length could delimit the *Geranium* group. The *Geranium* species highlighted morphological differences. We argue that such a dissimilarity was due to differences in quantitative and qualitative traits.

Present findings on morphological differences are in line with the previous studies (JEITER *et al*., 2015; SALIMI MOGHADAM *et al*., 2015; AEDO and PANDO, 2017). Polymorphic information content (PIC) values are useful to detect genetic diversity. The current study recorded average PIC values of 0.49. This value is sufficient to study genetic diversity in the population (KEMPF *et al*., 2016). High genetic diversity among the *Geranium* 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 *Geranium* species. This existence of shared alleles is related to self-pollination in *Geranium* (WILLIAMS *et al*., 2000). Some *Geranium* members are also pollinated by bees, flies, and honey bees (LEFEBVRE *et al*., 2019). Present findings revealed limited gene flow, and it is quite logical to report low gene flow. Similar low gene flow values were recorded while using RAPD markers (FISCHER *et al*., 2000). Other probable reasons for limited gene flow are geographical isolation (FISCHER *et al*., 2000) among the *Geranium* species and population. Low or limited gene flow results were according to the Mantel test results. The Mantel test indicated a positive correlation between genetic and geographical distances. Therefore, it is concluded that isolation by distance and limited gene determines the Geranium population genetic structure.

Molecular markers (RAPD) and morphometry analysis were useful to study genetic diversity and population structure in *Geranium* species identification. All the species had distinct genetic differentiation. Present results highlighted isolation and limited gene flow are the main deterministic factors that shape the Geranium population. We also reported high genetic diversity, which clearly shows the *Geranium* species can adapt to changing environments since high genetic diversity is linked to species adaptability.

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REFERENCES

AEDO, C. (1996): Revision of *Geranium* subgenus *Erodioidea* (Geraniaceae). Syst. Bot. Monogr., 49:1–104.

AEDO, C., J.J., ALDASORO, C., NAVARRO (1998): Taxonomic revision of *Geranium* L., sections *Divaricata* Rouy and *Batrachioidea* W.D.J. Koch (Geraniaceae). Ann. Missouri Bot. Gard., *85*:594–630.

AEDO, C., M.D.L., ESTRELLA (2006): Taxonomic revision of *Geranium* subsect. *Tuberosa* (Boiss.) Yeo. ISR J Plant Sci., *54*(1):19–44.

AEDO, C., M.L., ALARCON, J.J., ALDASORO, C., NAVARRO (2007): Taxonomic revision of *Geranium* subsect. *Mediterranea* (Geraniaceae). Syst. Bot., *32*:93–128.

AEDO, C., F., PANDO (2017): A distribution and taxonomic reference dataset of Geranium in the New World. Sci Data, *4*(1):170049.

CIRES, E., Y., DE SMET, C., CUESTA, P., GOETGHEBEUR, S., SHARROCK, D., GIBBS, S., OLDFIELD, A., KRAMER, M-S., SAMAIN (2013): Gap analyses to support ex situ conservation of genetic diversity in Magnolia, a flagship group. Biodivers Conserv., *22*(3):567-590.

ELLEGREN, H., N., GALTIER (2016): Determinants of genetic diversity. Nat. Rev. Genet., *17*(7):422-433.

ERBANO, M., G.S.E., SCHÜHLI, É.P.D., SANTOS (2015): Genetic variability and population structure of Salvia lachnostachys: implications for breeding and conservation programs. Int. J. Mol. Sci., *16*(4):7839-7850.

ESFANDANI-BOZCHALOYI, S., M.,SHEIDAI, M., KESHAVARZI, Z., NOORMOHAMMADI (2018c): Morphometric and ISSR-analysis of local populations of *Geranium molle* L. from the southern coast of the Caspian Sea. Cytol. Genet., *52*(4):309–321.

ESFANDANI-BOZCHALOYI, S., M., SHEIDAI (2018d): Molecular diversity and genetic relationships among *Geranium pusillum* and *G. pyrenaicum* with inter simple sequence repeat (ISSR) regions. Caryologia, *71*(4):1-14.

ESFANDANI-BOZCHALOYI, S., M, SHEIDAI, M. KALALEGH (2019): Comparison of DNA extraction methods from Geranium (Geraniaceae). Acta Bot. Hung., *61*(3-4):251-266.

ESFANDANI-BOZCHALOYI, S., M., SHEIDAI, M., KESHAVARZI, Z., NOORMOHAMMADI (2018a): Species Relationship and Population Structure Analysis In *Geranium* Subg. *Robertium* (Picard) Rouy With The Use of ISSR Molecular Markers. Act Bot. Hung., *60*(1–2):47–65.

ESFANDANI-BOZCHALOYI, S., M., SHEIDAI, M., KESHAVARZI, Z., NOORMOHAMMADI (2018b): Species Identification and Population Structure Analysis In *Geranium* Subg. *Geranium* (Geraniaceae) . Hacquetia, *17*(2):235–246.

ESFANDANI-BOZCHALOYI, S., M., SHEIDAI, M., KESHAVARZI, Z., NOORMOHAMMADI (2017): Genetic and morphological diversity in *Geranium dissectum* (Sec. Dissecta, Geraniaceae) populations. Biologia, *72*(10):1121- 1130.

EVANNO, G., S., REGNAUT, J., GOUDET (2005): Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol., *14*:2611–2620.

FISCHER, M., R., HUSI, D., PRATI, M., PEINTINGER, M., KLEUNEN, B., SCHMID (2000): RAPD variation among and within small and large populations of the rare clonal plant Ranunculus reptans (Ranunculaceae). Am. J. Bot., *87*(8):1128-1137.

FRANKHAM, R. (2005): Stress and adaptation in conservation genetics. J. Evol. Biol., *18*(4):750-755.

FREELAND, J.R., H., KIRK, S.D., PETERSON (2011): Molecular ecology (2nd ed). UK: Wiley-Blackwell.

GOMEZ, A., G.G., VENDRAMIN, S.C., GONZÁLEZ-MARTÍNEZ, R., ALIA (2005): Genetic diversity and differentiation of two Mediterranean pines (Pinus halepensis Mill. and Pinus pinaster Ait.) along a latitudinal cline using chloroplast microsatellite markers. Divers Distrib., *11*(3): 257-263.

HAMMER, O., D.A.T., HARPER, P.D., RYAN (2001): PAST: paleontological statistics software package for education and data analysis. Palaeontol. Electron., *4*(1):1-9.

HEIKRUJAM, M., J., KUMAR, V., AGRAWAL (2015): Genetic diversity analysis among male and female Jojoba genotypes employing gene targeted molecular markers, start codon targeted (SCoT) polymorphism and CAAT box-derived polymorphism (CBDP) markers. Meta Gene, *5*:90-97.

HUSON, D.H., D., BRYANT (2006): Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol., *23*:254–267.

ISMAIL, N.A., M.Y., RAFII, T.M.M., MAHMUD, M.M., HANAFI, G., MIAH (2019): Genetic Diversity of Torch Ginger Germplasm Revealed by ISSR and SSR Markers. BioMed Res., 2019:5904804.

JANIGHORBAN, M. (2005): Geraniaceae. In: Assadi M et al. editors Flora of Iran, Vol. 62; p. 136, Research Institute of Forests and Rangelands Publication, Tehran.

JEITER, J., H.H., HILGER, E.F., SMETS, M., WEIGEND (2017): The relationship between nectaries and floral architecture: a case study in Geraniaceae and Hypseocharitaceae. Ann. Bot., *120*(5):791-803.

KEMPF, K., M., MORA-ORTIZ, L.M.J., SMITH, R., KÖLLIKER, L., SKOT (2016):. Characterization of novel SSR markers in diverse sainfoin (Onobrychis viciifolia) germplasm. BMC Genet., *17*(1):124-124.

KURATA, S., S., SAKAGUCHI, M., ITO (2019): Genetic diversity and population demography of Geranium soboliferum var. kiusianum: a glacial relict plant in the wetlands of Japan. Conserv. Genet., *20*(3):431-445.

KHAYATNEZHAD, M., R. GHOLAMIN (2020). "A Modern Equation for Determining the Dry-spell Resistance of Crops to Identify Suitable Seeds for the Breeding Program Using Modified Stress Tolerance Index (MSTI)." Bioscience Biotechnology Research Communications *13*(4): 2114-2117.

KHAYATNEZHAD, M., R. GHOLAMIN (2021). "The Effect of Drought Stress on the Superoxide Dismutase and Chlorophyll Content in Durum Wheat Genotypes." Advancements in Life Sciences *8*(2): 119-123.

KHAYATNEZHAD, M., R. GHOLAMIN (2021). "Impacts of Drought Stress on Corn Cultivars (Zea mays L.) At the Germination Stage." Bioscience Research *18*(1): 409-414.

LEFEBVRE, V., C., DAUGERON, C., VILLEMANT, C., FONTAINE (2019): Empidine dance flies pollinate the woodland geranium as effectively as bees. Biol. Lett., *15*(7):20190230.

ONSORI, S., F., SALIMPOUR, A., MAZOOJI (2010): The new record of *Geranium linearilobum* Dc. based on anatomy and micromorphological study of pollen and seed, in Iran. J. Plant Environ. Physiol., *5*:21-30. [in Persian with English abstract]

PEAKALL, R., P.E., SMOUSE (2006): GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes, *6*:288–295.

PODANI, J. (2000): Introduction to the exploration of multivariate data [English translation]. Leide (Netherlands): Backhuyes.

SALIMI MOGHADAM, N., S., SAEIDI MEHRVARZ, A., AHMADIAN NAMIN, R.S., SHAVVON (2015): Micromorphology of fruits and seeds of Iranian Geranium (Geraniaceae), and its systematic significance. Nord. J. Bot., *33*(6):673-683.

SCHONBECK-TEMESY, E. (1970): Geraniaceae. In: Rechinger KH, editor. Flora Iranica, Vol. 69. Graz (Austria): Akademische Druck; p. 30–58.

SHEN, Z., K., ZHANG, L., MA, J., DUAN, Y., AO (2017): Analysis of the genetic relationships and diversity among 11 populations of Xanthoceras sorbifolia using phenotypic and microsatellite marker data. Electron. J. Biotechnol., *26*:33-39.

TURCHETTO, C., A.L.A., SEGATTO, G., MÄDER, D.M., RODRIGUES, S.L., BONATTO, L.B., FREITAS (2016): High levels of genetic diversity and population structure in an endemic and rare species: implications for conservation. AoB Plants, *8*:plw002.

WILLIAMS, C.F., M.A., KUCHENREUTHER, A., DREW (2000): Floral dimorphism, pollination, and self-fertilization in gynodioecious Geranium richardsonii (Geraniaceae). Am. J. Bot., *87*(5): 661-9.

WONDIMU, T., A., GIZAW, F.M., TUSIIME, C.A., MASAO, A.A., ABDI, Y., HOU, S., NEMOMISSA, C., BROCHMANN (2017): Genetic structuring, dispersal and taxonomy of the high-alpine populations of the Geranium arabicum/kilimandscharicum complex in tropical eastern Africa. Plos One, *12*(5):e0178208.

YEH, F.C., R., YANG, T., BOYLE (1999): POPGENE. Microsoft Windows-based freeware for population genetic analysis. Release 1.31. University of Alberta, 1-31.

YEO, P.F. (2008): Fruit-discharge-type in Geranium (Geraniaceae): its use in classification and its evolutionary implications. Bot. J. Linn Soc., *89*(1):1-36.

ZOHARY, M. (1972): Flora Palaestina. Platanaceae to umbelliferae. Jerusalem (Israel): The Israel Academy of Sciences and Humanities.

EVALUACIJA GENETIČKOG DIVERZITETA KOD VRSTE *Geranium* (Geraniaceae) POMOĆU RAPD MARKERA

Juan YIN[1*], Majid KHAYATNEZHAD[2], Abdul SHAKOOR[3,4]

[1]Koledž za šumarstvo, Xinyang koledž za poljoprivredu i šumarstvo, Xinyang, Henan, Kina
[2]Departman za životnu sredinu i inžinjering, Ogranak Ardabil, Islamski Azad Univerzitet, Ardabil, Iran
[3]Koledž za životnu sredinu i planiranje, Henan Univerzitet, Kaifeng, Henan, Kina
[4]Glavna laboratorija geoprostorne tehnologije za regione Srednja i Donja Žuta reka, Ministarstvo obrazovanja, Kaifeng, Henan, Kina

Izvod

Proučavanja genetičkog diverziteta su ključna za razumevanje očuvanja i upravljanja biljnim resursima u bilo kom okruženju. U Iranu je zabeleženo trinaest vrsta *Geranium*. Nisu sprovedena detaljnija RAPD proučavanja genetske raznolikosti Geraniuma. Stoga smo prikupili i analizirali trinaest vrsta iz devet provincija iranskih regiona. Ukupno je prikupljeno 125 biljnih primeraka. Cilj nam je bio 1) da procenimo genetsku raznolikost vrste *Geranium* 2) da utvrdimo da li postoji korelacija između genetske i geografske udaljenosti vrsta 3) utvrdimo genetsku strukturu populacija i taksona. Pokazali smo značajne razlike u kvantitativnim morfološkim svojstvima biljnih vrsta. Neponderisana metoda parnih grupa sa aritmetičkom sredinom i višedimenzionalnim skaliranjem podelila je vrste *Geraniuma* u dve grupe. *G. silvaticum* je prikazao nepristrasnu očekivanu heterozigotnost (UHe) u opsegu od 0,11. Šenonove informacije su bile visoke (0,38) u *G. columbinum*. *G. silvaticum* je pokazao najnižu vrednost, 0,14. Uočeni broj alela (Na) kretao se od 0,25 do 0,55 kod *G. persicum* i *G. tuberosum*. Neponderisana metoda parnih grupa sa aritmetičkom sredinom i višedimenzionalnim skaliranjem podelila je vrste geranijuma u dve grupe. G. silvaticum je prikazao nepristrasnu očekivanu heterozigotnost (UHe) u opsegu od 0,11. Šenonove informacije su bile visoke (0,38) u *G. columbinum. G. silvaticum* je pokazao najnižu vrednost, 0,14. Efektivni broj alela (Ne) bio je u opsegu od 1.020-1.430 za *G. tuberosum* i *G. collinum*. Protok gena (Nm) bio je relativno nizak (0,33) u *Geranium*. Mantelov test pokazao je korelaciju ($r = 0,27$, $p = 0,0002$) između genetske i geografske udaljenosti. Utvrdili smo veliku genetsku raznolikost, što jasno pokazuje da se vrste *Geranium* mogu prilagoditi promenljivom okruženju, jer je velika genetska raznolikost povezana sa prilagodljivošću vrsta. Ovi rezultati ukazali su na korisnost RAPD markera i morfometrijskih metoda za istraživanje genetske raznolikosti vrste *Geranium*.

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