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*Original scientific article*

# ASSESSMENT OF GENETIC STRUCTURE AND DIVERSITY OF *Erodium* (Geranaiceae) SPECIES

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*Erodium* Aiton (Geraniaceae) with 75 species is distributed in all continents except Antarctic. Its main diversification center is Mediterranean region with 62 species. The genetic diversity was assessed through Sequence-related amplified polymorphism. To uncover genetic diversity and species characteristics in *Erodium* species, were studied through a combination of morphological and molecular data. 70 individuals related to seven *Erodium* were collected in 7 provinces. A total of 96 (Number of total loci) (NTL) DNA bands were produced through polymerase chain reaction amplifications (PCR) amplification of seven *Erodium* species. These bands were produced with the combinations of six selective primers. The total number of amplified fragments ranged from 10 to 25. The genetic similarities between seven species are estimated from 0.70 to 0.85. Clustering results showed two major clusters. This study also detected a significant signature of isolation by distance (Mantel test results). Present results showed that sequence-related amplified polymorphism have the potential to identify and decipher genetic affinity in *Erodium* species. Current results have implications in biodiversity and conservation programs. Besides this, present results could pave the way for selecting suitable ecotypes for forage and pasture purposes in Iran.
*Key words:* Sequence-related amplified polymorphism, Gene Flow; Genetic Diversity, Morphometric Analysis, *Erodium* species

## INTRODUCTION

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

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population size (FRANKHAM, 2005; CHEN *et al.,* 2021; HAN *et al*., 2021). 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; HOU *et al*., 2021; JI *et al*., 2021; ZHANG *et al*., 2020b).

Sequence-related amplified polymorphism (SRAP) is PCR –based marker system. It is one of the efficient and simple marker systems to study gene mapping and gene tagging in plant species (LI and QUIROS, 2001; JI *et al*., 2020a, 2020b; SHEN *et al*., 2020), and SRAP are potential markers to assess plant systematics and genetic diversity studies (ROBARTS and WOLFE, 2014; JIANG *et al*., 2021; SUN *et al.,* 2021). Previously, WU *et al*. (2010) assessed genetic diversity and population structure in *Pogostemon cablin* with the aid of SRAP markers. SRAP markers were successfully implemented in Lamiaceae family to study natural populations and variations within the family (SAEBNAZAR and RAHMANI, 2013; TALEBI *et al*., 2015). These past studies showed that molecular markers, including SRAP markers, are efficient to investigate genetic diversity analyses and phylogenetic relationship among *Erodium* species in Geraniaceae family.

*Erodium* comprises 15 species in different parts of Iran (JANIGHORBAN, 2009: SCHÖNBECK-TEMESY, 1970; SHEN *et al*., 2021; WANG *et al.,* 2021; ZHANG *et al*., 2016). Most of these species are Irano-Touranian and Saharo-Sindian elements. Only one species is endemic in Hyrcanian region (JANIGHORBAN, 2009). Habit of *Erodium* species ranges from annuals (25 species) to perennials. Annuals are generally autogamous, actinomorphic, and lack attracting features, while most perennial species have an allogamous condition, zygomorphic flowers and striking floral structures (ALDASORO *et al*., 2000; ZHANG *et al*., 2020a; ZHANG *et al*., 2021a, b, c). Selfing plants are common among annuals and taxa colonizing temporary or disturbed habitats (BAKER, 1955, 1967; STEBBINS, 1957, 1970; ARMBRUSTER, 1993). Most of the 22 selfers in *Erodium* show large areas of distribution, clearly indicating an ability to disperse and establish, taking advantage of these reproductive characteristics (STEBBINS, 1957, 1970). Two main types of leaf venation were found in the Geraniaceae: palmate and pinnate. *California* and *Geranium* are generally palmate, whereas all *Erodium* species are subpinnate to pinnate. The degree of leaf division is arranged into three types: pinnatifid, pinnatipartite or pinnatisect (FIZ *et al*., 2006). Three fruit types based on surface ornamentation of the mericarp body were found in *Erodium* species: smooth (3), papillate (70), and foveate (1). *Erodium* species possess significant pharmacological and biological activities. The whole plant was used as astringent and haemostatic in uterine and other bleeding (AL-QURAN, 2008) and as abortifacient (LIS-BALCHINA and HARTB, 1994). Extracts of the plant were also used in traditional medicine as antidiarrheic, diuretic, stomachic and antihemorrhageic drugs (FECKA and CISOWSKI, 2014). The root and leaves were eaten by nursing mothers to increase the flow of milk.

There are limited chromosome records for *Erodium* in the world. Three basic chromosome numbers are considered because all counts in *Erodium* are multiples either of 8, 9, or 10 (FIZ *et al.*, 2006). We infer that most species of *Erodium* (55) share a basic chromosome number of x 5 10. Eight more species share a basenumber of x 5 9, and one (*E. stephanianum*) with x 5 8; whereas the number for three species (*E. guttatum, E. oxyrrhynchum, E. pelargoniflorum*) is compatible with both x 5 10 and x 5 9 (FIZ *et al.*, 2006).

MARTIN *et al*. (1997) report the use of RAPD markers to gain information about the genetic variability among and within populations of *E. paularense.* ALARCÓN *et al.* (2012)

examines the phylogeny of *Erodium* subsect. *Petraea*, a group of six morphologically and genetically very similar species from the mountains of the western Mediterranean. Combined trnL–F-ITS analysis was unable to determine the phylogenetic relationships of these species owing to sequence similarity. AFLP fragment analysis showed different populations to cluster in six closely related phylogroups that partially coincided with morphological species. Phylogenetic reconstructions in the Mediterranean genus *Erodium* are for the first time performed using two matrices: one with 96 trnL-trnF sequences from *Erodium* plus 23 morphological characters, using Maximum Parsimony (MP) and Bayesian Inference (BI) (FIZ *et al.*, 2006). Their result showed that trnLtrnF analysis of the 95 accessions of Geraniaceae and the combined data analysis indicate that *Erodium* and *California* together form a monophyletic group.

The present study investigated the molecular variation of seven species in Iran. Objectives of the study were; a) to estimate genetic diversity; b) to evaluate population relationships using WARD approaches. Current results have implications in breeding and conservation programs.

## MATERIAL AND METHODS

*Plants collection*

Seventy individuals were sampled. Seven *Erodium* species in west Azerbaijan, Mazandaran, Hamadan, Kurdistan, Esfahan, Razavi Khorasan, Semnan and Tehran Provinces of Iran were selected and sampled during July-August 2015-2020 (Table 1). Morphometric and SRAP analyses on 70 plant accessions were carried out. Five to ten samples from each population belonging to seven different species were selected based on other eco-geographic characteristics. Samples were stored at - 20 °C till further use. Detailed information about locations of samples and geographical distribution of species are mentioned (Table 1).

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

| No | Taxa | Locality | Latitude | Longitude | Altitude(m) |
|---|---|---|---|---|---|
| Sp1 | *E. gruinum* (L.) L'Hér. | Hamedan, 20km s of Nahavand | 37° 07' 48 " | 49° 54' 04" | 165 |
| Sp2 | *E. cicutarium* (L.) | Razavi Khorasan, Kashmar, Kuhsorkh District | 37° 07' 08" | 49°54' 11" | 159 |
| Sp3 | *E. moschatum* (L.) L'Hér. | Esfahan, ardestan on road to taleghan | 38 ° 52' 93" | 47 ˚25' 92" | 1133 |
| Sp4 | *E. ciconium* (L.) L'Hér. | Semnan, 20km NW of shahrud | 38˚52' 93" | 47 ˚25' 92" | 1139 |
| Sp5 | *E. oxyrrhynchum* M. Bieb. | Mazandaran, 40 km Tonekabon to janat abad | 35 ˚50' 36" | 51˚ 24' 28" | 2383 |
| Sp6 | *E. hoefftianum* C. A,Mey | West-Azarbaijan, Urumieh, Silvana | 35 ˚42'29" | 52 ˚20'51" | 2421 |
| Sp7 | *E. neuradifolium* Delile ex Godr. | Tehran, Damavand | 35 ˚42'29" | 52 ˚20'51" | 2421 |

*Morphological studies*

Each species was subjected to morphometric analysis and twelve samples per species were processed. Qualitative (8) and quantitative (28) morphological characters were studied (Table 2). Data were transformed before calculation. Different morphological characters of flowers, leaves, and seeds were studied. Ordination analyses were conducted while using Euclidean distance (PODANI, 2000).

*Table 2. Morphological characters in studied species*

| No | Characters | No | Characters |
|---|---|---|---|
| 1 | Plant height (mm) | 19 | Mericarp length (mm) |
| 2 | Length of stem leaves petiole (mm) | 20 | Mericarp width (mm) |
| 3 | Length of stem leaves (mm) | 21 | Mericarp length/width (mm) |
| 4 | Width of stem leaves (mm) | 22 | Seed length (mm) |
| 5 | Length / Width of stem leaves (mm) | 23 | Seed width (mm) |
| 6 | Number of segment stem leaves (mm) | 24 | Seed length/ width (mm) |
| 7 | Length of basal leaves petiole (mm) | 25 | Stipules length (mm) |
| 8 | Length of basal leaves (mm) | 26 | Stipules width (mm) |
| 9 | Width of basal leaves (mm) | 27 | Stipules length/ width (mm) |
| 10 | Length / Width of basal leaves (mm) | 28 | Bract length (mm) |
| 11 | Number of segment basal leaves | 29 | Petal shape : obovate (1), spathulate (2) |
| 12 | Calyx length (mm) | 30 | State of petal ligule: presence (1), absence (2) |
| 13 | Calyx width (mm) | 31 | Petal apex : emarginate or obtuse (1), obtuse or mucronate (2), |
| 14 | Calyx length/ width (mm) | 32 | State of petal ligule hair: ciliated at base (1), not ciliated at base (2) |
| 15 | Petal length (mm) | 33 | Stamen filament hair: ciliate (1), not ciliate (2) |
| 16 | Petal width (mm) | 34 | All organ plant hair density: 1-sparsly hairy 2-Glabrous |
| 17 | Petal length / width (mm) | 35 | Mericarp color: 1-yellowish-green; 2- brown; |
| 18 | Fruit length (mm) | 36 | Number of flowers per inflorescence |

*Sequence-related amplified polymorphism method*

Fresh leaves were used randomly from one to twelve plants. These were dried with silica gel powder. Genomic DNA was extracted while following previous protocol. SRAP assay was performed as described previously (LI and QUIROS, 2001). Six SRAP in different primer combinations were used (Table 3). A 25µl volume containing 10 mM of Tris-HCl buffer at pH 8; 50 mM of KCl; 1.5 mM of MgCl2; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of single primer; 20 ng of genomic DNA and 3 U of Taq DNA polymerase (Bioron, Germany) were subjected to PCR reactions. The overall reaction volume consisted of 25 µl. This PCR reaction was carried out in Techne thermocycler (Germany). The following cycles and programs were observed. The initial denaturation step was performed for 5 minutes at 94°C. The initial denaturation step was followed by 40 cycles for 1 minute at 94°C; 1 minute at 52-57°C, and 2 minutes at 72°C. The reaction was completed by a final extension step of 7-10 min at 72°C.

Staining was performed with the aid of ethidium bromide. DNA bands/fragments were compared against a 100 bp molecular size ladder (Fermentas, Germany).

*Table 3. SRAP primer information and results*

| Primer name | NTL[a] | NPL[b] | P[c] | PIC[d] | RP[e] |
|---|---|---|---|---|---|
| Em2-Me5 | 15 | 8 | 65.00% | 0.44 | 50.99 |
| Em3-Me4 | 12 | 12 | 100.00% | 0.41 | 42.24 |
| Em3-Me1 | 25 | 14 | 75.00% | 0.38 | 66.55 |
| Em4-Me1 | 19 | 19 | 100.00% | 0.44 | 44.23 |
| Em5-Me1 | 10 | 7 | 73.00% | 0.47 | 58.55 |
| Em5-Me2 | 12 | 12 | 100.00% | 0.32 | 49.65 |
| Mean | 17 | 14 | 87.00% | 0.43 | 55.43 |
| Total | 96 | 83 | | | 276.85 |

a: Number of total loci (NTL)

b: Number of polymorphic loci (NPL)

c: Polymorphic ratio(P %)

d: Polymorphic information content (PIC)

e: Resolving power (Rp)

*Data Analyses*

UPGMA (Unweighted paired group using average) ordination method was implemnented to assess morphological characters. ANOVA (Analysis of variance) was conducted to assess morphological differences among species. Principal component analysis (PCA) was implemented to identify variable morphological characters in *Erodium* species. Multivariate statistical analyses i.e., PC analysis, were performed in PAST software version 2.17 (HAMMER *et al.,* 2001).

*Molecular analyses*

Sequence-related amplified polymorphism (SRAP) bands were recorded. Presence and absence of bands were scored present (1) and absent (0), respectively. Total loci (NTL) and the number of polymorphism loci (NPL) for each primer were calculated. Furthermore, the polymorphic ratio was assessed based on NPL/NTL values. Polymorphism information content was calculated as previously suggested by ROLDAN-RUIZ *et al*. (2000); PREVOST and WILKINSON, 1999). 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 and SMOUSE, 2006). Nei's genetic distance among populations was used for Neighbor Joining (NJ) clustering and Neighbor-Net networking (FREELAND *et al*., 2011; HUSON and BRYANT, 2006). Mantel test checked the correlation between geographical and genetic 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) were used to show genetic difference of the populations. Gene flow was determined by (i) Calculating Nm an estimate of gene flow from Gst by PopGene ver. 1.32 (1997) as: Nm = 0.5(1 - Gst)/Gst. This approach considers the equal amount of gene flow among all populations.

## RESULTS

*Mophometery*

The ANOVA findings showed substantial differences (p<0.01) between the species in terms of quantitative morphological characteristics. Principal component analysis results explained 50% cumulative variation. The first PCA axis explained 38% of the total variation. The highest correlation (> 0.7) was shown by morphological characters such as calyx length, calyx width, corolla length, corolla color, leaf length and leaf width. The morphological characters of *Erodium* species are shown in PCA plot (Figure 1). Each species formed separate groups based on morphological characters. The morphometric analysis showed clear difference among *Erodium* species and separated each groups.

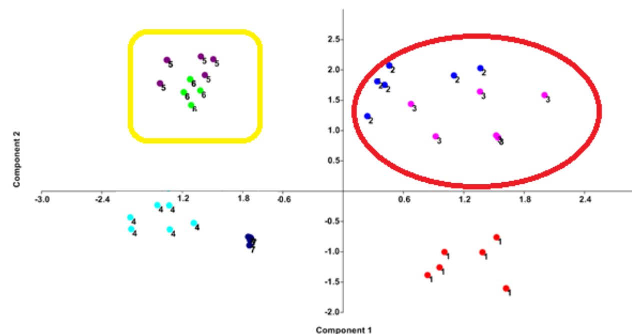


Fig 1. PCA plots of morphological characters revealing species delimitation in *Erodium*.

*Species identification and genetic diversity*

Six (6) suitable primer combinations (PCs), out of 15 PCs were screened in this research. Figure 2 illustrates the banding pattern of Em5-Me1 and Em2-Me5 primer by the SRAP marker profile. Eighty three (83) amplified polymorphic bands (number of polymorphic loci) were produced. These bands (fragments) had different range i.e. 100bp to 2000 bp. Maximum and minimum numbers of polymorphic bands were 19 and 7 for Em4-Me1 and Em5-Me1, respectively. Each primer produced 14 polymorphic bands on average. The PIC ranged from 0.32 (Em5-Me2) to 0.47 (Em5-Me1) for the 10 SRAP primers, with an average of 0.43 per

primer. RP of the primers ranged from 42.24 (Em3-Me4) to 66.55 (Em3-Me1) with an average of 55.43 per primer (Figure 2, Table 3). The calculated genetic parameters of *Erodium* species are shown (Table 4). The unbiased heterozygosity (H) varied between 0.018 (*E. neuradifolium*) and 0.30 (*E. moschatum*) with a mean of 0.21. Shannon's information index (I) was maximum in *Erodium moschatum* (0.39), where as we recorded minimum Shannon's information index in *Erodium neuradifolium* (0.015). The observed number of alleles (*N*a) ranged from 0.255 in *E. ciconium* to 1.345 in *E. cicutarium.* The significant number of alleles (*N*e) ranged from 1.021 (*E. neuradifolium)* to 1.537 (*E. moschatum*).

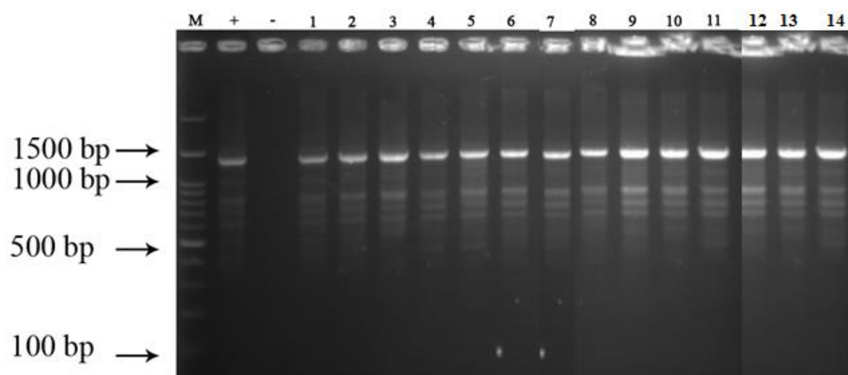


Fig 2. Electrophoresis gel of studied ecotypes from DNA fragments produced by SRAP profile; 1, 8: *E. gruinum,* 2, 9: *E. cicutarium*; 3,10: *E. moschatum*; 4, 11: *E. ciconium;* 5, 12: and *E. oxyrrhynchum* 6, 13: *E. hoefftianum;* 7, 14: *E. neuradifolium,* L = Ladder 100 bp

*Table 4. Genetic diversity parameters*

| SP | N | Na | Ne | I | He | UHe | %P |
|---|---|---|---|---|---|---|---|
| *E. gruinum* | 8.000 | 0.333 | 1.016 | 0.192 | 0.17 | 0.22 | 53.23% |
| *E. cicutarium* | 12.000 | 1.345 | 1.190 | 0.271 | 0.184 | 0.192 | 45.91% |
| *E. moschatum* | 5.000 | 0.358 | 1.537 | 0.39 | 0.35 | 0.30 | 63.50% |
| *E. ciconium* | 16.000 | 0.255 | 1.029 | 0.231 | 0.18 | 0.23 | 34.38% |
| *E. oxyrrhynchum* | 12.000 | 0.462 | 1.095 | 0.288 | 0.25 | 0.22 | 52.05% |
| *E. hoefftianum* | 10.000 | 0.358 | 1.117 | 0.18 | 0.15 | 0.12 | 34.30% |
| *E. neuradifolium* | 5.000 | 0.269 | 1.021 | 0.015 | 0.011 | 0.018 | 12.15% |

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

Analysis of Molecular Variance results in significant genetic difference ($p$ = 0.01) among *Erodium* species. The majority of genetic variation occurred among species. AMOVA

findings revealed that 66% of the total variation was between species and comparatively less genetic variation was recorded at the species level (Table 5). Genetic difference between *Erodium* species was highlighted by genetic statistics (Nei's $G_{ST}$), as evident by significant *p* values i.e. Nei's $G_{ST}$ (0.234, $p = 0.01$) and D_est values (0.985, $p = 0.01$).

Different clustering and ordination methods produced similar results therefore, Neighbor joining tree are presented here (Figure 3). In general, plant samples of each species belong to a distinct section, were grouped together and formed separate cluster (Figure 3). This result show that molecular characters studied can delimit *Erodium* species in two different major clusters or groups. In the studied specimens we did not encounter intermediate forms. In general, two major clusters were formed in Neighbor joining tree (Figure. 3), populations of: *E. moschatum; E. hoefftianum* and *E. neuradifolium* were placed in the first major cluster and were placed with great distance from the other species. The second major cluster included two sub-clusters. Plants of *E. ciconium* comprised the first sub-cluster, while plants of *E. gruinum; E. cicutarium* and *E. oxyrrhynchum* formed the second sub-cluster.

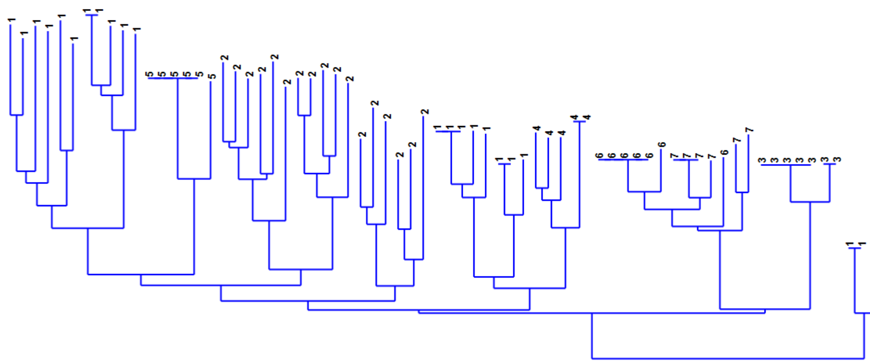

Fig 3. Neighbor joining tree of SRAP data in the studied *Erodium* species. sp1: *E. gruinum,* Sp2: *E. cicutarium,* sp3: *E. moschatum;* sp4: *E. ciconium,* sp5: *E. oxyrrhynchum* sp6: *E. hoefftianum,* sp7: *E. neuradifolium,*

*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 (Fig. not included). The STRUCTURE plot (Figure not included) produced more detailed information about the genetic structure of the species studied as well as shared ancestral alleles and / or gene flow among *Erodium* species. This plot revealed that the genetic affinity between *E. gruinum* and *E.*

*cicutarium* (similarly colored), as well as *E. hoefftianum* and *E. neuradifolium* (similarly colored) due to shared common alleles. This is in agreement with Neighbor joining dendrogram presented before. The other species are distinct in their allele composition and differed genetically from each other.

We detected strong correlation between geographical and genetic distances (r = 0.88, $p$=0.0002) and gene flow ($N_{m}$) score of 0.543 was reported among species. Detailed information about genetic distances and genetic identity (Nei's) are described (Supplementary Table). The findings suggested that there was the highest degree of genetic similarity (0.85) between *E. cicutarium* and *E. moschatum.* On the contrary to this, *E. hoefftianum* and *E. neuradifolium* (0.70) had lowest genetic resemblance.

## DISCUSSION

In the present study, we used morphological and molecular (SRAP) data to evaluate species relationships in *Erodium* species. Morphological analyses of *Erodium* species showed that quantitative indicators (ANOVA test results) and qualitative characteristics are well differentiated from each other. PCA analysis suggests that morphological characters such as leaf apex, corolla length, leaf length, leaf width, corolla shape, calyx shape, calyx length, bract length and leaf shape have the potentialsto identify and delimitate *Erodium* species. Principal component analysis results suggests the utilization of morphological characters to identify and delimitate *Erodium* species. Morphological characters including corolla length, leaf length, leaf width, corolla shape play key role in plant systematics and taxonomy. Our work also highlighted the significance of morphological characters and molecular data to identify and study species genetic diversity. In general, genetic relationships obtained from SRAP data coincides with morphometric results. This is in accordance with the parameters of AMOVA and genetic diversity results. SRAP molecular markers detected clear genetic difference among species. These results indicate that SRAP have potentials to study plant systematics and taxonomy in *Erodium* members.

The present research, revealed interesting data about its genetic variability, genetic stratification and morphological divergence in north and west part of Iran. Degree of genetic variability within a species is highly correlated with its reproductive mode, the higher degree of open pollination/ cross breeding brings about higher level of genetic variability in the studied taxon (MEUSEL *et al.,* 1965; XU *et al.,* 2021). 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 variability may be more directly related to the degree of polymorphism. Generally, PIC value between zero to 0.25 suggest 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). In this research, the SRAP primers' PIC values ranged from 0.32 to 0.47, with a mean value of 0.43, which indicated a mid-level ability of SRAP primers in determining genetic diversity among the species of *Erodium.* All of 6 primer pairs showed a good polymorphism in taxon of *Erodium* species. A total 83 alleles were recognized for the studied species. Total number of bands per primers ranged from 7 to 19 polymorphic bands and the mean of the allele number in loci was 14.

In most studies, population size is limited to several vegetative accession (MEUSEL *et al.,* 1965; UOTILA, 1996). This population could be showed genetic drift, whose effect are observed in the high level of $F_{IS}$ and low level of genetic diversity. The isolation of the population and absence the gene flow led to fragmentation of the *Erodium* populations. Between genetic diversity parameters and population size were showing positive correlations that confirmed various studies (LEIMU *et al.*, 2006). There are two reasons for the positive correlation between genetic diversity and population size (LEIMU *et al.,* 2006). 1- A positive correlation could imply the presence of an extinction vortex, where the drop-in population size lowers genetic diversity, which leads to inbreeding depression. The second reason is the fact that plant fitness differentiates populations based on variations in habitat quality (VERGEER *et al.,* 2003).

According to BOOY *et al.* (2000) the low levels of genetic diversity could reduce plant fitness and restrict a population's ability to respond to changing environmental conditions through selection and adaptation. Genetic diversity (34%) was obtained within populations, whereas 66% of genetic variation obtained between the evaluated populations. One of the key factors determining the distribution of genetic variation is the breeding system in plant species (DUMINIL, 2007). Couvet (BOOY *et al.,* 2000) revealed that one migrant per generation cannot be existed to guarantee long-term survival of small populations and that the number of migrants is demonstrate through life history characters and population genetic (VERGEER *et al.,* 2003; ZHAO *et al*., 2021).

Genetic variances between the three groups were very similar, but statistically important. There are two hypotheses for the absence of differences between isolated populations. The first hypothesis explained that genetic diversity within and between populations demonstrate gene flow processes, which led to the fragmentation of larger populations (DOSTÁLEK *et al.,* 2010). The second hypothesis presented that geographically proximate populations are more efficiently connected through gene flow than populations separated by greater distance.

AMOVA and STRUCTURE analysis revealed that the species of *Erodium* are genetically differentiated but have some degree of shared common alleles. Several trends in pollination mechanism can be observed in *Erodium* with gradual transition between them. Annuals are generally autogamous, actinomorphic, and lack attracting features, while most perennial species have an allogamous condition, zygomorphic flowers and striking floral structures (ALDASORO *et al*., 2000). Shifts in growth form may be part of adaptation to drought episodes in the Mediterranean during late Tertiary (BAKKER *et al*., 1998, 2000, 2004; RICHARDSON *et al*., 2000; BELL and DONOGHUE, 2005). Selfing plants are common among annuals and taxa colonizing temporary or disturbed habitats (BAKER 1955, 1967; STEBBINS 1957, 1970; ARMBRUSTER, 1993). Most of the 22 selfers in *Erodium* show large areas of distribution, clearly indicating an ability to disperse and establish, taking advantage of these reproductive characteristics (STEBBINS, 1957, 1970). This shows the high utilization of the SRAP technique to identify *Erodium* species. Our results have implications for conservation and breeding programs. Furthermore, it may identify suitable ecotypes for forage and pasture

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**PROCENA GENETIČKE STRUKTURE I DIVERZITETA VRSTE *Erodium* (Geranaiceae)**

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Izvod

*Erodium* Aiton (Geraniaceae) sa 75 vrsta rasprostranjen je na svim kontinentima osim na Antarktiku. Njegov glavni centar diverziteta je mediteranski region sa 62 vrste. Genetska raznolikost procenjena je na osnovu polimorfizma sekvenci. Za utvrđivanje genetske raznovrsnosti i karakteristika vrsta roda *Erodium*, korišćeni su morfološki i molekularni podaci. U 7 provincija prikupljeno je 70 individua osoba povezanih sa sedam *Erodium* vrsta. Ukupno 96 (Broj ukupnih lokusa) (NTL) DNK traka proizvedeno je PCR amplifikacijom sedam vrsta *Erodium*. Ove trake su proizvedene u kombinaciji šest selektivnih prajmera. Ukupan broj amplifikovanih fragmenata kretao se od 10 do 25. Genetske sličnosti između sedam vrsta procenjuju se od 0,70 do 0,85. Rezultati grupisanja pokazali su dva glavna klastera. Sadašnji rezultati pokazali su da pojačani polimorfizam povezan sa sekvencama ima potencijal da identifikuje i dešifruje genetski afinitet kod vrsta *Erodium*. Trenutni rezultati imaju implikacije na biodiverzitet i programe konzervacije. Pored toga, sadašnji rezultati mogli bi utrti put odabiru odgovarajućih ekotipova za krmu i pašnjake u Iranu.

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