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STUDY ON GENETIC DIVERSITY BETWEEN *Erodium* (Geranaiceae) SPECIES BASED ON INTER-SIMPLE SEQUENCE REPEAT MARKERS

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Identifying the accurate boundaries of a species is critical to have a better perspective of any biological studies. Therefore, species delimitation is a subject of extensive part of studies in the framework of biology. *Erodium* species possess significant pharmacological and biological activities. The whole plant was used as astringent and haemostatic in uterine and other bleeding Therefore, due to the importance of these plant species, we performed a molecular data analysis for this species. For this study, we used 60 randomly collected plants from 5 species in five provinces. Amplification of genomic DNA using 10 primers produced 52 bands, of which 50 were polymorphic (98.48%). The obtained high average PIC and MI values revealed high capacity of ISSR primers to detect polymorphic loci among *Erodium* species. The genetic similarities of five collections were estimated from 0.77 to 0.91. According to Inter-Simple sequence repeats (ISSR) markers analysis, *E. cicutarium* and *E. malacoides* had the lowest similarity and the species of *E. malacoides* and *E. oxyrrhynchum* had the highest similarity. The aims of present study are: 1) can ISSR markers identify *Erodium* species, and 2) to investigate the species inter-relationship? The present study revealed that ISSR markers can identify the species.

Keyword: Species Identification; Structure, *Erodium*, ISSR markers

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INTRODUCTION

Identifying the accurate boundaries of a species is critical to have a better perspective of any biological studies. Therefore, species delimitation is a subject of extensive part of studies in the framework of biology (COLLARD and MACKILL, 2009; WU *et al.*, 2013). However, defining the criterion which could address the boundaries of species is matter of debate (WU *et al.*, 2013). Wild relatives of crops contain genes with the great potential for use in breeding programs and constitute a part of their gene pool (PANDEY *et al.*, 2008). In addition, the study of intra-specific levels of genetic variation and investigation of genetic structure of wild populations is crucial for development of effective conservation strategies.

The genus *Erodium* Aiton (Geraniaceae) includes 74 species and is distributed on all continents, excluding Antarctica (FIZ *et al*., 2006). A major center of diversity is observed in the Mediterranean Basin (62 species). In the western Mediterranean, most species belong to the large subgenus *Barbata*, with c. 42 species in this area (30 perennials and 12 annuals, FIZ *et al*., 2006). *Erodium* comprises 15 species in different parts of Iran (JANIGHORBAN, 2009; SCHÖNBECK-TEMESY, 1970). 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. 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). The androecium consists of five fertile stamens and five staminodia in all species of *Erodium*, while in *California* it is reduced to five fertile stamens. This contrasts with the androecium of *Monsonia* (15 fertile stamens) and *Geranium* (10 fertile stamens). Pollen in Geraniaceae displays four ornamentation types taking into account supratectal structures and tectum shape. Sixty-eight of the 74 species of *Erodium* distributed in the four subclades display striate pollen (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).

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 (COWLING *et al*., 1996; BAKKER *et al*., 1998; 2000; 2004; RICHARDSON *et al*., 2000; BELL and DONOGHUE, 1996). 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)

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).

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.

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.

Molecular markers provide a powerful tool for studying the genetic diversity. Among advanced genetic markers, Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) markers have been widely used for diversity analyses (PHARMAWATI *et al*., 2004). RAPD technique is quick, easy and requires no prior sequence information. The technique detects nucleotide sequence polymorphism using a single primer of arbitrary nucleotide sequence (MORENO *et al*., 1998). ISSR marker involves PCR amplification of DNA by a single 16-18 bp. long primer composed of a repeated sequence anchored at the 3' or 5' end of 2-4 arbitrary nucleotides. The technique is rapid, simple, inexpensive and more reproducible than RAPD (COLLARD and MACKILL, 2009; WU *et al.*, 2013).

The present investigation has been carried out to evaluate the genetic diversity and relationships among different *Erodium* species using new gene-targeted molecular markers, i.e ISSR markers. This is the first study on the use of ISSR markers in *Erodium* genus. Therefore, we performed molecular study of 60 collected specimens of five *Erodium* species.

MATERIALS AND METHODS

Plant materials

A total of 60 individuals were sampled representing 5 geographical populations belonging to 5 *Erodium* species in Lorestan, Guilan, Mazandaran, Esfahan and Kohgiluyeh and Boyer-Ahmad Provinces of Iran during July-Agust 2016-2019 (Table 1). For ISSR analysis we used 60 plant accessions (Five to twelve samples from each populations) belonging to 5 different populations with different eco-geographic characteristics were sampled and stored in -20 till further use. More information about geographical distribution of accessions are in Table 1.

Table 1. Voucher details of Erodium species in this study from Iran.

No	Sp.	Locality	Latitude	Longitude	Altitude (m)
Sp1	*E. cicutarium* (L.) L'Hér.	Kohgiluyeh and Boyer-Ahmad	38 ˚ 52'37"	47 ˚ 23' 92"	1144
Sp2	*E. ciconium* (L.) L'Hér.	Mazandaran, Haraz road, Emam Zad-e-Hashem	32°50′03″	51°24′28″	1990
Sp3	*E. malacoides* Bové ex Decne.	Guilan, Sangar, Road sid	29◦20′07‴	51° 52′08″	1610
Sp4	*E. oxyrrhynchum* M. Bieb.	Esfahan:, Ghameshlou, Sanjab	38 ˚ 52'373	47 ˚ 23' 92"	1144
Sp5	*E. hoefftianum* C. A,Mey	Lorestan, Oshtorankuh, above Tihun village	33° 57′12″	47° 57′32″	2500

Morphological studies

Five to twelve samples from each species were used for Morphometry. In total 17 morphological (10 qualitative, 7 quantitative) characters were studied. Data obtained were standardized (Mean= 0, variance = 1) and used to estimate Euclidean distance for clustering and ordination analyses (PODANI 2000). Morphological characters studied are corolla shape, bract shape, calyx shape, calyx length, calyx width, calyx margins, bract length, corolla length, corolla width, corolla apex, leaf length and leaf width, leaf apex, leaf margins, leaf shape, leaf gland and bract margins.

DNA Extraction and ISSR Assay

Fresh leaves were used randomly from one to twelve plants in each of the studied populations. These were dried by silica gel powder. CTAB activated charcoal protocol was used to extract genomic DNA. The quality of extracted DNA was examined by running on 0.8% agarose gel. For the ISSR analysis, 22 primers from UBC (University of British Columbia) series were tested for DNA amplification. Ten primers were chosen for ISSR analysis of genetic variability, based on band reproducibly (Table 2). PCR reactions were carried in a 25µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM $MgCl_2$; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of a single primer; 20 ng genomic DNA and 3 U of *Taq* DNA polymerase (Bioron, Germany). The amplifications, reactions were performed in Techne thermocycler (Germany) with the following program: 5 min initial denaturation step 94°C, followed by 40 cycles of 1 min at 94°C; 1 min at 52-57°C and 2 min at 72°C. The reaction was completed by final extension step of 7-10 min at 72°C. The amplification products were observed by running on 1% agarose gel, followed by the ethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany).

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

Primer name	Primer sequence (5'-3')	TNB	NPB	PPB	PIC	PI	EMR	MI
ISSR-1	DBDACACACACACACACA	15	15	100.00%	0.66	5.86	10.55	5.45
ISSR-2	GGATGGATGGATGGAT	9	8	84.99%	0.43	2.91	17.43	6.85
ISSR-3	GACAGACAGACAGACA	11	11	100.00%	0.54	3.34	10.88	4.44
ISSR-4	AGAGAGAGAGAGAGAGYT	5	5	100.00%	0.57	3.88	16.56	3.85
ISSR-5	ACACACACACACACACC	12	12	100.00%	0.45	5.23	7.23	7.47
Mean		10	9	98.48%	0.50	5.7	12	5.6
Total		52	50					

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 CAAT box- derived polymorphism (CBDP) primers

Data Analyses

Morphological Studies

Morphological characters were first standardized (Mean = 0, Variance = 1) and used to establish Euclidean distance among pairs of taxa (PODANI, 2000). For grouping of the plant specimens, The UPGMA (Unweighted paired group using average) ordination methods were used (PODANI, 2000). ANOVA (Analysis of variance) were performed to show morphological difference among the populations while, PCA (Principal components analysis) biplot was used to identify the most variable morphological characters among the studied populations (PODANI, 2000). PAST version 2.17 (HAMMER *et al.* 2012) was used for multivariate statistical analyses of morphological data.

Molecular Analyses

ISSR bands obtained were coded as binary characters (presence = 1, absence = 0) and used for genetic diversity analysis. Discriminatory ability of the used primers was evaluated by means of two parameters, polymorphism information content (PIC) and marker index (MI) to characterize the capacity of each primer to detect polymorphic loci among the genotypes (POWELL *et al.*, 1996). MI is calculated for each primer as MI = PIC × EMR, where EMR is the product of the number of polymorphic loci per primer (*n*) and the fraction of polymorphic fragments (β) (HEIKRUJAM *et al.*, 2015). The number of polymorphic bands (NPB) and the effective multiplex ratio (EMR) were calculated for each primer. Parameter like Nei's gene diversity (H), Shannon information index (I), number of effective alleles, and percentage of polymorphism (P% =number of polymorphic loci/number of total loci) were determined (WEISING *et al.*, 2005; FREELAND *et al.*, 2011). Shannon's index was calculated by the formula: H' = -Σpiln pi. Rp is defined per primer as: Rp = ∑ Ib, were "Ib" is the band informativeness, that takes the values of 1-(2x [0.5-p]), being "p" the proportion of each genotype containing the band. The percentage of polymorphic loci, the mean loci by accession and by population, UHe, H' and PCA were calculated by GenAlEx 6.4 software (PEAKALL 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

Species identification and inter-relationship

Morphometry

ANOVA showed significant differences (P <0.01) in quantitative morphological characters among the species studied. In order to determine the most variable characters among the taxa studied, PCA analysis has been performed. It revealed that the first three factors comprised over 61% of the total variation. In the first PCA axis with 44% of total variation, such characters as corolla shape, calyx shape, calyx length, bract length and leaf shape have shown the highest correlation (>0.7), leaf apex, corolla length, leaf length, leaf width were characters influencing PCA axis 2 and 3 respectively. Different clustering and ordination methods produced similar results therefore, PCA plot of morphological characters are presented here (Figs 1). In general, plant samples of each species were grouped together and formed separate groups. This result show that both quantitative and qualitative morphological characters separated the studied species into distinct groups. In the studied specimens we did not encounter intermediate forms.

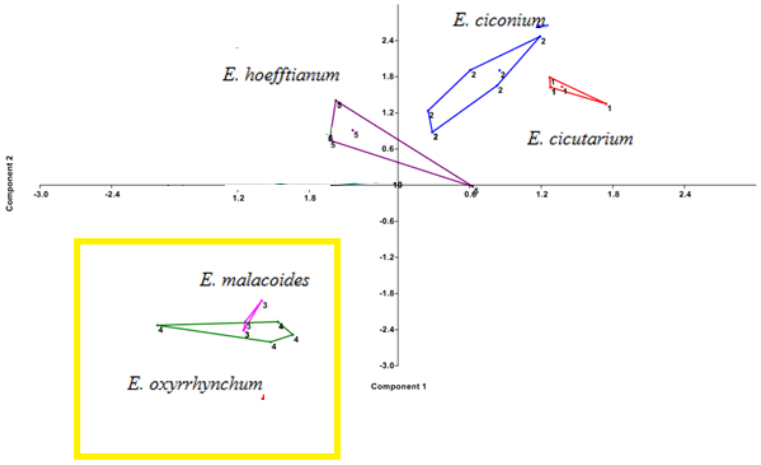

Figure 1. PCA plots of morphological characters revealing species delimitation in the *Erodium*.

Species Identification and Genetic Diversity

Five ISSR primers were screened to study genetic relationships among *Erodium* species; all the primers produced reproducible polymorphic bands in all 5 *Erodium* species. A total of 50 amplified polymorphic bands were generated across 5 *Erodium* species. The size of the amplified fragments ranged from 200 to 3000 bp. The highest and lowest number of polymorphic bands was 15 for ISSR-1 and 8 for ISSR-2, on an average of 9 polymorphic bands per primer. The PIC of the 5 ISSR primers ranged from 0.43 (ISSR-2) to 0.66 (ISSR-1) with an average of 0.50 per primer. MI of the primers ranged from 3.85 (ISSR-4) to 7.47 (ISSR-5) with an average of 5.6 per primer. EMR of the ISSR primers ranged from 7.23 (ISSR-5) to 17.43 (ISSR-2) with an average of 12 per primer (Table 2). The primers with the high EMR values were considered to be more informative in distinguishing the genotypes.

The genetic parameters were calculated for all the 5 *Erodium* species amplified with ISSR primers (Table not included). Unbiased expected heterozygosity (*H*) ranged from 0.19 (*E. malacoides*) to 0.36 (*E. hoefftianum*), with a mean of 0.25. A similar pattern was observed for Shannon's information index (*I*), with the highest value of 0.37 observed in *E. hoefftianum* and the lowest value of 0.10 observed in *E. malacoides* with a mean of 0.23. The observed number of alleles (*Na*) ranged from 0.255 in *E. hoefftianum* to 0.799 in *E. cicutarium*. The effective number of alleles (*Ne*) ranged from 1.024 (*E. ciconium*) to 1.096 (*E. hoefftianum*).

AMOVA test showed significant genetic difference (P = 0.001) among studied species. It revealed that 70% of total variation was among species and 30% was within species (Table not included) Moreover, genetic differentiation of these species was demonstrated by significant Nei's GST (0.99, P = 0.001) and D_est values (0.732, P = 0.001). These results revealed a higher distribution of genetic diversity among *Erodium* species compared to within species.

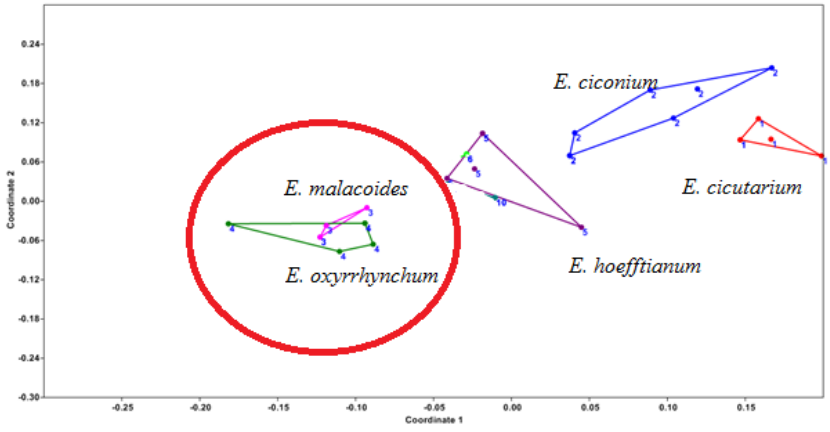

Figure 2. PCoA plots of ISSR data revealing species delimitation in the *Erodium*.

Different clustering and ordination methods produced similar results therefore, UPGMA clustering and PCoA plot are presented here (Figure 2 and 3). In general, plant samples of each species belong to a distinct section, were grouped together and formed separate cluster (Figure 2). 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 UPGMA tree (Figure. 3), populations of *E. ciconium* 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. cicutarium* comprised the first sub-cluster, while plants of *E. malacoides*, *E. oxyrrhynchum* and *E. hoefftianum* formed the second sub-cluster.

In general, relationships obtained from ISSR data agrees well with species relationship obtained from morphological. This is in agreement with AMOVA and genetic diversity parameters presented before. The species are genetically well differentiated from each other. These results indicate that ISSR molecular markers can be used in *Erodium* species taxonomy. The Nm analysis by Popgene software also produced mean Nm= 0.855, that is considered very low value of gene flow among the studied species.

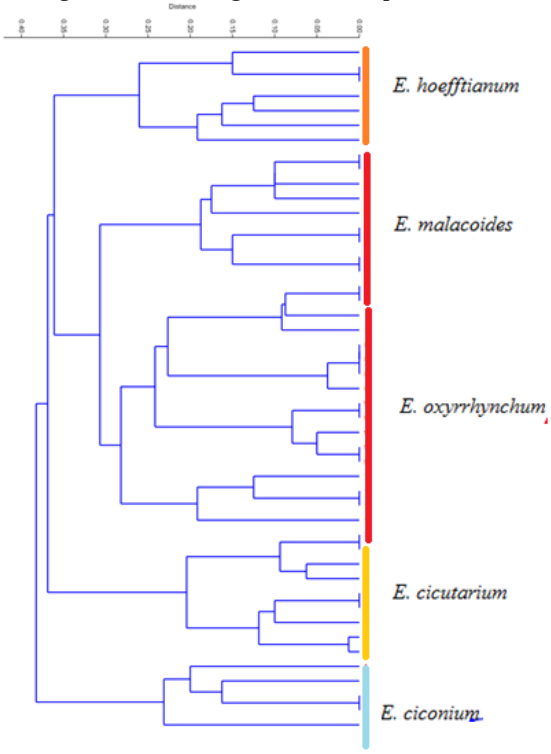

Figure 3. UPGMA tree of ISSR data revealing species delimitation in the *Erodium*: *E. cicutarium*, sp2: *E. ciconium*, sp3: *E. malacoides,: E. oxyrrhynchum*, *E. hoefftianum.*

Mantel test with 5000 permutations showed a significant correlation (r = 0.55, p=0.0002) between genetic distance and geographical distance, so isolation by distance (IBD) occurred among the *Erodium* species studied.

Nei's genetic identity and the genetic distance determined among the studied species (Table not included). The results showed that the highest degree of genetic similarity (0.91) occurred between *E. malacoides* and *E. oxyrrhynchum.* The lowest degree of genetic similarity occurred between *E. cicutarium* and *E. malacoides* (0.77). The low Nm value (0.855) indicates limited gene flow or ancestrally shared alleles between the species studied and indicating high genetic differentiation among and within *Erodium* species.

DISCUSSION

Genetic diversity is an important role in biology of long-term evolution of a taxon or a population. The basis of existence, growth, and evolution of taxon. Thus, the study of genetic diversity of taxon is fundamental to recognize the taxonomy, origin, and evolution of taxon. Moreover, such research will provide a theoretical basis for the germplasm resource conservation, development, utilization, and breeding (LUBBERS *et al.*, 1991).

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). 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 ISSR primers' PIC values ranged from 0.43 to 0.66, with a mean value of 0.50, which indicated a mid-level ability of ISSR primers in determining genetic diversity among the species of *Erodium.* All of 10 primer pairs showed a good polymorphism in taxon of *Erodium* species. A total 52 alleles were recognized for the studied species. Total number of bands per primers ranged from 8 to 15 polymorphic bands and the mean of the allele number in loci was 9.

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 (30%) was obtained within populations, whereas 70% 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).

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)

The methods we used are indirect estimation of gene flow and if it is identified to occur among species may be either due to ancestral shared alleles or ongoing gene flow. The Nm value obtained based on ISSR data, revealed very limited amount of gene flow among the studied species that was also supported by STRUCTURE analysis as *Erodium* species mostly had distinct genetic structure. Reticulation analysis also showed some degree of gene flow for ISSR. We did not observe any intermediate forms in our extensive plant collection, but morphological variability within each species did occur to some extent. In conclusion, the results of this study showed that to evaluate the genetic diversity of the *Erodium genus*, the primers derived from ISSR were more effective than the other molecular markers. Also, *Erodium* species were clearly separated from each other in the dendrogram and PCA, indicating the higher efficiency of ISSR technique in *Erodium species* identification.

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GENETIČKI DIVERZITET *Erodium* (Geranaiceae) VRSTA ZASNOVAN NA ISSR MARKERIMA

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Izvod

Utvrđivanje tačnih granica vrste je presudno za bolju perspektivu bilo kojih bioloških studija. Stoga je razgraničenje vrsta predmet opsežnog dela studija u okviru biologije. Vrste *Erodium* imaju značajne farmakološke i biološke aktivnosti. Cela biljka je korišćena kao adstringentno i hemostatično sredstvo kod materice i kod drugih krvarenja. Zbog važnosti ovih biljnih vrsta izvršili smo njenu molekularnu analizu. Za ovu studiju koristili smo 60 nasumično sakupljenih biljaka iz 5 vrsta u pet provincija. Amplifikacija genomske DNK pomoću 10 prajmera proizvela je 52 trake, od kojih je 50 polimorfnih (98,48%). Dobijene visoke prosečne vrednosti PIC i MI otkrile su visok kapacitet ISSR prajmera za otkrivanje polimorfnih lokusa među vrstama *Erodium*. Genetske sličnosti pet kolekcija procenjene su od 0,77 do 0,91. Prema analizi ISSR markera najmanju sličnost imali su *E. cicutarium* i *E. malacoides*, a najveću sličnost imale su vrste *E. malacoides* i *E. okirrhinchum*. Ciljevi ovog istraživanja bili su su: 1) da se utvrdi mogu li ISSR markeri identifikovati vrste *Erodium* i 2) istražiti međusobni odnos vrsta. Ova studija je otkrila da ISSR markeri mogu identifikovati vrstu.

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