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POPULATION GENETIC STRUCTURE AND GENE FLOW IN *Alcea aucheri* (BOISS.) ALEF.: A POTENTIAL MEDICINAL PLANT

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The genus *Alcea*, a member of Malvaceae family consists of approximately 75 species worldwide distributing mainly in South-West Asia. Among these, 33 species grow in Iran. Plants of the *Alcea* (or *Althaea*) are among important medicinal plants in Iranian traditional medicine. They have long been used in the treatment of health problems and diseases. *Alcea aucheri* (Boiss.) Alef. species are distributed in different habitats of Iran. There is no information on its population genetic structure, genetic diversity, and morphological variability in Iran. Therefore, due to the importance of these plant species, we performed a combination of morphological and molecular data for this species. For this study, we used 118 randomly collected plants from 10 geographical populations in 5 provinces. AMOVA test revealed significant genetic difference among the studied populations and also revealed that, 74% of total genetic variability was due to within population diversity while, 26% 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 revealed some degree of gene flow among these populations.

Keyword: Alcea aucheri, Gene flow, Genetic differentiation, Inter Simple Sequence Repeats (ISSR).

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INTRODUCTION

Genetic diversity is a basic component of biodiversity and its conservation is essential for long term survival of any species in changing environments (MILLS and SCHWARTZ, 2005; TOMASELLO et *al.*, 2015). Among different populations, genetic diversity is non randomly distributed and is affected by various factors such as geographic variations, breeding systems, dispersal mechanisms, life span, etc. Change in environmental conditions often leads to variation in genetic diversity levels among different populations and populations with low variability are generally considered less adapted under adverse circumstances (FALK and HOLSINGER, 1991; OLIVIERI *et al.*, 2016). Most of the authors agree that genetic diversity is necessary to preserve the long-term evolutionary potential of a species (FALK and HOLSINGER, 1991). In the last decade, experimental and field investigations have demonstrated that habitat fragmentation and population decline reduce the effective population size. In the same way, most geneticists consider population size as an important factor for maintaining genetic variation (ELLEGREN and GALTIER, 2016; TURCHETTO et *al.*, 2016). This is very important in fragmented populations because are more vulnerable due to the loss of allelic richness and increased population differentiation by genetic drift (decreases heterozygosity and eventual fixation of alleles) and inbreeding depression (increases homozygosity within populations; FRANKHAM, 2005). Therefore, knowledge of the genetic variability and diversity within and among different populations is crucial for their conservation and management (e.g. CIRES *et al.*, 2012, 2013; ESFANDANI-BOZCHALOYI *et al*., 2018a, 2018b, 2018c, 2018d).

The genus *Alcea*, a member of Malvaceae family consists of approximately 75 species worldwide distributing mainly in South-West Asia. Among these, 33 species grow in Iran (ESCOBAR *et al*., 2009) while 16 species are endemic to the country (PAKRAVAN, 2008). Regarding Iran, there are only a few studies on the taxonomy of the genus, since the report of *Alcea* in Flora Iranica (RIEDEL, 1976). However, because of the special geographical situation of Iran, further species could be expected to occur (PAKRAVAN and GHAHREMAN, 2003). Given its striking appearance and geometry, *Alcea* could be easily distinguished from other genera; the flowers are either solitary or arranged in racemes or fascicles on an unbranched upright stem rising to over 2 m at a fast rate. This genus is also characterized by having long notched petals ranging in color from white and yellow to pink and purple. The leaves usually appear on long petioles and are often lobed or toothed, and adorn the whole parts of the herbage (AZIZOV *et al*., 2007). From an industrial and medicinal point of view, *Alcea* is among the most important genera recognized in the family Malvaceae. Molecular-phylogenetic data also support the monophyly and distinctness (as suggested by morphological data) of *Alcea* but they are of limited use in determining relationships between species and species delimitations (ESCOBAR *et al*., 2012). *Alcea* exhibits a considerable taxonomic complexity (ZOHARY, 1963a,b; RIEDL, 1976; TOWNSEND, 1980).

Alcea aucheri (Boiss.) Alef. is a perennial plant with 30 to 50 cm height, covered by high densely hairs and dingy appearance. Flowers are pink, whity-pink or dingy pink and appear among June and July, last up to first severe cold in autumn. The plant with acceptable appearance and low water requirement could be a good choice to use in xeriscaping in arid and semiarid regions. Its seed germination is inherently poor and becomes unpredictable, especially in semi-arid regions characterized by low rainfall (SHAHEEN *et al*., 2010).

Plants of the *Alcea* (or *Althaea*) genus from Malvaceae family are among important medicinal plants in Iranian traditional medicine. They have long been used in the treatment of health problems and diseases. The flowers of *Alcea spp*. have been widely used as mucilage for treatment of irritated oral and pharyngeal mucosa, respiratory, and gastrointestinal disorders, as well as urinary complaints and skin inflammations. They have been also used as a diuretic agent and sedative remedy (BLUMENTHAL *et al*., 2000). Some of species of the *Alcea* genus with medicinal activities include *Alcea rosea* L. (hollyhock), *A. officinalis* L. (marshmallow), and *A. aucheri* (Boiss.) Alef. (South marshmallow) (BLUMENTHAL *et al*., 2000).

Molecular markers play a significant role in protection of biodiversity, identification of promising cultivars, quantitative trait loci (QTL) mapping, etc. Different PCR based dominant markers such as ISSR, SCoT, SRAP, etc. have been effectively used for quantification of genetic diversity (GEORGE *et al*., 2006). Recent ISSR studies of natural populations have demonstrated the hypervariable nature of these markers and their potential use for population-level studies (HULTE´N and FRIES, 1986). Limitations of the ISSR technique, as is the case for Random Amplification of Polymorphic DNA (RAPD; (ESFANDANI-BOZCHALOYI *et al*., 2019), are that bands are scored as dominant markers and that genetic diversity estimates are based on diallelic characters. In the present study, ISSR markers were employed to analyze genetic diversity in 118 *Alcea aucheri* accessions belonging to 10 different populations for the first time in the Iran.

MATERIALS AND METHODS

Plant materials

A total of 118 individuals were sampled representing 10 natural populations of *Alcea aucheri* in Kermanshah, Esfahan, Tehran, Fars, Yazd and Kerman Provinces of Iran during July-Agust 2019-2020 (Table 1).

Table 1. Voucher details and diversity within Iranian populations of A. aucheri in this study

No	Subspecies	Locality	Latitude	Longitude	Altitude (m)
Pop1	var. *aucheri* Boiss.	Esfahan:Ghameshlou, Sanjab	36 ˚ 52'37"	52 ˚ 23' 92"	122
Pop2	var. *aucheri* Boiss.	Tehran, Damavand	37°50′03″	49°24′28″	-6
Pop3	var. *aucheri* Boiss.	Fars, Estahban	36◦20′07″	50° 52′08″	13
Pop4	var. *aucheri* Boiss.	Kermanshah, Islamabad	36 ˚ 52'373	54 ˚ 23' 92"	155
Pop5	var. *aucheri* Boiss.	Tehran, Karaj, Malard	36° 57′12″	53° 57′32″	5
Pop6	var. *lobata* Bornm.	Esfahan:Najafabad	36 ˚ 52'373	51 ˚ 23' 92"	180
Pop7	var. *lobata* Bornm.	Yazd: Khormiz, 5km SW of Mehriz, NE of Kuh-e Khoseh.	31 ˚ 52'373	54 ˚ 23' 92"	1700
Pop8	var. *lobata* Bornm.	Fars, Darab	36°50′03″	53°24′28″	-3
Pop9	var. *lobata* Bornm.	Fars, Shiraz	36°14′14″	52°18′07″	-14
Pop10	var. *lobata* Bornm.	Kerman , Bam	36◦36′93″	52°27′90″	44

For morphometric and ISSR analysis we used 118 plant accessions (four to twelve samples from each populations) belonging to 10 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 and Fig. 1. The specimens were

identified using the identification keys and descriptions of the *Alcea* species in the relevant floras [Taxonomical Studies in *Alcea* of South-western Asia (ZOHARY, 1963a, b), Flora Orientalis (BOISSIER, 1967), Flora Palestina (ZOHARY, 1972), Flora Iranica (RIEDL, 1976), Flora of Iraq (TOWNSEND *et al*., 1980). Vouchers were deposited at the herbarium of Islamic Azad University, Science and Research Branch, Tehran, Iran (IAUH).

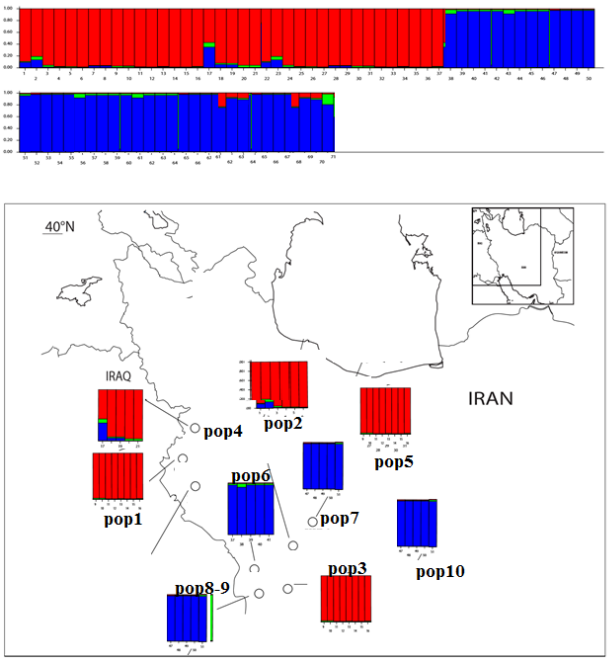

Fig. 1. Distribution map of the studied populations.

DNA extraction and ISSR assay

Fresh leaves were used randomly from four 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 (ESFANDANI-BOZCHALOYI *et al*., 2019). The quality of extracted DNA was examined by running on 0.8% agarose gel. 10 ISSR primers; $(AGC)_5GT$, $(CA)_7GT$, $(AGC)_5GG$, UBC810, $(CA)_7AT$, $(GA)_9C$, UBC807, UBC811, $(GA)_9T$ and $(GT)_7CA$ commercialized by UBC (the University of British Columbia) were used. 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).

Data analyses

Morphological studies

In total 29 morphological quantitative characters were studied. Four to twelve samples from each population were randomly studied for morphological analyses (Appendix 1). 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) were used (PODANI, 2000). PAST version 2.17 (HAMMER *et al.*, 2012) was used for multivariate statistical analyses of morphological data.

Molecular analyses

The ISSR 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 (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) 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 clustering method based on the Bayesian-model implemented in the software program STRUCTURE (PRITCHARD *et al*., 2000) was used on the same data set to better detect population substructures. This clustering method is based on an algorithm that assigns genotypes to homogeneous groups, given a number of clusters (K) and assuming Hardy-Weinberg and linkage equilibrium within clusters, the software estimates allele frequencies in each cluster and population memberships for every individual (PRITCHARD *et al*., 2000). The number of potential subpopulations varied from two to ten, and their contribution to the genotypes of the accessions was calculated based on 50,000 iteration burn-ins and 100,000 iteration sampling periods. The

most probable number (K) of subpopulations was identified following 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 (Nm) which were calculated using POPGENE (version 1.31) program (YEH *et al*., 1999). Gene flow was estimated indirectly using the formula: Nm = 0.25(1 - FST)/FST. In order to test for a correlation between pair-wise genetic distances (FST) and geographical distances (in km) between populations, a Mantel test was performed using Tools for Population Genetic Analysis (TFPGA; MILLER, 1997) (computing 999 permutations). This approach considers equal amount of gene flow among all populations. (ii) Population assignment test based on maximum likelihood as performed 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

Populations' genetic diversity

Genetic diversity parameters determined in 10 geographical populations of *Alcea aucheri* populations are presented in Table 2. The highest value of percentage polymorphism (45.38%) was observed in Fars, Shiraz (population No.9) which shows high value for gene diversity (0.27). and Shanon' information index (0.33). Population Tehran, Damavand (No.2) has the lowest value for percentage of polymorphism (18.82%) and the lowest value for Shanon, information index (0.10), and He (0.070).

Table 2. Genetic diversity parameters in the studied populations Alcea aucheri

Pop	N	Na	Ne	I	He	UHe	%P
Pop1	10	0.835	1.206	0.179	0.119	0.132	35.12
Pop2	14	0.541	1.118	0.104	0.070	0.084	18.82
Pop3	8	0.718	1.162	0.147	0.097	0.106	29.41
Pop4	15	0.918	1.225	0.197	0.132	0.159	35.29
Pop5	10	0.452	1.089	0.23	0.22	0.15	35.05%
Pop6	14	0.333	1.006	0.122	0.12	0.22	43.23%
Pop7	8	1.247	1.35	0.271	0.184	0.192	35.91%
Pop8	15	0.258	1.017	0.174	0.11	0.12	34.30%
Pop9	14	0.258	1.029	0.331	0.27	0.29	45.38%
Pop10	11	0.452	1.089	0.18	0.22	0.25	42.05%

N = number of samples, Na = No. of Different Alleles, Ne = No. of Effective Alleles = 1 / (p^2 + q^2)

I = Shannon's Information Index = -1* (p * Ln (p) + q * Ln(q)), He = Expected Heterozygosity = 2 * p * q

UHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He, *P%= percentage of polymorphism, populations*

Where for Diploid Binary data and assuming Hardy-Weinberg Equilibrium, q = (1 - Band Freq.)^0.5 and p = 1 - q.

UHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He

Where for Diploid Binary data and assuming Hardy-Weinberg Equilibrium, q = (1 - Band Freq.)^0.5 and p = 1 - q.

Population genetic differentiation

AMOVA (PhiPT = 0.356, P = 0.010), revealed significant difference among the studied populations (Table 3). It also revealed that, 74% of total genetic variability was due to within population diversity and 26% was due to among population genetic differentiation.

Table 3. Analysis of molecular variance (AMOVA) of the studied species.

Source	df	SS	MS	Est. Var.	%	*ΦPT*
Among Pops	20	416.576	35.327	5.082	26%	26%
Within Pops	62	444.767	7.530	7.530	74%	
Total	82	831.342		12.543	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).

Stat	Value	P(rand >= data)
PhiPT	0.793	0.010

Probability, P(rand>=data), for PhiPT is based on permutation across the full data set.

PhiPT = AP / (WP + AP) = AP / TOT

Key: AP = Est. Var. Among Pops, WP = Est. Var. Within Pops

The pairwise comparisons of 'Nei genetic identity' among the studied populations *Alcea aucheri* (Table 4) have shown a higher a genetic similarity (0.89) between populations Tehran, Damavand (pop. No 2) and Fars, Estahban (pop. No 3), while the lowest genetic similarity value (0.65) occurs between Esfahan: Najafabad (pop. No.6) and Fars, Shiraz populations (pop. No. 9).

Table 4. Pairwise Population Matrix of Nei Unbiased Genetic Identity

pop1	pop2	pop3	pop4	pop5	pop6	pop7	pop8	pop9	pop10	
1.000										pop1
0.745	1.000									pop2
0.707	0.897	1.000								pop3
0.757	0.866	0.797	1.000							pop4
0.837	0.772	0.804	0.752	1.000						pop5
0.731	0.716	0.781	0.727	0.824	1.000					pop6
0.756	0.725	0.768	0.719	0.806	0.772	1.000				pop7
0.768	0.863	0.800	0.760	0.836	0.755	0.831	1.000			pop8
0.765	0.769	0.812	0.736	0.783	0.650	0.826	0.797	1.000		pop9
0.755	0.849	0.823	0.744	0.781	0.727	0.772	0.732	0.873	1.000	pop10

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 Yazd: Khormiz, 5km SW of Mehriz, NE of Kuh-e Khoseh (pop. No 7) and Fars, Darab; Fars, shiraz and Kerman, Bam (pop. No 8,9,10) (*A. aucheri* var. *lobate*) are distinct and stand separate from the other populations with great distance. The populations 1 and 2, as well as populations 3 and 5 (*A. aucheri* var. *aucheri*) show closer genetic affinity and are placed close to each other. In general, the description here about Figure 2 is more or less consistent with Figure 3.

Genetic divergence and separation of populations 1-5, as well as 6 - 10 from the other populations is evident in MDS plot of ISSR 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 ($r = 0.987$, $P = 0.001$). Therefore, the populations that are geographically more distant have less amount of gene flow, and we have isolation by distance (IBD) in *Alcea aucheri.*

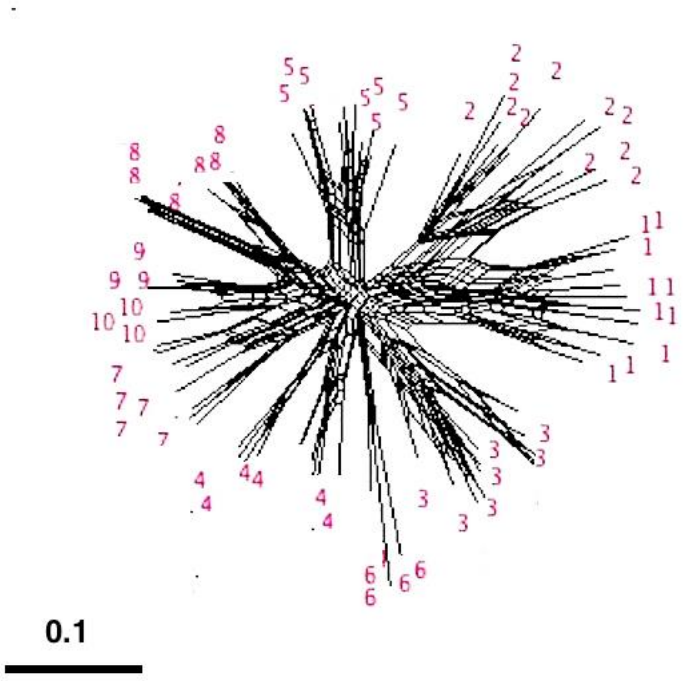

Fig.2. Neighbor-Net network of populations in *Alcea aucheri* based on ISSR data.

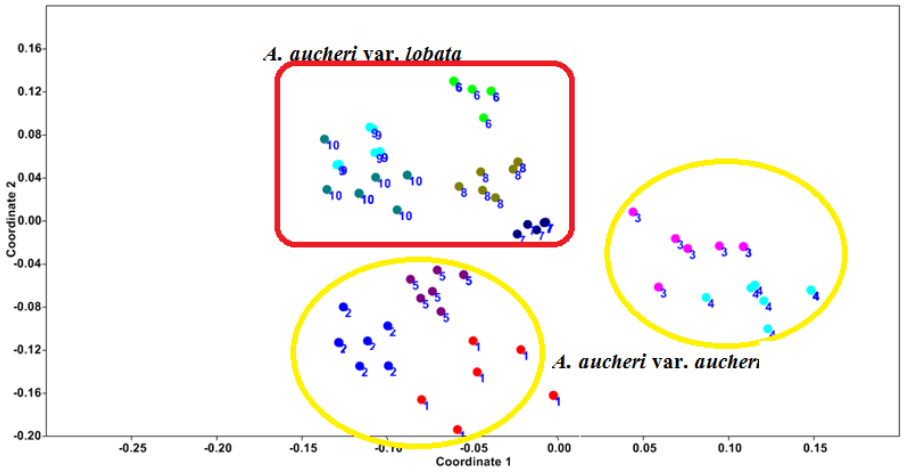

Fig. 3. MDS plot of populations in *Alcea aucheri* based on ISSR data.

Populations genetic structure

K = 2 reveal the presence of 2 genetic group. Similar result was obtained by Evanno test performed on STRUCTURE analysis which produced a major peak at k = 2 (Figure.4, Table 5). Both these analyses revealed that *Alcea aucheri* populations show genetic stratification.

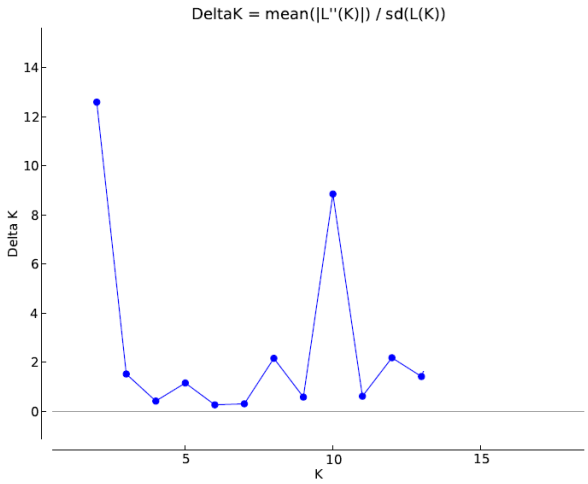

Fig. 4**.** Delta k plot of Evanno's test based on STRUCTURE analysis.

Table 5. K-Means clustering result. (Best clustering according to Calinski and Harabasz' pseudo-F: k = 2. Best clustering according to Bayesian Information Criterion: k = 6).*

k	SSD(T)	SSD(AC)	SSD(WC)	r-squared	pseudo-F	AIC	BIC	Rho
1	1119.354	0	0	0	0	216.38	580.088	0
2*	1119.354	545.8	573.5	0.488	10.061	177.47	556.102	0.472
3	1119.354	210.5	908.9	0.188	9.147	203.56	571.822	0.263
4	1119.354	292.3	827.1	0.261	9.189	198.04	568.493	0.303
5	1119.354	367.5	751.9	0.328	9.409	192.49	565.084	0.363
6	1119.354	438.8	680.5	0.392	9.801	186.65	561.316	0.4
7	1119.354	498.3	621	0.445	10.03	181.54	558.22	0.436
8	1119.354	697.1	422.3	0.623	9.493	165.74	553.028	0.576
9	1119.354	581	538.4	0.519	9.846	174.81	555.325	0.496
10&	1119.354	719.8	399.5	0.643	9.425	164.12	552.895	0.592

* Best clustering according to Calinski & Harabasz' pseudo-F: k = 2

& Best clustering according to Bayesian Information Criterion: k = 10

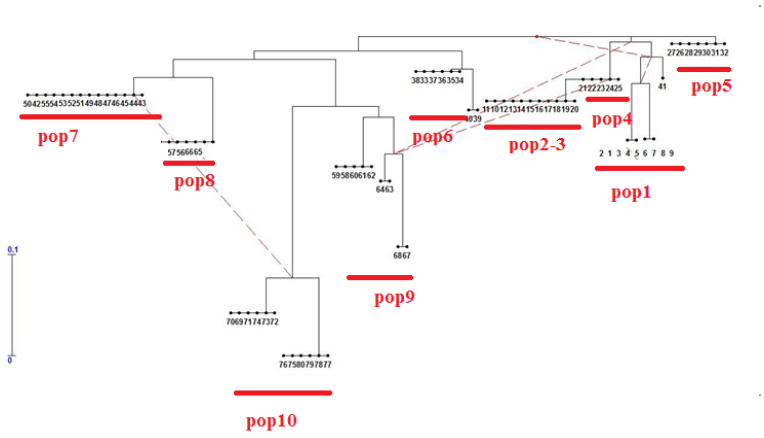

Fig. 5. Reticulogram of *Alcea aucheri* populations based on least square method analysis of ISSR data. (Population numbers are according to Table 1.

STRUCTURE plot based on k = 2 (Figure 1, Table 5), revealed genetic affinity between populations 1-5 (similarly colored), as well as populations 6-10.

The mean Nm = 0.354 was obtained for all ISSR loci, which indicates low amount of gene flow among the populations and supports genetic stratification as indicated by K-Means and STRUCTURE analyses. However, reticulogram obtained based on the least square method

(Figure. 5), revealed some amount of shared alleles among populations 10 and 7, and between 9 and 1-4. This result is in conflict 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 not in agreement in showing high degree of genetic stratification within *Alcea aucheri* populations.

Morphometric analyses

In present study we used 118 plant accessions (four to twelve samples from each populations) belonging to 10 different populations. PCA plot of *Alcea aucheri* populations based on morphological characters produced similar results (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 (Number of segment stem leaves (mm) and shape basal leaves and stem leaves, separated population No. 7-10, character (Width of basal leaves) separated population No. 1,3,4, while character Calyx width, separated populations 2 and 5-6 from the other populations.

A consensus tree was obtained for both ISSR and morphological trees (Figure not included), 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.

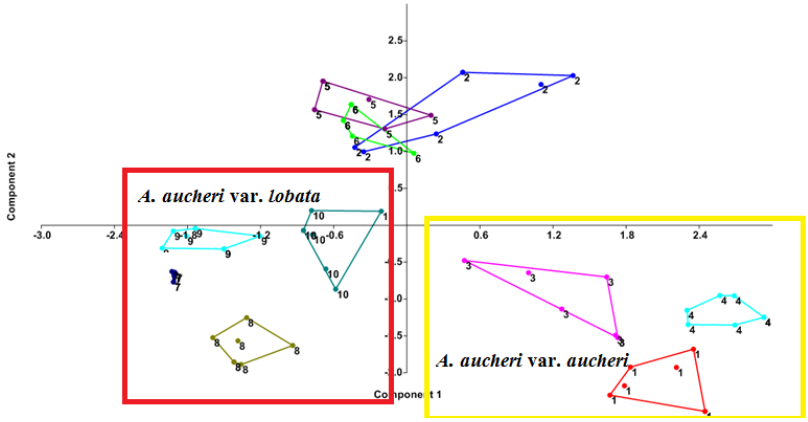

Fig. 6. PCA plot of *Alcea aucheri* populations based on morphological characters.

DISCUSSION

A well-suited system for investigating radiations in the Irano-Turanian region is the genus *Alcea* L. (Malvaceae). It includes approximately 50 species, which are mainly of Irano-Turanian distribution with extensions into the Caucasus and the eastern Mediterranean (ZOHARY, 1963b). *Alcea* comprises mostly tall-growing hemicryptophytes with simple, lobed,

palmatipartite or palmatisect leaves with a more or less pronounced indumentum of stellate and fasciculate hairs. *Alcea* is considered one of the most complicated and challenging genera of the Middle Eastern flora (ZOHARY, 1963b; RIEDL, 1976; TOWNSEND, 1980; PAKRAVAN, 2001).

The present study revealed interesting data about its genetic variability, genetic stratification and morphological divergence in south and west part of Iran. The studied populations have a low level of genetic diversity (He = 0.034- 0.199). 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 (GUITTONNEAU, 1972). 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 (KNUTH, 1908).

Low genetic variability may also occur due to small size of the populations and genetic drift (DAHLGREN, 1980). These species tend to perform inbreeding as also evidenced by very low Nm value and IBD obtained for the studied species. However, limited gene flow was not solely due to geographical distance among the species, but some of the species, which grew in adjacent areas with overlapping zones, did not form any hybrids or intermediate forms as evidenced by morphological and ISSR clusters obtained (WEBB and CHATER, 1968).

In our study, 10 primer combinations could amplify 98 discrete bands of which 90 were polymorphic (95% polymorphism). This value appeared to be relatively high, similar to the other ISSR based studies, e.g. orchid (80.52%; CAI *et al*., 2011), *Salvia miltiorrhiza* (90%; SONG *et al*., 2010), and coffee species (93%; MISHRA *et al*., 2011). This result implies that ISSR markers are efficient for analyzing polymorphism level in *Alcea*. Occurrence of high polymorphism could be explained for species in different climatic zones with varying selection pressure during the course of evolution (MISHRA *et al*., 2011). Genetic diversity is affected by a number of evolutionary factors including mating system, gene flow and seed dispersal, geographic range, as well as natural selection (HAMRICK and GODT, 1989). The geographic range of species appears to influence the levels of genetic diversity greatly. Generally, small geographic range of species leads to less genetic diversity than geographically widespread species (HAMRICK and GODT, 1989). Based on this assumption, a high level of genetic diversity within species is expected in *Alcea*. Our genetic similarity analysis revealed a wide degree of variation from 0.17 to 0.68, which reflects sufficient amount of diversity among *Alcea* species in Iran.

According to BADRKHANI *et al*. (2014) sequence-related amplified polymorphism (SRAP) marker was employed to assess the genetic diversity and genetic similarity relationships among14 species of *Alcea* collected from northwest of Iran. Seventeen SRAP primer combinations generated 104 fragments, of which 97 (93%) were polymorphic, with an average of 5.7 polymorphic fragments per primer. Percentage of polymorphism ranged from 50% (ME2-EM6) to a maximum of 100%, and mean polymorphism information content value obtained was 0.3. The lowest genetic similarity (0.17) was observed between *A. sophiae* and *A. flavovirens*, while the highest was found between *A. digitata* and *A. longipedicellata* (0.68). Two main clusters were detected using UPGMA, which did not correspond to geographical origin of the species. Their study indicates that SRAP markers could be good candidates for assessing genetic variation in *Alcea*.

Iranian *Alcea* species have only been characterized with morphological data, so far. However, the genus has a complicated taxonomy due to small number of characters. Based on study of PAKRAVAN (2008) on *Alcea*, only examination of the leaf sequence and configuration of the carpels would represent valuable characters. For example, *A. flavovirens* and *A. glabrata* differ only in the size of the carpel and width of wing (PAKRAVAN, 2008).

ESCOBAR *et al*. (2012) with using three molecular markers (nrDNA ITS and the plastid spacers *psbA-trnH* and *trnL-trnF*), showed that a phylogeny of *Alcea* and test previous infrageneric taxonomic hypotheses as well as its monophyly with respect to *Althaea*, a genus with which it has often been merged. They additionally discuss morphological variation and the utility of morphological characters as predictors of phylogenetic relationships. Their results show that while molecular data unambiguously support the circumscription of *Alcea* inferred from morphology, they prove to be of limited utility in resolving interspecific relationships, suggesting that *Alcea*'s high species diversity is due to rapid and recent radiation. Their work provides the first phylogeny of *Alcea* and aims to set the scene for the study of processes underlying species radiation in the Irano-Turanian region.

Alcea aucheri is of wide spread in our country and it has several medicinal applications (SHAHEEN *et al*., 2010), however we had no information on its genetic structure and detailed taxonomic information. Our results revealed interesting data about its genetic variability, genetic stratification and morphological divergence in south and west part of Iran.

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GENETIČKA STRUKTURA POPULACIJE I PROTOK GENA KOD *Alcea aucheri* (BOISS.) ALEF.: POTENCIJALNA LEKOVITA BILJKA

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Izvod

Rod *Alcea*, član familije Malvaceae, sastoji se od približno 75 vrsta širom sveta koje su raspoređene uglavnom u jugozapadnoj Aziji. Među njima, 33 vrste rastu u Iranu. Biljke *Alcee* (ili *Althee*) spadaju među važne lekovite biljke u iranskoj tradicionalnoj medicini. Dugo se koriste u lečenju zdravstvenih problema i bolesti. *Alcea aucheri* (Boiss.) Alef. vrste su rasprostranjene u različitim staništima Irana. Nema podataka o genetskoj strukturi njegove populacije, genetskoj raznolikosti i morfološkoj varijabilnosti u Iranu. Zbog toga smo zbog važnosti ovih biljnih vrsta izvršili kombinaciju morfoloških i molekularnih podataka za ovu vrstu. Za ovu studiju koristili smo 118 nasumično prikupljenih biljaka iz 10 geografskih populacija u 5 provincija. AMOVA test otkrio je značajnu genetsku razliku među proučavanim populacijama, a takođe je otkrio da je 74% ukupne genetske varijabilnosti bilo posledica populacione raznolikosti, dok je 26% bilo genetičke diferencijacije populacije. Mantel test je pokazao pozitivnu značajnu korelaciju između genetske udaljenosti i geografske udaljenosti proučavanih populacija. Umrežavanjem, analize STRUKTURE otkrile su određeni stepen protoka gena među ovim populacijama.

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