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Original scientific article

POPULATION DIFFERENTIATION AND GENE FLOW IN *Erodium cicutarium*: A POTENTIAL MEDICINAL PLANT

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Erodium (Geranaiceae) species are distributed in different habitats of Iran. Some species are of medicinal importance while some are well known weeds. In arid and semi-arid regions, *E. cicutarium* has had some importance as a forage plant and is an important grazing plant and source of protein supplements to straw for ruminants in semi deserts and wastelands of the Middle East. There is no information on its population genetic structure, genetic diversity, and morphological variability in Iran. Due to the medicinal importance of this species, a genetic variability and populations' structure study is performed studying 15 geographical populations of *E. cicutarium* Therefore, we used six inter-retrotransposon amplified polymorphism (IRAP) markers and 15 combined IRAP markers to reveal within and among population genetic diversity in this plant. AMOVA test produced significant genetic difference (PhiPT = 0.39, P = 0.010) among the studied populations and also revealed that, 55% of total genetic variability was due to within population diversity while, 45% was due to among population genetic differentiation. Mantel test showed positive significant correlation between genetic distance and geographical distance of the studied populations. Networking, STRUCTURE analyses and population assignment test revealed some degree of gene flow among these populations. PCoA plot of populations based on morphological characters was in agreement with MDS plot of molecular data. These results indicated that geographical

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populations of *E. cicutarium* are well differentiated both in genetic content as well as morphological characteristics. Consensus tree based on morphological and genetic data separated some of these populations from the others suggesting the existence of ecotypes within this species.

Keywords: *E. cicutarium***,** Gene flow, Genetic differentiation, IRAP

INTRODUCTION

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 Iran, *Erodium* is classified in two sections; *Plumosa* Boiss. and *Erodium* Boiss. and three subsections; *Absinthioidea* Brumhard, *Malacoides* Lange and *Cicutaria* Lange (SCHÖNBECK-TEMESY, 1970). *Erodium* species are found in different parts of Iran (JANIGHORBAN, 2005; SCHÖNBECK-TEMESY, 1970; ESFANDANI-BOZCHALOYI *et al.* 2017a, 2017b, 2017c, 2017d). Most of these species are Irano-Touranian and Saharo-Sindian elements. Only one species is endemic in Hyrcanian region (JANIGHORBAN, 2005). Genus *Erodium* comprises 15 species in different parts of Iran (SCHONBECK – TEMESY, 1970).

Erodium cicutarium is distinguished from other members of its genus by its lobed cotyledons, with sinuses almost reaching the midvein; deeply incised pinnate leaflets, always divided more than halfway to the midrib; mostly actinomorphic flower petals; and dense appressed hairs on the mericarp (DAHLGREN, 1980). The tricolpate pollen grains have a striate-reticulate exine morphology (VERHOEVEN and VENTER, 1987; PERVEEN and GAISER, 1999; SHEHATA, 2008).

Erodium cicutarium is best adapted to Mediterranean climates characteristic of its native habitats, but is found globally in temperate areas with hot summers, most commonly in semi-arid rangelands and prairies of North and South America, South Africa and Australia (GREUTER *et al.* 1986; HULTE´N and FRIES, 1986). Although the species requires moisture from rainfall or irrigation for optimal germination, established plants are drought tolerant and can survive periodic arid conditions (BLACKSHAW and HARKER, 1998a; BUSSO *et al.*, 1998; BROOKS and BERRY, 2006). In arid and semi-arid regions, *E. cicutarium* has had some importance as a forage plant on ranges in California and Arizona (ANONYMOUS, 1939; BUSSO *et al.* 1998; GEORGE *et al*. 2006); and is an important grazing plant and source of protein supplements to straw for ruminants in semi deserts and wastelands of the Middle East (AL-MASRI, 2007). In Turkey, the species is gathered as a food plant in the Aegean region (BILGIR, 1982). According to ALI ESMAIL AL-SNAFI (2017) showed that *Erodium cicutarium* contained Tannin, catechins, gallic and elagic acids, sugars (glucose, galactose, fructose), amino acids (glycine, alanine, proline, histidine, tryptophan, tyrosine, glutamic acid), vitamins K and C, and wide range of essential and volatile oils.

Chromosome counts of 2n=20, 36, 40, 48, and 54 have been reported for the species. The most commonly reported counts from western Europe and non-native areas, where the species has become naturalized, have been n=10, 20; 2n=20, 40 (e.g. WARBURG, 1938; HEISER and WHITAKER 1948; LARSEN, 1958). The flowers of *E. cicutarium* subsp. *cicutarium* are mostly homogamous or slightly protogynous, so that self-pollination is most likely to occur, but flowers

with dark markings serving as guides to the concealed nectar may be protandrous and insect pollinated (KNUTH, 1908).

Considered an aggregate species, *E. cicutarium* has been split into poorly understood segregates with a probable history of hybridization and polyploidization (WARBURG, 1938; GUITTONNEAU, 1972). The north-west European taxon *E. danicum* K. Larsen, with a count of 2n_60, was interpreted as an amphidiploid hybrid between *E. cicutarium* and *E. glutinosum* Dumort. (LARSEN, 1958). Many hybrids between species in the genus were artificially created by GUITTONNEAU (1972).

Considerable morphological and genetic variability has been found within the type subspecies (WEBB and CHATER, 1968; DAHLGREN, 1980). Three subspecies have been recognized in Europe (WEBB and CHATER, 1968): subsp. *cicutarium*, found throughout the range of the species; subsp. *Bipinnatum* Tourlet, a usually less robust plant with violet or white flowers and petals lacking a dark basal patch and subsp. *Jacquinianum* (Fisch., C.A. Mey. and Ave´-Lall.) Brig., a robust plant with very dense glandular hairs, petals without a dark patch and very long beaks (40_70 mm) (WEBB and CHATER, 1968). The two latter subspecies have been reported to occur in the United States (USDA, NRCS, 2012).

E. cicutarium exhibits great extent of morphological variability and form many geographical populations in Iran. These geographical populations have variable eco-geographical features, some of which are in close vicinity, while some others are distributed in distant regions. We have no information on genetic variability, gene flow and genetic structure of *E. cicutarium* populations. Moreover, due to extensive morphological variability of this species in the country, there is possibility of having infra-specific taxonomic forms in this species. Therefore, we carried out population genetic analysis and morphometric study of 15 geographical populations for the first time in the country.

Different methods of DNA fingerprinting have proved to be a useful tool with a wide range of applications in plant population studies, such as detection of genetic variation within and between populations, characterization of clones, analysis of breeding systems, and analysis of ecogeographical variation (DAHLGREN, 1980).

For genetic study, we used the inter-retrotransposon amplified polymorphism (IRAP) method that displays insertional polymorphisms by amplifying the segments of DNA between two retrotransposons. It has been used in numerous studies of genetic diversity (SMYKAL *et al.*, 2011).

MATERIALS AND METHODS

Plant materials

A total of 102 individuals were sampled representing 15 natural populations of *E. cicutarium* in East Azerbaijan, Lorestan, Kermanshah, Gilan and Ardabil Provinces of Iran during July-Agust 2018 (Table 1). Fresh leaves of 1-7 individuals from each population, were collected, and immediately dried in Silica Gel (ESFANDANI-BOZCHALOYI *et al.*, 2018a) (Table 1). Different references were used for the correct identification of species (*E. cicutarium*) (DAVIS, 1967; SCHÖNBECK-TEMESY, 1970; ZOHARY, 1972; JANIGHORBAN, 2005). Details of sampling sites are mentioned in Table 1 and Fig.1. Vouchers were deposited at the herbarium of Islamic Azad University, Science and Research Branch, Tehran, Iran (IAUH).

Table 1. Populations studied their locality and ecological features.

Pop.no	Locality	Alt.(m)	Coordinates	Voucher no.
1	Lorestan: Sepid-Dasht, 5 km from Sepid-Dasht to Khorram-Abad	1300	48° 51.778’ E; 33° 13.175’ N	IAUH-14977
2	Lorestan: Sepid-Dasht,	1280	48° 50.649’ E; 33° 13.292’ N	IAUH-14978
3	Lorestan: Khorram-Abad, Shorab	1100	48° 10.286’ E; 33° 27.407’ N	IAUH-14923
4	Lorestan: Khorram-Abad, 60 km from Pol-Dokhtar to Khorram-Abad	1370	48° 15.886’ E; 33° 98.327’ N	IAUH-14945
5	Lorestan: Pol-Dokhtar, 85 km from Pol-Dokhtar to Khorram-Abad	110	47° 49.748’ E; 33° 18.168’ N	IAUH-14558
6	Lorestan: Pol-Dokhtar, 5 Km from Darre-Shahr to Pol-Dokhtar	670	47° 30.663’ E; 33° 4.840’ N	IAUH-15019
7	Lorestan: Khorram-Abad, 35 km from Khorram-Abad to Pol-Dokhtar	940	47° 57.328’ E; 33° 57.121’ N	IAUH-14959
8	Lorestan: Pol-Dokhtar, 5 km from Pol-Dokhtar to Andimeshk	800	47° 42.448’ E; 33° 6.480’ N	IAUH-15004
9	Kermanshah: Ghasre-Shirin, Ghasre-Shirin	360	45° 34.376’ E; 34° 29.661’ N	IAUH-14953
10	Kermanshah: Ghasre-Shirin, 5 km from Paveh to Nusod	1474	46° 20.252’ E; 35° 3.777’ N	IAUH-14955
11	Kermanshah: Paveh, Paveh Shahid Kazemi Forest Park	1400	46° 20.396’ E; 35° 1.812’ N	IAUH-14984
12	Ardabil: Germi, 20 km from Germi to Pars-Abad	380	48° 5.222’ E; 39° 10.859’ N	IAUH-15029
13	Gilan: Totkabon, 10 km from Totkabon to Ammarlo	230	49° 33.188’ E; 36° 51.654’ N	IAUH-14976
14	Gilan: Foman, 15 km Foman to Masole	250	49° 8.158’ E; 37° 10.483’ N	IAUH-15046
15	Azarbaijan (E): Ahar, 45 Km from Meshkin-Shahr to Ahar	1250	47° 17.038’ E; 38° 23.792 ’ N	IAUH-15038

Fig. 1. Distribution map of the studied populations.

DNA extraction and IRAP assay

Fresh leaves were used randomly from 5-10 plants in each of the studied populations. These were dried by silica gel powder. CTAB activated charcoal protocol was used to extract genomic DNA (ESFANDANI-BOZCHALOYI and SHEIDAI, 2019). The quality of extracted DNA was examined by running on 0.8% agarose gel. A set of six outward-facing LTR primers (SMYKAL *et al.*, 2011; Table 2) were used for IRAP analysis. We also used 15 different combinations of outward-facing LTR pair primers. PCR reactions were carried in a 25µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM MgCl2; 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 thermal program was carried out with an initial denaturation for 1 min at 94°C, followed by 40 cycles in three segments: 35 s at 95°C, 40s at 47°C and 55s at 72°C. Final extension was performed at 72°C for 5 min. 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. Erodium IRAP primers based on SMYKAL et al. (2011) study

IRAP	Sequence (5´-3´)
GU735096	ACCCCTTGAGCTAACTTTTGGGGTAAG
GU980589	AGCCTGAAAGTGTTGGGTTGTCG
GU929878	GCATCAGCCTGGACCAGTCCTCGTCC
GU735096	CACTTCAAATTTTGGCAGCAGCGGATC
GU929877	TCGAGGTACACCTCGACTCAGG
GU980590	ATTCTCGTCCGCTGCGCCCCTACA

Data analyses

Morphological studies

In total 60 morphological (24 qualitative, 36 quantitative) characters were studied. Five-Ten plant specimens were randomly studied for morphological analyses (Table 3).

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) and Ward (Minimum spherical characters) as well as ordination methods of MDS (Multidimensional scaling) and PCoA (Principal coordinate analysis) 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.

Table 3. Evaluated morphological characters

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	Bract width (mm)
12	Calyx length (mm)	30	Bract length / width (mm)
13	Calyx width (mm)	31	Pedicel length (mm)
14	Calyx length/ width (mm)	32	Peduncle length (mm)
15	Petal length (mm)	33	Rostrum length (mm)
16	Petal width (mm)	34	Style length (mm)
17	Petal length / width (mm)	35	Stamen filament length (mm)
18	Fruit length (mm)	36	Number of flowers per inflorescence
37	Type root: tuberculate (1), not tuberculate (2)	49	Bract outline: linear-lanceolate (1), lanceolate (2), ovate-lanceolate (3)
38	Vegetation-forms::annual (1), annual or biennial (3), perennial rootstock (4)	50	Stipules outline: lanceolate (1), elliptic-obtuse or oblong (2)
39	State of stem strength : erect or decumbent (1), erect-ascending (2)	51	Seed surface ornamentation: 1-micro-reticulate; 2-reticulate; 3- bi- reticulate
40	State of stem branches:bifurcating at middle (1), bifurcating from the collar (2), bifurcating upper than middle of stem (3)	52	Shape of calyx: oval-lanceolate (1), angled (2)
41	Leaf outline : polygonal, cordate (1), sub orbicular to reniform (2),	53	Sepals Apical arista : presence (1), absence (2)
42	Phyllotaxy: alternate (1), opposite (2)	54	Petal shape : obovate (1), spathulate (2)
43	Leaf tips: presence (1), absence (2)	55	State of petal ligule: presence (1), absence (2)
44	Mericarp hair:1-absence 2-non-glandular short and medium hairs 3-glandular short hairs	56	Petal apex : emarginate or obtuse (1), obtuse or mucronate (2),
45	Seed outline: circular; 2- narrow-elliptical; 3- elliptical	57	State of petal ligule hair: ciliated at base (1), not ciliated at base (2)

46	Seed color: brown; 2-reddish- brown; 3-blackish- brown	58	Stamen filament hair: ciliate (1), not ciliate (2)
47	Stem hair:1-absence 2-non-glandular short and medium hairs 3-glandular short hairs	59	All organ plant hair density: 1-sparsly hairy 2-Glabrous
48	Petioles and Leaf hair: 1-absence 2-non-glandular short and medium hairs 3-glandular short hairs	60	Mericarp color: 1-yellowish-green; 2- brown;

Molecular analyses

The IRAP profiles obtained for each samples were scored as binary characters. Parameter like Nei's gene diversity (H), Shannon information index (I), number of effective alleles, and percentage of polymorphism were determined (WEISING *et al.*, 2005; FREELAND *et al.*, 2011).

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 distance of the studied populations (PODANI, 2000). These analyses were done by PAST ver. 2.17 (HAMMER *et al.*, 2012), DARwin ver. 5 (2012) and SplitsTree4 V4.13.1 (2013) software.

AMOVA (Analysis of molecular variance) test (with 1000 permutations) as implemented in GenAlex 6.4 (PEAKALL and SMOUSE, 2006), and Nei's Gst analysis as implemented in GenoDive ver.2 (2013) (MEIRMANS and VAN TIENDEREN, 2004) were used to show genetic difference of the populations. Moreover, populations' genetic differentiation was studied by G'ST est = standardized measure of genetic differentiation (HEDRICK, 2005), and D_est = Jost measure of differentiation (JOST, 2008).

The genetic structure of populations was studied by Bayesian based model STRUCTURE analysis (PRITCHARD *et al*. 2000), and maximum likelihood-based method of K-Means clustering of GenoDive ver. 2. (2013). For STRUCTURE analysis, data were scored as dominant markers (FALUSH *et al*. 2007). The Evanno test was performed on STRUCTURE result to determine proper number of *K* by using delta *K* value (EVANNO *et al.*, 2005). In K-Means clustering, two summary statistics, pseudo-F, and Bayesian Information Criterion (BIC), provide the best fit for k (MEIRMANS, 2012).

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 equal amount of gene flow among all populations. (ii) Population assignment test based on maximum likelihood as performed in Genodive ver. in GenoDive ver. 2. (2013). The presence of shared alleles was determined by drawing the reticulogram network based on the least square method by DARwin ver 5. (2012).

RESULTS AND DISCUSSION

Populations' genetic diversity

Genetic diversity parameters determined in 15 geographical populations of *E. cicutarium* are presented in Table 4. The highest value of percentage polymorphism (56.41%)

was observed in Lorestan: Sepid-Dasht, (population No.2) which shows high value for gene diversity (0.199). and Shanon, information index (0.29). Population Kermanshah: Ghasre-Shirin, 5 km from Paveh to Nusod (No.10) has the lowest value for percentage of polymorphism (9.24%) and the lowest value for Shanon, information index (0.034), and He (0.050).

Table 4. Genetic diversity parameters in the studied populations E. cicutarium (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).

Pop	Na	Ne	I	He	UHe	%P
Pop1	1.094	1.309	0.235	0.182	0.189	42.35
Pop2	1.176	1.327	0.299	0.199	0.241	56.41
Pop3	0.647	1.182	0.152	0.103	0.111	27.06
Pop4	0.506	1.104	0.090	0.061	0.067	18.47
Pop5	0.694	1.131	0.126	0.081	0.087	27.06
Pop6	0.482	1.090	0.077	0.052	0.059	14.12
Pop7	0.459	1.115	0.089	0.062	0.068	12.29
Pop8	0.329	1.036	0.087	0.079	0.021	45.71
Pop9	0.388	1.081	0.068	0.046	0.056	19.76
Pop10	0.318	1.058	0.050	0.034	0.045	9.24
Pop11	0.835	1.206	0.179	0.119	0.132	35.12
Pop12	0.541	1.118	0.104	0.070	0.084	18.82
Pop13	0.718	1.162	0.147	0.097	0.106	29.41
Pop14	0.918	1.225	0.197	0.132	0.159	35.29
Pop15	0.576	1.144	0.122	0.083	0.095	29.18

Population genetic differentiation

AMOVA (PhiPT = 0.45, P = 0.010), and Gst analysis (0.469, p = 0.001) revealed significant difference among the studied populations (Table 5). It also revealed that, 55% of total genetic variability was due to within population diversity and 45% was due to among population genetic differentiation. Pairwise AMOVA produced significant difference among the studied populations. Moreover, we got high values for Hedrick standardized fixation index after 999 permutation (G'st = 0.465, P = 0.001) and Jost, differentiation index (D-est = 0.278, P = 0.001). These results indicate that the geographical populations of *E. cicutarium* are genetically differentiated from each other.

Table 5. Analysis of molecular variance (AMOVA) of the studied species.

Source	df	SS	MS	Est. Var.	%	*ΦPT*
Among Pops	14	396.576	28.327	4.082	45%	45%
Within Pops	58	494.767	8.530	8.530	55%	
Total	72	891.342		12.613	100%	

df: degree of freedom; SS: sum of squared observations; MS: mean of squared observations; EV: estimated variance; *Φ*PT: proportion of the total genetic variance among individuals within an accession, (P < 0.001).

Populations' genetic affinity

NJ tree and Neighbor-Net network produced similar results therefore only Neighbor-Net network is presented and discussed (Figure. 2). We have almost complete separation of the studied population in the network, supporting AMOVA result. The populations 3 and 4 are distinct and stand separate from the other populations with great distance. The populations 7 and 8, as well as populations 10 and 15 show closer genetic affinity and are placed close to each other.

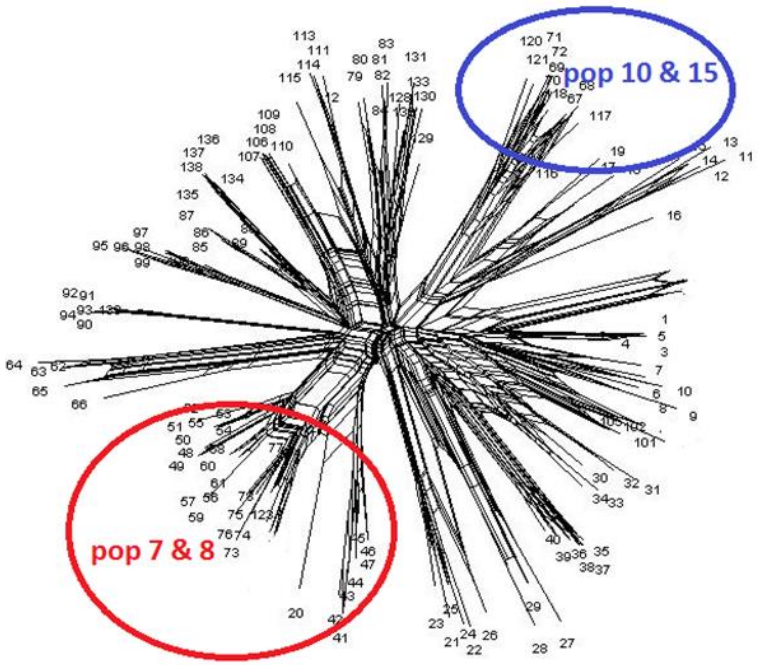

Fig.2. Neighbor-Net network of populations in *E. cicutarium* based on IRAP data.

Genetic divergence and separation of populations 1-5, as well as 9 and 11 from the other populations is evident in MDS plot of IRAP data after 900 permutations (Figure.3). The other populations showed close genetic affinity. Mantel test after 5000 permutations produced significant correlation between genetic distance and geographical distance in these populations ($r = 0.32$, $P = 0.001$). Therefore, the populations that are geographically more distant have less amount of gene flow, and we have isolation by distance (IBD) in *E. cicutarium.*

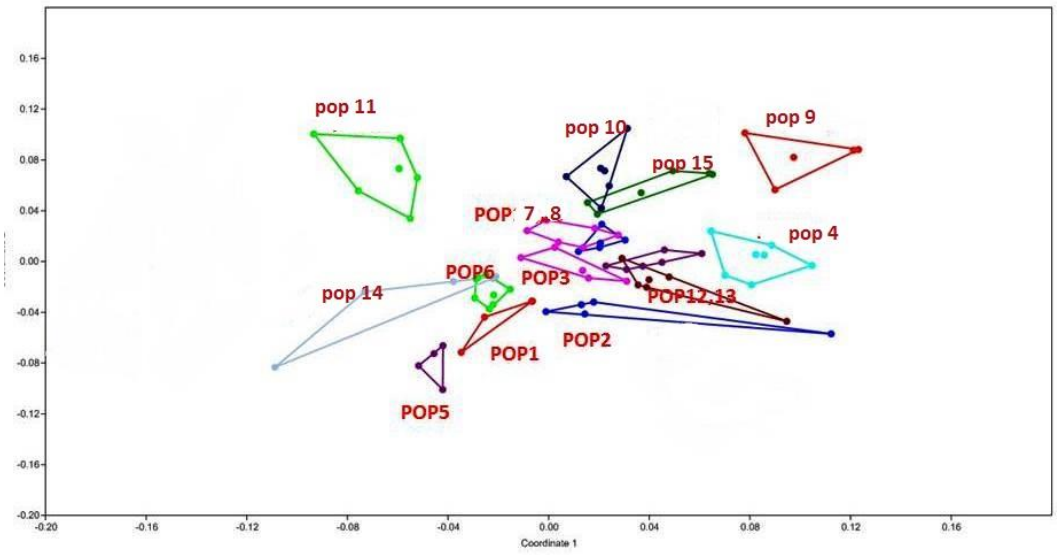

Fig. 3. MDS plot of populations in *E. cicutarium* based on IRAP data.

Populations genetic structure

K = 10 reveal the presence of 10 genetic group. Similar result was obtained by Evanno test performed on STRUCTURE analysis which produced a major peak at k = 10 (Figure.4). Both these analyses revealed that *E. cicutarium* populations show genetic stratification.

STRUCTURE plot based on k = 10, revealed genetic difference of populations 2 and 3 (differently colored), as well as 6 and 7. But it showed genetic affinity between populations 4 and 5 (similarly colored), populations 8 and 9-10, as well as populations 12-13. The mean Nm = 0.28 was obtained for all IRAP loci, which indicates low amount of gene flow among the populations and supports genetic stratification as indicated by K-Means and STRUCTURE analyses. Population assignment test also agreed with Nm result and could not identify significant gene flow among these populations. However, reticulogram obtained based on the least square method (Figure. 5), revealed some amount of shared alleles among populations 1 and 5, and between 13 and 6 and 7, also between 8, and 9. This result is in agreement with grouping we obtained with MDS plot, as these populations were placed close to each other. As evidenced by STRUCTURE plot based on admixture model, these shared alleles comprise very limited part of the genomes in these populations and all these results are in agreement in showing high degree of genetic stratification within *E. cicutarium* populations.

In total 90 IRAP bands (loci) were obtained, out of which 16 bands were private. Populations 1-7, 8, 14 and 15 contained 1-4 private bands.

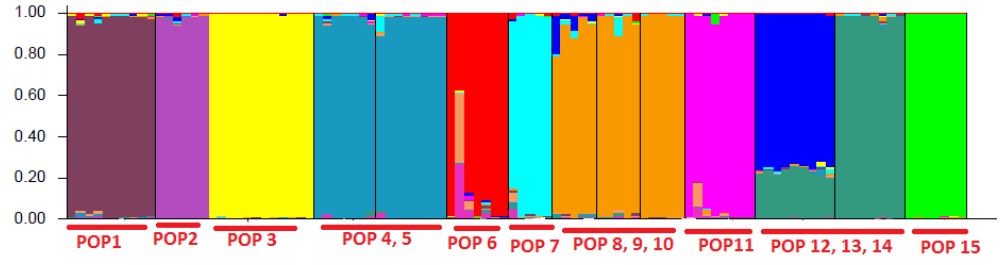

Fig. 4. STRUCTURE plot of *E. cicutarium* populations based on k = 10 of IRAP data.

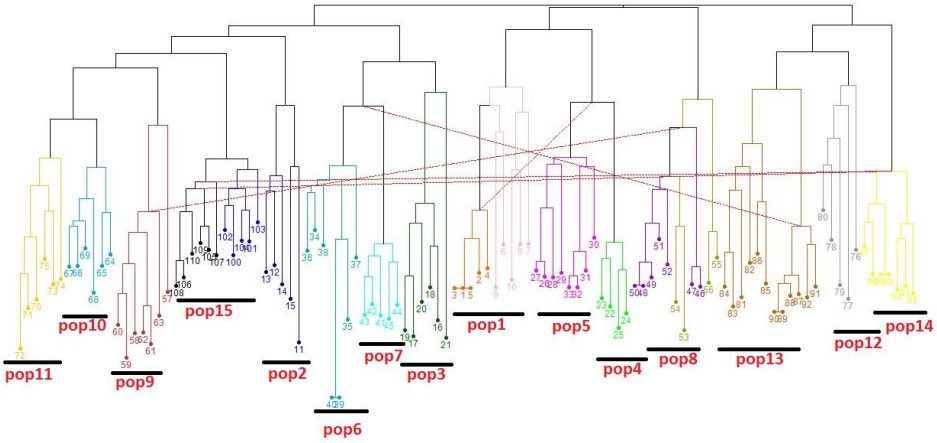

Fig. 5. Reticulogram of *E. cicutarium* populations based on least square method analysis of IRAP data. (Population numbers are according to Table 1).

Morphometric analyses

In present study 102 plant samples were collected from 15 geographical populations. ANOVA test revealed significant difference in quantitative morphological characters among the studied populations ($P < 0.05$). Clustering and PCoA plot of *E. cicutarium* populations based on morphological characters produced similar results therefore only PCA plot is presented and discussed (Figure. 6). The result showed morphological difference/ divergence among most of the studied populations. This morphological difference was due to quantitative characters only. For example, character (Peduncle length), separated population No. 2, character (Width of basal leaves) separated population No. 6, while character Calyx width, separated populations 14 and 15 from the other populations.

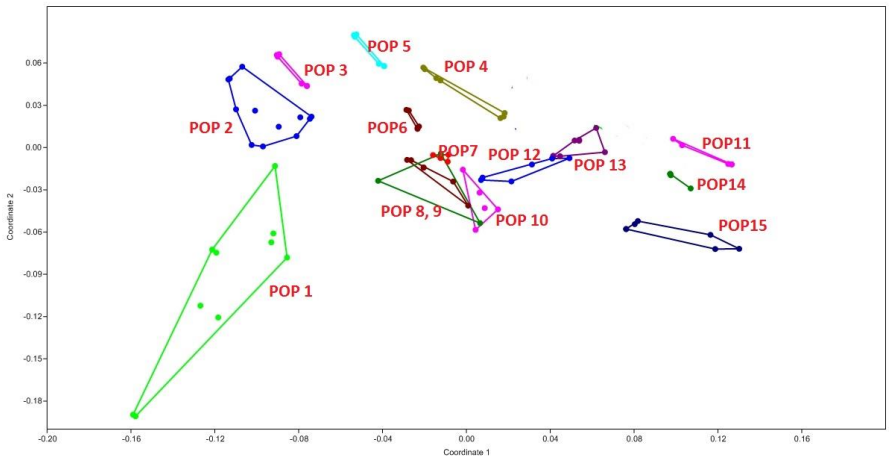

Fig. 6. PCOA plot of *E. cicutarium* populations based on morphological characters.

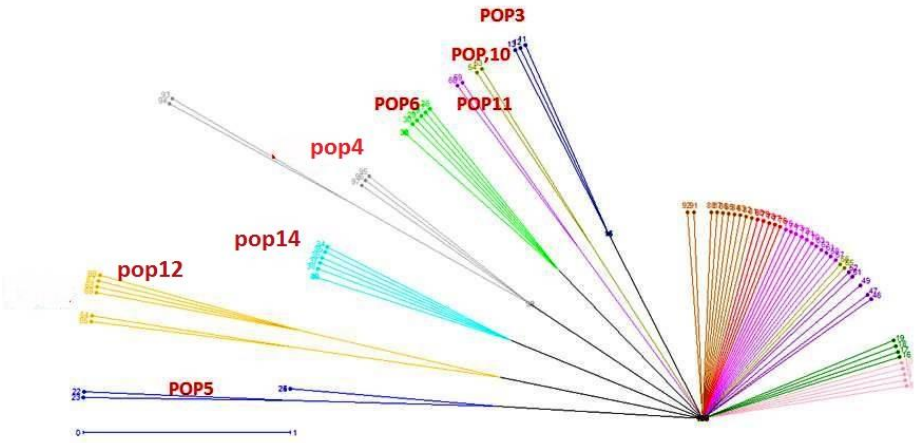

Fig. 7. Consensus tree of morphological and molecular data in *E. cicutarium* populations.

A consensus tree was obtained for both IRAP and morphological trees (Figure. 7), to reveal the populations that are diverged based on both morphological and molecular features. Interesting enough, it showed divergence of almost all populations at molecular level as well as morphological characteristics. Detailed comparison of the characteristics in these populations

revealed that, for example, population No. 2, has the longest peduncle length (10-15 mm), the highest pedicle length (2.56 mm), and the largest ratio of length/width of petal (6-7 mm), among the studied populations. Similarly, population No. 6 had, the longest stem-leaf length (45 mm) and the broadest basal-leaf width (57 mm). Population No. 14 had, the narrowest peduncle width (1-3 mm), and the highest ratio of pedicle length/width.

CONCLUSIONS

Population genetics analyses are important in genetic and breeding studies. They provide information on the levels of genetic variation, partitioning of genetic variability within/between populations, inbreeding or outcrossing, effective population size and population bottleneck (ELLIS and BURKE, 2007). The advent of molecular markers has greatly improved population genetic studies. These markers have been used to identify potentially novel genotypes among the many *Erodium* accessions (MARTIN *et al*.,1997). In recent years, molecular marker systems such as randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR), simple sequence repeat (SSR) and inter-retrotransposon amplified polymorphism (IRAP) have been used to measure genetic variation and relationships in cultivars and landraces (EVERAERT *et al.*, 2001; WIESNEROVA and WIESNER, 2004). Transposable elements, particularly retrotransposons, comprise most of plant genomes. Their replication generates genomic diversity and makes them an excellent source of molecular markers (SMYKAL *et al*., 2011). The inter-retrotransposon amplified polymorphism (IRAP) method displays insertional polymorphisms by amplifying the segments of DNA between two retrotransposons. It has been used in numerous studies of genetic diversity (SMYKAL *et al.*, 2011).

According to MARTIN *et al*., (1997) showed that Genetic diversity within and among populations of a threatened species: *Erodium paularense* Fern. Gonz. & Izco. They report the use of RAPD markers to gain information about the genetic variability among and within populations of *E. paularense.* According to ALARCÓN *et al*. (2012) AFLP variation suggests that this might have led to their differentiation into groups and speciation during inter glacials, but it probably also provided the basis for recurrent recolonisations and the mixing of neighbouring populations at the last glacial maxima. Their results showed that genetic diversity of the two *Erodium* lineages suggests two migration episodes took place from southern Iberia towards the north, with one lineage migrating via western Iberia and the other via eastern Iberia. The patterns of genetic diversity observed in populations of 56 European species (27 genera) leads to the hypothesis that disparate proportions of unique polymorphic fragments are the result of the evolutionary histories of their mountain populations irrespective of the currently recognised species. Geography appears to play an important role in isolation by distance, particularly for Mediterranean plants. Reductions in gene flow may lead to the appearance of new species or subspecies, with isolation in glacial refugia as a major promoter of such diversification (ESFANDANI-BOZCHALOYI *et al.*, 2018a; 2018b; 2018c; 2018d). *E. cicutarium* is of wide spread in our country and it has several medicinal applications (WIESNEROVA and WIESNER, 2004), however we had no information on its genetic structure and detailed taxonomic information. The present study revealed interesting data about its genetic variability, genetic stratification and

morphological divergence in north and west part of Iran. The studied populations had low to moderate level of genetic diversity. The Genetic diversity is of fundamental importance in the continuity of a species as it is used to bring about the necessary adaptation to the cope with changes in the environment (ÇALIŞKAN, 2012). 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 (FREELAND *et al*., 2011). *E. cicutarium* is mainly self-pollinating species (BAKER, 1955), therefore, low level of genetic variability within populations in this species might be related to the closer nature of breeding in this taxon. Another well-known feature of self-pollinating species is high among-population genetic and morphological divergence. This happens due to limited amount of gene flow or its complete absence among geographical population in a single species (FREELAND *et al*., 2011). The present study also revealed significant morphological and genetic difference among *E. cicutarium* populations, quite in agreement with the mentioned assumption. This is particularly supported by STRUCTURE plot that identified 10 separate genetic groups within this population and by consensus tree of both morphological and genetic data. Different mechanisms like isolation, drift, founder effects and local selection may act to bring about among population differentiation and therefore, populations differ in phenotypic traits and allelic composition (JOLIVET and BERNASCONI, 2007). We should state that, the studied populations differed in quantitative morphological characters and we do not know how much of the morphological difference among the studied populations is genetically controlled; they may be under influence of environmental conditions. Therefore, we do not attempt to suggest new taxonomic forms bellow the species level for this taxon and consider them as different ecotypes only. The present population divergence may be under influence of isolation-by distance across the distribution range of the studied *E. cicutarium* populations. The dispersal of these populations might be constrained by distance and gene flow is most likely to occur between neighboring populations. As a result, more closely situated populations tend to be more genetically similar to one another (SLATKIN, 1993; HUTCHISON and TEMPLETON, 1999; MEDRANO and HERRERA, 2008).

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DIFERENCIJACIJA POPULACIJA I PROTOK GENA U *Erodium cicutarium*: POTENCIJALNA LEKOVITA BILJKA

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Izvod

Erodium (Geranaiceae) vrste su rasprostranjene u različitim staništima Irana. Neke vrste su od medicinskog značaja, dok su neke dobro poznati korov. U sušnim i polusušnim regionima *E. cicutarium* je imao određenu važnost kao krmna biljka i važna je biljka za ispašu i izvor proteinskih dodataka slami za preživare u polu pustinjama i pustarama Bliskog Istoka. Nema podataka o genetskoj strukturi njegove populacije, genetskoj raznolikosti i morfološkoj varijabilnosti u Iranu. Zbog medicinske važnosti ove vrste, sprovodena je studija genetske varijabilnosti i strukture populacija koja proučava 15 geografskih populacija *E. cicutarium*. Zbog toga smo koristili šest IRAP markera i 15 kombinovanih IRAP markera kako bismo otkrili unutar i među populacijski genetički diverzitet ove biljke. AMOVA test je stvorio značajnu genetičku razliku (PhiPT = 0,39, P = 0,010) među ispitivanim populacijama i takođe je otkrio da je 55% ukupne genetičke varijabilnosti nastalo zbog unutar populacijske raznovrsnosti, dok je 45% bilo zbog genetičke diferencijacije između populacija. Mantel test je pokazao signifikatnu pozitivnu korelaciju između genetičke i geografske distance proučavanih populacija. Umrežavanje, STRUKTURE analiza i test rasporeda populacije otkrili su određeni stepen protoka gena između ovih populacija. Plot PCoA populacija na osnovu morfoloških karakteristika bila je u saglasnosti sa MDS grafikom molekularnih podataka. Ovi rezultati su pokazali da su geografske populacije E. cicutarium dobro diferencirane kako u genetskom sadržaju, tako i po morfološkim karakteristikama. Stablo zasnovano na morfološkim i genetičkim podacima razdvojilo je neke od ovih populacija od ostalih, što sugeriše postojanje ekotipova unutar ove vrste.

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