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Population Differentiation and Gene Flow of *Salicornia persica* Akhani (Chenopodiaceae)

Xiaoju Zhang[1], Li Bai[2,*], Somayeh Esfandani-Bozchaloyi[3]

[1] *College of Humanities and Management, Xi'an Traffic Engineering Institute, Xi'an 710300, Shaanxi, China*
[2] *Department of Mechanical and Electrical Technology, XiJing University, Xi'an 710123, Shaanxi, China*
[3] *Department of Plant Sciences, university Shahid Beheshti, Iran*
*Corresponding author. E-mail: baili891066611@163.com

Abstract. The genus *Salicornia* (Amaranthaceae) was established by Linnaeus. Commonly known as 'glassworts', the species of the genus are articulated succulent herbs with cortical palisade, opposite decussate scale-leaves, thyrsoid cymes, flowers packed in cauline depressions and the diaspore composed of l-seeded utricle. Therefore, due to the importance of the plant species, we performed a combination of morphological and molecular data analyses on this species. A total of 72 randomly collected plants from 8 natural populations in 2 provinces were evaluated 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. A total number of 158 bands were detected by ISSR primers, of which 144 (89%) bands with an average of 14.4 bands per primer were polymorphic. The Percentage of Polymorphic Bands (PPB) ranged from 70% (ISSR-7) to 100% (ISSR-1, ISSR-4 and ISSR-5). The average Polymorphic Information Content (PIC), Shannon's Information index (I), and Number of effective alleles (Ne) were 0.49, 0.28, and 1.09, respectively.

Keywords: genetic diversity, gene flow, genetic differentiation, *Salicornia persica*, Inter Simple Sequence Repeat (ISSR).

INTRODUCTION

Genetic diversity is a basic component of biodiversity and its conservation is essential for survival of any species in the changing environments (Si *et al*. 2020; Liu *et al.* 2021). Most authors agree that genetic diversity is necessary to preserve the long-term evolutionary potential of a species (Peng *et al.* 2021; Ma *et al.* 2021). This is very important in fragmented populations, because they are more vulnerable due to the loss of allelic richness and increased population differentiation by genetic drift (decreased heterozygosity and eventual fixation of alleles) and inbreeding depression (increased homozygosity within populations; Chen *et al.* 2021; Bi *et al.* 2021). Therefore,

understanding the genetic variability and diversity within and among different populations is crucial for their conservation and management (e.g., Esfandani-Bozchaloyi *et al.,* 2018a, 2018b, 2018c).

The genus *Salicornia* (Amaranthaceae) was established by Linnaeus (1753). Commonly known as 'glassworts', the species of the genus are articulated succulent herbs with cortical palisade, opposite decussate scale-leaves, thyrsoid cymes, flowers packed in cauline depressions and the diaspore composed of l-seeded utricle. Many species are green, but their foliage turns red in autumn. The hermaphrodite flowers are wind pollinated. The species inhabit saline habitats such asinland salt-marshes, saline seasonal river banks and tidal coastlines, but all tidal coasts and salines are not home to glassworts. *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.

The halophyte *Salicornia* supports soil microbial growth and boosts the TPHs degradation in saline oil-contaminated soils. Combining *Salicornia* and *P. aeruginosa* accelerates TPH degradation and reduces saline oil-contaminated soils phytotoxicity (Ebadi *et al.* 2018).

The first comprehensive account of family Chenopodiaceae in Flora Iranica (Hedge *et al.* 1997) provides very useful and fundamental data on the arid flora of the Old World. The genus *Salicornia* is among the most diverse genera of the Salicornieae tribe. The genus currently comprises 25 to 30 species (Kadereit *et al.* 2007). The taxonomy of the genus *Salicornia* is still far from satisfactory, although numerous species aggregates, species and microspecies have been described over the last 250 years. Frequently the name *Salicornia europaea* is used in a very broad sense to include most of the species of the genus. Additionally, the plants show a high level of phenotypic plasticity (Ingrouille and Pearson 1987). The salinity of their habitats fluctuates greatly due to different factors - tidal cycles, evapotranspiration, precipitation and availability of fresh groundwater. This is the reason why *Salicornia* develops high physiological plasticity which causes phenotypic variation (Kadereit *et al.* 2007). Morphological distinction between the taxa is only possible when the plants are fresh, between flowering and fruiting (Gehu *et al.* 1979). Morphometric studies using all phenotypic differences available, irrespective of whether they have a genetic basis or not, could not reveal distinct taxa even on a small regional scale (Ingrouille and Pearson 1987).

Salicornia plants tend to have phenotypic variations depending on environmental conditions such as temperature, quality of soil, concentration of salt and population density. Ball and Akeroyd (1993) suggested that the specific limits of classification of the *Salicornia* plants based on morphological features, especially those of dried *Salicornia* plants, are obscure. To prove the relevance between the genotype and phenotype in *Salicornia* plants, genetic variability was analyzed by RAPD fingerprinting. The use of molecular markers is considered to be the best for genetic diversity analysis since it has proved to be non-invasive in the sense that there are no negative effects on the stage of development, environment or management practices. Furthermore, these kinds of studies can be applied even on dead plants when the genomic DNA is extractable (Choudhury *et al.* 2001).

Molecular markers play a significant role in the protection of biodiversity, identification of promising cultivars, quantitative trait loci (QTL) mapping, etc. Various PCR-based markers such as ISSR, SCoT, SRAP, etc. have been effectively used for the quantification of genetic diversity. Recent ISSR studies of natural populations have demonstrated the hypervariable nature of the markers and their potential use for the population-level studies (Hultén and Fries 1986). In the present study, the ISSR markers and morphologic traits were used for the first time in Iran to analyze the genetic diversity in 72 *Salicornia persica* accessions belonging to 8 different populations.

MATERIALS AND METHODS

Plant materials

For the morphometric and ISSR analyses, we used 72 plant accessions (four to twelve samples from each population) belonging to 8 different populations of *Salicornia persica* in Esfahan and Tehran Provinces of Iran during July-Agust 2018-2020 (Table 1). More information about the geographical distribution of the accessions are given in Table 1 and Fig. 1. The plant individuals were identified morphologically using different literature (Kadereit *et al.* 2007; Akhani 2003).

DNA extraction and ISSR analysis

Fresh leaves were randomly used from four to twelve samples for each of the studied populations. They dried by silica gel. The CTAB-activated charcoal protocol was used to extract genomic DNA (Esfandani-Bozchaloyi *et al.* 2019). For the ISSR analysis, 22 primers from the UBC (University of British Columbia) series were tested

Table 1. Voucher details and diversity within Iranian populations and subspecies of *Salicornia persica* in this study.

No	Subspecies	Locality
Pop1	subsp. *persica* Akhani	Esfahan,the river of Zayanderud at Varzaneh
Pop2	subsp. *persica* Akhani	Esfahan,Nain, it is 70km to Varzaneh
Pop3	subsp. *persica* Akhani	Fars, Tashk lake
Pop4	subsp. *persica* Akhani	Esfahan, northern coasts of Batlaq-e Gavkhooni
Pop5	subsp. *rudshurensis* Akhani	Tehran ;60 km west of Tehran, 25 km SE of Karaj
Pop6	subsp. *rudshurensis* Akhani	Tehran: ca. 60 km W Tehran, Mardabad salt flats, along Rude Shur
Pop7	subsp. *rudshurensis* Akhani	Tehran, Karaj located in 25 km NW Rude Shur
Pop8	subsp. *rudshurensis* Akhani	Tehran ; Rude-Shur River, which is located 40 km west of Tehran

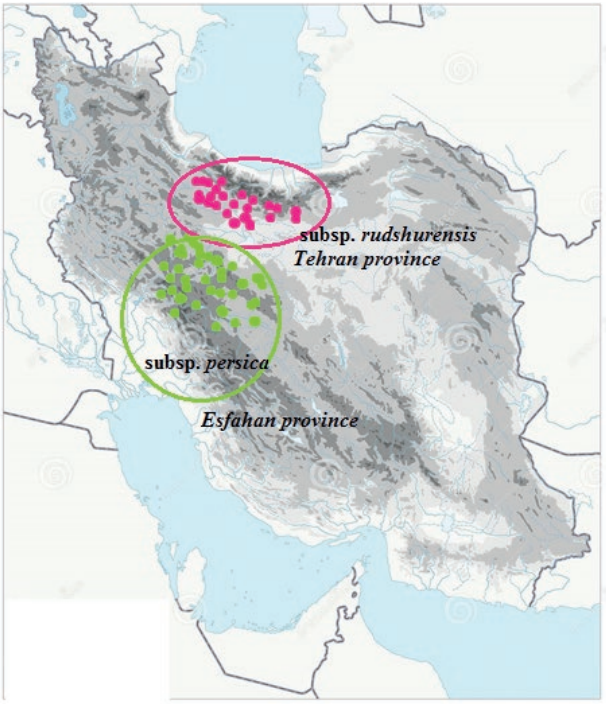

Figure 1. Distribution map of studied populations of *Salicornia persica* in Iran.

for the DNA amplification. Ten primers were chosen for the ISSR analysis of genetic variability based on band reproducibility (Table 2). The PCR reactions were carried out in a 25µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM $MgCl_2$; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of a single primer; 20 ng of 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 at 95°C, followed by 37 one-minute cycles at 95°C, 1 min at 50-56°C, and 1 min at 72°C. The reaction was completed by the final 5-10 min extension step 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 using a 100bp molecular size ladder (Fermentas, Germany).

DATA ANALYSIS

Morphological studies

A total of 19 metric and 6 multistate characterers were used for measurements in different combinations (Table not included), 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). The data obtained were standardized (mean= 0, variance = 1) and used to estimate the Euclidean distance for clustering and ordination analyses (Podani 2000). To group the plant specimens, The UPGMA (unweighted pair group with arithmetic mean), Ward's (Minimum spherical traits), and MDS (multidimensional scaling) ordination methods were used (Podani 2000). PAST software version 2.17 (Hammer *et al.* 2012) was used for the multivariate statistical analyses of the morphological data.

Molecular analysis

The ISSR profiles obtained for each sample were scored as binary traits. The discriminating capability of the used primers was evaluated by means of two param-

Table 2. Details about the banding pattern revealed by ISSR primers.

Primer name	Primer sequence (5'-3')	TNB	NPB	PPB	PIC	PI
ISSR-1	DBDACACACACACACACA	10	10	100.00%	0.28	5.11
ISSR-2	GGATGGATGGATGGAT	9	7	93.00%	0.38	6.41
ISSR-3	GACAGACAGACAGACA	24	20	87.00%	0.56	4.34
ISSR-4	AGAGAGAGAGAGAGAGYT	10	10	100.00%	0.49	3.88
ISSR-5	ACACACACACACACACC	15	15	100.00%	0.41	5.66
ISSR-6	GAGAGAGAGAGAGAGARC	11	9	94.00%	0.25	4.99
ISSR-7	CTCTCTCTCTCTCTCTG	13	7	70.00%	0.64	4.21
ISSR-8	CACACACACACACACAG	13	9	82.00%	0.32	4.32
ISSR-9	GTGTGTGTGTGTGTGTYG	12	10	93.00%	0.25	6.56
ISSR-10	CACACACACACACACARG	25	21	91.00%	0.57	4.11
	Average	15.8	14.4	89.00%	0.49	5.12
	Total	158	144			

Note: TNB - the number of total bands, NPB: the number of polymorphic bands, PPB (%): the percentage of polymorphic bands, PI: polymorphism index, PIC, polymorphism information content for each of ISSR primers.

eters, polymorphism information content (PIC) and marker index (MI), to characterize the capacity of each primer to detect polymorphic loci among the genotypes. The number of polymorphic bands (NPB) was calculated for each primer. The parameters like Nei's genetic diversity (H), Shannon's 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 following formula: H' = -Σpiln pi. Rp is defined per primer as: Rp = ∑ Ib, where "Ib" is the band informativeness, which takes the values of 1-(2x [0.5-p]), and "p" is 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 the 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). The 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) implemented in GenAlex 6.4 (Peakall and Smouse 2006) and Nei's GST analysis implemented in GenoDive ver.2 (2013) were used to show the genetic difference of the populations. Moreover, the populations' genetic differentiation was studied by $G(ST)_{est}$ = standardized measure of genetic differentiation (Hedrick 2005), and D_{est} = Jost measure of differentiation.

To assess the population structure of the *Salicornia persica* accessions, a heuristic method based on the Bayesian clustering algorithms was utilized. The clustering method based on the Bayesian model implemented in the STRUCTURE software (Falush et al., 2007) was used on the same data set to better detect the population substructures. This clustering method is based on an algorithm that assigns genotypes to homogeneous groups based on the number of clusters (K). Assuming Hardy-Weinberg and linkage equilibrium within the clusters, the software estimates allele frequencies in each cluster and population membership for each 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 burnins 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. Gene flow (Nm) was calculated using POPGENE (version 1.31) software. Gene flow was estimated indirectly using the following 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) among the populations, Mantel test was performed using Tools for Population Genetic Analysis (TFPGA; Miller, 1997) (computing 999 permutations). This approach considers an equal amount of gene flow among all populations. Population assignment test based on maximum likelihood was 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

Morphometry

The morphological evaluation revealed considerable variations among the accessions for spike characteristics. Based on the botanical traits, 41 out of 72 evaluated accessions were identified as subsp. *persica* and 31 accessions as subsp. *rudshurensis* (Fig. 1).

ANOVA showed significant differences ($P < 0.01$) in quantitative morphological traits among the populations under study. In order to determine the most variable traits among the taxa studied, the PCA analysis was performed. It revealed that the first three factors comprised over 76% of the total variations. In the first PCA axis with 52% of total variations, such traits as Length of visible part of central flower of cyme, 2nd fertile segment; Width of 2nd fertile segment at base showed the highest correlation (>0.7). Length of 2nd fertile segment measured over the central; Length of spike and Number of fertile segments on longest were the traits influencing the PCA axes 2 and 3, respectively. Different clustering and ordination methods produced similar results and, therefore, the PCA plot of morphological traits are presented here (Fig. 2). In general, the PCA plot of the studied populations did entirely delimit the studied populations and revealed that the plants in these populations are not intermixed.

Populations' genetic diversity

In the present study, 10 out of 22 selected ISSR primers amplified 158 clear discernible bands, of which 144 (80 %) were polymorphic, showing the high discriminative and resolving power of the used ISSRs in the studied germplasm. The total number of bands per primer ranged from 9 (ISSR-2) to 25 (ISSR-10), with an average of 15.8. The number of polymorphic bands per primer varied from 7 (ISSR-2, ISSR-7) to 21 (ISSR-10), with an average of 14.4. The band sizes of the amplified products were found between 100 and 3,000 bp. To characterize the capacity of each primer to detect polymorphism and to evaluate the discriminating capability of each primer in the studied germplasm, various diversity indices such as the highest percentage of polymorphic bands, *Ne, I* and, *PIC* were calculated. The highest percentage of polymorphic bands was produced by primers ISSR-1, ISSR-4 and ISSR-5 (100%), while primer ISSR-7 produced the lowest percentage of polymorphic bands (70%). The *PIC* values across all primers averaged 0.49. ISSR-7 showed the highest (0.64) and ISSR-6, ISSR-9 the lowest (0.25) *PIC* value, respectively (Table 2).

The genetic diversity parameters determined in 8 geographical populations of *Salicornia persica* are presented in Table 3. The highest value of polymorphism percentage (52.15%) was observed in Esfahan, northern coasts of Batlaq-e Gavkhooni (population No. 4, subsp. *persica*), which shows a high value for the genetic diversity (0.38) and Shannon's information index (0.45). The population of Tehran*;* Rude-Shur River, which is located 40 km west of Tehran (No. 8, subsp. *rudshurensis*) has the lowest value for the percentage of polymorphism (15.91%) and the lowest value for Shannon's information index (0.17) and He (0.18).

Populations' genetic differentiation

AMOVA (PhiPT = 0.88, P = 0.0010) revealed significant difference among the studied populations (Table 4). It also revealed that 45% of total genetic variations was due to the diversity within the population and 55% was due to the genetic differentiation among the populations. The pairwise comparison of Nei's genetic identity among the studied populations *Salicornia persica* (Table

Table 3. Genetic diversity parameters in the studied *Salicornia persica* populations.

SP	N	Na	Ne	I	He	UHe	%P
Pop1	16.000	0.892	1.168	0.321	0.251	0.265	34.63%
Pop2	6.000	0.344	1.035	0.31	0.23	0.25	40.53%
Pop3	11.000	0.441	1.036	0.33	0.25	0.27	42.53%
Pop4	8.000	0.247	1.021	0.45	0.38	0.33	52.15%
Pop5	5.000	0.290	1.024	0.23	0.25	0.18	24.30%
Pop6	10.000	0.452	1.089	0.29	0.22	0.25	45.05%
Pop7	10.000	0.333	1.006	0.222	0.22	0.22	33.23%
Pop8	9.000	1.247	1.275	0.171	0.184	0.142	15.91%

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

Table 4. Analysis of molecular variance (AMOVA) of the studied *Salicornia persica*.

Source	df	SS	MS	Est. Var.	%	*ΦPT*
Among Pops	56	1221.364	52.789	21.154	55%	55%
Within Pops	120	114.443	12.905	12.905	45%	
Total	176	1365.807		33.060	100%	

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

Table 5. Pairwise Population Matrix of Nei Unbiased Genetic Identity.

pop1	pop2	pop3	pop4	pop5	pop6	pop7	pop8	
1.000								pop1
0.833	1.000							pop2
0.810	0.933	1.000						pop3
0.875	0.873	0.830	1.000					pop4
0.818	0.896	0.874	0.812	1.000				pop5
0.852	0.858	0.844	0.838	0.884	1.000			pop6
0.712	0.846	0.800	0.796	0.881	0.794	1.000		pop7
0.779	0.855	0.809	0.794	0.874	0.752	0.862	1.000	pop8

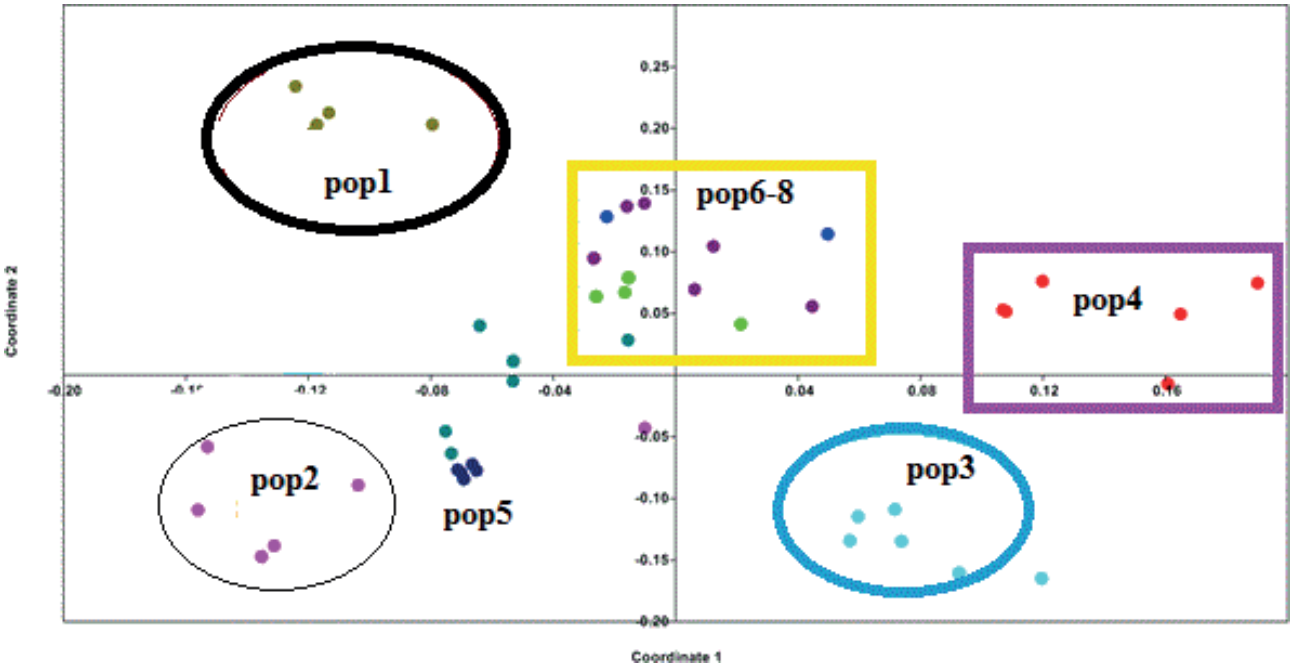

Figure 2. PCA plot of *Salicornia persica* populations based on morphological traits.

5) showed a higher genetic similarity (0.93) between the populations of Esfahan, Nain, it is 70km to Varzaneh (pop. No. 2) and Fars, Tashk lake (pop. No. 3), while the lowest genetic similarity value (0.712) occurred between Esfahan, the river of Zayanderud at Varzaneh (pop. No. 1) and Tehran, Karaj located in 25 km NW Rude Shur (pop. No. 7).

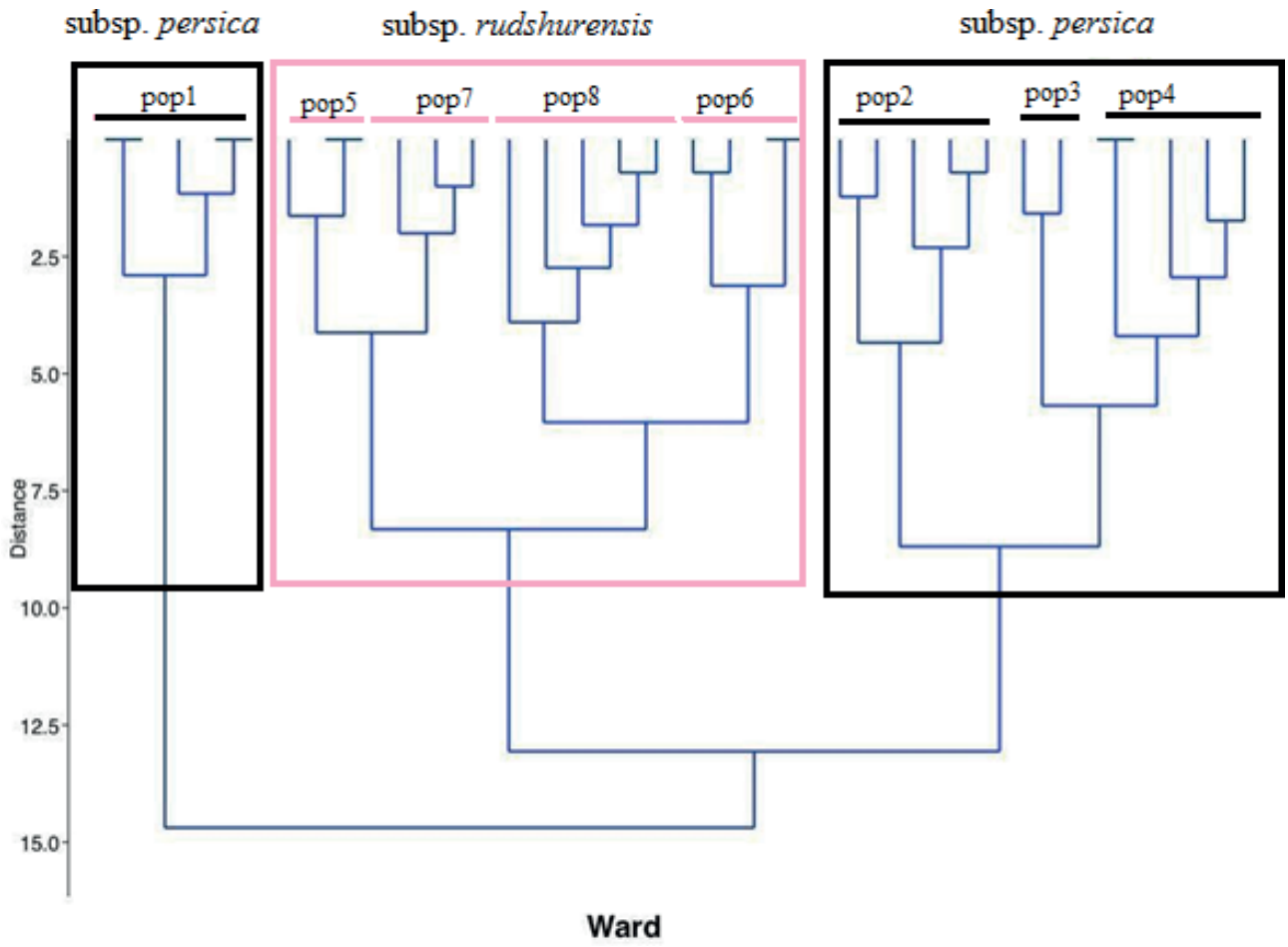

Figure 3. UPGMA plot of populations in *Salicornia persica* populations based on ISSR data (Population numbers according to Table 1).

Populations' genetic affinity

Two major clusters were formed in the UPGMA tree (Fig. 3). The first major cluster contained two sub-clusters: the population of Esfahan, Nain, it is 70km to Varzaneh; Fars, Tashk lake and Esfahan, northern coasts of Batlaq-e Gavkhooni (pop. No. 2,3,4, subsp. *persica*) is distinct and remains separate from the other populations with a great distance and comprises the first sub-cluster. The second sub-cluster was formed by the other populations from subsp. *rudshurensis*, (pop. No. 5-8) which showed close genetic affinity. The second major cluster contained only subsp. *persica,* which separated from the other studied populations and joined the others with a great distance. These results show that the plant specimens of each studied subspecies were grouped together, indicating that the subspecies are delimited based on the ISSR molecular markers. Therefore, this result confirms our morphology results. The Nm analysis by Popgene software also produced mean Nm= 0.17, which is considered a very low value of gene flow among the studied species. Mantel test after 5000 permutations produced significant correlation between genetic distance and geographical distance in these populations (r = 0.33, P = 0.001).

Populations' genetic structure

K = 2 reveal the presence of 2 genetic groups. Similar results were obtained by Evanno test performed on STRUCTURE analysis, which produced a major peak at k = 2. Both analyses revealed that *Salicornia persica* populations show genetic stratification. The STRUCTURE plot based on k = 2 revealed the genetic difference in population of Esfahan and Fars province (pop. No. 1-4; subsp. *persica*) (red colored) with other populations. However, it showed genetic affinity between populations 5-8 of Tehran province (subsp. *rudshurensis*) (green colored)

The mean Nm = 0.17 was obtained for all ISSR loci, which indicates the low amount of gene flow among the populations, and supports the genetic stratification as indicated by K-Means and STRUCTURE analyses. However, the reticulogram obtained based on the least square method (Figure 4) revealed some amount of shared alleles among populations 1 and 2, and populations 3 and 8. This result agrees with the grouping obtained with UPGMA tree, as the populations were placed close to each other. As evidenced by STRUCTURE plot based on the admixture model, the shared alleles comprise a very limited part of the genomes in the populations, and all the results consistently show a high degree of genetic stratification within *Salicornia persica* populations.

DISCUSSION

The present study revealed interesting data about the genetic variability, genetic stratification, and morphological divergence in the central parts of Iran. The genetic diversity is of fundamental importance in the continuity of a species, as it is used to bring about the necessary adaptation to cope with the changes in the environment (Wang *et al.* 2021; Yin *et al.* 2021; Zhao *et al.* 2021). The degree of genetic variability within a species highly correlates with its reproduction mode. The higher degree of open pollination/cross breeding brings about a higher level of genetic variability in the studied taxon (Jia *et al.* 2020; Shi *et al.* 2021; Zheng *et al.* 2021; Zhu *et al.* 2021).

The PIC and MI characteristics of a primer help to

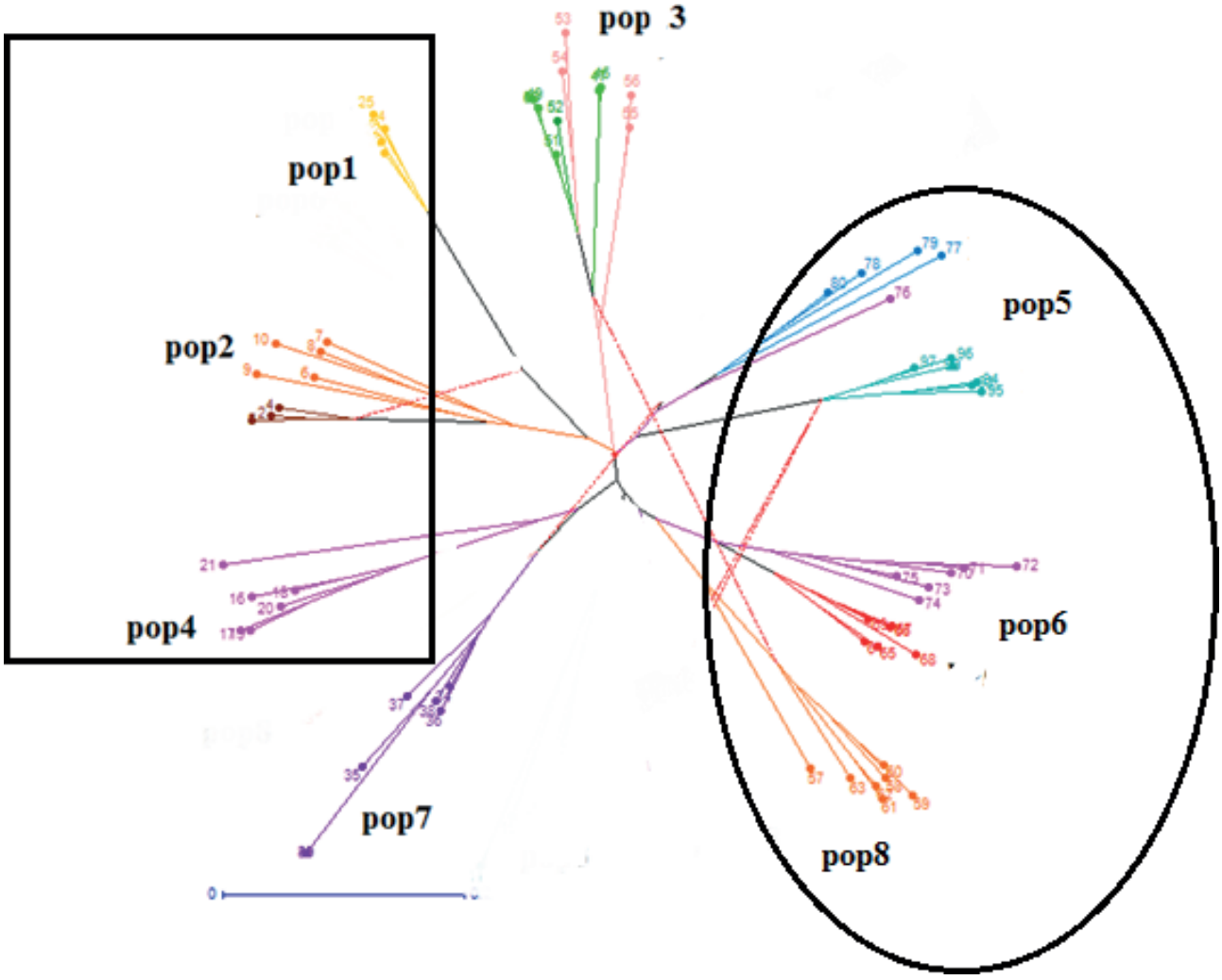

Figure 4. Reticulogram of *Salicornia persica* populations based on least squares method analysis of ISSR data (Population numbers according to Table 1).

determine its effectiveness in the genetic diversity analysis. Sivaprakash *et al.* (2004) suggested that the ability of a marker technique to resolve genetic diversity may be more directly related to the degree of polymorphism. Generally, the PIC value between 0 and 0.25 imply a very low genetic diversity among genotypes, the value between 0.25 and 0.50 shows a mid-level genetic diversity, and the value ≥0.50 suggests a high level of genetic diversity. In this research, the ISSR primers' PIC values ranged from 0.25 to 0.64, with a mean value of 0.49, which indicated the high capability of ISSR primers for determining the genetic diversity among the *Salicornia persica* accessions.

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 the range of species in *Salicornia* is imperfectly known, it is rather premature to evaluate their plant geographical importance. However, based on present data two species groups can be distinguished: The first are Central and South-Central Iranian species including *S. persica* subsp. *persica*, *S. persica* subsp. *rudshurensis*, *S. perspolitana*, *S. iranica* and *S. x tashkensis* and the second group consisted only of *S. sinus-persica* which is endemic around the Persian Gulf. The Central and South-Central Iran possess several endemic species of desert and arid flora with remarkable phytogeographic importance. Furthermore the area is part of the Zagros Mountains which is known as a very important plant diversity center in SW Asia (Akhani, 2008). Sagane *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.

The genetic diversity of 102 individuals of *S. persica* (15 populations) were studied using 10 Start Codon Targeted (SCoT) markers (Liu & Esfandani-Bozchaloyi 2022). Their result showed high polymorphic bands (94.18%), polymorphic information content (0.27), and allele number (1.38) showed SCoT as a reliable marker system for genetic analysis of this species. According to Chatrenoor & Akhani (2021) an integrated morpho-molecular study of *Salicornia* (Amaranthaceae-Chenopodiaceae) in Iran proves Irano-Turanian region the major center of diversity of annual glasswort species. Their results (1) confirm the efficiency of plastid sequences comparing to ETS sequences for clarifying species-level phylogeny of *Salicornia*; (2) identify the *S. persica* clade as a monophyletic Irano-Turanian endemic lineage; (3) recognize nine origins of the Irano-Turanian *Salicornia* based on nuclear ETS sequences; (4) approve the monophyly of tetraploid species using plastid sequences.

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. 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). The other study using RAPD technique showed correlations between DNA polymorphism and geographical distribution in *S. ramosissima*. 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.

In conclusion, the results of this study showed that to evaluate the genetic diversity of *Salicornia persica,* the primers derived from ISSR were more effective than the other molecular markers.

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