Genetic Diversity of *Halocnemum strobilaceum* (Pall.) M.Bieb. (Amaranthaceae) in South-West Iran: Insights from Molecular Markers and DNA Barcoding

Running Title: Genetic Diversity of *Halocnemum strobilaceum* in South-West Iran

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ABSTRACT

The Earth hosts a complex and diverse array of plant species, and analyzing their genetic diversity is essential for advancing biodiversity conservation. In this study, we investigated the genetic variation of *Halocnemum strobilaceum* in Khuzestan Province, located in southwestern Iran, a region characterized by extreme heat and belonging to the Saharo-Sindian floristic zone. To assess the genetic structure of the species, we employed ISSR, SCoT, and DNA barcoding markers (ITS and trnH-psbA). Accessions were categorized based on ecogeographic differences between inland saline and coastal marsh habitats. Both marker systems revealed considerable variation, and barcoding identified three distinct ITS and two trnH-psbA haplotypes. These results enhance our understanding of genetic differentiation in *H. strobilaceum* and inform conservation planning. As a halophyte with potential medicinal and economic uses, preserving its genetic resources is vital for sustainable utilization. In the face of escalating environmental threats, such integrative studies are key to protecting vulnerable plant taxa in extreme habitats.

Keywords: Halophytes, Molecular variation, Haplotypes analysis, Conservation genetics

INTRODUCTION

Biodiversity loss is rapidly accelerating, with recent global assessments estimating that about one million species, many of which are plants, are at risk of extinction due to human activities, habitat destruction, and climate change [1]. In this context, identifying and utilizing plant species that have both ecological importance and economic value is essential, especially as global temperatures rise and human populations continue to grow.

Halocnemum strobilaceum (Pall.) M.Bieb. (Amaranthaceae; formerly Chenopodiaceae) is a salt-tolerant halophyte species with significant potential in medicinal and pharmacological uses [2, 3], phytoremediation [4, 5], oil production [6], and livestock fodder [7]. It is also a promising candidate for halophyte-based technologies. This species is considered a vital part of Iran's halophytic flora [8], showing remarkable adaptation to extreme salinity and high temperatures. *H. strobilaceum* is classified as a pluri-regional species [9], found across both the Irano-Turanian floristic region, which is known as a center for the diversification of salt-tolerant lineages within the Caryophyllales [10], and the Saharo-Sindian region [11]. Due to its broad ecological range and extensive geographic distribution, studying the genetic diversity of *H. strobilaceum* is crucial to protect its adaptability and conservation amid increasing environmental changes.

Genetic diversity plays a pivotal role in conservation biology, especially as preserving biodiversity becomes increasingly urgent in light of global environmental shifts [12, 13]. This diversity underpins the resilience of populations, communities, and ecosystems [14], particularly under the influence of climate change and its associated stressors. Genetic variation provides a reservoir of novel traits that can facilitate adaptation to both biotic and abiotic challenges [15].

In this context, molecular markers provide valuable tools for evaluating genetic variation and managing plant genetic resources [16]. ISSR (inter-simple sequence repeat) markers, which amplify regions between microsatellite sequences using primers that target simple sequence repeats (SSRs), are effective in detecting polymorphisms through differences in fragment length [17]. This method has been widely used for diversity analysis, DNA fingerprinting, and genome mapping in various plant species [18], playing an important role in conservation genetics [19].

Additionally, the start codon targeted (SCoT) marker system, introduced by Collard & Mackill (2009), is a gene-targeted approach that amplifies conserved regions flanking the ATG start codon. SCoT markers are recognized for their high reproducibility, cost-effectiveness, and ability to detect genetic diversity without prior genomic information. They have proven useful for evaluating genetic relationships, cultivar identification, and population structure in numerous species [21]. Complementing these genomic fingerprinting methods, DNA barcoding enables precise species identification and offers enhanced resolution for evaluating intraspecific genetic variation [22]. The trnH-psbA plastid region, a highly variable non-coding intergenic spacer, is widely used for plant identification and phylogenetic studies due to its high interspecific variability and ease of amplification [23, 24]. The nuclear ribosomal internal transcribed spacer (ITS) region, meanwhile, serves as a reliable phylogenetic marker for resolving relationships at taxonomic levels and has been endorsed as a core plant barcode [25, 26].

The objective of this study is to assess the genetic diversity of *H. strobilaceum* accessions in Khuzestan Province, a region characterized by harsh environmental conditions, including extreme heat, salinity, and anthropogenic pressures. The findings aim to contribute to the understanding of this species' genetic structure and to inform future conservation and sustainable utilization strategies.

MATERIAL AND METHODS
Plant Materials

Initially, 200 accessions of *H. strobilaceum* were collected from various saline habitats across Khuzestan Province and evaluated for morphological variation. As the preliminary assessment revealed no significant differences in morphological traits, a subset of 15 representative accessions was selected for molecular analysis (Table 1; Fig. 1), based on distinct ecogeographic characteristics and maximum environmental heterogeneity. Sampling criteria included proximity to saline or brackish water bodies, soil salinity gradients, industrial activity, road accessibility, vegetation density, and elevation.

To account for the species' clonal growth habit via rhizomatous ramets [27], linear transects were established at each site, and individuals were sampled at sufficient distances to minimize redundancy and avoid collecting genetically identical clones. While distinct ecogeographic differences guided site selection, logistical constraints prevented the quantitative measurement of environmental parameters such as soil salinity, pH, and moisture.

Based on these ecogeographic criteria, the selected accessions were grouped into two categories reflecting the main habitat types and their general geographic locations: inland saline (central) and coastal marsh (southern) regions. This approach ensured representative sampling that captured both ecological diversity and genetic variability across the region.

Table 1 Geographical data of *Halocnemum strobilaceum* collection sites

| Groups	Code	Latitude (N)	Longitude (E)
Central accessions	hal 1-4	31°14'55.36"	48°58'22.47"
	hal 5-1	30°59'19.55"	48°57'53.35"
	hal 17-4	30°45'2.58"	48°26'14.84"
	hal 18-4	31°21'52.38"	48°42'55.81"
	hal 19-2	31°22'16.58"	48°39'44.32"
	hal 19-5	31°22'14.29"	48°39'42.86"
	hal 19-6	31°22'16.19"	48°39'47.28"
	hal 20	31°22'10.81"	48°40'13.13"
Southern accessions	hal 10-1	30°32'30.74"	49° 8'45.72"
	hal 11-3	30°12'42.82"	49°47'31.75"
	hal 12-3	30°25'30.56"	49° 9'38.10"
	hal 13-4	30°24'56.35"	49° 9'16.98"
	hal 15-3	30°25'40.79"	49°11'22.30"
	hal 15-4	30°25'32.01"	49°11'56.75"
	hal 16-4	30°15'31.61"	49° 7'28.65"
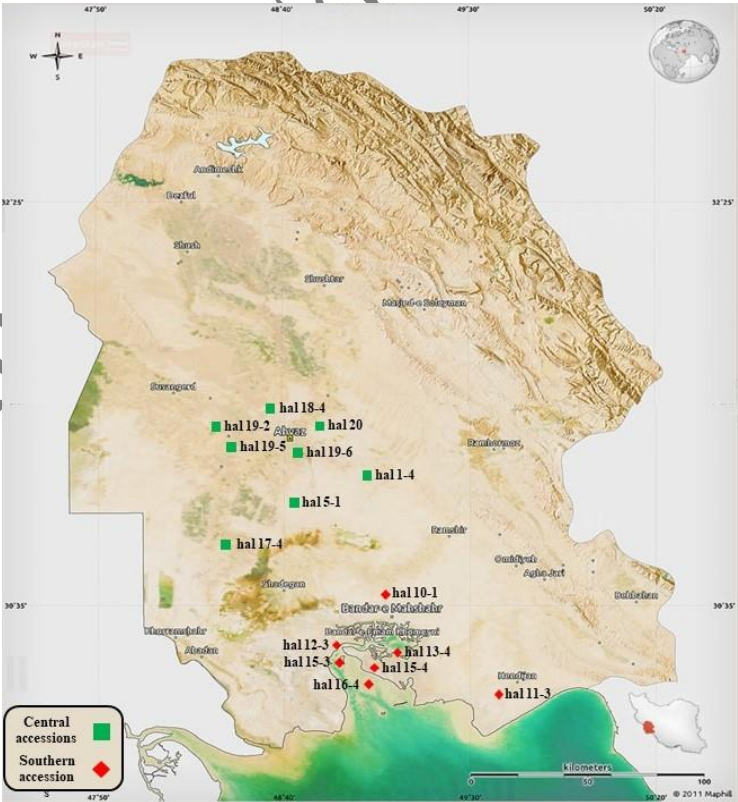
Fig. 1 Geographical distribution of the collected accessions in Khuzestan Province, southwest Iran (see Table 1)

DNA Isolation

DNA was extracted from the silica gel-dried tissues using the column method (DNA Extraction Kit, AnaCell, Iran) following the manufacturer's instructions. The extracted DNA was evaluated using 0.8% agarose gel electrophoresis for quantification and a spectrophotometer for quality assessment.

ISSR and SCoT Amplification

Twelve ISSR and twelve SCoT [20] primers (TAG Copenhagen, Denmark) were selected based on their reported polymorphism in related halophytic species, optimized, and then used in polymerase chain reactions (PCR) with a reaction volume of 25 μl. Each PCR mixture contained 12.5 μl of 2X PCR master mix (Ampliqon, Denmark), 1 μl of primer (10 pmol/μl), 1 μl of DNA (30 ng), and 10.5 μl of double-distilled water (ddH$_2$O). Details of the amplification program are provided in Supplementary Table S1. The PCR products of the ISSR and SCoT markers were visualized by electrophoresis on 1.5% agarose gels and stained with YTA safe stain (Yekta Tajhiz, Iran).

A pre-screening test was performed on a subset of representative DNA samples to evaluate the clarity, reproducibility, and polymorphism of each primer. Only those that showed consistent and informative amplification patterns during initial optimization were retained for further analysis. However, one SCoT primer (SCoT3) did not produce scorable bands in the full dataset.

Amplification of DNA Barcodes and Sequencing

The PCR mixture consisted of 10 μl 2X PCR master mix, 0.8 μl of each primer (10 pmol/μl), 1 μl of DNA (30 ng), and 7.4 μl of double-distilled water (ddH$_2$O). Primer sequences and specific amplification conditions for ITS and trnH-psbA are provided in Supplementary Table S2. The purified PCR products were sequenced in both directions using the same primers on an ABI 3730xl DNA analyzer.

Data Analysis
ISSR and SCoT

All amplified products were analyzed using CLIQS 1D Pro (USA). Band presence or absence was scored as '1' and '0', respectively. A dendrogram was generated through the unweighted pair group method with arithmetic mean (UPGMA), based on the Jaccard coefficient, using the SAHN clustering module in NTSYS-PC version 2.1 [28]. The average polymorphic information content (PIC) was calculated using the formula described by Serrote *et al.* (2020), while the marker index (MI) was estimated according to [30]. Principal coordinate analysis (PCoA) was conducted in GenAlEx version 6 [31], employing a standardized genetic distance matrix.

Sequence Analysis

The ITS and trnH-psbA sequences were aligned using the MUSCLE algorithm implemented in MEGA version 11.0.13 [32]. Sequence identity was assessed through BLASTn searches against the GenBank database (https://www.ncbi.nlm.nih.gov/BLAST). The number of haplotypes was calculated using DnaSP version 6 [33] based on aligned sequences generated in this study. To visualize relationships among sequences, a haplotype network for the ITS region was constructed using DARwin version 6.0.021 [34], while a phylogenetic tree for the trnH-psbA region was generated in MEGA [32]. Aligned sequences were also visually inspected and manually verified in BioEdit version 7.2 [35].

RESULTS
Polymorphism of ISSR Markers

The twelve ISSR primers generated a total of 128 bands, ranging in size from 200 to 3100 bp (Table 2). Among these, 114 bands (89%) were polymorphic. The number of bands per primer varied from 4 (ISSR8) to 17 (ISSR1), while the number of polymorphic bands ranged from 3 (ISSR4 and ISSR6) to 17 (ISSR1). Notably, 7 out of the 12 primers exhibited 100% polymorphism.

The polymorphic information content (PIC) values ranged from 0.18 (ISSR6) to 0.47 (ISSR8), with a mean of 0.32. Marker index (MI) values varied from 0.27 (ISSR6) to 6.67 (ISSR1), averaging 3.08.

Table 2 ISSR primer details and calculated marker parameters

Primer	Primer sequence 5'→ 3'	GC%	NB	NP	P%	PIC	MI	SB
ISSR1	CACACACACACAGG	57.1	17	17	100	0.39	6.67	300-3000
ISSR2	CACACACACACAAC	50	12	12	100	0.35	4.16	500-3000
ISSR3	CACACACACACAAG	50	13	10	76	0.26	2.03	350-2500
ISSR4	AGAGAGAGAGAGAGAGT	47.1	6	3	50	0.20	0.29	300-1500
ISSR5	AGAGAGAGAGAGAGAGC	52.9	8	7	87	0.31	1.86	400-2000
ISSR6	CACACACACACACAAGG	52.6	6	3	50	0.18	0.27	400-1100
ISSR7	ACACACACACACACACG	52.9	15	15	100	0.40	5.99	200-2000
ISSR8	TATTCCGACGCTGAGGCAG	57.9	4	4	100	0.47	1.87	800-2300
ISSR9	GGAGAGGAGAGGAGA	60	14	10	71	0.27	1.94	350-2500
ISSR10	GAGAGAGAGAGAGAGAGT	50	11	11	100	0.36	4.09	300-2700
ISSR11	GAGAGAGAGAGAGAGATC	50	13	13	100	0.41	5.38	450-3100
ISSR12	CCAACGATGAAGAACGCAGC	55	9	9	100	0.28	2.51	500-3000
			128	114		0.32	3.08	

NB: Number of total bands; NP: Number of polymorphic bands; PIC: Polymorphic information content; MI: Marker index; SB: Size range of bands

Polymorphism of SCoT markers

As one of the tested primers, SCoT3 failed to yield scorable bands, likely due to the absence of suitable binding sites or mismatches. It was therefore excluded from the analysis.

The remaining primers generated a total of 88 bands, of which 76 (86%) were polymorphic, with sizes ranging from 200 to 3150 bp. The number of amplified bands per primer varied from 2 (SCoT34) to 12 (SCoT1), while the number of polymorphic bands ranged from 2 (SCoT5 and SCoT34) to 12 (SCoT1).

PIC values ranged from 0.14 (SCoT5) to 0.38 (SCoT19), with an average of 0.27. MI values ranged from 0.19 (SCoT5) to 4.20 (SCoT1), with a mean of 1.94.

Table 3 SCoT primer details and calculated marker parameters

Primer	Primer sequence 5'→ 3'	GC%	NB	NP	P%	PIC	M	SB
SCoT1	CAACA<u>ATGG</u>CTACCACCA	50	12	12	100	0.35	4.2	400-1800
SCoT5	CAACA<u>ATGG</u>CTACCACGA	55.6	3	2	66	0.14	0.19	1000-3000
SCoT11	AAGCA<u>ATGG</u>CTACCACCA	50	9	3	66	0.27	1.06	400-2500
SCoT13	ACGAC<u>ATGG</u>CGACCATCG	50	11	10	91	0.32	2.95	200-3150
SCoT14	ACGAC<u>ATGG</u>CGACCACGC	61.1	7	6	85	0.26	1.31	250-3000
SCoT19	ACC<u>ATGG</u>CTACCACCGGC	66.7	11	10	90	0.38	3.46	450-3000
SCoT20	ACC<u>ATGG</u>CTACCACCGCG	66.7	10	10	100	0.31	3.07	500-3000
SCoT21	ACGAC<u>ATGG</u>CGACCCACA	66.7	7	5	71	0.18	0.65	500-3000
SCoT26	ACC<u>ATGG</u>CTACCACCGTC	61.1	6	6	100	0.28	1.65	600-2800
SCoT34	ACC<u>ATGG</u>CTACCACCGCA	61.1	2	2	100	0.23	0.45	700-2800
SCoT35	C<u>ATGG</u>CTACCACCGGCCC	61.1	10	10	100	0.26	2.62	300-3100
			88	76		0.27	1.96	

NB: Number of total bands; NP: Number of polymorphic bands; PIC: Polymorphic information content; MI: Marker index; SB: Size range of bands

Cluster Analysis

UPGMA dendrograms generated from ISSR and SCoT markers revealed two major clusters that generally corresponded to the geographic origins of the *H. strobilaceum* accessions; coastal marshes (southern group) and inland saline habitats (central group) (Supplementary Fig. S1). Although the overall clustering patterns were broadly consistent between the two marker systems, some minor discrepancies were noted. Specifically, accession Hal1-4 clustered with the coastal group in the ISSR dendrogram, despite being collected from an inland site. Similarly, Hal5-1, also of inland origin, grouped with coastal accessions. In the SCoT dendrogram, Hal16-4 appeared in an unexpected position relative to its collection site, possibly reflecting marker-specific variability or sampling-related genetic divergence.

PCoA Analysis

Principal coordinate analysis (PCoA) was conducted to evaluate genetic differentiation among accessions from inland saline (central) and coastal marsh (southern) habitats. For ISSR markers, the first two principal coordinates explained 28.52% and 11.19% of the total genetic variation, respectively (Fig. 2a). In contrast, for SCoT markers, Coord1 and Coord2 accounted for 27.74% and 24.17% of the variation, respectively (Fig. 2b), indicating a more balanced contribution of the axes and enhanced resolution.

Both marker systems produced clustering patterns that broadly aligned with the geographical origin of the accessions. However, the SCoT-based PCoA plot (Fig. 2b) demonstrated a more distinct genetic separation between the inland group (green squares) and the coastal group (red diamonds), suggesting that the SCoT markers are more sensitive in detecting habitat-associated genetic differentiation. These results are in agreement with the clustering observed in the UPGMA dendrograms.
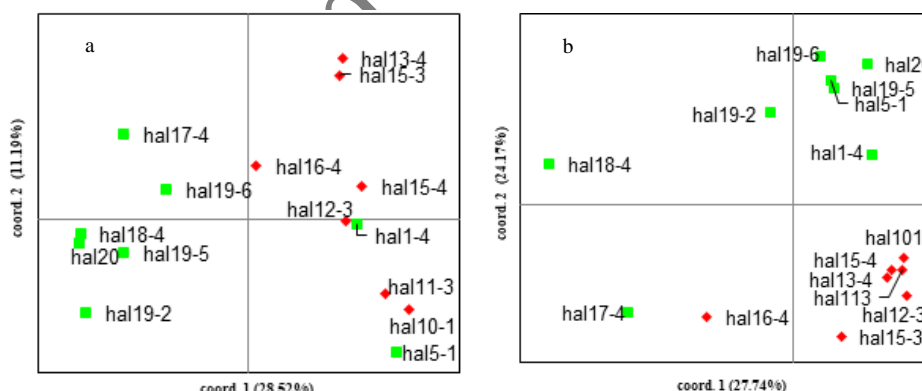

Fig. 2 PCoA plots based on (a) ISSR and (b) SCoT markers, showing genetic separation between central group (■ green squares; inland saline) and southern group (◆ red diamonds; coastal marsh).
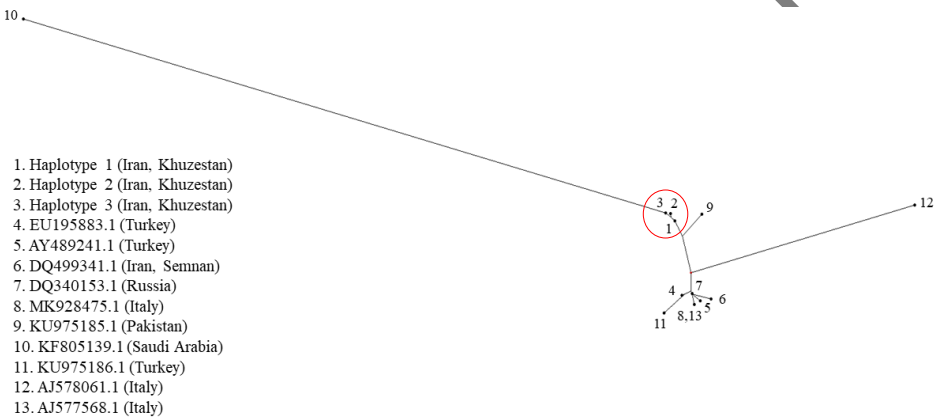
DNA barcoding

The amplified DNA fragments of ITS and trnH-psbA were 646 bp and 484 bp in length, respectively. The sequences generated in this study have been deposited in the NCBI GenBank under accession numbers OR264649 to OR264661 for ITS and OR162338 to OR162347 for trnH-psbA (Supplementary Table S3). The ITS analysis revealed three distinct haplotypes among the studied accessions. Haplotype 1 included hal12-3, hal16-4, hal19-2, hal19-5, hal19-6, hal17-4, hal15-4, hal13-4, and hal11-3. Haplotype 2 was represented solely by hal10-1, while hal5-1 formed a distinct haplotype as Haplotype 3. These haplotypes, along with their observed mutations (Fig. 3a), highlight the presence of genetic variability within *H. strobilaceum* in Khuzestan Province. The ITS sequences were aligned and compared using BLAST searches against the NCBI database to assess sequence identity with global records (Fig. 4).

For the trnH-psbA region, two distinct haplotypes were identified. Haplotype 1 included hal1-4, hal5-1, hal10-1, hal12-3, hal13-4, hal15-3, hal15-4, hal19-2, and hal20, while haplotype 2 was represented solely by hal19-5. This accession exhibited notable genetic divergence from haplotype 1, including 47 nucleotide substitutions and several indels (Fig. 3b). Interestingly, hal19-5 was collected from a southern coastal habitat characterized by elevated salinity levels and proximity to anthropogenic disturbance, which may partially explain its distinct genetic profile. A phylogenetic tree was constructed using trnH-psbA sequences with over 95% query coverage and sequence identity from the NCBI database to assess its relationship with global accessions (Fig. 5). This study presents the first report of the trnH-psbA intergenic spacer in *H. strobilaceum*.

a

b
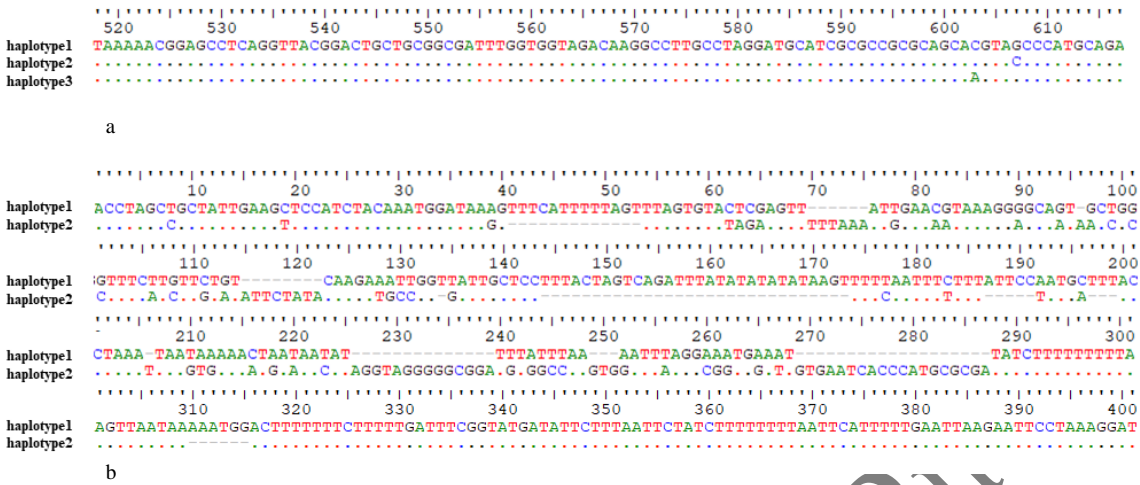
Fig. 3 Polymorphic sites among *H. strobilaceum* haplotypes from Khuzestan based on (a) ITS and (b) trnH-psbA regions.

1. Haplotype 1 (Iran, Khuzestan)
2. Haplotype 2 (Iran, Khuzestan)
3. Haplotype 3 (Iran, Khuzestan)
4. EU195883.1 (Turkey)
5. AY489241.1 (Turkey)
6. DQ499341.1 (Iran, Semnan)
7. DQ340153.1 (Russia)
8. MK928475.1 (Italy)
9. KU975185.1 (Pakistan)
10. KF805139.1 (Saudi Arabia)
11. KU975186.1 (Turkey)
12. AJ578061.1 (Italy)
13. AJ577568.1 (Italy)

Fig. 4 Genetic relationships among *H. strobilaceum* haplotypes based on ITS sequences. The red circle indicates haplotypes identified in Khuzestan.
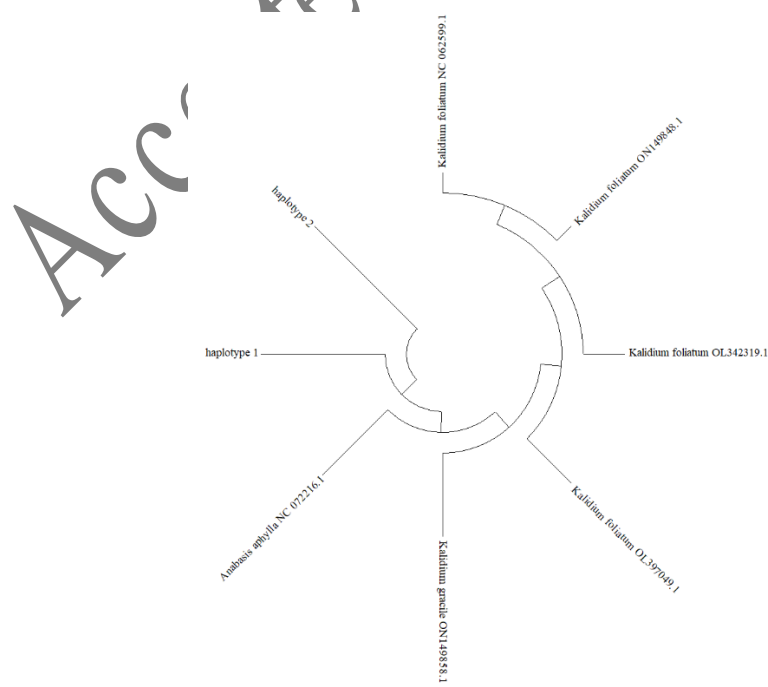
Fig. 5 Neighbor-joining tree based on trnH-psbA sequences of *H. strobilaceum* and related species retrieved from BLAST-NCBI.

DISCUSSION

Genetic diversity plays a fundamental role in conservation, enhancing a species' adaptability to environmental changes such as drought, heat, submergence, and salinity, which are stressors expected to intensify under climate change [13]. In this study, ISSR and SCoT markers, alongside DNA barcoding, were employed to assess the genetic diversity of *Halocnemum strobilaceum*. The two marker systems produced 89% and 86% polymorphic bands, respectively, reflecting substantial genetic variation among accessions. In contrast, their PIC values (0.32 for ISSR and 0.27 for SCoT) suggest moderate informativeness of the primers used. These findings underscore the value of using complementary marker types for reliable assessment of genetic diversity in halophytic species.

Our results are consistent with earlier reports of high genetic polymorphism in Egyptian halophytes, as determined by ISSR analysis [36]. While those studies described genus-level clustering across broad geographic regions, our intraspecific analysis of *H. strobilaceum* revealed finer-scale genetic structuring associated with ecological gradients, including soil salinity and anthropogenic disturbance. Similarly, the effectiveness of SCoT markers for detecting genetic variation under saline conditions has been validated in other halophyte species [37]. Patterns of adaptive divergence shaped by salinity gradients were also observed in *H. strobilaceum* from Egypt, where ISSR and RAPD markers revealed moderate to high polymorphism and grouping patterns corresponding to variation in soil salinity [38].

In our study, both ISSR- and SCoT-based dendrograms revealed clustering patterns that were generally consistent with the geographical origin of the accessions, suggesting a relationship between genetic similarity and habitat characteristics. The grouping based on SCoT markers appeared more distinct and informative compared to that obtained with ISSR markers. Principal coordinate analysis (PCoA) further supported this pattern, with SCoT markers explaining a greater proportion of total genetic variance across the first two axes (51.91%) compared to ISSR markers (39.71%). This enhanced resolution is likely due to the ability of SCoT primers to target functional genomic regions, providing deeper insights into genetic differentiation, as reported in previous study [39].

While the clustering patterns were generally coherent, some inconsistencies were also detected. Similar mismatches have been reported in other halophyte species, where genetic structure expected from habitat-based selection was masked or modified by factors such as clonal propagation or anthropogenic disturbance [40, 41]. These findings suggest that local gene flow and external stressors may sometimes obscure the signal of ecological divergence, highlighting the need to integrate molecular data with detailed ecological variables in future research.

Field observations offer further support for this interpretation. Coastal accessions were collected from small islands periodically inundated by seawater, whereas inland accessions were sampled from areas near oil extraction sites affected by brine discharge and hydrocarbon pollution. These distinct environmental conditions likely imposed selective pressures, contributing to the observed genetic divergence. Our findings align with those of a previous study conducted in Golestan province, where ISSR markers revealed substantial genetic differentiation among *H. strobilaceum* samples, with clustering patterns reflecting their geographic origin [42]. However, unlike that study, our research focuses on accessions selected based on distinct ecogeographic characteristics in southwestern Iran, employing ISSR, SCoT, and DNA barcoding markers to reveal habitat-associated genetic structuring and adaptive divergence.

Similar patterns of habitat-associated genetic differentiation have been observed in other halophytes such as *Aeluropus lagopoides* [40], *Tamarix chinensis* [43], and *Phragmites australis* [44], where salinity gradients, rather than geographic distance, emerged as the primary driver of genetic structure. These findings highlight the role of environmental heterogeneity in promoting adaptive divergence.

Prior studies have also highlighted the impact of factors such as industrial pollution, soil salinity, and edaphic characteristics (e.g., moisture content, contamination) on plant genetic diversity [45]. In a comparable context, halophyte communities in Jiaozhou Bay exhibited altered diversity patterns in response to petroleum contamination [41]. Taken together, these insights highlight the important role of local environmental factors, both natural and anthropogenic, in shaping the genetic structure of *H. strobilaceum*. As noted by Hulshof & Spasojevic (2020), "Soil variations impact the significance of speciation, dispersal, ecological drift, niche selection, and the interactions among these processes". Although this study recorded broad habitat categories, detailed ecological parameters (e.g., salinity, pH, and soil moisture) were not systematically quantified due to logistical constraints. Future research should incorporate such data to more accurately identify the environmental drivers underlying genetic differentiation in *H. strobilaceum*.

DNA barcoding is a powerful molecular tool for assessing genetic diversity, as it enables accurate species identification, reveals haplotype-level variation, and provides insights into both intra- and interspecific genetic patterns. Such information is essential for biodiversity conservation and ecological management [47]. Among the most widely used barcoding markers, the ITS and trnH-psbA regions have proven reliable across a broad range of angiosperms [24].

In the present study, three distinct ITS haplotypes were identified (Fig. 3a). Comparison with sequences from other regions (Fig. 4) revealed that the Khuzestan haplotypes (codes 1, 2, and 3) are genetically distinct. The Semnan haplotype (code 6), although also from Iran, differs notably from those of Khuzestan, likely due to contrasting ecological conditions. Semnan is situated in the Irano-Turanian phytogeographic region, whereas Khuzestan lies within the Saharo-Sindian zone. Climatic differences between these two regions may have contributed to the observed genetic divergence. The Saudi Arabian haplotype (code 10; Fig. 4) exhibited the highest level of dissimilarity, likely due to geographic distance and substantial environmental variation. Interestingly, the Khuzestan haplotypes appear to form a genetic continuum extending toward the Saudi Arabian haplotype, reflecting their geographic proximity within the broader Saharo-Sindian region.

The genetic structuring observed in *H. strobilaceum* accessions from different phytogeographic regions (e.g., Saharo-Sindian vs. Irano-Turanian) aligns with the historical biogeographic diversification of the Salicornioideae lineage, which has adapted to arid and saline environments since the Oligocene [48]. These regionally differentiated haplotypes underscore the importance of conserving genetically distinct accessions, particularly those originating from ecologically diverse habitats. Conservation strategies should consider these phylogeographic patterns to safeguard the adaptive potential of *H. strobilaceum* in response to anticipated environmental changes.

Two distinct haplotypes were identified based on the trnH-psbA barcode. Notably, haplotype 2 differed significantly from haplotype 1 (Fig. 3b). NCBI BLAST analysis yielded no matches for *H. strobilaceum*, suggesting that this may be the first report of trnH-psbA

sequences for the species. However, the ITS sequences confirmed their identity as *H. strobilaceum*. Although no obvious morphological differences were observed in the field, the pronounced sequence divergence in trnH-psbA may indicate cryptic genetic variation. Similar patterns have been documented in other halophytes, where low morphological variability masked underlying genetic differentiation [49]. These findings further emphasize the genetic complexity of *H. strobilaceum* in Khuzestan and highlight the utility of plastid barcodes such as trnH-psbA in detecting hidden genetic diversity.

Although the number of accessions analyzed was limited due to logistical constraints and the harsh environmental conditions of Khuzestan Province, the inclusion of accessions from both inland saline and coastal marsh habitats enabled the detection of substantial genetic variation. Recent studies on Egyptian halophytes have similarly emphasized the importance of characterizing and preserving genetic diversity to ensure the sustainable use of halophytes under increasing salinity stress [36]. Nevertheless, expanding the sample size in future studies could yield a more comprehensive understanding of population structure and help detect rare alleles.

In light of the observed genetic diversity, evidenced by three distinct ITS haplotypes and a unique trnH-psbA haplotype, this study highlights the need for targeted, site-specific conservation measures. We recommend prioritizing genetically distinct accessions for seed banking and ex-situ propagation. Furthermore, conservation units should reflect the ecological divergence between inland and coastal habitats. Such actions, combined with long-term genetic monitoring and active involvement of local communities, are critical to safeguarding the adaptive resilience of *H. strobilaceum* amid intensifying environmental challenges.

CONCLUSION

This study represents the first molecular investigation of *Halocnemum strobilaceum* in Khuzestan Province, southwestern Iran, using ISSR, SCoT, and DNA barcoding markers. The application of both ISSR and SCoT markers revealed substantial genetic diversity among accessions, highlighting the influence of ecological variation, particularly between inland salt marshes and coastal habitats. In addition, the identification of three ITS haplotypes and two trnH-psbA haplotypes provides new insights into the genetic structure of *H. strobilaceum*. These findings have important implications for future conservation strategies, especially in the context of climate change. Given the observed genetic variation and the complex ecological conditions of the region, further research is warranted. We recommend future studies with broader sampling across diverse floristic regions, along with detailed investigations of the species' mating system. Such efforts will improve our understanding of gene flow, population connectivity, and adaptive potential, thereby supporting more effective conservation planning.

Conflict of Interests

The authors have not declared any conflict of interests.

Acknowledgments

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