

## GENETIC DIVERSITY AND GENE-POOL OF *Salicornia sinus-persica* Akhani BASED ON RETROTRANSPOSON-BASED MARKERS

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*Salicornia Sinus-Persica* Akhani has been discovered in southern Iran, namely in Khuzestan, Bushehr, and Hormozgan, along salty and brackish rivers draining into the Persian Gulf well as estuaries and coastal habitats. Genetic diversity is an essential component of biological variability for conservation methods, particularly for rare and narrowly endemic species. Iran's folk's genetic structure, diversity, and morphological distinctions are unknown. Because of the plant's medicinal value, genetic diversity and folk structure were studied in six regional folks of *Salicornia Sinus-Persica* Akhani. To emphasize genetic variability within and across people in this plant, we employed six inter-retrotransposon amplified polymorphism (IRAP) markers and 15 combined IRAP markers. The AMOVA test indicated a substantial genetic difference across the folks investigated ( $\Phi_{PT} = 0.41$ ,  $P = 0.010$ ), as well as the fact that within-folk variation accounted for 80% of overall genetic diversity and genetic divergence between folks accounted for 20%. The Mantel test revealed a substantial positive connection between genetic distance and geographical distance across all groups investigated. According to STRUCTURE analysis and folk assignment tests, these folks exhibit some degree of gene flow. The UPGMA classification of molecular data matched with the PCoA plot of

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people. Based on (IRAP) markers, these findings revealed that the regional folks of *Salicornia Sinus-Persica* are incredibly distinct.

**Key words:** Gene flow, IRAP, *Salicornia sinus-persica*; Population Differentiation

## INTRODUCTION

*Salicornia* is a halophytic plant that thrives in salt marshes and salty wetlands in Asia, North America, the Middle East, and near coastlines. *Salicornia* is one of the most varied genera in the Salicornieae family. Approximately 25 to 30 species in the genus (KADEREIT *et al.*, 2007). *Salicornia* species are succulent plants with a jointed horizontal main stem and upright lateral branches that develop to be less than 30 cm tall. Because of the microscopic, scale-like leaves, the plant may seem leafless. Many plants are green throughout the summer, but their leaves change crimson in the fall. The breeze pollinates the hermaphrodite blooms, and the fruit is tiny and tasty, with just one seed.

*Salicornia* is a worldwide genus whose taxonomy has been described as "a horrific nightmare" by a recent study (KADEREIT *et al.*, 2007). Only *Salicornia europaea* L. has been found in Iran, according to HEDGE (1997) and other works on Iranian flora. However, the author's subsequent extensive field studies in nearly all *Salicornia* folks in Iran and Turkey, as well as molecular studies (in preparation), co-cultivation experiments, and ecological data (GHAFFARI *et al.*, 2006), revealed rejection of *S. Europaea's* presence in Iran and the occurrence of a remarkable diversity in Iranian *Salicornia*. There are at least five other taxa in Central and Southern Iran and the recently identified endemic species *S. persica* (AKHANI, 2003, AKHANI, 2008). (1) The diploid Symphytum *Iranica* (Iranian Symphytum) Akhani is found in the salines of central Iran. The upright stance and small opposing top spikes define this species. (2) *S. sinus-persica* In the provinces of Khuzestan, Bushehr, and Hormozgan in southern Iran, Akhani thrives in salty and brackish rivers that flow into the Persian Gulf, as well as estuaries and coastal environments. (3) *S. persopolitana* Akhani which is found in the salines near the Tashk and Bakhtegan hypersaline lakes in South-Central Iran, is linked to Akhani. It has a yellowish color and a broad branching, ascending to loosely prostrate habit. This is the first species in the genus *Salicornia* with leaf-like bracts; (*A. persica* subsp. *rudshurensis* is a horizontal plant with leaf-like bracts. Slender spikes with obtuse center bloom separate Akhani, a taxon occurring in the Tehran region of North-Central Iran, from common subspecies; and (5) *S. x tashkensis* Akhani hybrid is a rumored hybrid that can only be found in Tashk Lake in South-Central Iran.

Due to the tremendous morphological heterogeneity of this species throughout the nation, infra-specific taxonomy variations of this species may occur. As a result, we conducted folk genetic research and morphometric studies on six regional groups for the first time in the country.

The inter-retrotransposon amplified polymorphism (IRAP) approach, which finds insertional polymorphisms by amplifying DNA segments between two retrotransposons, was utilized for genetic research. It's been used in several research on genetic diversity (SMYKAL *et al.*, 2004).

The goal of this study was to use the inter-retrotransposon amplified polymorphism (IRAP) approach to examine genetic diversity across *Salicornia Sinus-Persica* cultivars/folks

from various geographical origins, as well as to use IRAP markers to detect genetic variation among and within materials.

## MATERIALS AND METHODS

### *Plant materials*

In July and August 2019-2020, 40 individuals from six natural folks of *Salicornia Sinus-Persica* were sampled in the salty and brackish rivers that flow into Iran's the Persian Gulf in the provinces of Khuzestan, Bushehr, and Hormozgan (Table 1). Fresh leaves were collected from 5-8 people in each group and dried in Silica Gel right away (Table 1). The species (*Salicornia sinus-persica*) was identified correctly using many sources (KADEREIT *et al.*, 2007; 2012; AKHANI, 2003). Table 1 and Figure 1 show the locations of the samples.

Table 1. Populations studied their locality and ecological features.

Pop.no	Locality	Alt.(m)	Coordinates
1	Bushehr: 16 km N Borazjan towards Konartakhteh	1300	48° 10.778' E; 33° 18.175' N
2	Bushehr: 10 km NW of Borazjan, along Dalaki River,	1280	48° 50.690' E; 33° 18.292' N
3	Bushehr: 4 km S of Delvar, shores of Persian Gulf, near Mohamad Ameri,	1100	48° 10.286' E; 33° 17.27.407' N
4	Bushehr: 17 km from Borazjan towards Bandare-Genaveh, Dalaki River margin,	1370	48° 15.890' E; 33° 18.98.327' N
5	Khuzestan: Bandare Mahshar, coastal area near the Mahshar Petrochemical Complex, near Pol-e Sazandegi	110	47° 49.748' E; 33° 18.168' N
6	Hormozgan: Between Bastak and Lar, near Moradnow village	670	47° 30.663' E; 33° 4.840' N



Fig. 1. Distribution map of the studied populations. (Population numbers are according to Table 1).

#### DNA extraction and IRAP assay

Fresh leaves were randomly picked from each research group's 5-10 plants. Silica gel powder was used to dry them. To extract genomic DNA, the CTAB activated charcoal approach was applied (ESFANDANI-BOZCHALOYI *et al.*, 2019). A 0.8 percent agarose gel was used to evaluate the purity of the isolated DNA.

#### Molecular analyses

Binary characters were assigned to the IRAP profiles acquired for each sample. We calculated the number of effective alleles, Nei's gene diversity (H), Shannon information index (I), and polymorphism percent (WEISING *et al.*, 2005; FREELAND *et al.*, 2011).

### RESULTS

#### Populations genetic diversity

Table 2 shows the genetic diversity characteristics of six regional folks of *Salicornia Sinus-Persica*. The largest percentage polymorphism (65.05 percent) was observed in Bushehr, 10 kilometers northwest of Borazjan, along the Dalaki River (folk No. 2) indicating a high value for gene diversity (0.34). & Shanon, index of information (0.37). The folk of Khuzestan has the lowest degree of polymorphism (21.53 percent) in Bandare Mahshar, a coastal region near the Mahshar Petrochemical Complex, near Pol-e Sazandegi (No.5) and Shanon, information index (0.10), and Hezbollah, information index (0.10). (0.10). (0.12).

Table 2. Genetic diversity parameters in the studied populations *Salicornia sinus-persica*

Pop	Na	Ne	I	He	UHe	%P
Pop1	0.341	1.058	0.29	0.32	0.31	23.75%
Pop2	0.455	1.077	0.377	0.34	0.32	65.05%
Pop3	0.499	1.067	0.29	0.23	0.24	29.26%
Pop4	0.555	1.020	0.22	0.25	0.28	33.53%
Pop5	0.431	1.088	0.11	0.12	0.15	21.53%
Pop6	0.255	1.02	0.25	0.28	0.22	37.15%

(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).

#### Population genetic differentiation

Gst analysis (0.887, p = 0.001) and AMOVA (PhiPT = 0.41, P = 0.010) revealed significant differences between the groups (Table 3). It was also discovered that genetic variation within folks accounted for 20% of overall genetic diversity, whereas genetic differences between groups accounted for 80%. Pairwise AMOVA results indicated a substantial difference between the two groups. After 999 permutations, we also got strong scores for the Hedrick standardized fixation index (G'st = 0.887, P = 0.001) and the Jost differentiation index (D-est = 0.556, P = 0.001). These findings suggest that regional folks of *Salicornia Sinus-Persica* differ genetically.

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

Source	df	SS	MS	Est. Var.	%	$\Phi_{PT}$
Among Pops	70	446.576	31.327	7.082	80%	
Within Pops	50	84.767	19.530	3.520	20%	80%
Total	120	511.342		10.613	100%	

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

#### Populations genetic affinity

Plant samples from each folk were grouped in the UPGMA tree, resulting in various groupings. There were no transitional forms among the specimens we examined. These findings revealed that two *Salicornia sinus-persica* folk clusters or groups might be recognized using IRAP data (Figure 2). Hormozgan: Between Bastak and Lar, near Moradnow hamlet (No.6); Khuzestan: Bandare Mahshar, the coastal region near the Mahshar Petrochemical Complex, near Pol-e Sazandegi; Hormozgan: Between Bastak and Lar, near Moradnow hamlet (No.6); Hormozgan: Between Bastak and Lar, near Moradnow hamlet (No.6); Hor (No.5). Similarly, the second central cluster included two sub-clusters: the first contained Bushehr, situated along the Dalaki River 10 kilometers northwest of Borazjan. The second sub-cluster had Bushehr: 4 km from Delvar, Persian Gulf coasts near Mohamad Ameri and Bushehr: 17 km from Borazjan towards Bandare-Genaveh, Dalaki River margin (No.2-4), while the third sub-cluster included Bushehr: 16 km N Borazjan towards Konartakhteh (Pop 1).

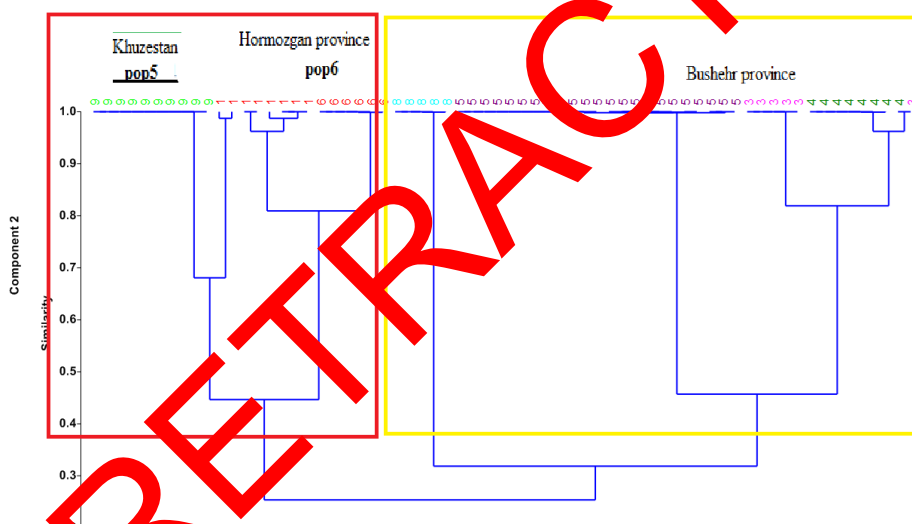


Fig.2. UPGMA clustering of populations in *Salicornia sinus-persica* based on IRAP data. Bootstrap value from 1000 replicates are indicated below branches (Population numbers are according to Table 1).

Separation of folks and genetic divergence Hormozgan: Between Bastak and Lar, close to Moradnow village (No.6); Hormozgan: Between Bastak and Lar, close to Moradnow village (No.6); Hormozgan: Between Bastak and Lar, close to Moradnow village (No.6); Hormozgan: Between Bastak and Lar, close to Moradnow village (No.6); Hormozgan: Between Bast ( After 900 permutations, the PCoA figure of IRAP data distinguishes Bandare Mahshar, a seaside site near the Mahshar Petrochemical Complex, near Pol-e Sazandegi (No.5), from other folks (Figure.3). A lot of genetic information was shared amongst the other folks. The Mantel test revealed a significant link between genetic distance and geographical distance ( $r = 0.38$ ,  $P = 0.001$ ) after 5000 permutations. As a result, when *Salicornia sinus-persica* are separated geographically, folk isolation by distance (IBD) develops.

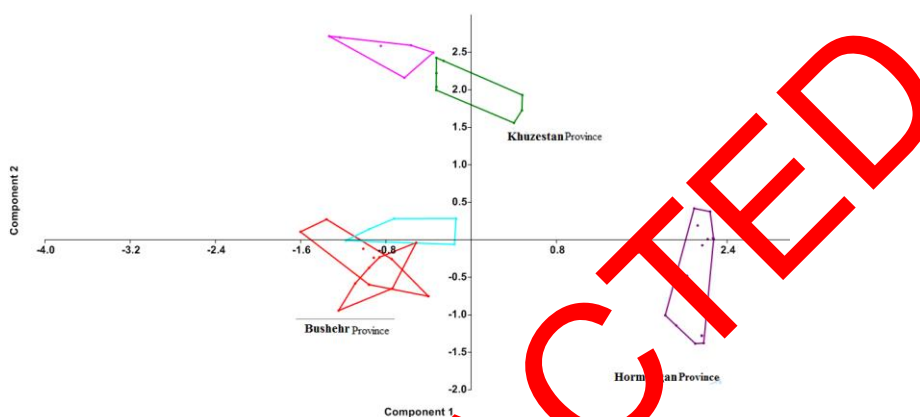


Fig. 3. PCoA plot of populations in *Salicornia sinus-persica* based on IRAP data.

#### DISCUSSION

In genetic and breeding studies, folk genetics evaluations are crucial. Genetic diversity, genetic variability, partitioning within and across folks, inbreeding and outcrossing, folk bottlenecks, and effective folk size are all covered (BI *et al.*, 2021; CHENG *et al.*, 2021). The use of molecular markers has substantially aided folk genetic investigations. These markers have been used to identify possible genotype differences among the several *Salicornia* accessions (MA *et al.*, 2021; PENG *et al.*, 2021; SI *et al.*, 2021). In recent years, RAPD, AFLP, inter simple sequence repeat (ISSR), simple sequence repeat (SSR), and inter-retrotransposon amplified polymorphism (IRAP) has been used to assess genetic variation and relationships in cultivars and landraces (WIESNEROVA and WIESNER, 2004; SALARI *et al.*, 2013; YIN *et al.*, 2021). The majority of plant genomes are transposable elements, predominantly retrotransposons. Because their replication produces genetic variation, they are a rich source of molecular markers (SMYKAL *et*

*et al.*, 2011). The inter-retrotransposon amplified polymorphism (IRAP) approach reveals insertional polymorphisms by boosting DNA segments between two retrotransposons. It's been utilized in several research on genetic diversity (ZHENG *et al.*, 2021; ZHU *et al.*, 2021).

*Salicornia Sinus-persica* distributed has the same distribution as some other endemic species found in the Persian Gulf and Oman. *Bienertia sinus persici* was recently a fascinating example (AKHANI *et al.*, 2005). Most of the endemics of the Persian Gulf area have an Irano-Turanian origin, as recently described on the phytogeographical importance of *Heliotropium* species (AKHANI, 2007), providing good evidence for ZOHARY (1973) and LÉONARD's consideration of this area as part of the Irano-Turanian area rather than Sudanian or Saharo-Sindian (1989). This research aimed to assess the genetic diversity of Iranian *Salicornia Sinus-Persica* to conserve the species' germplasm. The data acquired on genetic diversity across and within folks will pave the way for effective conservation strategies. The current study discovered that Iranian- *Salicornia Sinus-Persica* is exceptionally diversified due to local genetic origins, breeding pressure, and/or restricted genetic material exchange. The unique nature of the Iranian *Salicornia Sinus-Persica* germplasm is highlighted by our findings, emphasizing the need for more extensive characterization, conservation, and breeding techniques. IRAP markers were quite helpful in determining genetic diversity among Iranians. Table 3 shows the findings of the molecular technique to fingerprint the 6 *Salicornia Sinus-Persica* folks. The 6 primers amplified 77 bands, with an average of 6 bands per primer and 60 (88%) polymorphic bands. The overall number of amplified fragments was between 5 and 12, whereas polymorphic fragments were between 4 and 12.

A species' genetic variety is critical to its survival (HENDRIX, 2002; ESFANDANI-BOZCHALOYI *et al.*, 2017a; 2017b,c,d; JIA *et al.*, 2021; JI *et al.*, 2020a, 2020b). Because *Salicornia Sinus-Persica* is a selfing plant with regeneration occurring within the species folk, which confers diversity, AMOVA analysis shows that percentages within and among people are relative. Because *Salicornia Sinus-Persica* is a selfing plant with regeneration occurring within the species folk, this causes Drift, founder effect, and local selection may all play a role in folk phenotypic and allelic variability (ESFANDANI-BOZCHALOYI *et al.*, 2018a; 2018b,c,d).

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## GENETIČKI DIVERZITET I GENSKI FOND *Salicornia sinus-persica* Akhani NA OSNOVU RETROTRANSPON MARKERIMA

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### Izvod

*Salicornia sinus-persica* Akhani je opisana od južnog Irana u provincijama Chuzestan, Bushehr i Hormozgan duž slanih i bočatih reka koje vode do Persijskog zaliva i estuara i obalnih staništa. Genetski diverzitet je jedan aspekt biološke raznovrsnosti koji je izuzetno važan za strategije očuvanja, posebno retkih i usko endemičnih vrsta. Nema informacija o njegovoj populacijskoj genetičkoj strukturi, genetskoj raznolikosti i morfološkoj varijabilnosti u Iranu. Zbog medicinskog značaja ove vrste, sprovedeno je proučavanje genetske varijabilnosti i strukture populacija na šest geografskih populacija *Salicornia sinus-persica* Akhani. Korišćeno je šest markera inter-retrotranspon pojačanog polimorfizma (IRAP) i 15 kombinovanih IRAP markera da bismo otkrili unutar i među populacijama genetsku raznovrsnost u ovoj biljci. AMOVA test je proizveo značajnu genetsku razliku ( $\Phi_{PT} = 0,41$ ,  $P = 0,010$ ) među proučavanim populacijama i takođe je otkrio da je 80% ukupne genetske varijabilnosti posledica unutar populacijske raznolikosti, dok je 20% posledica genetske diferencijacije među populacijama. Mantel test je pokazao pozitivnu značajnu korelaciju između genetske udaljenosti i geografske udaljenosti ispitivanih populacija. Analiza STRUSTRUCTURE i test raspodele populacije otkrili su određeni stepen protoka gena među ovim populacijama. PCoA dijagram populacija bio je u saglasnosti sa UPGMA grupisanim molekularnim podacima. Ovi rezultati su pokazali da su geografske populacije *Salicornia sinus-persica* dobro diferencirane na osnovu IRAP markera.

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