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Genetic Characterization of *Salicornia persica* Akhani (Chenopodiaceae) Assessed Using Random Amplified Polymorphic DNA

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Abstract. *Salicornia* is a wild and annual (biennial, in some species) halophytic plant from Chenopodiaceae family that grows near salt marshes and salted wetlands or the vicinity of coastal areas in Asia, North America, and the Middle East. It is also grown in semi-arid areas of Iran such as Isfahan, Fars, and Yazd provinces. Genetic variability and populations' structure were studied in 10 geographical populations of *Salicornia persica*. Genetic diversity parameters were determined in these populations. 10 of 20 random amplified polymorphic DNA (RAPD) primers produced 146 reproducible bands with average of 14.6 bands per primer and 88.67% of polymorphism. OPA10 primer showed the highest number of effective allele (Ne), Shannon index (I) and genetic diversity (H). The highest values of genetic diversity in RAPD markers were obtained in Esfahan, Nain, it is 70km to Varzaneh populations. WARD trees of RAPD data grouped the populations in two different clusters/groups, indicating their genetic difference which is discussed in details. The results of this study showed that the level of genetic variation in *Salicornia persica* is relatively low. WARD-based dendrogram showed a close relationship between members of Esfahan, Varzaneh and Esfahan, the river of Zayanderud at Varzaneh while the Yazd, Meybud and Yazd, Nádüshan protected population differ the most from the other populations. Principal component analysis, however, showed some minor differences with WARD-based dendrograms.

Keywords: gene flow, *Salicornia persica*, Random Amplified Polymorphic DNA (RAPD).

INTRODUCTION

Genetic diversity is one aspect of biological diversity that is extremely important for conservation strategies, especially in rare and narrowly endemic species (Wang *et al*., 2021; Yin *et al*., 2021; Zhao *et al*., 2021). Most of the authors agree that genetic diversity is necessary to preserve the long-term evolutionary potential of a species (Jia *et al.* 2020; Shi *et al*., 2021; Zheng *et al.,* 2021; Zhu *et al*., 2021).

Salicornia is a wild and annual (biennial, in some species) halophytic plant from Chenopodiaceae family that grows near salt marshes and salted

wetlands or the vicinity of coastal areas in Asia, North America, and the Middle East. It is also grown in semi-arid areas of Iran such as Isfahan, Fars, and Yazd provinces (Akhani, 2003). *Salicornia* varieties are often multipurpose (Al-Oudat and Qadir, 2011; SI *et al*, 2020) and it is very rich in vitamins, minerals and highly unsaturated oils. *Salicornioideae* species are also rich in dietary fiber and many bioactive substances, such as phytosterols, polysaccharides, and phenolic compounds mainly flavonoids and phenolic acids (Choi *et al*., 2014). *Salicornia*, commonly called Saloni is a member of Chenopodiaceae (Amaranthaceae). A family that constitute among it vegetables like spinach and beets etc. *Salicornia* is a halophyte which tolerates extreme saline conditions and grows along the coastlines and feeds on salt water. It is a leaf less annual succulent salt marsh halophyte considered to be a potential alternative crop of seawater agriculture, due to its economic potential (Glenn *et al.* 1991). The *Salicornia* species are small, usually less than 30 cm tall, succulent herbs with a jointed horizontal main stem and erect lateral branches. The leaves are small and scale-like and as such the plant may appear leafless. Many species are green, but their foliage turns red in autumn. The hermaphrodite flowers are wind pollinated, and the fruit is small and succulent and contains a single seed. *Salicornia* species can generally tolerate immersion in salt water. *Salicornia* has leafless stems with branches that resembles asparagus, hence the plants other common name sea asparagus. Common names for the genus include glasswort, pickleweed, and marsh samphire. According to a molecular phylogenetic study by Kadereit *et al.* (2006), *Salicornia* is monophyletic and nested within the morphologically and ecologically closely related *Sarcocornia*. *Salicornia* and *Sarcocornia* differ from all other Salicornioideae by seeds that lack perisperm (Shepherd *et al*., 2005). *Salicornia* split from *Sarcocornia* during the Middle Miocene (14.2– 9.4 mya), but its extant lineages started to diversify only in the Early Pleistocene (1.4–1.8 mya; Kadereit *et al*., 2006; Liu *et al*., 2021).

According to Zhang and Bai (2022) A total of 72 randomly collected plants from 8 natural populations *Salicornia persica* Akhani in 2 provinces were evaluated using ISSR markers and morphological traits.

Salicornia persica is frequent along several salt marshes, river salt margins, and salty river estuaries in the Provinces Esfahan, Fars and Yazd. However, populations in these areas are threatened because many important wetlands (e.g., Gavkhooni salt swamp) and rivers (e.g., Zayandeh Rud) are drying out. observations around Tashk lake show that the species is grazed by goats. The species is characterized by having ascending habit, verticillate inflorescence branches, and reversed pentagonal central flowers that are truncated at the apex and reach to the upper segments. The molecular markers are extensively used in germplasm characterization, fingerprinting, genetic analysis, linkage mapping, and molecular breeding. RAPD (Random Amplified Polymorphic DNA) analysis using PCR in association with short primers of arbitrary sequence has been demonstrated to be sensitive in detecting variation among individuals. The advantages of this technique are: a) a large number of samples can be quickly and economically analyzed using only micro-quantities of material; b) the DNA amplicons are independent from the ontogenetic expression; and c) many genomic regions can be sampled with a potentially unlimited number of markers (PENG *et al.,* 2021; MA *et al.,* 2021 Esfandani- Bozchaloyi *et al.,* 2017a, b, c, d). We have no information on genetic variability, gene flow and genetic structure of *Salicornia persica* 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 10 geographical populations for the first time in the country.

MATERIALS AND METHODS

Plant materials

A total of 60 individuals were sampled representing 10 natural populations of *Salicornia persica* in Esfahan, Fars and Yazd Provinces of Iran during May-Agust 2016-2020 (Table 1, Fig. 1). According to previous references, all the population were identified (Kadereit *et al*., 2006; Akhani 2003).

Morphological studies

A total of 19 metric and 6 multistate characterers were used for measurements in different combinations (Table 2), modified from the character list detailed by Ingrouille and Pearson (1987). Of these 25 characters, 15 covered the overall vegetative morphology, and 10 were characteristics of the fertile spike, fertile spike segments and flowers. Though vegetative morphology may be partly uninformative due to the wide phenotypic plasticity, both vegetative and fertile spike characteristics were used, because some vegetative traits have been shown useful in separating populations and taxa at least in single cases (Ingrouille and Pearson 1987,).

Table 1. Location and herbarium accession numbers of the studied populations of *Salicornia persica* in Iran.

Pop. no	Locality
1	Esfahan, Varzaneh
2	Esfahan, the river of Zayanderud at Varzaneh
3	Esfahan, Nain, it is 70km to Varzaneh
4	Esfahan; Varzaneh to Kashan
5	Fars, Tashk lake
6	Fars, Lake Bakhtegan
7	Fars, Abadeh Tashk
8	Yazd, Kavire Marvast
9	Yazd, Meybud
10	Yazd, Nádüshan

Table 2. Evaluated morphological characters for populations of *Salicornia persica*

1. Height of plant from rooting point to apex, mm
2. Number of sterile internodes below the terminal spike
3. Number of 1st order branches
4. Number of 2nd order branches
5. Number of 3rd order branches
6. Number of 4th order branches
7. Length of longest basal 1st order branch, mm
8. Number of sterile internodes on longest basal 1st order branch
9. Number of fertile segments on main axis of longest basal 1st order branch
10. Branching angle of longest basal 1st order branch,
11. Branching habit of longest basal 1st order branch,
12. Length of longest (regular) ultimate 1st order branch, mm
13. Number of fertile segments on longest (regular) ultimate 1st order branch
14. Branching angle of longest (regular) ultimate 1st order
15.Branching habit of longest (regular) ultimate 1st order
16. Length of spike, mm
17. Number of fertile segments
18. Length of 2nd fertile segment measured over the central flower of cyme, mm
19. Height of triangular apex of 2nd fertile segment, mm
20. Width of 2nd fertile segment at base, mm
21. Maximum width of 2nd fertile segment, mm
22. Place of the widest point of 2nd fertile segment,
23. Length of visible part of central flower of cyme, 2nd fertile segment, mm
24. Width of central flower of cyme, 2nd fertile segment, mm
25. Place of the widest point of central flower of 2nd

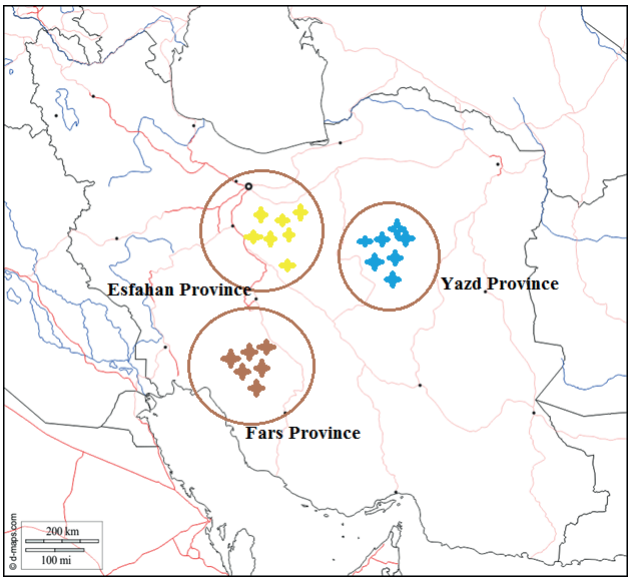

Figure 1. Map of distribtion of populations *Salicornia persica*.

DNA extraction and RAPD 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 *et al.,* 2019). The quality of extracted DNA was examined by running on 0.8% agarose gel. 22 decamer RAPD primers of Operon technology (Alameda, Canada) belonging to OPA, OPB, OPC, OPH, OPI, OPM sets were used in this study. 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 amplifications reactions were performed in Techne thermocycler (Germany) with the following program: 5Min initial denaturation step 94°C, followed by 40 cycles of 1min 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).

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) 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.

Random Amplified Polymorphic DNA (RAPD) analysis

RAPD bands obtained were treated as binary characters and coded accordingly (presence =1, absence = 0). 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,). 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. The genetic structure of populations was studied by Bayesian based model STRUCTURE analysis (Pritchard *et al.* 2000; Chen *et al.* 2021 ; BI *et al.* 2021), and maximum likelihood-based method of K-Means clustering of GenoDive ver. 2. (2013). For STRUCTURE analysis, data were scored as dominant markers . 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 .

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

Morphometric analyses

In present study 60 plant samples were collected from 10 geographical populations. ANOVA test revealed significant difference in quantitative morphological characters among the studied populations ($P < 0.05$). Clustering and PCA plot of *Salicornia persica* populations based on morphological characters produced similar results therefore only PCA plot is presented and discussed (Fig. 2). The result showed morphological difference/ divergence among most of the studied populations. This morphological difference was due to quantitative characters only. For example, character (Length of longest basal 1st order branch), separated population No. 1-4, character (Length of longest (regular) ultimate 1st order branch) separated population No. 8, while character Length of 2nd fertile segment measured over the central flower of cyme, separated populations 5 and 6,7 from the other populations.

RAPD analysis

Out of 20 RAPD primers used, 10 primers produced reproducible bands. In total, 146 RAPD bands (loci) were obtained with average of 14.6 bands per primer and only 10 bands ranging in size from 100– 2000 bp were common in all 10 populations studied and 129 bands were polymorph.

The mean percentage of polymorphism was 88.67%. RAPD primers including OPB-05, OPA-13, produced the highest polymorphism (85-100%), while OPA-10, OPC-01 produced the lowest percentage of polymorphism (69-77%). The mean value of effective alleles, Shannon index and genetic diversity of RAPD loci studied were 1.06, 0.20 and 0.25, respectively, while OPA-10 primers showed the highest number of effective alleles (1.098), Shannon index (0.377) and genetic diversity (0.34) (Table 3).

Among the primers used OPA-10 produced the highest number of bands (24), while primers OPA-04 produced the lowest number of bands (7). The Primer OPA-04 also produced the highest number of polymorphic bands (22).

The primer OPC-01 produced 8 specific bands, primer OPH-07 produced 6 specific bands, while some

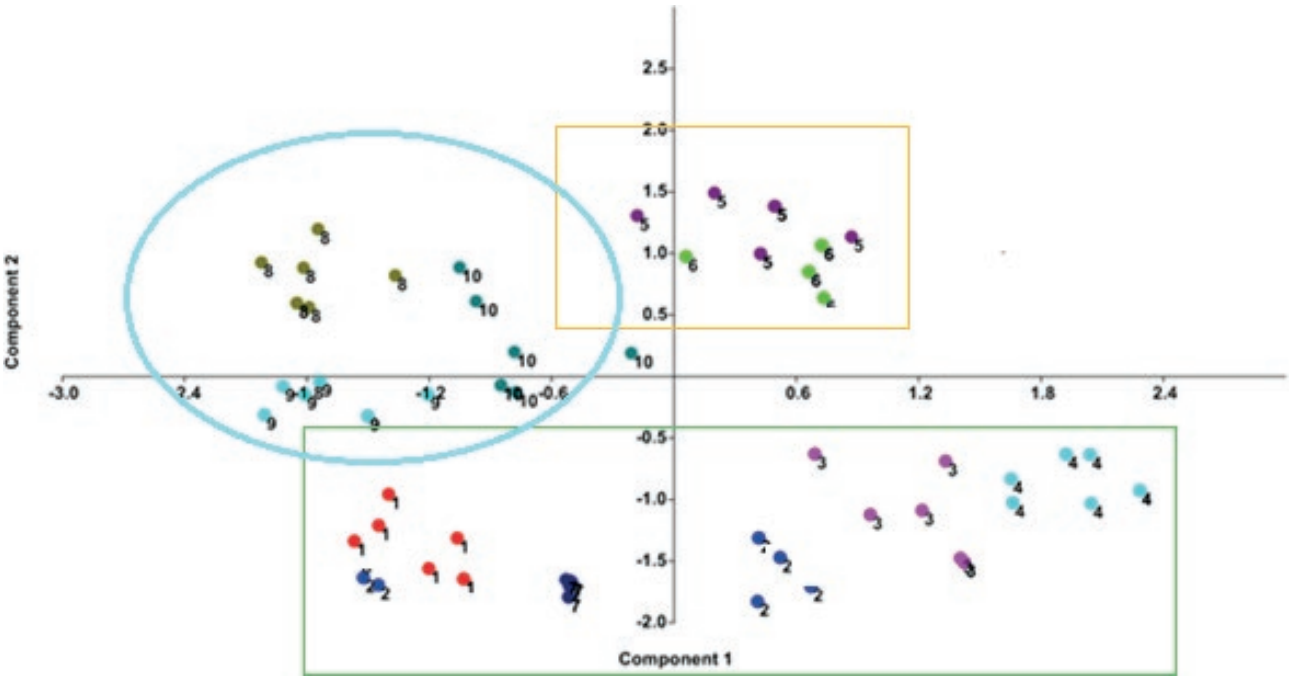

Figure 2. PCA plot of morphological data in *Salicornia persica* populations studied; 1. Esfahan, Varzaneh ; 2. Esfahan, the river of Zayanderud at Varzaneh; 3. Esfahan, Nain, it is 70km to Varzaneh; 4. Esfahan; Varzaneh to Kashan, 5. Fars, Tashk lake 6. Fars, Lake Bakhtegan; 7. Fars, Abadeh Tashk.8. Yazd, Kavire Marvast .9. Yazd, Meybud. 10. Yazd, Nádüshan.

Table 3. Genetic diversity parameters in the studied populations of *Salicornia persica* (N = number of samples, Ne = number of effective alleles, I= Shannon's information index, He = gene diversity, UHe = unbiased gene diversity, P%= percentage of polymorphism, populations).

RAPD Loci	Total band	Na	Ne	I	He	UHe	%P
OPB05	19	0.201	1.00	0.29	0.21	0.22	42.23%
OPA04	7	0.341	1.058	0.24	0.20	0.20	53.75%
OPA10	24	0.455	1.098	0.377	0.34	0.32	55.05%
OPM10	10	0.499	1.067	0.24	0.23	0.24	49.26%
OPH07	11	0.555	1.020	0.22	0.24	0.28	43.53%
OPA13	15	0.431	1.088	0.29	0.25	0.25	41.53%
OPA05	20	0.255	1.021	0.23	0.28	0.22	47.15%
OPC01	11	0.724	1.067	0.143	0.095	0.180	38.74%
OPI12	16	1.115	1.003	0.257	0.251	0.280	52.57%
OPA09	12	1.215	1.075	0.157	0.271	0.270	40.57%
Mean	14.6	0.77	1.05	0.28	0.25	0.25	49.13%

other primers produced 4 and 3 specific bands and the primers OPA-04, OPM-10 and OPA-10 produced 1 specific band.

Some of the populations showed the presence of specific bands, for example only Esfahan, the river of Zayanderud at Varzaneh population had band 1100 bp of RAPD primer OPA-10, band 900 bp of the primer OPM-10 and band 730 bp of the primer OPA-04. The Fars, Lake Bakhtegan population showed specific bands of 420 bp of the primer OPB-05, while Esfahan, Nain, it is 70km to Varzaneh population was the only population having band 150 bp of the primer OPA-09, band 635 bp of the primer OPC-01 and 220 bp of OPH-07. Yazd, Kavire Marvast population had the band of 370 bp of the primer OPA-13. Some bands were present in all except one population, for example bands 930 bp of the

Table 4. Analysis of molecular variance (AMOVA) of the studied species.

Source	df	SS	MS	Est. Var.	%	*ΦPT*
Among Pops	18	226.576	22.327	19.082	59%	59%
Within Pops	12	104.767	18.530	21.530	41%	
Total	30	330.342		40.613	100%	

primer OPA-05 and 870 bp of OPM-10 were absent only in Yazd, Kavire Marvast population.

Population genetic differentiation

AMOVA (PhiPT = 0.66, P = 0.010), and Gst analysis (0.279, p = 0.001) revealed significant difference among the studied populations (Table 4). It also revealed that, 41% of total genetic variability was due to within population diversity and 59% 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 = 0.279, P = 0.001) and Jost, differentiation index (D-est = 0.777, P = 0.001). These results indicate that the geographical populations of *Salicornia persica* are genetically differentiated from each other.

Populations genetic affinity

UPGMA tree and Neighbor-Net network produced similar results therefore only Neighbor-Net network is presented and discussed (Fig. 3). 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 1 and 2, as well as populations 6 and 7 show closer genetic affinity and are placed close to each other.

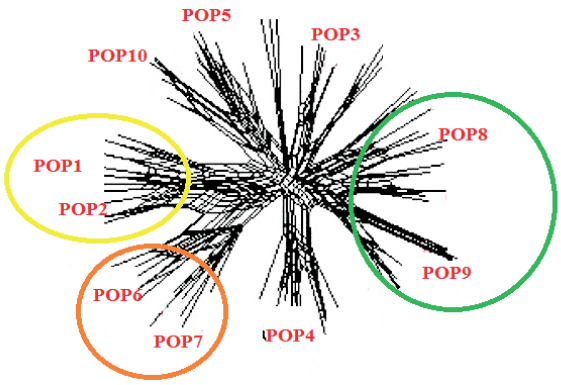

Figure 3. Neighbor-Net of RAPD data in *Salicornia persica* populations studied. 1. Esfahan, Varzaneh ; 2. Esfahan, the river of Zayanderud at Varzaneh; 3. Esfahan, Nain, it is 70km to Varzaneh; 4. Esfahan; Varzaneh to Kashan, 5. Fars, Tashk lake 6. Fars, Lake Bakhtegan; 7. Fars, Abadeh Tashk.8. Yazd, Kavire Marvast .9. Yazd, Meybud. 10. Yazd, Nádüshan.

Nei's genetic identity and genetic distance for RAPD data determined among the *Salicornia persica* are given in Table 5. The value of genetic identity varied from 0.552 between Esfahan, Varzaneh and Yazd, Meybud to 0.918 between Esfahan, Nain, it is 70km to Varzaneh and Esfahan; Varzaneh to Kashan. The mean value of genetic identity for RAPD markers was 0.75.

WARD and NJ dendrograms of RAPD data produced similar results supported by PCA ordination plot (Fig. 2). The Cophenetic correlation of WARD tree was higher (r = 0.95) and therefore, it is discussed below. In general, two major clusters were obtained. The populations of Esfahan, Varzaneh ; 2. Esfahan, the river of Zayanderud at Varzaneh; 3. Esfahan, Nain, it is 70km

Table 5. Nei's genetic identity (above diagonal) for RAPD data among *Salicornia persica.*

pop1	pop2	pop3	pop4	pop5	pop6	pop7	pop8	pop9	pop10	
1.000										pop1
0.882	1.000									pop2
0.838	0.755	1.000								pop3
0.883	0.683	0.918	1.000							pop4
0.594	0.591	0.714	0.741	1.000						pop5
0.719	0.591	0.808	0.871	0.750	1.000					pop6
0.652	0.737	0.764	0.735	0.609	0.794	1.000				pop7
0.706	0.573	0.837	0.744	0.621	0.784	0.724	1.000			pop8
0.552	0.737	0.764	0.735	0.609	0.729	0.875	0.868	1.000		pop9
0.534	0.581	0.714	0.641	0.621	0.639	0.661	0.635	0.829	1.000	pop10

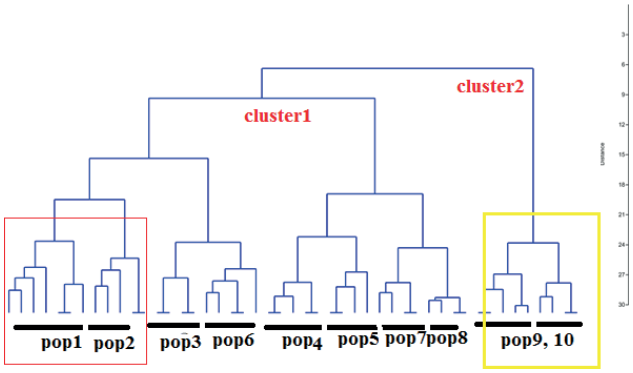

Figure 4. NJ tree of RAPD data in *Salicornia persica* populations studied. 1. Esfahan, Varzaneh ; 2. Esfahan, the river of Zayanderud at Varzaneh; 3. Esfahan, Nain, it is 70km to Varzaneh; 4. Esfahan; Varzaneh to Kashan, 5. Fars, Tashk lake 6. Fars, Lake Bakhtegan; 7. Fars, Abadeh Tashk.8. Yazd, Kavire Marvast .9. Yazd, Meybud. 10. Yazd, Nádüshan.

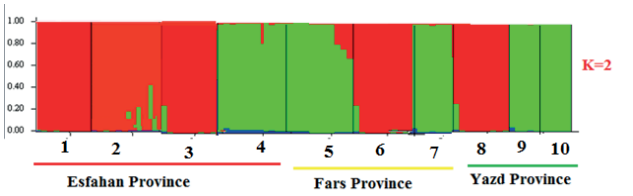

Figure 5. STRUCTURE plot of RAPD data in *Salicornia persica* populations studied. 1. Esfahan, Varzaneh ; 2. Esfahan, the river of Zayanderud at Varzaneh; 3. Esfahan, Nain, it is 70km to Varzaneh; 4. Esfahan; Varzaneh to Kashan, 5. Fars, Tashk lake 6. Fars, Lake Bakhtegan; 7. Fars, Abadeh Tashk.8. Yazd, Kavire Marvast .9. Yazd, Meybud. 10. Yazd, Nádüshan.

to Varzaneh; 4. Esfahan; Varzaneh to Kashan, 5. Fars, Tashk lake 6. Fars, Lake Bakhtegan; 7. Fars, Abadeh Tashk.8. Yazd, Kavire Marvast protected formed the first major cluster, out of which, Esfahan, Varzaneh and Esfahan, the river of Zayanderud at Varzaneh show more RAPD similarity and the Esfahan; Varzaneh to Kashan, 5. Fars, Tashk lake 6. Fars, Lake Bakhtegan; 7. Fars, Abadeh Tashk.8. Yazd, Kavire Marvast protected population differ the most from the other populations.

Two other populations form the second major cluster, which, Yazd, Meybud and Yazd, Nádüshan showed close genetic affinity. Mantel test after 5000 permutations produced significant correlation between genetic distance and geographical distance in these populations ($r = 0.55$, $P = 0.001$). Therefore, the populations that are geographically more distant have less amount of gene flow, and we have isolation by distance (IBD) in the *Salicornia persica*.

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 (Fig. 5). Both these analyses revealed that *Salicornia persica* populations show genetic stratification.

STRUCTURE plot based on k = 2, revealed genetic affinity between populations 1: Esfahan, Varzaneh; 2. Esfahan, the river of Zayanderud at Varzaneh; 3. Esfahan, Nain, it is 70km to Varzaneh; (red colored), also population 9: Yazd, Meybud. 10. Yazd, Nádüshan (green colored), as well as populations 5 and 7 (green colored). The mean Nm = 0.33 was obtained for all RAPD 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 not included), revealed some amount of shared alleles among populations 7 and 8, and between 10 and 6 and 7, also between 8, and 9. This result is in agreement with grouping we obtained with PCA 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 *Salicornia persica* populations.

DISCUSSION

Nature is having a hard time where human activities, global environmental changes, habitat loss and species extinction often lead to a loss of biodiversity. For example, habitat fragmentation and population decline could reduce the effective population size and threaten the viability of the target species (Falk and Holsinger, 1991; Paul *et al.* 2021; Salami *et al.* 2021; Wasana *et al.* 2021). Many biologists argue that establish correct conservation strategies minimizing biodiversity lossand a good example is conserve geographically-rare species (Esfandani-Bozchaloyi et al., 2018a, 2018b, 2918c, 2018d). Programs to conserve rare and endemic plants should take into account the use of molecular markers because can contribute to the setting of conservation priorities (Frankham *et al.*, 2004). Unfortunately, limited information is available regarding the population genetics of rare, endemic, threatened or endangered species. Endemic (and rare) plants with narrow distri-

bution range have been analyzed traditionally within the framework of the theoretical predictions of small populations. In these taxa, the lowest population genetic diversity levels are expected, and many study cases confirm such predictions (Cole, 2003). Our study of the genetic structure of *Salicornia persica* populations has important implications for the conservation and management of this narrowly distributed.

Molecular analyses

Salicornia species can generally tolerate immersion in salt water. They use the C 4 to take in carbon dioxide from the surrounding atmosphere. *Salicornia* has leafless stems with branches that resembles asparagus. Taxonomic studies of the genus by Moss (1954), Duval-Jouve (1868), Scott (1977), Ball (1964) and others Ball and Tutin (1959), Ball and Brown (1970) and the recent revision of Eurasian *Salicornias* by Kadereit and her school (Kadereit *et al*.,2012), are notable. There are three regional studies of *Salicornia* that were based on molecular data (Papini *et al.,* 2004; Murakeözy *et al.,* 2007;). Papini *et al.* (2004) found that diploid and tetraploid accessions of *Salicornia* resolved as sister clades. The study was based on ITS sequences of twelve samples of *Salicornia* (all but one from Italy) representing four species (three tetraploid, one diploid). The presence of RAPD polymorphic bands in the populations studied indicates the presence of genetic polymorphism in these populations. Moreover, the occurrence of specific bands/loci only in some of the populations illustrates the occurrence of unique insertion/deletion in DNA material of these genotypes. As stated before, there were bands which occurred in some of the populations but were absent in the others. Since, even single base change at the primer annealing site is manifested as appearance or disappearance of RAPD and ISSR bands, these bands may indicate the occurrence of genetic changes in the genome of the populations through the loss or rearrangement of some of their nucleotides (Smith *et al*. 1996; Dadzie *et al.* 2021; Fikirie *et al.* 2020; Hindersah & Kalay 2021; Hindersah *et al*. 2021; Mieso & Befa 2020). Both RAPD and morphological trees obtained are in general agreement separating the populations studied in two groups, indicating their genetic difference, which is also partly supported by ISSR analysis.

Diversity Study in Salicornia *species*

In field growing plants morphological variations can be observed in growth pattern, plant canopy and number of other features. Genetic diversity of the parental lines is good indicator of progeny performance. Success through hybridization and subsequent selection depends primarily on the selection of parents having high genetic variability for various agronomic traits. Sanane *et al.* (2003) conducted a study in Japan on identification of *Salicornia* population through morphological and RAPD fingerprinting. They observed variations in plant length, segment number, length and number of branches, and incidence of the secondary branches etc. on the basis of genotype based on the RAPD marker they identified five groups in three selected populations. Gohil and Pandya (2006) conducted study to find out the degree and the nature of genetic divergence among *Salicornia brachiata* (Roxb.) genotypes. Gohil and Pandya (2006) found a significant difference amongst the *salicornia* genotypes for all the phenological characters, (like height, canopy, main branch, segment, spike length, spike/branch and seed yield) indicating high genetic variability present in the population. The genotypes under study were grouped into five clusters, indicating wide diversity in the material for majority of the characters. Zhang and Bai (2022) studied in Iran on population differentiation and gene flow of *Salicornia persica* using ISSR markers and morphological traits. Analysis of molecular variance (AMOVA) test revealed significant genetic difference among the studied populations, and also showed that 45% of total genetic variability was due to the diversity within the population, while 55% was due to the genetic differentiation among populations.

Previous results from molecular studies imply near 100% inbreeding in *Salicornia*, which certainly contributes greatly to the taxonomic difficulties in the group because of inbreeding lines with minute but fixed phenotypic differences (Noble *et al*., 1992). DNA polymorphism was detected among the three Spanish populations of *Salicornia* using Random Amplification of Polymorphic DNA (RAPD) approach (Luque *et al*., 1995). The other study using RAPD technique showed correlations between DNA polymorphism and geographical distribution in *S. ramosissima* (Kruger *et al*., 2002). According to Kadereit *et al.* (2007), the main reason for the taxonomic confusion are the young age of the extant lineages, the rampant dispersal of *Salicornia* which has led to widespread genotypes with high phenotypic plasticity. This is the reason why *Salicornia* plants have different names in different regions, and morphological parallelism resulted in the fact that different genotypes have the same name in one region. Anita K. Badlani, (2011) was undertaken to assess the genetic diversity among germplasm of *Salicornia* collected from 11 different locations using RAPD and ISSR marker system.

This study will provide the genetic back ground of *S. brachiata* populations and extent of molecular diversity existing among them. The characterized diversity and identified polymorphic markers can be a good source of plant genetic resources and can be further exploited for genetic improvement of the species through marker assisted breeding.

CONCLUSIONS

This study proved the potential of RAPD marker analyses in the identification and evaluation of genetic relationships between populations of *Salicornia persica*. This is the first report about the application of these techniques to estimate the genetic variation and genetic characterization of *Salicornia persica* accessions. The results of this study can be useful in breeding programs, planning of conservation strategies, germplasm collection, and taxonomy of the genus.

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REFERENCES

Akhani H. 2003. *Salicornia persica* Akhani (Chenopodiaceae), a remarkable new species from Central Iran. *Linz. Biol. Beit.,* 35: 607-612.

Al-Oudat, M. Qadir, M. 2011. The halophytic flora of Syria. International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria.

Ball, P. W. 1964. A taxonomic review of *Salicornia* in Europe. Feddes Repert. 69: 1–8.

Ball, P. W. and Tutin, T. G. 1959. Notes on annual species of *Salicornia* in Britain. Watsonia 4: 193–205.

Ball, P. W. and Brown, K. G. 1970. A biosystematic and ecological study of *Salicornia* in the Dee Estuary. Watsonia 8: 27–40.

Bi, D., C. Dan, M. Khayatnezhad, Z. Sayyah Hashjin, Z. Y. Ma. 2021. Molecular Identification And Genetic Diversity In Hypericum L.: A High Value Medicinal Plant Using Rapd Markers Markers. Genetika 53(1): 393-405.

Chen, W., Khayatnezhad, M., Sarhadi, N. 2021. Protok Gena I Struktura Populacije Kod Allochrusa (Caryophylloideae, Caryophyllaceae) Pomocu Molekularnih Markera Genetika 53(2): 799-812.

Cole, C.T. 2003. Genetic variation in rare and common plants. Annu. Rev. Ecol. Evol. Syst. 34: 213-237.

Choi, D., Lim, G. S., Piao, Y. L., Choi, O. Y., Cho, K. A., Park, C. B., Chang, Y. C., Song, Y. I., LEE, M. K. & Cho, H. 2014. Characterization, stability, and antioxidant activity of *Salicornia herbaciea* seed oil. Korean Journal of Chemical Engineering, 31, 2221-2228.

Dadzie, R. G., Amoah, R. S. ., Ampofo-Asiama, J., Quaye, B., Kizzie-Hayford, N., & Abano, E. E. (2021). Improving the Storage Quality of Eggplants (Solanum Aethiopicum L.) Fruit using Aloe Vera Gel Coating . *Journal of Food Technology Research*, *8*(2), 58–66.

Duval-Jouve, M.J., 1868. Des Salicornia de l'Herault. Bull. Soc. Bot. France 15, 165–178.

Evanno, G, S., Regnaut, J., Goudet 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol., 14:2611-2620.

Esfandani-Bozchaloyi S, Sheidai M, Keshavarzi M, Noormohammadi Z 2017a. Genetic Diversity and Morphological Variability In *Geranium Purpureum* Vill. (Geraniaceae) Of Iran. Genetika 49: 543 - 557. https://doi.org/10.2298/GENSR1702543B

Esfandani-Bozchaloyi S, Sheidai M, Keshavarzi M, Noormohammadi Z 2017b. Species Delimitation In *Geranium* Sect. *Batrachioidea*: Morphological and Molecular. Act Bot Hung 59(3–4):319–334. doi: 10.1556/034.59.2017.3-4.3

Esfandani-Bozchaloyi S, Sheidai M, Keshavarzi M, Noormohammadi Z 2017c. Genetic and morphological diversity in *Geranium dissectum* (Sec. Dissecta, Geraniaceae) populations. Biologia 72(10): 1121- 1130. DOI: 10.1515/biolog-2017-0124

Esfandani-Bozchaloyi S, Sheidai M, Keshavarzi M, Noormohammadi Z 2017d. Analysis of genetic diversity in *Geranium robertianum* by ISSR markers. Phytologia Balcanica 23(2):157–166.

Esfandani-Bozchaloyi S, Sheidai M, Keshavarzi M, Noormohammadi Z 2018a. Species Relationship and Population Structure Analysis In *Geranium* Subg. *Robertium* (Picard) Rouy with The Use of ISSR Molecular Markers. Act Bot Hung, 60(1–2), pp. 47–65.

Esfandani-Bozchaloyi S, Sheidai M, Keshavarzi M, Noormohammadi Z 2018b. Species Identification and Population Structure Analysis In *Geranium* Subg. *Geranium* (Geraniaceae). Hacquetia, 17/2 , 235–246 DOI: 10.1515/hacq-2018-0007

Esfandani -Bozchaloyi S, Sheidai M, Keshavarzi M, Noormohammadi Z 2018c. Morphometric and ISSR-analysis of local populations of *Geranium molle* L. from the southern coast of the Caspian Sea. Cytology and genetics, 52, 4, 309–321.

Esfandani -Bozchaloyi S, Sheidai M, 2018d. Molecular diversity and genetic relationships among *Geranium pusillum* and *G. pyrenaicum* with inter simple sequence repeat (ISSR) regions, Caryologia, 71,4,1-14.https://doi.org/10.1080/00087114.2018.1503500

Esfandani-Bozchaloyi S, Sheidai M, 2019. Comparison Of Dna Extraction Methods From *Geranium* (Geraniaceae), *Acta Botanica Hungarica* 61(3–4),251–266

Falk, D.A. Holsinger K.E. (Eds.). 1991. Genetics and conservation of rare plants. Oxford Univ. Press, New York.

Fikirie, K., Bezu, A., Eshetu, M., Bekele, D., & Rabo, M. (2020). Evaluate Technical Standards of Implemented Soil Bund in Central Rift Valley of Ethiopia: The Case of Adama, Lume and Dodota Districts. *Agriculture and Food Sciences Research*, *7*(1), 51–57.

Freeland, Jr., H., Kirk, S.D., Peterson 2011. Molecular Ecology, 2nd Ed. Wiley-Blackwell, Chichester, 464 pp.

Frankham, R., Ballou, J.D., Briscoe, D.A. & McInnes, K.H. 2004. A Primer of conservation genetics. Cambridge Univ. Press, Cambridge.

Gohil R. H and Pandya J. B. 2006.genetic divergence in salicornia (salicornia brachiata roxb.) Indian j. Genet., 66(1): 75-76.

Glenn, E. P., Oleary, J. W., Watson, M. C., Thompson, T. L. & Kuehl, R. O. 1991. *Salicornia bigelovii* Torr.: an oilseed halophyte for seawater irrigation. Science, 251 (4997): 1065-1067.

Hindersah, R., & Kalay, A. M. (2021). Azotobacter and Total Bacterial Population in Enriched Compost: Preliminary Study. *Current Research in Agricultural Sciences*, *8*(1), 31–36. https://doi.org/10.18488/journal.68.2021.81.31.36

Hindersah, R., Fitriatin, B. N., Setiawati, M. R., & Risanti, R. R. (2021). Effect of Beneficial Soil Microbes on Growth and Yield of Celery in Volcanic Soil of West Java. *Current Research in Agricultural Sciences*, *8*(2), 90–96.

Hammer, Q., Harper, Dat., Ryan, P. D., 2012.PAST: Paleontological Statistics software package for education and data analysis. Palaeonto. Electro. 4: 9.

Ingrouille, M.J., and J., Pearson 1987. The pattern of the morphological variation in the *Salicornia europaea* L. aggregate (Chenopodiaceae). *Watsonia* 16, 269-281.

Jia, Y., M. Khayatnezhad, S. Mehri 2020. Population differentiation and gene flow in Rrodium cicutarium: A potential medicinal plant. Genetika 52(3): 1127-1144.

Kruger, A.M., Hellwig, F.H., and C., Oberprieler 2002. Genetic diversity in natural and antropogenic inland populations of salt-tolerant plants: random amplified polymorphic DNA analysis of *Aster tripolium* L. (Compositae) and *Salicornia ramosissima* Woods (Chenopodiaceae). *Mol.* Ecol. 11, 1647-1655.

Kadereit, G., P. Ball, S. Beer, L. Mucina, D. Sokoloff, P. Teege, A.E. Yaprak, Freitag, H. 2012. A taxonomic nightmare comes true: phylogeny and biogeography of glassworts (*Salicornia* L., Chenopodiaceae). Taxon, 56: 1143-1170.

Kadereit, G., Mucina, L. Freitag, H. 2006. Phylogeny of Salicornioideae (Chenopodiaceae): diversification, biogeography, and evolutionary trends in leaf and flower morphology. Taxon 55: 617–642.

Luque, T., Ruiz, C., Avalos, J., Calderon, I.L., and M.E., Figueroa 1995. Detection and analysis of genetic variation in *Salicornieae* (Chenopodiaceae) using random amplified polymorphic DNA (RAPD) markers. Taxon 44, 53-63.

Li, A. Mu, X. Zhao, X. Xu, J. Khayatnezhad, M. Lalehzari, R. 2021. Developing the non-dimensional framework for water distribution formulation to evaluate sprinkler irrigation; Irrigation And Drainage; 70,4: 659-667.

Liu, S., Wang, Y., Song, Y., Khayatnezhad, M., & Minaeifar, A. A. 2021. Genetic variations and interspecific relationships in Salvia (Lamiaceae) using SCoT molecular markers. Caryologia, 74(3), 77-89.

Ma, S., M. Khayatnezhad, A. A. Minaeifar. 2021. Genetic diversity and relationships among Hypericum L. species by ISSR Markers: A high value medicinal plant from Northern of Iran. Caryologia, 74(1): 97-107.

Moss, C.E., 1954. The species of Arthrocnemum and Salicornia in Southern Africa. J. South Afr. Bot. 20, l–22.

Mieso, B., & Befa, A. (2020). Physical Characteristics of the Essential Oil Extracted from Released and Improved Lemongrass Varieties, Palmarosa and Citronella Grass. *Agriculture and Food Sciences Research*, *7*(1), 65–68.

Murakeozy, E. P. et al. 2007. Phylogenetic relationships and genetic diversity of the *Salicornieae* (Chenopodiaceae) native to the Atlantic coasts of France. – Plant Syst. Evol. 264: 217–237.

Noble, S.M., Davy, A.J. and R.M., Oliver 1992. Ribosomal DNA variation and population differentiation in *Salicornia* L. New Phytol. 122, 553–565.

Papini, A. Trippanera, G.B. Maggini, F., Filigheddu, R., Biondi, E. 2004. New insights in Salicornia L. and allied genera (Chenopodiaceae) inferred from nrDNA sequence data. Plant Biosyst.

Podani, J., 2000. Introduction to the Exploration of Multivariate Data English translation., Leide, Backhuyes publisher, 2000.

Peng, X., M. Khayatnezhad, L. Ghezeljehmeidan. 2021. Rapd profiling in detecting genetic variation in stellaria l. (caryophyllaceae). Genetika-Belgrade, *53*(1): 349-362.

Paul, S., Ara, R., Ahmad, M. R., Hajong, P., Paul, G., Kobir, M. S., & Rahman, M. H. (2021). Effect of Blanching Time and Drying Method on Quality of Black Pepper (Piper nigrum). *Journal of Food Technology Research*, *8*(1), 18–25.

Peakall, R., Smouse, P. E., 2006.GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research, Mol. Ecol. Notes., 2006,6, 288-295.

Pritchard, J.K., M., Stephens, P., Donnelly 2000. Inference of population structure using multilocus genotype Data. Genetics, 155: 945–959.

Smith JF, Burke CC, Wogner WL 1996. Interspecific hybridization in natural populations of cyrtandra (Generiaceae) on the Hawaiian Islands: evidence from RAPD markers. Plant Syst. Evol. 200: 61-77.

Si, X., L., Gao, Y. Song, M, Khayatnezhad, A.A. Minaeifar 2020. Understanding population differentiation using geographical, morphological and genetic characterization in *Erodium cicunium*. Indian J. Genet., 80(4): 459-467.

Shi, B., Khayatnezhad, M., Shakoor, A. 2021. The interacting effects of genetic variation in Geranium subg. Geranium (Geraniaceae) using scot molecular markers. Caryologia, 74(3), 141-150.

Salami, H. A., Matemilola, S., Fasasi, S. A., Ahmed, M. O., Adigun, I. A., Adeleke, A. A., Fashina, S. M., & Olowosokedile, O. (2021). Towards Achieving Food Security in Nigeria: A Fuzzy Comprehensive Assessment of Heavy Metals Contamination in Organic Fertilizers. *Current Research in Agricultural Sciences*, *8*(2), 110–127.

Sagane, Y., Sato, K., and S., Momonoki 2003. Identification of Salicornia Populations: Comparison between Morphological Characterization and RAPD Fingerprinting. Plant Production Science 4 (6), 287-294.

Shepherd, K.A., Macfarlane, T.D. & Colmer, T.D. 2005. Morphology, anatomy and histochemistry of Salicornioideae (Chenopodiaceae) fruits and seeds. Ann. Bot. 95: 917–933.

Weising, K., Nybom, H., Wolff, K. and Kahl, G. , 2005. DNA Fingerprinting in Plants. Principles, Wang, C., Y. Shang, M. Khayatnezhad 2021. Fuzzy Stress-based Modeling for Probabilistic Irrigation Planning Using Copula-NSPSO. Water Resources Management. 35, 4943–4959

Wang, J., Ye, Q., Zhang, T., Shi, X., Khayatnezhad, M., Shakoor, A. 2021. Palynological analysis of genus Geranium (Geraniaceae) and its systematic implications using scanning electron microscopy. Caryologia, 74(3), 31-43.

Wasana, W. L. N., Ariyawansha, R., & Basnayake, B. (2021). Development of an Effective Biocatalyzed Organic Fertilizer Derived from Gliricidia Sepium Stem Biochar. *Current Research in Agricultural Sciences*, *8*(1), 11–30.

Yin, J., M. Khayatnezhad, A. Shakoor 2020. Evaluation of genetic diversity in Geranium (*Geraniaceae*) using rapd marker. Genetika, 53(1): 363-378.

Zheng, R., S. Zhao, M. Khayatnezhad, S, Afzal Shah 2021. Comparative study and genetic diversity in Salvia (Lamiaceae) using RAPD Molecular Markers. Caryologia, 74(2): 45-56.

Zhu, K., L. Liu, S. Li, B. Li, M. Khayatnezhad and A. Shakoor. 2021. "Morphological method and molecular marker determine genetic diversity and population structure in *Allochrusa*." Caryologia 74(2): 121-130.

Zhao Y, Wang H, Liang W, Khayatnezhad, M, Faisal. 2021. Genetic Diversity And Relationships Among Salvia Species By Issr Markers; Genetika-Belgrade, 53(2): 559-574.