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COMPARISON OF INDIVIDUAL BASED APPROACHES USING RAPD MARKERS FOR IDENTIFYING GENETIC RELATIONSHIPS IN *Erodium* (Geranaiceae)

Xixi YAO[1], Rui ZHOU[2*], Mohsen FARSHADFAR[3]

[1]College of Agriculture and Animal Husbandry, Qinghai University, Xining,Qinghai, 810016, China
[2]Academy of animal husbandry and Veterinary Sciences, Qinghai University, Xining, Qinghai, 810016, China
3 Department of Agriculture, Payame Noor University (PNU), Tehran, Iran

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Genetic diversity research is required to understand the conservation and management of plant resources in all ecosystems. In Iran, there are 15 different *Erodium* species. *Erodium* genetic diversity has not been studied extensively using Random Amplified Polymorphic DNA (RAPD). As a result, six species were gathered and learned from six different Iranian locales. A total of 82 plant specimens were collected. Our objectives were to assess *Erodium* species genetic diversity and to identify *Erodium* species genetic diversity. 2) Is there a link between a species's genetic makeup and geographic location? 3) Population and taxon genetic structure. *Erodium* species were separated into two groups using an unweighted pair group technique with arithmetic mean and principal component analysis. In *Erodium*, gene flow (Nm) was relatively modest (0.33). The Mantel test revealed a link between genetic and geographical distances (r = 0.77, p=0.0001). We identified many genetic variations in the *Erodium* species, which indicates that it can adapt to changing circumstances. According to recent findings, RAPD markers and morphometry approaches may investigate *Erodium* species genetic diversity.

Keywords Gene flow, Random Amplified Polymorphic DNA (RAPD), *Erodium* species

INTRODUCTION

In Iran, there are 15 species of *Erodium* (ESFANDANI-BOZCHALOYI *et al.*, 2017; SCHNBECK-TEMESY, 1970). Irano-Touranian and Saharo-Sindian components make up the majority of these species. The Hyrcanian area is home to just one species (JANIGHORBAN, 2009). *Erodium* species grow in various settings, from annuals (25 species) to perennials. In the

Corresponding author: Rui Zhou, College of Agriculture and Animal Husbandry, Qinghai University, Xining, Qinghai, 810016, China. E-mail: 13893632442@163.com; jamshidman389@gmail.com;

Geraniaceae, there are two forms of leaf venation: palmate and pinnate. Except for *California* and *Geranium*, which are palmate, *Erodium* species vary from subpinnate to pinnate. Pinnatifid, pinnatipartite, and pinnatisect are the three forms of leaf division (FIZ *et al*., 2006). In all Erodium species, the androecium has five fertile stamens and five staminodia, but only five fertile stamens in California. In comparison, Monsonia's androecium (15 fertile stamens) and Geranium's androecium (15 fertile stamens) have less fertile stamens (10 fertile stamens). Pollen from the Geraniaceae family has four unique ornamentation styles based on supratectal features and tectum morphology. Striate pollen was found in 68 of the 74 Erodium species identified in the four subclades (FIZ *et al*., 2006). The three varieties of Erodium species are based on the surface ornamentation of the mericarp body: smooth (3), papillate (70), and foveate fruit (1).

Annuals are often autogamous, actinomorphic, and lack attractive features, while perennials are allogamous, have zygomorphic blooms, and have exceptional floral architecture (ALDASORO *et al.*, 2000). Drought adaptation in the Mediterranean during the late Tertiary may have resulted in changes in growth morphology (BAKKER *et al.*, 1955). Annuals and species that establish themselves in transient or disturbed environments are prone to selfing (BAKER, 1955; STEBBINS, 1957). Most *Erodium's* 22 selfers have large distribution regions, indicating that they may spread and establish themselves via these reproductive traits (STEBBINS, 1957). The use of RAPD markers to acquire information on genetic diversity within and among populations of *E. paularense* was described by MARTIN *et al.* (1997). The *Erodium* subsect phylogeny. ALARCN *et al*. study Petraea, a morphologically and genetically related group of six species from the western Mediterranean Alps (2012). Due to sequence similarities, the combined trnL–F-ITS analysis could not establish the evolutionary relationships between these taxa. Molecular markers are often used in genetic diversity research. Molecular markers are a powerful tool for identifying evolutionary links between species and populations. RAPD (Random Amplified Polymorphic DNA) is a sensitive genetic technique or marker for detecting individual variation in a species (MA *et al*., 2021; PENG *et al*., 2021; SHAT *et al*., 2021) The RAPD approach is low-cost and may be used for small sample sets. RAPD may also amplify and target attractive genetic regions and markers (ESFANDANI-BOZCHALOYI *et al*., 2018a; 2018b; 2018c; 2018d; YIN *et al.*, 2021).

In the past, taxonomic systematic investigations were used to identify *Erodium* species. To the best of our knowledge, no RAPD data on genetic diversity research has been found in Iran. We looked at 82 different samples. Our objectives were to 1) determine the genetic diversity of *Erodium* species and 2) determine the genetic diversity of *Erodium* species. 2) Is there a link between species and distance from home? 3) Population and taxon genetic structure 4) Is it possible for *Erodium* species to share genes?

MATERIALS AND METHODS

Plant materials

Six *Erodium* species have been detected in Iran in various locations (Table 1). These species were studied using molecular methods. The random amplified polymorphic DNA analysis process used a total of 82 samples. All species were recognized using primary sources (BAKER, 1955; STEBBINS, 1957).

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

Taxa	Locality	LAtitude	Longitude	Altitude(m)
E. malacoides Bové ex Decne.	Kordestan, Sanandaj	37° 07' 48"	49° 54' 04"	165
E. gruinum (L.) L'Hér.	Razavi Khorasan, Kashmar, Kuhsorkh District	37° 07' 08"	49°54' 11"	159
E. glaucophyllum (L.) L'Hér.	Esfahan, ardestan on road to taleghan	38 ˚ 52' 93"	47 ˚25' 92"	1133
E. hoefftianum C. A,Mey	Semnan, 20km NW of shahrud	38˚52' 93"	47 ˚25' 92"	1139
E. neuradifolium Delile ex Godr.	Mazandaran, 40 km Tonekabon to janat abad	35 ˚50' 36"	51˚ 24' 28"	2383
E. moschatum (L.) L'Hér.	West-Azarbaijan, Urumieh, Silvana	35 ˚42'29"	52 ˚20'51"	2421

Random Amplified Polymorphic DNA

Fresh leaves were used to obtain DNA. Before being utilized, the leaves had been dried. The DNA extraction procedure was followed just as previously (ESFANDANI-BOZCHALOYI *et al.*, 2019). An agarose gel was used to test the DNA purity. RAPD primers amplify the DNA (Operon technology, Alameda, Canada). These primers were from OPA, OPB, OPC, and OPD primer sets. Six primers were selected based on their ability to produce distinct bands and polymorphisms (Table 2).

Table 2. RAPD primers and other parameters.

Primer name	Primer sequence (5'-3')	TNB	NPB	PPB	PIC	PI	EMR	MI
OPA-05	5′-AGGGGTCTTG-3′	17	15	93.14%	0.29	5.22	8.11	3.11
OPB-01	5′-GTTTCGCTCC-3′	14	14	100.00%	0.57	4.11	7.55	2.22
OPB-02	5′-TGATCCCTGG-3′	13	7	66.19%	0.69	6.33	6.34	3.55
OPC-04	5'-CCGCATCTAC-3′	2	2	100.00%	0.32	2.25	7.11	3.14
OPD-02	5′-GGACCCAAGG-3′	5	5	100.00%	0.31	4.66	11.44	4.45
OPD-08	5′-GTGTGCCCCA-3′	19	19	100.00%	0.30	4.18	9.11	3.12
Mean		14	13	92.18%	0.47	4.6	8.3	3.5
Total		84	77					

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 ISCBDP primers.

RESULTS

Species Identification and Genetic Diversity

Plant DNA (*Erodium* species) may be amplified using the primers OPB-01-02 and OPD-02-08 (Figure 1). We produced and amplified 77 polymorphic bands. The items were amplified between 100 and 3000 times their original volume. In OPD-08, we detected the most polymorphic bands. The polymorphic bands in OPB-02 were the smallest. Each primer included an average of 13 polymorphic bands. The samples' polymorphic information content (PIC)

ranged from 0.29 to 0.69 (OPA-05) (OPB- 02). The average polymorphism information content of primers was 0.47.

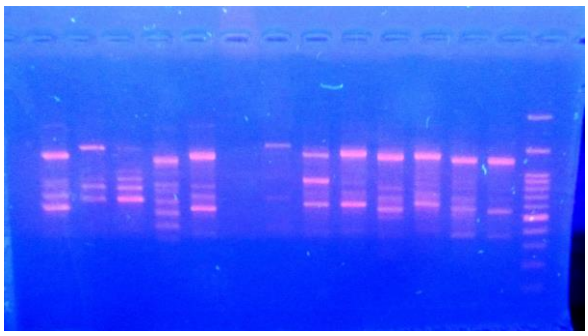

Figure 1. Gel Electrophoresis image of DNA fragments of *Erodium* species.

MI values ranged from 2.22 (OPB-01) to 4.45 (OPD-02), with an average of 3.5 for each primer. Deals of the effective multiplex ratio (EMR) are essential for distinguishing genotypes. EMR values ranged from 6.34 (OPB-02) to 11.44 (OPD-02) in our research. EMR readings per primer were 8.3 on average (Table 2). The genetic traits of six *Erodium* species have been computed and are shown (Table 3). Unbiased anticipated heterozygozygosity (UHe) in *E. hoefftianum* was in the range of 0.13. A 0.34 was found in *E. neuradifolium*. In the *Erodium* species as a whole, UHe value heterozygosity had a mean value of 0.22. In *E. neuradifolium*, Shannon information was high (0.266). The lowest value, 0.14, was found in *E. hoefftianum*. Shannon's information had a mean value of 0.2[illegible]. In *E. gruinum* and *E. neuradifolium*, the observed number of alleles (Na) varied from 0.31 to 0.422. For *E. glaucophyllum* and *E. neuradifolium*, the adequate number of alleles (Ne) ranged between 1.012-1.133.

The AMOVA (Analysis of Molecular Variance) test was used to find genetic variations between *Erodium* species (P = 0.001). According to AMOVA, 60% of genetic variation occurs across species. There was significantly less variety within the species (40 percent). Genetic similarity and dissimilarity, as evaluated by Genetic statistics (GST), had Dest values of (0.155, P = 0.001) and (0.155, P = 0.001), respectively, indicating considerable disparities.

Because the results of several clustering and ordination techniques were identical, UPGMA clustering is presented here (Figure 2). Plant samples from each species's specific area were grouped and formed separate clusters in general (Figure 2). The molecular traits analyzed in this research seem to separate *Erodium* species into two distinct sets or groupings. We didn't find any transitional forms among the specimens we looked at. The UPGMA tree (Figure 2) yielded two large clusters, with populations of *E. glaucophyllum*, *E. neuradifolium*, and *E. moschatum* grouped in the first and separated by a significant distance from the other species in the second. Two sub-clusters made up the second central cluster. The first sub-cluster consisted of *E. gruinum* plants, whereas the second sub-cluster consisted of *E. malacoides* and *E. hoefftianum* plants.

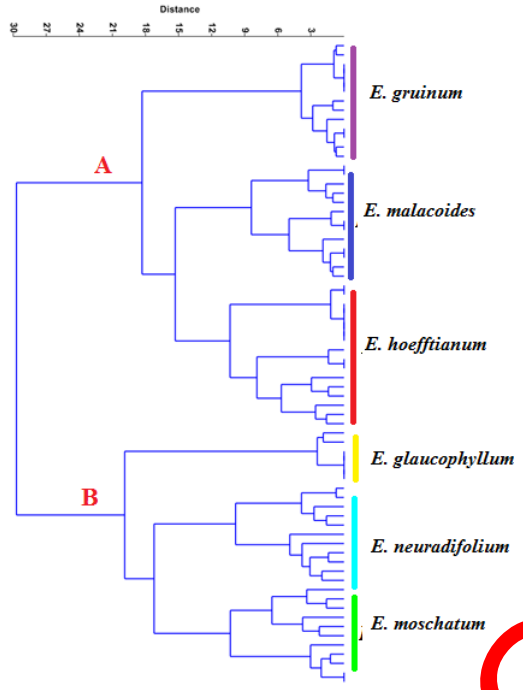

Figure 2. UPGMA clusters of RAPD data revealing species delimitation in *Erodium* species.

Table 3. Genetic diversity variables of Erodium species

Taxon	N	Na	Ne	I	He	UHe	%P
E. malacoides Bové ex Decne.	14.000	1.232	1.042	0.20	0.23	0.20	47.53%
E. gruinum (L.) L'Hér.	15.000	0.316	1.034	0.25	0.26	0.29	31.83%
E. glaucophyllum (L.) L'Hér.	12.000	0.358	1.012	0.22	0.21	0.18	20.38%
E. hoefftianum C.A.Mey	13.000	0.411	1.049	0.14	0.18	0.13	18.38%
E. neuradifolium Delile ex Godr.	10.000	0.422	1.133	0.266	0.30	0.34	52.05%
E. moschatum (L.) L'Hér.	15.000	0.399	1.033	0.24	0.25	0.29	49.11%

(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).

In *Erodium* species, gene flow (Nm) was shallow (0.33). *Erodium* members' genetic identity and phylogenetic distance are investigated. *E. hoefftianum* and *E. malacoides* were genetically related (0.83). Due to their minimal genetic resemblance, *E. moschatum* and *E.*

gruinum were shown to be different (0.61). The mantel test revealed a link between genetic and geographical distances (r = 0.77, p=0.0001).

The Evanno test yielded K = 6 (Figure not included) and indicated the *Erodium* species' genetic characteristics. According to STRUCTURE research, *E. hoefftianum* and *E. malacoides* are closely connected to common alleles. Due to diverse allelic structures, the remaining *Erodium* species are genetically distinct. A similar effect was seen on the plot next door. K-Means and STRUCTURE studies backed up the limited gene flow findings. We found no evidence of significant gene exchange between *Erodium* species. Because these populations were near one another, this result corresponds to the grouping we found using UPGMA (Figure 2). According to the STRUCTURE plot based on the admixture model, these common alleles make up a minuscule proportion of the genomes in diverse populations. These data suggest a high degree of genetic stratification among *Erodium* groups.

DISCUSSION

Erodium is a taxonomic category having several morphologic[al] tra[its] that make it challenging to identify and categorize *Erodium* species (FIZ *et al.,* 20[20]). Other techniques to improve the central taxonomic methodology must be investigated because of the complexity (ERBANO *et al*., 2015; ZOU *et al.,* 2019; JI *et al*., 2020a, 2020b; [LI]A *et al.,* 2021; ZHENG *et al*., 2021; ZHU *et al.,* 2021). Plant taxonomists may now use molecular techniques to explore plant groupings thanks to advances in molecular technology (ERB[A]NO *et al*., 2015; BALARI *et al.,* 2013, 2020; BI *et al.,* 2021; CHENG *et al.,* 2021). Molecular approaches were used to look at the genetic diversity of *Erodium* species. To study genetic diversity and genetic affinity in *Erodium* species, we mainly employed RAPD markers. Similar patterns emerged from our grouping and ordination techniques.

For the first time, genetic diversity in six *Erodium* species groups is described in this work. This research aimed to identify diagnostic traits that may be used to distinguish *Erodium* species in Iran. PIC values (polymorphic information content) help detect genetic diversity. The average PIC values in this investigation were 0.[3]. This number is adequate for evaluating population genetic diversity (KEM[P] *et al.,* 2016). According to the current research, the *Erodium* population contains a lot of genetic variabilities. STRUCTURE genomic research and molecular variance analysis revealed genetic variation between the two species.

Surprisingly, STRUCTURE research revealed that *Erodium* species had common alleles. Self-pollination is linked to common alleles in *Erodium* (WILLIAMS *et al.,* 2000). According to the available data; hence, reporting minimal gene flow is reasonable. When utilizing RAPD markers, gene flow values were similar (MARTIN *et al*., 1997). Reduced gene exchange among *Erodium* species and populations might potentially be attributed to physical isolation (FISCH[ER] *et al.,* 2000). According to the Mantel test findings, there was little or no gene flow. The Mantel test found a strong link between genetic and geographical distances. As a result, the genetic structure of the *Erodium* population is determined by gene limitation and separation by distance. The use of RAPD markers to acquire information on genetic diversity within and among people of *E. paularense* was described by MARTIN *et al*. (1997). ALARCN *et al.* study the phylogeny of the *Erodium* subsect. *Petraea*, a morphologically and genetically related group of six species from the western Mediterranean Alps (2012). Due to sequence similarities,

the combined trnL–F-ITS analysis could not establish the evolutionary relationships between these taxa. Different populations clustered into six closely related phylogroups that were somewhat associated with morphological species, according to AFLP fragment analysis. For the first time, phylogenetic reconstructions in the Mediterranean genus *Erodium* are carried out using two matrices: one with 96 *Erodium* trnL-trnF sequences plus 23 morphological characters, and the other with 96 Erodium trnL-trnF sequences plus 23 morphological characters, using Maximum Parsimony (MP) and Bayesian Inference (BI) methods, respectively (FIZ *et al.*, 2006). Based on a trnLtrnF investigation of 95 Geraniaceae accessions and combined data analysis, their findings showed that *Erodium* and California are monophyletic.

The genetic diversity and population structure of *Erodium* species were investigated using molecular markers (RAPD). There were genetic differences among all of the species. According to the current findings, isolation and restricted gene flow are the vital deterministic traits that define the *Erodium* population. We also discovered that the *Erodium* species has a lot of genetic diversity, suggesting that it can adapt to changing environments since genetic diversity is connected to species adaptability.

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POREĐENJE INDIVIDUALNIH PRISTUPA KORIŠĆENJEM RAPD MARKERA ZA IDENTIFIKACIJU GENETSKIH VEZA U *Erodium* (Geranaiceae)

Xixi YAO[1], Rui ZHOU[2*], Mohsen FARSHADFAR[3]

[1]Visoka poljoprivredna i stočarska škola, Qinghai Univerzitet, Xining, Qinghai, 810016, Kina
[2]Akademija stočarstva i veterine, Qinghai Univerzitet, Xining, Qinghai, 810016, Kina
3 Department za poljoprivredu, Payamee Noor Univerzitet (PNU), Tehran, Iran

Izvod

Studije genetičke raznovrsnosti su od suštinskog značaja za razumevanje očuvanja i upravljanja biljnim resursima u bilo kom okruženju. U Iranu je prijavljeno 15 vrsta *Erodium*. Nisu sprovedena detaljna RAPD proučavanja da bi se proučavala genetska raznolikost *Erodium* vrste. Stoga smo prikupili i analizirali šest vrsta iz šest provincija Irana. Ukupno su prikupljena 82 primerka biljaka. Naši ciljevi su bili: 1) da procenimo genetičku raznovrsnost među vrstama *Erodium* 2) da utvrdimo da li postoji korelacija između genetske i geografske udaljenosti vrsta? 3) da se utvrdi genetička struktura populacija i taksona. Metoda grupe neponderisanih parova sa analizom aritmetičke sredine i glavne komponente podelila je vrste *Erodium* u dve grupe. Protok gena (Nm) je bio relativno nizak (0,33). Mantelov test je pokazao korelaciju (r = 0,77, p=0,0001) između genetičke i geografske udaljenosti. Prijavili smo visoku genetsku raznovrsnost, što jasno pokazuje da se vrste *Erodium* mogu prilagoditi promenljivim sredinama jer je visoka genetska raznolikost povezana sa prilagodljivošću vrsta. Sadašnji rezultati su istakli korisnost RAPD markera i metoda morfometrije za istraživanje genetske raznolikosti vrsta *Erodium*.

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