J Genet Resour2025; 11(1): 126-131 *Homepage: http://sc.journals.umz.ac.ir/*

RESEARCH ARTICLE *DOI: 10.22080/jgr.2025.28944.1434*

J|G|R

Molecular Characterization of *Cressa cretica* L. Using SCoT and ISSR Markers

Mozhdeh Jahangir and Fatemeh Nasernakhaei

Department of Plant Production Engineering and Genetics, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran

ARTICLE INFO

Article history:
Received 12 October 2024
Accepted 14 January 2025
Available online 31 January 2025

Keywords:
Alkali weed
Genetic diversity
Genetic structure
Molecular markers
Polymorphism

Corresponding authors:
✉ F. Nasernakhaei
f.nasernakhaei@scu.ac.ir

p-ISSN 2423-4257
e-ISSN 2588-2589

ABSTRACT

Cressa cretica L. (Convolvulaceae) is a halophytic species with remarkable adaptability to saline environments, holding significant ecological and economic value. In this study, we conducted a comprehensive assessment of genetic diversity and structure in thirteen accessions collected from the eastern and western sides of the Karun River in Ahvaz, Iran. Two types of molecular markers, start codon targeted (SCoT) and inter-simple sequence repeat (ISSR), were employed to detect genetic variation and patterns of differentiation between geographical groups. Ten SCoT primers produced 92 amplified fragments, 87.9% of which were polymorphic, while ten ISSR primers generated 114 fragments with 85.6% polymorphism. Most of the genetic variation was found within groups rather than between them, indicating low genetic differentiation. Although both UPGMA and PCoA analyses grouped the accessions into two main clusters, these clusters did not correspond clearly to their geographical origins. Bayesian model-based grouping revealed three genetic subgroups with SCoT data, while ISSR data supported two subgroups, suggesting a higher resolution of SCoT markers in detecting subtle genetic subdivisions. Although ISSR markers displayed higher average values for polymorphism information content, Nei's gene diversity, and Shannon's index, the ability of SCoT markers to uncover finer genetic structures highlights their complementary role. These results indicate moderate differentiation with significant gene flow among accessions, likely influenced by both reproductive strategies and local environmental conditions. The combined use of both marker systems provided a more comprehensive picture of genetic variation in this salt-tolerant species. These findings provide valuable baseline information for future conservation efforts and sustainable utilization of *C. cretica* in saline ecosystems.

Please cite this paper as: Jahangir, M., & Nasernakhaei, F. (2025). Molecular characterization of *Cressa cretica* L. using SCoT and ISSR markers. *Journal of Genetic Resources,* 11(1), 126-131. doi: 10.22080/jgr.2025.28944.1434

Introduction

Cressa cretica L. (Alkali weed), a salt-tolerant perennial herbaceous member of the Convolvulaceae family, grows in saline habitats including coastal sands and alkaline soils across the Mediterranean basin, Middle East, Africa, and South Asia, employing both sexual (seed) and asexual (rhizome) reproductive strategies (Austin, 2000). Phytochemical analyses have identified valuable compounds in *C. cretica*, such as coumarins, flavonoids, glycosides, tannins, and sterols (Al-Snafi, 2016; El-Alfy *et al*., 2019). This medicinal plant exhibits antibacterial, antifungal, antidiabetic, and anti-inflammatory properties, and is traditionally used to treat asthma, ulcers, and digestive disorders (Priyashree *et al*., 2010). Additionally, its extract shows potential anticancer effects, though further research is needed (Chintaluri, 2025). *C. cretica*, with its high seed oil content

(23%) and suitable engine performance parameters, is a promising source of biodiesel (Weber *et al*., 2007; Abideen *et al*., 2015). This plant is also valuable as animal feed (Alvarez Cruz, 2013; Pirasteh-Anosheh *et al*., 2023) and has demonstrated effectiveness in stabilizing soil in coastal areas (Agha, 2009). Due to its remarkable adaptability to saline environments, *C. cretica* is a valuable resource for sustainable land management and environmental restoration (Naz *et al*., 2024). It also serves as an effective halophyte for saline agriculture, soil desalination, and phytoremediation, helping reclaim degraded lands and reduce heavy metal pollution in affected regions (Joshi *et al*., 2020).

Preserving genetic diversity across multiple levels forms the basis for population monitoring and efficient conservation strategies. Given that genetic variation occurs between individuals of a species, proper evaluation necessitates examining entire populations (Salgotra and Chauhan, 2023). Molecular markers with high precision and resolution have become indispensable tools for DNA-based differentiation, assessing variability, and analyzing population structure (Hussain and Nisar, 2020). Among these, start codon targeted (SCoT) and inter-simple sequence repeat (ISSR) markers have been effectively employed in numerous plant genetic studies. The SCoT marker system amplifies conserved regions around the ATG start codon in plant genes, generating reliable genetic data (Collard and Mackill, 2009), with recent applications demonstrating its effectiveness (Atapour *et al*., 2022). In contrast, ISSR markers amplify regions between microsatellite repeats, mainly in non-coding areas, providing a broader overview of genetic variability across the genome (Zietkiewicz *et al*., 1994; Ng and Tan, 2015; Gemmill and Grierson, 2021), as evidenced by studies such as Nazarzadeh *et al*. (2020). The high polymorphism, robustness, and reproducibility of ISSR markers have contributed to their widespread use in population genetics research.

Despite its ecological and medicinal significance, the genetic diversity of *C. cretica* remains largely unexplored. For the first time, this study employs SCoT and ISSR markers to assess the genetic diversity of *C. cretica* accessions in Ahvaz. The findings will enhance understanding of its genetic variation, supporting conservation strategies and potential genetic improvement for medicinal and ecological applications.

Materials and Methods

Plant materials

This study randomly collected 200 accessions of *C. cretica* from various habitats across Ahvaz to capture the species' ecological and geographical diversity. A representative subset was carefully selected to reflect the observed variation based on preliminary morphological analyses (Jahangir and Nasernakhaei, 2020) that showed low phenotypic variation and suggested clonal reproduction. The selected accessions encompassed different microhabitats on both sides of the Karun River, capturing potential environmental and spatial variation. From the broader set of collected accessions, 13 were purposefully selected for molecular analyses based on their ability to represent the observed diversity, while also meeting the technical requirements for DNA quality. This selection strategy balanced biological relevance with methodological considerations, aiming to maximize informative value while avoiding redundancy (Fig.1 and Table 1).

DNA extraction

Genomic DNA was isolated using silica-dried leaves with a DNA extraction kit (Bio Basic Inc., Canada). The purified total DNA was quantified by 0.8% agarose gel electrophoresis and verified by spectrophotometer.

PCR amplification

This study used 12 SCoT primers (Collard and Mackill, 2009). Twelve ISSR primers were also tested for DNA amplification. The complete list of primers is provided in Table 2. The PCR reaction was performed in a total volume of 20 µL, consisting of 10 µL of 2X ready-to-use PCR master mix (Ampliqon Co., Denmark), 7.2 µL of double-distilled water (ddH_2O), 0.8 µL of each primer (10 pmol/µL), and 2 µL of extracted DNA from each accession at a concentration of 25-50 ng.

The ISSR-PCR amplification protocol included an initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at primer-specific temperatures (optimized via gradient PCR, ranging from 46°C to 51.1°C) for 45 sec, and extension at 72°C for 2 min. A final extension step was performed at 72°C for 7 min. For SCoT-PCR amplification, the initial denaturation was carried out at 94°C for 4 min, followed by 35 cycles, each consisting of denaturation at 94°C for 50 sec, annealing at 50°C for 45 sec, and elongation at 72°C for 2 min. A final extension step was performed at 72°C for 10 min. Amplification products were separated using 1.5% agarose gel electrophoresis and visualized using YTA safe stain (Yekta Tajhiz Azma; Iran).

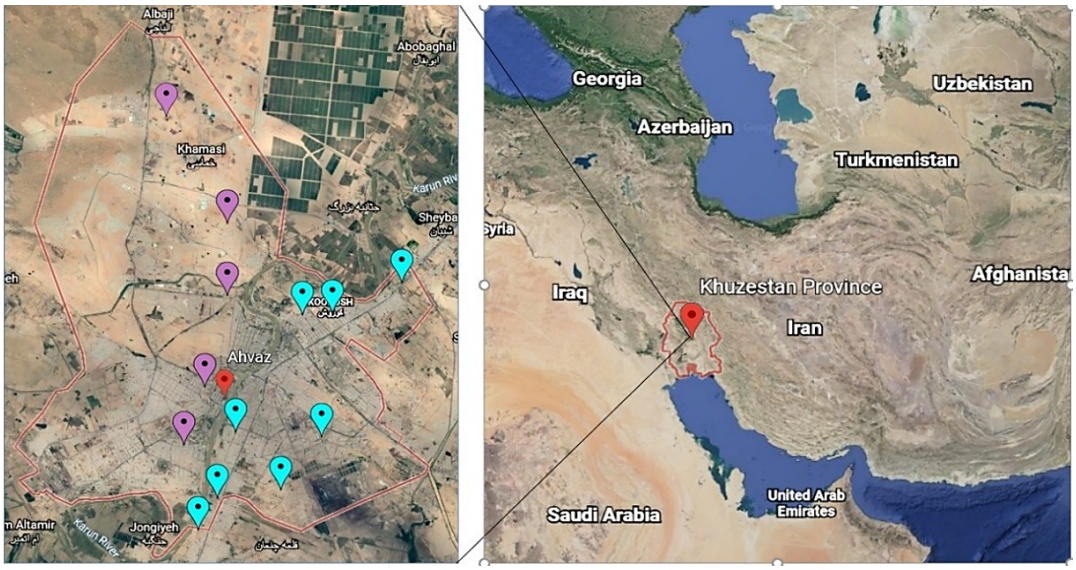

Fig. 1. Geographical distribution of *C. cretica* accessions in the east (green) and west (pink) of the Karun River, Ahvaz, Iran.

Table 1. Geographical coordinates and accession information of *C. cretica* accessions collected from Ahvaz.

Geographical groups	Number of accessions	Code	Latitude (N)	Longitude (E)
West of the Karun River	5	Cre.2	31°17′45.164″	48°39′9.460″
		Cre.9	31°17′53.320″	48°38′42.625″
		Cre.180	31°24′26.629″	48°43′50.666″
		Cre.191	31°22′23.818″	48°38′55.544″
		Cre.200	31°22′21.189″	48°39′2.292″
East of the Karun River	8	Cre.45	31°13′44.710″	48°38′50.914″
		Cre.53	31°14′0.655″	48°37′22.593″
		Cre.63	31°13′52.340″	48°37′16.788″
		Cre.71	31°13′39.518″	48°38′53.600″
		Cre.72	31°12′8.277″	48°38′19.153″
		Cre.136	31°23′21.228″	48°47′6.351″
		Cre.159	31°21′58.230″	48°43′5.726″
		Cre.161	31°22′10.632″	48°43′20.864″

Table 2. List of SCoT and ISSR primers used for genetic analysis of *C. cretica.*

Primer name	Primer sequence	%GC	Primer name	Primer sequence	%GC
SCoT1	CAACAATGGCTACCACCA	50	ISSR1	CACACACACACAGG	57.1
SCoT3	CAACAATGGCTACCACCG	56	ISSR2	CACACACACACAAC	50
SCoT5	CAACAATGGCTACCACGA	50	ISSR3	CACACACACACAAAG	50
SCoT11	AAGCAATGGCTACCACCA	50	ISSR4	AGAGAGAGAGAGAGAGT	47.1
SCoT13	ACGACATGGCGACCATCG	61	ISSR5	AGAGAGAGAGAGAGAGC	52.9
SCoT14	ACGACATGGCGACCACGC	67	ISSR6	CACACACACACACACAAGG	52.6
SCoT19	ACCATGGCTACCACCGGC	67	ISSR7	ACACACACACACACACG	52.9
SCoT20	ACCATGGCTACCACCGCG	67	ISSR8	TATTCCGACGCTGAGGCAG	57.9
SCoT21	ACGACATGGCGACCCACA	61	ISSR9	GGAGAGGAGAGGAGA	60
SCoT26	ACCATGGCTACCACCGTC	61	ISSR10	GAGAGAGAGAGAGAGAGT	50
SCoT34	ACCATGGCTACCACCGCA	61	ISSR11	GAGAGAGAGAGAGAGATC	50
SCoT35	CATGGCTACCACCGGCCC	72	ISSR12	CCAACGATGAAGAACGCAGC	55

Data analysis

Visible and reproducible bands generated by SCoT and ISSR markers were scored as "1" for the presence and "0" for the absence. polymorphism information content (PIC), marker index (MI), and resolving power (Rp) were calculated to assess the informativeness of the markers in differentiating accessions. PIC was calculated according to Roldan-Ruiz *et al*. (2000), as PIC= $2f_i$ $(1\text{-}f_i)$ where fi is the frequency of the i allele. MI was determined as the product of PIC and the effective multiplex ratio (EMR), as described by Nagaraju *et al*. (2001) (*i.e.,* MI= PIC× EMR).

The Rp of each primer was calculated using $R_p= \Sigma I_b$ where I_b is band informativeness (the I_b can be represented on a scale of 0- 1 by the following formula: Ib= 1- [2× |0.5- p|] where p is the proportion of individuals containing the band) (Prevost and wilkins,1999).

To assess genetic differentiation, the selected accessions were categorized into two groups based on geographical origin: (1) East of the Karun River and (2) West of the Karun River (Fig. 1 and Table 1). Analysis of molecular variance (AMOVA) was performed using GenAlEx version 6.5 (Peakall and Smouse, 2006) to evaluate the distribution of genetic variation within and between groups. Additionally, genetic variation indices, including the percentage of polymorphic bands (PPB), the observed numbers of alleles (Na) and effective numbers of alleles (Ne), Nei's gene diversity (h), and Shannon's information index (I), were estimated using POPGENE version 1.32.

A genetic similarity matrix was generated using NTSYS-pc version 2.2 (Rohlf, 2000), and a dendrogram was constructed based on Jaccard's coefficient. Principal coordinate analysis (PCoA) was performed using GenAlEx version 6.5 (Peakall and Smouse, 2006) to visualize genetic relationships in two-dimensional space. The first three coordinates were interpreted based on their cumulative contribution to total variance. STRUCTURE analysis was inferred using a Markov Chain Monte Carlo (MCMC) simulation approach implemented in STRUCTURE HARVESTER version 0.6.94 (Earl and vonHoldt, 2012). This analysis employed an admixture model with correlated allele frequencies and evaluated a range of K values (representing the number of clusters) from 2 to 10. For each K value, a burn-in period of 100,000 MCMC iterations was followed by three replication iterations, with ten independent runs conducted per K value, and the most probable number of clusters (K) was determined using the ΔK method (Evanno *et al*., 2005).

Results

Polymorphism of amplified products

To evaluate genetic diversity, 12 SCoT primers were screened, with 10 producing reproducible amplification patterns (Table 3). The 92 bands were generated, of which 84 (87.9%) were identified as polymorphic. Most primers exhibited 100% polymorphism, except for SCoT21 (40%), SCoT11 (57.14%), SCoT35 (87.5%), and SCoT14 (94.44%). The number of amplified fragments per primer ranged from 4 (SCoT20) to 18 (SCoT14), with an average of 9.2 bands, 8.4 of which were polymorphic. Fragment sizes varied from 140 bp to 3000 bp. PIC values ranged from 0.10 (SCoT21) to 0.36 (SCoT13), averaging 0.24. SCoT14 demonstrated the highest resolving power (Rp= 8.66), while SCoT13 recorded the highest marker index (MI= 5.44).

For ISSR analysis, 12 primers were tested, and 10 produced reproducible and scorable banding patterns, which were subsequently used in the analysis. 114 scoreable bands were obtained, with 98 (85.62%) identified as polymorphic. ISSR8 showed 100% polymorphism, while the remaining primers ranged from 60% (ISSR10) to 93.33% (ISSR4 and ISSR9). The number of amplified bands per primer varied from 5 (ISSR8) to 15, with an average of 11.4 bands, including 9.8 polymorphic bands. Fragment sizes ranged from 250 bp to 3 kb. PIC values ranged from 0.22 (ISSR11) to 0.40 (ISSR4), with an average of 0.31. ISSR4 exhibited the highest resolving power (Rp= 11.33) and marker index (MI= 5.27).

Genetic diversity

Analysis of molecular variance (AMOVA) revealed that most genetic variation was distributed within geographical groups rather than between them. This accounted for 90%

Table 3. Banding pattern characteristics of SCoT and ISSR markers in *C. cretica.*

	Primer	TNB	NPB	PPB	Rp	PIC	MI	SZ (bp)
SCoT Primers	SCoT1	9	9	100	7.33	0.35	3.46	400-2400
	SCoT3	5	5	100	6.8	0.24	1.2	600-3000
	SCoT5	7	7	100	4.28	0.29	2.08	510-3000
	SCoT11	7	4	57.14	6.85	0.16	0.38	400-2500
	SCoT13	15	15	100	7.2	0.36	5.44	180-2700
	SCoT14	18	17	94.44	8.66	0.31	5.1	200-3000
	SCoT19	12	12	100	5.83	0.29	3.55	410-3000
	SCoT20	4	6	100	0.0	0.14	0.85	140-2800
	SCoT21	5	2	40	5.2	0.1	0.08	280-2200
	SCoT35	8	7	87.5	6.0	0.24	1.48	300-2600
Total		92	84	-	-	-	-	-
Average		9.2	8.4	87.9	5.81	0.24	2.36	-
ISSR Primers	ISSR1	11	10	90.9	8.36	0.3	2.73	250-2000
	ISSR2	13	11	84.61	9.07	0.34	3.18	250-2700
	ISSR3	12	11	91.66	6.0	0.38	3.87	250-2700
	ISSR4	15	14	93.33	11.33	0.4	5.27	250-3000
	ISSR5	14	12	85.71	6.0	0.32	3.33	300-2500
	ISSR7	10	9	90.0	8.4	0.32	2.66	300-2700
	ISSR8	5	5	100	4.8	0.28	1.44	300-2700
	ISSR9	15	14	93.33	8.26	0.36	4.8	300-2700
	ISSR10	10	6	60	8.8	0.3	1.09	300-3000
	ISSR11	9	6	66.66	4.88	0.22	0.88	500-2500
Total		114	98	-	-	-	-	-
Average		11.4	9.8	85.62	7.59	0.32	2.92	-

TNB= Total number of bands; NPB= Number of polymorphic bands; PPB= Percentage of polymorphic bands; Rp= Resolving power; PIC= Polymorphism information content; MI= Marker index; SZ= Band size range.

Table 4. Analysis of molecular variance (AMOVA) in *C. cretica* accessions based on SCoT/ISSR markers.

		SCoT				ISSR			
Source	**df**	**SS**	**MS**	**Est. Var.**	**Var%**	**SS**	**MS**	**Est. Var.**	**Var%**
Among the geographical group	1	22.23	22.23	1.47	10%	25.34	25.34	0.89	4%
Within accessions	11	145.00	13.18	13.18	90%	218.35	19.85	19.85	96%
Total df	12	167.23	-	14.65	100%	243.69	-	20.74	100%

df= degrees of freedom; SS= Sum of squares; MS= Mean squares; Est. Var= Estimated variance components; Var%= Percentage of total variance.

and 96% of the total variance based on SCoT and ISSR markers, respectively (Table 4). These findings indicate low genetic differentiation between the east and west groups of the Karun River, with most variation occurring within these groups (Table 5). For SCoT markers, the observed number of alleles (Na) ranged from 1.261± 0.094 in the western group (west of the Karun River) to 1.707± 0.061 in the eastern group (east of the Karun River). The effective number of alleles (Ne) varied between 1.318± 0.038 (western group) and 1.382± 0.038 (eastern group). Nei's gene diversity (h) was highest in the eastern group (0.229± 0.185) and lowest in the western group (0.189± 0.193). Similarly, Shannon's information index (I) showed its highest value in the eastern group (0.353± 0.027) and the lowest in the western group (0.287± 0.029). The ISSR marker analysis showed slightly different results. Na values ranged from 1.711± 0.048 in the eastern group to 1.623± 0.062 in the western group. The Ne values were similar across groups, varying between 1.503± 0.035 (eastern group) and 1.495± 0.037 (western group). Nei's gene diversity (h) reached its highest value in the

eastern group (0.287± 0.195) and its lowest in the western group (0.279± 0.200). Shannon's information index (I) showed minimal difference between groups. The coefficient of genetic differentiation (Gst), estimated using SCoT and ISSR markers, was 0.14 and 0.11, respectively. These values indicate that only 14% (SCoT) and 11% (ISSR) of the total genetic variability was attributed to differences between groups, while 86% (SCoT) and 89% (ISSR) of the variation occurred within groups. The estimated gene flow (Nm) between the two groups was 2.92 for SCoT markers and 3.85 for ISSR markers, suggesting moderate genetic exchange.

Table 5. Genetic diversity indices and differentiation parameters of *C. cretica* accessions based on SCoT and ISSR markers.

Marker	Geographical groups	N	Na ±SD	Ne ± SD	h ± SD	I ± SD	Pp (%)	Gst	Nm
SCoT	West of the Karun River	5	1.261 ± 0.094	1.318 ± 0.038	0.189 ± 0.193	0.287 ± 0.029	59.52		
	East of the Karun River	8	1.707 ± 0.061	1.382 ± 0.038	0.229 ± 0.185	0.353 ± 0.027	77.17		
	Average		1.484± 0.058	1.350 ± 0.027	0.247 ± 0.170	0.320 ± 0.020	66.85	0.146	2.924
ISSR	West of the Karun River	5	1.623 ± 0.062	1.495 ± 0.037	0.279 ± 0.200	0.411 ± 0.026	71.93		
	East of the Karun River	8	1.711 ± 0.048	1.503 ± 0.035	0.287 ± 0.195	0.422 ± 0.026	73.68		
	Average		1.667± 0.039	1.499 ± 0.025	0.319 ± 0.167	0.416 ± 0.018	72.81	0.114	3.854

N= Number of accessions; Na= Observed number of loci; Ne= Effective number of alleles; h= Nei's gene diversity; I= Shannon's information index; Pp= Percentage of polymorphic loci; Gst= Diversity between geographical groups; Nm= Estimated gene flow (0.5 (1- Gst)/Gst).

Cluster and principal coordinate analysis

Cluster analysis using UPGMA and Jaccard's coefficient grouped the 13 accessions into two main clusters (I and II) for both SCoT (Fig. 2A) and ISSR (Fig. 2B) markers. These clusters were defined based on the node with the highest genetic dissimilarity, as indicated by the vertical dashed line. However, the clustering patterns did not align clearly with geographical groupings, as accessions from the eastern and western sides of the Karun River were intermixed within Cluster I. Cluster II contained only a single accession in both marker systems, further confirming the weak spatial structuring. This suggests that the clustering reflects genetic similarity rather than spatial separation. Principal coordinate analysis (PCoA) further supported these findings. The two-dimensional scatter plot (Fig. 3) reflected the UPGMA clustering and revealed two main groups. However, there was considerable overlap between accessions from both geographical areas.

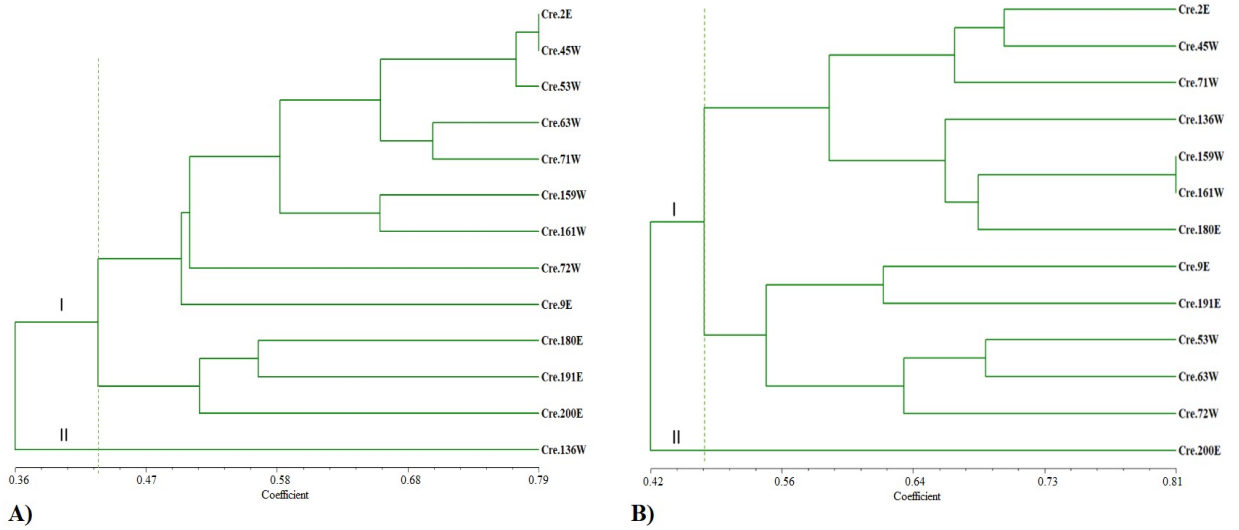

Fig. 2. UPGMA dendrograms showing genetic relationships among 13 *C. cretica* accessions using: A) SCoT and B) ISSR markers, based on Jaccard's coefficient (Accessions are labeled by geographic origin: E (East) and W (West) of the Karun River; Clusters I and II are defined by branch dissimilarity).

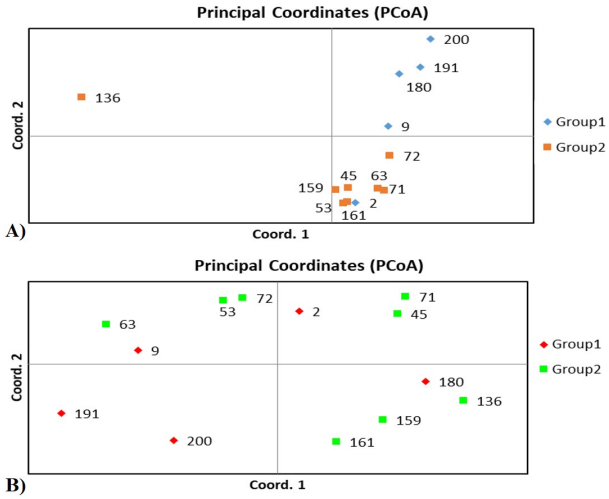

Fig. 3. Principal Coordinates Analysis (PCoA) of 13 *C. cretica* accessions based on genetic distance matrices derived from (A) SCoT and (B) ISSR markers (Group 1= East of the Karun River, Group 2= West of the Karun River).

In the SCoT-based analysis, the first three principal components explained 44.91%, 17.91%, and 14.88% of the total genetic variation, respectively, with the first two axes accounting for 62.82% of the total variance. The ISSR-based analysis explained a higher proportion of variation, with the first three axes contributing 70.82% (axis 1: 37.11%, axis 2: 22.57%, axis 3: 11.13%).

Structure analysis

The Bayesian clustering analysis using STRUCTURE revealed distinct patterns of genetic grouping depending on the marker system used. For the SCoT dataset, the highest likelihood was observed at K= 3 (Fig. 4A), resulting in three inferred genetic groups. While some accessions were clearly assigned to distinct clusters, others (e.g., Cre.9 and Cre.2) exhibited admixture patterns. In contrast, the ISSR dataset indicated that K= 2 was the most probable number of clusters (Fig. 4B), exhibiting clearer group separation and minimal admixture among accessions.

Discussion

Genetic diversity is pivotal for species adaptation and survival. This study evaluated *C. cretica* accessions from two geographical groups using SCoT (targeting functional gene regions) and ISSR (amplifying random genomic loci) markers. These markers revealed complementary and marker-specific patterns of genetic variation: ISSR markers, as demonstrated in *Luffa cylindrica* (Tyagi *et al*., 2020), captured genome-wide diversity, while SCoT markers, as shown in *Salvia* spp. (Etminan *et al*., 2018), resolved fine-scale genetic structures in functionally important regions. The rationale for using both marker types was to combine the broad genome coverage of ISSR with the gene-targeted specificity of SCoT, thereby enhancing the resolution of genetic variation and structure in *C. cretica*.

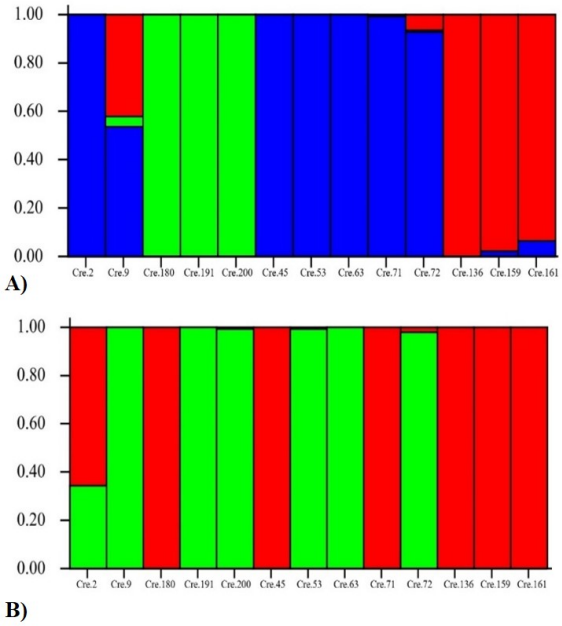

Fig. 4. Genetic structure analysis of *C. cretica* accessions using STRUCTURE: A) Three genetic clusters inferred from SCoT markers (K= 3); B) Two genetic clusters inferred from ISSR markers (K= 2).

To elucidate the genetic relationships among *C. cretica* accessions, we employed three complementary clustering approaches: UPGMA, PCoA, and STRUCTURE (Bayesian) analyses. Cluster analyses based on UPGMA and PCoA consistently indicated weak genetic structuring, with only partial or ambiguous separation between eastern and western accessions. This suggests that the Karun River may not serve as a strict genetic barrier, possibly due to ongoing gene flow and shared environmental pressures. Interestingly, STRUCTURE analysis revealed marker-dependent patterns, emphasizing how methodological differences can influence

interpretations of genetic structure (Fig. 4). The SCoT marker analysis identified three genetic clusters (K= 3), with several accessions, including Cre.9 and Cre.2, exhibiting evidence of admixture. These admixed accessions may indicate recent gene exchange. In contrast, ISSR markers demonstrated a simpler genetic structure with only two clusters (K= 2). The discrepancy between SCoT (K= 3) and ISSR (K= 2) findings highlights the critical impact of marker selection on structure inference. The greater resolution provided by SCoT markers, likely due to their gene-targeted nature, appears more sensitive for detecting fine-scale genetic patterns and recent admixture events. These findings collectively suggest that while some genetic structuring exists in *C. cretica*, it does not strictly correlate with geographical divisions. The weak clustering patterns and marker-dependent results emphasize: 1) the need for careful interpretation of genetic structure analyses, 2) the importance of employing multiple marker systems to gain comprehensive insights, and 3) the role of gene flow in shaping genetic relationships in plant species. Therefore, comparisons between model-based and distance-based clustering approaches should be made with care, as their outcomes may not always be consistent.

The reproductive system of *C. cretica* appears to play a significant role in shaping its genetic structure. A mixed reproductive strategy, combining sexual reproduction (likely through cross-pollination) and asexual propagation via rhizomes, may account for the high within-group diversity and low regional differentiation. Similar strategies have been reported in species such as *Satureja rechingeri* (Hadian *et al*., 2015) and *Cynodon dactylon* (Akbari *et al*., 2018), indicating outcrossing tendencies. The genetic differentiation coefficient (Gst) was 0.146 for SCoT and 0.114 for ISSR, indicating moderate differentiation between the two geographical groups (Nei, 1973). Gene flow (Nm), estimated as 0.5(1–Gst)/Gst (Slatkin, 1985), was 2.924 for SCoT and 3.854 for ISSR, suggesting extensive inter-group genetic exchange despite the presence of the Karun River as a physical boundary. This may result from ecological variation and multiple dispersal pathways, including movement by animals (e.g., water buffaloes and birds), floodwaters, and human-related changes such as land use and soil disturbance. The observed higher genetic diversity in the eastern group (Table 5) may reflect more heterogeneous habitats or increased anthropogenic activity, such as land use changes (Jump *et al*., 2009). Similar to findings in *Ambrosia artemisiifolia* (Kropf *et al*., 2018), our results suggest that human-mediated disturbances may enhance gene flow and disrupt spatial genetic patterns, sometimes even more than natural dispersal processes. These mechanisms may help maintain genetic connectivity and adaptive potential in *C. cretica*, with direct implications for conservation planning.

The SCoT markers, being gene-targeted, demonstrated a higher capacity for detecting subtle genetic structures. STRUCTURE analysis based on SCoT data identified three genetic clusters (K= 3), suggesting the presence of fine-scale genetic differentiation. Furthermore, the analysis of molecular variance (AMOVA) using SCoT markers revealed 10% of the total variation among geographical regions and 90% within accessions, which supports the presence of sub-structuring within regions. Despite their lower overall polymorphism (PIC= 0.24, h= 0.247, I= 0.320), SCoT markers captured the structural nuances of *C. cretica* accessions more effectively. In contrast, ISSR markers displayed higher levels of polymorphism and diversity, with PIC= 0.31, h= 0.319, and I= 0.416. These results indicate that ISSR markers are more efficient for assessing overall genetic variability across the species' range. STRUCTURE analysis with ISSR data resolved two major clusters (K= 2), corresponding broadly to the eastern and western accessions divided by the Karun River. Additionally, AMOVA based on ISSR markers showed only 4% variation among geographic regions and 96% within accessions, highlighting strong intra-regional diversity and limited inter-regional divergence. This suggests that ISSR markers better capture genome-wide polymorphisms rather than detailed genetic structuring among accessions. Together, these findings demonstrate the complementary nature of both marker systems. SCoT markers are more effective in identifying recent divergence and localized differentiation, whereas ISSR markers provide a broader picture of genome-wide

diversity. The integration of both systems offers a more complete perspective on the genetic architecture and adaptive potential of *C. cretica*.

Conclusion

This study presents the first integrated assessment of genetic diversity in *C. cretica* using both SCoT and ISSR molecular markers. The findings reveal substantial genetic variation, particularly within geographical groups (accessions), and highlight the species' ecological adaptability and mixed reproductive strategy as key drivers of its genetic structure. By employing a combination of clustering methods (UPGMA, PCoA, and STRUCTURE), we uncovered complex but weak genetic structuring that does not correspond strictly with geographical divisions, likely due to extensive gene flow across the Karun River. The contrast between ISSR and SCoT results underscores the value of using complementary marker systems to capture both genome-wide diversity and gene-associated variation. Although the apparent lack of strong genetic differentiation might reflect biological realities, it may also partly result from the scale and scope of sampling. Future research using additional markers and broader geographic coverage is essential to confirm these patterns.
These insights provide a foundation for conservation planning and potential breeding programs for this ecologically important halophyte.

Conflict of interests

The authors declare that they have no conflicts of interest.

References

Abideen, Z., Qasim, M., Rizvi, R. F., Gul, B., Ansari, R., & Khan, M. A. (2015). Oilseed halophytes: a potential source of biodiesel using saline degraded lands. *Biofuels*, 6(5-6), 241-248. https://doi.org/10.1080/17597269.2015.1090812

Agha, F. (2009). Seasonal variation in productivity of *Cressa cretica* from coastal population along the arabian sea. *Pakistan Journal of Botany*, 41(6), 2883-2892. https://www.pakbs.org/pjbotnew/index.php

Akbari, M., Salehi, H., & Niazi, A. (2018). Evaluation of diversity based on morphological variabilities and ISSR molecular markers in Iranian *Cynodon dactylon* (L.) Pers. accessions to select and introduce cold-tolerant genotypes. *Molecular Biotechnology*, 60, 259-270. https://doi.org/10.1007/s12033-018-0068-5

Al-Snafi, A. E. (2016). The chemical constituents & therapeutic importance of *Cressa cretica*-A review. *IOSR Journal of Pharmacy*, 6(6), 39-46. https://www.iosrjournals.org/IOSR-PHR.html

Alvarez Cruz, N. S. (2013). *Cressa cretica* L. In G. H. Schmelzer & A. Gurib-Fakim (Eds.), *PROTA 11(2): Medicinal plants/Plantes médicinales 2*. PROTA.

Atapour, Y., Ghanbari, A., Estaji, A., & Haghjooyan, R. (2022). Evaluation of genetic diversity of twenty-eight sweet cherry genotypes by morphological traits & SCoT markers in the Northwest of Iran. *Journal of Genetic Resources*, 8(2), 138-146. https://doi.org/10.22080/jgr.2022.22815.1293

Austin, D. F. (2000). A revision of *Cressa* L. (Convolvulaceae). *Botanical Journal of the Linnean Society*, 133(1), 27-39. https://doi.org/10.1111/j.1095-8339.2000.tb01535.x

Chintaluri, P. G., Ganapathy Vilasam Sreekala, A., Gupta, K. K., Sivadasan, A., & Nathan, V. K. (2025). Network pharmacological evaluation of *Cressa cretica* L.-an integrated approach of modern & ancient pharmacology. *Journal of Biomolecular Structure and Dynamics*, 1-16. https://doi.org/10.1080/07391102.2025.2472403

Collard, B. C., & Mackill, D. J. (2009). Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Molecular Biology Reporter*, 27, 86-93. https://doi.org/10.1007/s11105-008-0060-5

Earl, D. A., & VonHoldt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output & implementing the Evanno method. *Conservation Genetics Resources*, 4, 359-361. https://doi.org/10.1007/s12686-011-9548-7

El-Alfy, T. S., Ammar, N. M., Al-Okbi, S. Y., Salama, M. M., Aly, H. F., & Amer, A. A. (2019). *Cressa cretica* L. growing in Egypt: phytochemical study & potential antioxidant & hepato-protective activities. *Journal of*

Applied Pharmaceutical Science, 9(1), 046-057. https://doi.org/10.7324/JAPS.2019.S106

Etminan, A., Pour-Aboughadareh, A., Noori, A., Ahmadi-Rad, A., Shooshtari, L., Mahdavian, Z., & Yousefiazar-Khanian, M. (2018). Genetic relationships & diversity among wild *Salvia* accessions revealed by ISSR and SCoT markers. *Biotechnology and Biotechnological Equipment*, 32(3), 610-617. https://doi.org/10.1080/13102818.2018.1447397

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14(8), 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Gemmill, C. E., & Grierson, E. R. (2021). Inter-simple sequence repeats (ISSR), microsatellite-primed genomic profiling using universal primers. *Molecular Plant Taxonomy: Methods and Protocols*, 249-262. https://doi.org/10.1007/978-1-0716-0997-2_14

Hadian, J., Karami, A., Azizi, A., & Khadivi-Khub, A. (2015). Ubiquitous genetic diversity among & within wild populations of *Satureja rechingeri* assessed with ISSR markers. *Plant Systematics and Evolution*, 301, 923-930. https://doi.org/10.1007/s00606-014-1126-5

Hussain, H., & Nisar, M. (2020). Assessment of plant genetic variations using molecular markers: a review. *Journal of Applied Biology and Biotechnology*, 8(5), 99-109. http://dx.doi.org/10.7324/JABB.2020.80514

Jahangir, M., & Nasernakhaei, F. (2020, January). Morphological variation of *Cressa cretica* L. in Ahvaz. 16th National Iranian Crop Science Congress, Ahvaz, Iran. https://doi.org/10.13140/RG.2.2.28084.19842

Joshi, A., Kanthaliya, B., Rajput, V., Minkina, T., & Arora, J. (2020). Assessment of phytoremediation capacity of three halophytes: *Suaeda monoica*, *Tamarix indica* & *Cressa critica*. *Biologia Futura*, 71, 301-312. https://doi.org/10.1007/s42977-020-00038-0

Jump, A. S., Marchant, R., & Peñuelas, J. (2009). Environmental change & the option value of genetic diversity. *Trends in Pant Science*, 14(1), 51-58. https://doi.org/10.1016/j.tplants.2008.10.002

Kropf, M., Huppenberger, A. S., & Karrer, G. (2018). Genetic structuring & diversity patterns along rivers-local invasion history of *Ambrosia artemisiifolia* (Asteraceae) along the Danube River in Vienna (Austria) shows non-linear pattern. *Weed Research*, 58(2), 131-140. https://doi.org/10.1111/wre.12284

Nagaraju, J., Reddy, K. D., Nagaraja, G. M., & Sethuraman, B. N. (2001). Comparison of multilocus RFLPs & PCR-based marker systems for genetic analysis of the silkworm, Bombyx mori. *Heredity*, 86(5), 588-597. https://doi.org/10.1046/j.1365-2540.2001.00861.x

Naz, N., Asghar, A., Basharat, S., Fatima, S., Hameed, M., Ahmad, M. S. A., ... & Ashraf, M. (2024). Phytoremediation through microstructural & functional alterations in alkali weed (*Cressa cretica* L.) in the hyperarid saline desert. *International Journal of Phytoremediation*, 26(6), 913-927. https://doi.org/10.1080/15226514.2023.2282044

Nazarzadeh, Z., Onsori, H., & Akrami, S. (2020). Genetic diversity of bread wheat (*Triticum aestivum* L.) genotypes using RAPD & ISSR molecular markers. *Journal of Genetic Resources*, 6(1), 69-76. https://doi.org/10.22080/jgr.2020.18262.1172

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences*, 70(12), 3321-3323. https://doi.org/10.1073/pnas.70.12.3321

Ng, W. L., & Tan, S. G. (2015). Inter-simple sequence repeat (ISSR) markers: are we doing it right. *ASM Science Journal*, 9(1), 30-39. https://www.akademisains.gov.my/asmsj/

Peakall, R. O. D., & Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. population genetic software for teaching and research. *Molecular Ecology Notes*, 6(1), 288-295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Pirasteh-Anosheh, H., Ranjbar, G., Akram, N. A., Ghafar, M. A., & Panico, A. (2023). Forage potential of several halophytic species grown on saline soil in arid environments. *Environmental Research*, 219, 114954. https://doi.org/10.1016/j.envres.2022.114954

Prevost, A., & Wilkinson, M. J. (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and Applied Genetics*, 98, 107-112. https://doi.org/10.1007/s001220051046

Priyashree, S., Jha, S., & Pattanayak, S. P. (2010). A review on *Cressa cretica* Linn.: a

halophytic plant. *Pharmacognosy Reviews*, (8), 161. http://dx.doi.org/10.4103/0973-7847.70910

Rohlf, F. J. (2000). *NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.2* [Computer software]. Exeter Software.

Roldan-Ruiz, I., Dendauw, J., Van Bockstaele, E., Depicker, A., & De Loose, M. A. F. L. P. (2000). AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). *Molecular Breeding*, 6, 125-134. https://doi.org/10.1023/A:1009680614564

Salgotra, R. K., & Chauhan, B. S. (2023). Genetic diversity, conservation, and utilization of plant genetic resources. *Genes*, 14(1), 174. https://doi.org/10.3390/genes14010174

Slatkin, M. (1985). Gene flow in natural populations. *Annual Review of Ecology and Systematics*, 393-430. https://www.annualreviews.org/content/journals/ecolsys

Tyagi, R., Sharma, V., Sureja, A. K., Das Munshi, A., Arya, L., Saha, D., & Verma, M. (2020). Genetic diversity and population structure detection in sponge gourd (*Luffa cylindrica*) using ISSR, SCoT and morphological markers. *Physiology and Molecular Biology of Plants*, 26, 119-131. https://doi.org/10.1007/s12298-019-00723-y

Weber, D. J., Ansari, R., Gul, B., & Khan, M. A. (2007). Potential of halophytes as source of edible oil. *Journal of Arid Environments*, 68(2), 315-321. http://dx.doi.org/10.1016/j.jaridenv.2006.05.010

Zietkiewicz, E., Rafalski, A., & Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, 20(2), 176-183. https://doi.org/10.1006/geno.1994.1151