Genetic Diversity and Population Genetic Structure of *Tamarix dubia* (Tamaricaceae) from Iran

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Abstract

The genus *Tamarix* (Tamaricaceae Link), commonly known as tamarisk, comprises 54 to 90 species and is known for its complex taxonomy and molecular phylogeny. This genus represents a complex group where multiple evolutionary mechanisms have contributed to its evolution. However, there has been no previous report on the genetic diversity and structure of *Tamarix dubia* populations. This study aimed to illustrate the genetic diversity and differentiation of the species in a geographical context in Iran. A total of 112 plants from 23 populations across four geographical regions in Iran were sampled for molecular investigations. Using 100 SRAP loci for genetic analyses, the data showed polymorphism ranging from 16.00% to 30%, indicating low genetic variability. The Analysis of Molecular Variance AMOVA test revealed significant genetic differences among the populations, and the Fst value was significant for most populations, suggesting notable genetic differentiation. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram and MDS plot clustered samples from each population separately, reflecting high intra-population genetic similarity. The heat map further differentiated the studied populations. Additionally, as evident in the Assignment test, genetic admixture was mainly observed between populations 20 and 21, as well as among populations 10, 11, 16, and 23. This admixture has contributed to the overall low gene flow, measured at 0.26. In conclusion, the *T. dubia* populations exhibited low level of genetic polymorphism, which is likely attributed to factors such as low effective population size (Ne) and random genetic drift.

Keywords: Population genetics, Population structure, geographical regions, Polymorphism percentage, *Tamarix dubia*

Introduction

The genus *Tamarix* L. (Tamaricaceae), commonly known as tamarisk, comprises approximately 54 to 90 species. This plant is known as a group characterized by significant complexity in terms of taxonomy

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and molecular phylogeny (Bahramsoltani et al., 2020).

The genus Tamarix, belonging to the family Tamaricaceae, is represented by 55 species distributed worldwide. Members of this genus are primarily found in temperate and subtropical regions. This plant is native to Mediterranean countries, southern Europe, China, India, Mongolia, North Africa, and southwestern Africa. Tamarix species encompass shrubs, semi-shrubs, and tall trees, with some individuals attaining heights of up to 18 meters. This genus is categorized as halophytic or xerophytic, characterized by its multiple stems and slender branches (Arianmanesh et al., 2016).

Diverse evolutionary mechanisms including polyploidy, gene flow, and inter-specific introgression, have been suggested to contribute to the evolution of the *Tamarix* genus (Villar et al., 2018; Sheidai et al., 2018). A recent investigation also suggested the occurrence of ancient hybridization and gene flow within this generic complex (Sheidai & Koohdar, 2022).

Due to their valuable phytochemicals, *Tamarix* species possess medicinal properties and are used to treat infections, wounds, liver and spleen issues, digestive disorders, diabetes, and dental problems. Some species also play an important role in preventing deforestation (Bahramsoltani et al., 2020).

Genetic diversity information can be valuable for establishing successful management strategies for indigenous and invasive species. In natural populations, gene flow serves as the main sources for generating novel polymorphisms. It has the potential to enhance theeffective population size, thereby counteracting the effects of random genetic drift. Such new gene combinations may influence by local selection pressures and the divergence of geographical populations (McCormick et al., 2016; Mayonde et al., 2019). Also, examining the mentioned cases can assist in the management of habitats at both regional and global levels (Su et al., 2017).

A diverse array of molecular markers ranging from multilocus SNPs (Single-nucleotide polymorphism), SSRs (Simple Sequence Repeats), AFLP (Amplified Fragments Length Polymorphism), and SCoT markers (Start Codon Targeted), have been used in genetic diversity studies including *Tamarix* species (see for example, Mayonde et al., 2019; Sheidai et al., 2019; Juybari et al., 2021; Zhang et al., 2019; Cheng et al., 2021). In the current study, we employed sequence-relate amplified polymorphism (SRAP) markers that target the amplifion of open reading frames (ORFs ((Li and Quiros, 2001). This particular marker has been frequently utilized within the genus *Tamarix*; for instance: Sheidai et al. (2019) applied this marker to differentiate the populations of *Tamarix mascatensis*. The results of the later study revealed that almost all of the populations have been separated into distinct clusters.

T. dubia is a type of tree that grows mainly in desert areas and among dry shrubs. This plant is native to the central and southern regions of Iran, particularly in Khuzestan, Bushehr, Sistan and Baluchestan, Hormozgan, and Fars provinces (Azadi, 2016), as well as Afghanistan. It has recently been included

in the IUCN red list and introduced as an endangered species in 2020 (POWO, 2023). Analyzing the genetic diversity of plant populations, while considering various factors such as fertilization systems, types of pollination, seed dispersal, and human impacts provides valuable insight into conservation efforts and population dynamics. In other words, methods for studying genetic diversity and the genetic structure of populations lead to effective conservation approaches (Escudero et al., 2003).

The study of *Tamarix dubia* is of significant importance due to its role in understanding genetic diversity, population structure, and evolutionary mechanisms. As a species found in dry and semi-arid regions, it plays a crucial ecological role in soil stabilization and preventing erosion. Moreover, examining its genetic makeup provides valuable insights into gene flow patterns and biogeographic processes, contributing to our knowledge of species distribution and evolutionary dynamics. Understanding the genetic variability of *T. dubia* is also essential for conservation efforts, as it aids in the protection of biodiversity and the development of sustainable management strategies for plant species in vulnerable environments. This research aims to enhance our comprehension of plant adaptation and the mechanisms that promote biodiversity in arid environments by investigating its evolutionary and ecological importance.

Though variou population genetic studies and evolutionary investigations have been carried out within the genus *Tamarix*, there has yet to be a report addressing the genetic diversity and genetic structure of T. dubia populations. Therefore, the aim of the present study was to illustrate genetic diversity versus genetic differentiation of this species within a geographical context in Iran.

Materials and methods

Plant sampling

A total of 115 plant specimens from 23 populations across four geographical regions in Iran—Sistan and Baluchestan, Kerman, Yazd, and South Khorasan—were collected for molecular investigations. Specimens were determined using the Flora of Iran (Assadi, 1988). Voucher specimens of each leaf have been deposited at the FUMH herbarium (The Herbarium of the Ferdowsi University of Mashhad).

Molecular studies

DNA extraction and PCR reaction

Silica-dried leaves of 5-10 plants per population were randomly utilized in the molecular study. Genomic DNA was extracted following the CTAB (Cetyltrimethylammonium bromide) activated charcoal protocol (Krizman et al., 2006). The quality of extracted DNA was examined by running on 0.8 % agarose gel. Amplification of sequences associated with sequence-related amplified polymorphism (SRAP) was done in a 25 μL reaction containing 10x PCR reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP (Bioron, Germany), 0.2 μM of each primer, 20 ng genomic DNA, and 1 U of *Taq* DNA polymerase (Bioron, Germany). Five primer pairs were used including forward (Me1, Me2, Me3, Me4, Me5) and reverse (Em1, Em2, Em3, Em4, Em5) primers (Feng et al.,

2014) (Table 2).

The amplification reactions were performed under the following programs: 5 min initial denaturation step at 94 °C was followed by five cycles of 94 °C for 1 min (denaturation step), 35 °C for 1 min (annealing step), 72 °C for 2 min (elongation step). The reactions were continued by 35 cycles at 94 °C for 1 min (denaturation step), 50 °C for 1 min (annealing step), 72 °C for 2 min (elongation step), and a final extension at 72 °C for 7 min. The amplification products were observed by running on 1 % agarose gel, followed by the ethidium bromide staining. The fragment size was estimated using a 100 bp molecular size ladder (Fermentas, Germany).

Data analysis

Genetic diversity analyses

SRAP bands obtained were treated as binary data (1 = presence, 0 = absence). The produced data matrix was used for genetic diversity analyses. Genetic diversity parameters were determined for the populations under study as performed

Table 1. *Tamarix dubia* populations and their localities

Population	Longitude	Latitude	Altitude	Province	Locality
1	612611	288646	1332	Sistan and Baluchestan	28 kilometers from Mirjaveh - Khash after Ladiz
2	607208	287581	1908	Sistan and Baluchestan	90 kilometers from Zahedan-Kash-Mirabad
3	607301	288361	1942	Sistan and Baluchestan	82 kilometers of Zahedan-Kash-Kam Zard
4	603491	282247	1338	Sistan and Baluchestan	Noukabad - Bezman after Goharkoh
5	578768	293852	1708	Kerman	125 kilometers Bam-Kerman
6	559925	302045	1917	Kerman	Rafsanjan-Mes Sarcheshme
7	560024	30253	1740	Kerman	Rafsanjan-Mes Sarcheshme
8	550093	303934	2188	Kerman	Shahrbabak-Jozem
9	551376	305993	1922	Kerman	Jozem -Anar
10	553682	306648	1610	Kerman	Anar
11	544843	315681	1445	Yazd	The beginning of Mehriz-Anar road
12	555643	330352	1196	Yazd	Yazd-Tabas
13	582905	32992	1212	South Khorassan	Dihuk-Khusuf before Til Khor
14	584929	329211	1154	South Khorassan	Dihuk-Khusuf then Til Khor
15	58637	328649	1302	South Khorassan	Dihuk-Khusuf then Til Khor
16	587118	328565	1461	South Khorassan	Dihuk-Khusuf then Til Khor
17	590715	328539	1489	South Khorassan	Khosuf-Birjandan Rakat road
18	590715	327486	1903	South Khorassan	Khosuf-Birjandan Rakat road
19	60318	324302	1384	South Khorassan	Sarbisheh – Darh
20	605746	323116	1241	South Khorassan	Darh - Mahi Rood
21	608275	321693	939	South Khorassan	Darh - Mahi Rood
22	598265	323164	1610	South Khorassan	25 kilometers Sarbisheh-Nehbandan
23	600427	316594	1196	South Khorassan	Shusf-Nehbandan

in GenAlex 6.4 (Peakall & Smouse, 2006). These parameters were: the percentage of allelic polymorphism, allele diversity (Weising et al., 2005), Nei' gene diversity (He), and Shannon information index (I) (Weising et al., 2005). Genetic differentiation of the populations was studied by calculating Fst from AMOVA (Analysis of molecular variance), as implemented in GeneAlex 6.4. Non-metric multi-dimensional scaling Jaccard similarity index (Podani, 2000) and UPGMA clustering were done using PAST ver. 3.01 (Hammer et al., 2012).

A heat map was constructed to reveal how SRAP loci are correlated and differentiated in various populations under study. This analysis was done using an online web tool for visualizing clustering for multivariate data (BETA) at https://biit.cs.ut.ee/clustvis. Gene flow among the studied cultivars was determined by Nm value, an estimate of gene flow from GST using PopGene ver. 1.32. (Yeh & Boyle, 1997; Yeh et al., 1999). The discriminating power of SRAP loci was determined by estimating the Gst value of the loci using POPGENE ver. 3.1 (Yeh and Boyle, 1997; Yeh et al., 1999).

To analyze the genetic structure across populations, we employed Bayesian model-based clustering using STRUCTURE version 2.3. This program applies a model-driven clustering approach to deduce population structure from genotype data with unlinked markers. The underlying model presupposes the existence of KKK distinct clusters. This framework is useful for detecting population structure, and assigning individuals to one or more populations based on genotype data, particularly in cases of admixed individuals (Sheidai et al., 2013).

Phylogeography and reconstruction of ancestral area

We employed RASP package ver. 4.2 (Yu et al., 2015) to investigate dispersal versus vicariance events of the populations. For these analyses, we performed the both S-DIVA and Bayesian binary MCMC (BBM) methods. DIVA (Dispersal-Vicariance Analysis) and S-DIVA reconstruct ancestral distributions and the direction of dispersal events among areas. Briefly, DIVA searches for the optimal reconstruction of ancestral areas by assuming current distribution using the result of the vicariant events (i.e., allopatric speciation), while recognizing that dispersal and extinction are possible (Yu et al., 2015). The most well-known and commonly used event-based method of biogeographic inference is Dispersal–Vicariance Analysis (DIVA). Bayesian approach to DIVA (Bayes-DIVA) was applied in which biogeographic reconstructions were averaged over a sample of highly probable Bayesian trees. The S-DIVA method represents an advancement of Bayes-DIVA and has been elaborately detailed in prior discussions.

Results

Genetic diversity and the population's genetic structure

In total 100 SRAP bands were recorded across 112 *T. dubia* individuals of *T. dubai*, representing 23 distinct populations. These bands were systematically coded and utilized for further analyses. Details of SRAP band occurrence are provided in Table 3. Private bands occurred only in three populations of 1, 16, and 19.

Table 3. Frequency of SRAP bands in the studied *T. dubia* populations

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
No. Bands and No. Bands Freq. >= 5%	59	55	50	53	52	54	61	63	61	56	59	55	69	54	61	61	55	53	62	60	58	64	61
No. Private Bands	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0
No. LComm Bands (<=25%)	2	4	1	3	4	5	5	7	3	3	3	2	6	1	1	1	2	4	2	1	3	1	3
No. LComm Bands (<=50%)	8	9	7	10	10	11	11	13	12	8	12	7	15	5	11	8	8	11	12	10	8	11	9

No. Bands = No. of Different Bands, No. Bands Freq. >= 5% = No. of Different Bands with a Frequency >= 5%, No. Private Bands = No. of Bands Unique to a Single Population, No. LComm Bands (<=25%) = No. of Locally Common Bands (Freq. >= 5%) Found in 25% or Fewer Populations, No. LComm Bands (<=50%) = No. of Locally Common Bands (Freq. >= 5%) Found in 50% or Fewer Populations

Table 4. Genetic diversity parameters determined in the studied in *T. dubia* populations

Pop	Na	Ne	I	He	uHe	%P
Pop1	0.820	1.148	0.127	0.086	0.095	23.00%
Pop2	0.730	1.128	0.106	0.072	0.080	18.00%
Pop3	0.670	1.115	0.097	0.066	0.073	17.00%
Pop4	0.710	1.139	0.109	0.075	0.084	18.00%
Pop5	0.710	1.121	0.103	0.069	0.077	19.00%
Pop6	0.800	1.135	0.128	0.083	0.093	26.00%
Pop7	0.870	1.175	0.147	0.100	0.111	26.00%
Pop8	0.850	1.126	0.113	0.075	0.083	22.00%
Pop9	0.860	1.141	0.128	0.084	0.094	25.00%
Pop10	0.770	1.126	0.111	0.074	0.082	21.00%
Pop11	0.810	1.151	0.127	0.086	0.096	22.00%
Pop12	0.790	1.140	0.126	0.084	0.093	24.00%
Pop13	1.000	1.199	0.170	0.115	0.127	31.00%
Pop14	0.700	1.110	0.092	0.062	0.071	16.00%
Pop15	0.830	1.150	0.125	0.085	0.094	22.00%
Pop16	0.940	1.226	0.185	0.126	0.140	33.00%
Pop17	0.700	1.128	0.097	0.068	0.078	15.00%
Pop18	0.750	1.127	0.115	0.076	0.085	22.00%
Pop19	0.820	1.119	0.106	0.070	0.078	20.00%
Pop20	0.800	1.117	0.105	0.069	0.077	20.00%
Pop21	0.870	1.177	0.154	0.103	0.114	29.00%
Pop22	0.940	1.183	0.160	0.107	0.119	30.00%
Pop23	0.860	1.159	0.136	0.091	0.104	25.00%

Abbreviations: Ne = No. of Effective Alleles = 1 / (p^2 + q^2), I = Shannon's Information Index = -1* (p * Ln (p) + q * Ln(q)), He = Expected Heterozygosity = 2 * p * q, and uHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He

The genetic diversity parameters determined in the studied populations are provided in Table 4. Polymorphism percentage varied from 16 % in population 14 (South Khorassan/ Dihuk-Khusuf then Til Khor) to 33 % in population 16. The average polymorphism percentage across these populations was 22.78, suggesting a low level of genetic variability. Likewise, a low level of gene diversity He = 0.09 occurred in these accessions.

AMOVA test showed significant genetic differences (P= 0.001) among the studied populations. It revealed that 57% of the total variation occurred among populations while 43 % of the genetic variability was within the population. These data indicate that *T. dubia* geographical populations have significant genetic differences (Table 5).

The UPGMA dendrogram and MDS plot of the SRAP data nearly grouped the samples of each population into separate clusters. This indicates a high level of genetic similarity within populations, which differentiates the studied *Tamarix dubia* populations from one another (Fig. 1). UPGMA dendrogram also reveals that the studied *Tamarix* accessions are genetically distributed in four major clusters or groups.

The MDS plot followed by a minimum spanning tree identified populations that are scattered in the four genetic groups and also showed their genetic affinity (Fig. 2).

Heat map (Fig. 3) constructed based on SRAP data shows how different SRAP loci are correlated and together can differentiate the studied trees from each other. For example, SRAP loci 5, 10, 13, 20, 42, 68, and 85, are highly correlated. Similarly, SRAP loci 17, 35, 36, 73, 81, and 89, are correlated and act to differentiate *T. dubia* populations. The same holds for loci 1, 9, 58, and 70.

Discriminating SRAP loci with high Gst value (> 0.70) (Govindaraju, 1988) are presented in Table 5. These loci are among SRAP loci which differentiated geographical populations and may have an adaptive potential value, which will be discussed in the coming paragraphs. Twenty-seven SRAP loci out of 100 loci have high discriminating power and therefore are efficient molecular markers for the genetic diversity of *T. dubia* (Table 6).

The mean value for Gst in all populations was 0.65, while the mean gene flow (Nm) value was 0.26. As evident in the Assignment test, genetic admixture occurs mainly between populations 20 and 21, and among populations 10, 11, 16, and 23. As stated, these populations contribute to the mean low gene flow of 0.26.

The Mantel test was performed between genetic distance and geographical distance was highly significant ($r = 0.21$, $p < 0.0001$) after 10000 permutations.

Table 5. AMOVA test of *T. dubia*

Source	df
Among Pops	57%
Within Pops	43%
Total	100%

Phylogeography results

RASP tree obtained based on MCMC binary method as well as S-DIVA produced similar results (Fig. 5). RASP tree reveals that most probably, the spread of genes and dispersal has occurred from Sistan and Baluchestan province (A in Fig. 5) to Kerman region (B in Fig. 5), and also from Kerman to Yazd province (C in Fig. 5), via gene flow and genetic admixture. However, accessions of Southern Khorassan are genetically distinct and exhibit separate and distinct vicariance events. Details of the phytogeography analysis are provided below (Table 7). Based on the phytogeography results, we suggest the gene dispersal route from Sistan and Baluchestan to South Khorasan, as presented in Figure 6.

Discussion

The present study reveals that a large number of SRAP bands can be obtained in *T. dubia* populations and these loci can differentiate geographical populations from each other as most of these loci have a high discriminating power. Moreover, SRAP private bands occur in some of the geographical populations (1, 16 & 19), which may have local adaptive potential.

For population genetic analyses we used various computational approaches to through light on detailed aspects of *T. dubia* population genetic structure and populations' genetic divergence versus gene flow. These methods include different clustering and ordination approaches along with reconstruction of ancestral distribution. In the present study, the populations showed a small amount of genetic polymorphism, which is probably due to the small effective population size (Ne) of the population, or random genetic drift. This result is consistent with the results of Mayonde et al. (2019) who reported low to moderate diversity.

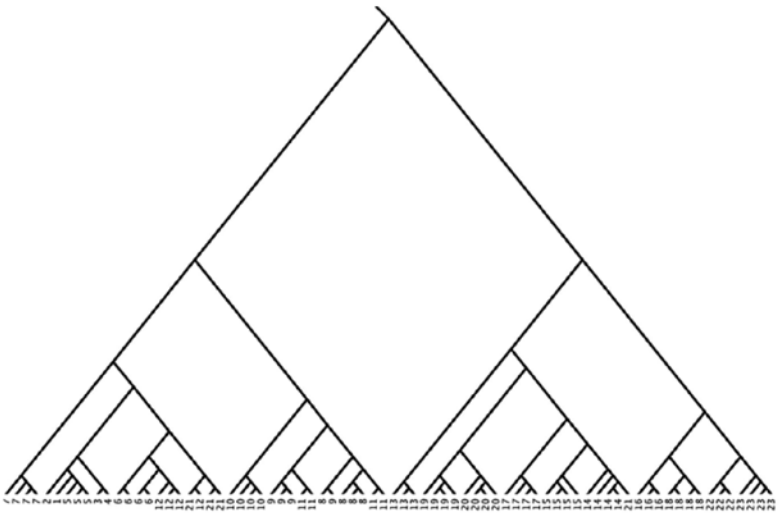

Fig. 1. UPGMA dendrogram of *T. dubia* populations based on genetic similarity, showing almost separation of the samples in each population from the other (Populations 1-23 indicated in Table 1) (1-4: Sistan and Baluchestan/ 5-10: Kerman/ 11-12: Yazd/ 13-23: South Khorasan)

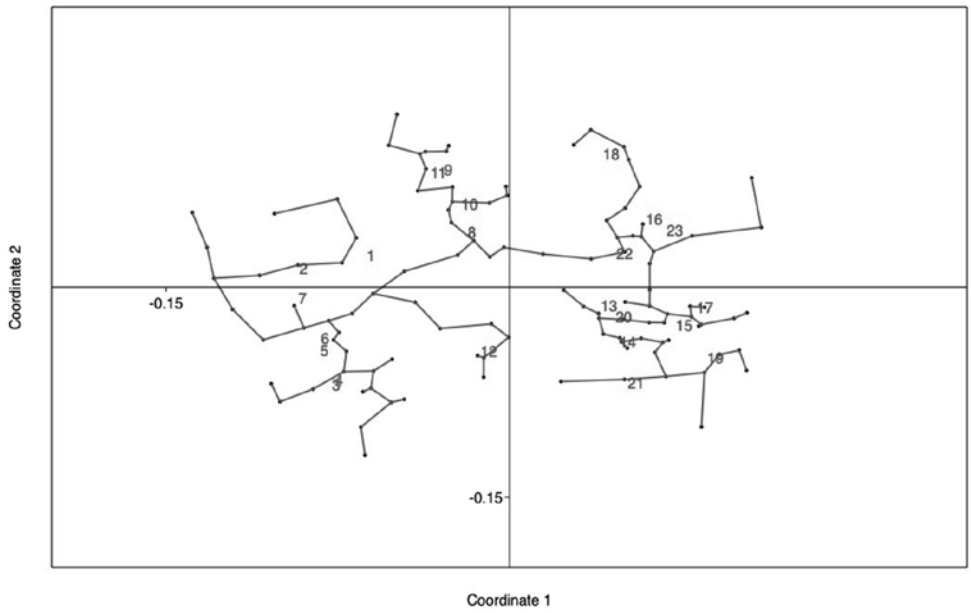

Fig. 2. MDS plot of *T. dubia* populations showing the populations scattered in four genetic groups and also reveals the populations' genetic affinity

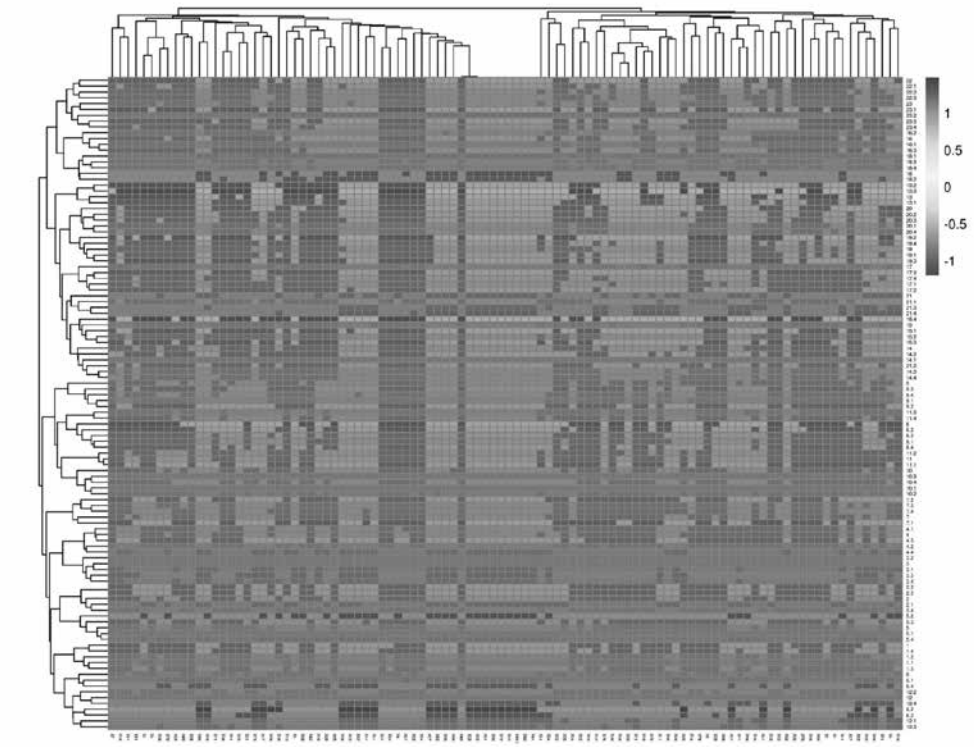

Fig. 3. Heat map showing SRAP loci which are correlated and differentiate *T. dubia* trees of the studied populations from each other (populations 1-23 indicated in Table 1, X1-X100 are SRAP loci)

Table 6. Gst value of SRAP loci in *T. dubia* populations (loci with Gst >0.7) are provided (Ht = total gene diversity, Hs = gene diversity at sub-population level, Gst = analog of Fst for the loci, Nm = rate of migration)

Locus	Sample Size	Ht	Hs	Gst	Nm*
Locus1	112	0.2612	0.0215	0.9177	0.0448
Locus7	112	0.1299	0.03	0.7694	0.1499
Locus9	112	0.1683	0.0101	0.9401	0.0319
Locus11	112	0.4884	0.0868	0.8222	0.1081
Locus15	112	0.2217	0.0417	0.8119	0.1159
Locus17	112	0.3427	0.0477	0.8607	0.0809
Locus18	112	0.4887	0.067	0.8628	0.0795
Locus19	112	0.371	0.0297	0.9199	0.0435
Locus20	112	0.4845	0.0803	0.8343	0.0993
Locus21	112	0.4971	0.1147	0.7694	0.1499
Locus22	112	0.3856	0	1	0
Locus24	112	0.339	0.0499	0.8527	0.0863
Locus29	112	0.4985	0.0632	0.8732	0.0726
Locus30	112	0.4823	0.0468	0.903	0.0537
Locus35	112	0.4	0.0733	0.8167	0.1122
Locus36	112	0.1338	0.0297	0.778	0.1427
Locus37	112	0.1588	0	1	0
Locus52	112	0.3013	0.0651	0.784	0.1378
Locus53	112	0.3194	0.0923	0.711	0.2033
Locus77	112	0.4716	0.1338	0.7163	0.1981
Locus78	112	0.4975	0.0771	0.8451	0.0916
Locus79	112	0.1416	0.0379	0.7323	0.1828
Locus84	112	0.4929	0.0929	0.8115	0.1161
Locus86	112	0.4075	0.0215	0.9472	0.0278
Locus87	112	0.4774	0.0916	0.8081	0.1188
Locus96	112	0.4593	0.0538	0.883	0.0663
Locus97	112	0.1874	0.0354	0.8111	0.1164

Table 7. Vicariance event, details in *T. dubia* populations

Dispersal Between areas	Speciation within areas	Dispersal within	Table:	From	to
A->B:1	A:10	A	1.00	2.00	10
B->A:2	B:16	B	4.00	2.00	16
B->C:2	C:4	C	0.00	2.00	4
D->B:1	D:32	D	1.00	0.00	32

Global Cost: Global Dispersal: 12, Global Vicariance: 6, Global Extinction: 0

Fig. 6. Gene flow and dispersal map of *T. dubia* in Iran

In the mentioned study, the low genetic diversity observed in the invasive *Tamarix chinensis* is attributed to its self-pollinating nature (autogamy). Additionally, genetic drift caused by population fragmentation is considered a significant factor contributing to its moderate genetic diversity. The researchers also discovered private alleles unique to the native species in certain remote, unpolluted areas of the Northern Cape.

The studied tree samples in each population were placed in a distinct cluster due to a high intra-population genetic similarity (Fig. 1). This high genetic affinity may also be due to genetic drift and limited crossing individuals between populations.

In general, the studied populations were grouped into four main genetic groups (Fig. 1). Populations sampled from Sistan and Baluchistan, Yazd, and Kerman provinces comprise the first major genetic group, while accessions collected from Southern Khorasan province were genetically differentiated and were placed in the second genetic group. Interestingly, based on the pairwise Fst of AMOVA the local populations were significantly differentiated (Table 4). This may indicate that local geographical selection and lack of frequent gene flow among these populations are the main reasons for genetic differences in the studied populations (Zhang et al., 2019; Segovia et al., 2020). The Mantel test conducted in this study yielded a correlation coefficient of r= 0.21 with a p-value of less than 0.0001. This indicates a significant correlation between genetic and geographical distance among the studied populations. These findings support our hypothesis that gene flow between these populations may be infrequent, primarily due to the considerable distances that separate them. As a result, the limited movement of genes between populations may hinder genetic exchange, leading to greater genetic divergence. These values

clearly indicate a good level of genetic differentiation versus a very low gene flow among the studied *T. dubia* (Govindaraju, 1988). Mantel test performed in this study ($r = 0.21$, $p < 0.0001$) also showed a significant correlation between genetic distance and geographical distance which also supports our suggestion that the gene flow may not occur frequently due to long distances among the studied populations. This indicates that the population genetic distance increases with an increase in geographical distance.

As observed in the Assignment test, genetic admixture is particularly evident between populations 20 and 21, as well as among populations 10, 11, 16, and 23. This admixture suggests that these populations have experienced substantial gene flow across their respective ranges. These findings align with the overall low mean gene flow of 0.26, as previously discussed, indicating limited but notable genetic exchange between these populations. The patterns of admixture observed in this study highlight the complex dynamics of gene flow within the species, which could be influenced by factors such as geographic proximity, ecological barriers, or historical events that shaped the population structure.

Yang (2017) studied the genetic diversity of 32 individuals of *Tamarix chinensis* using ISSR molecular markers. She obtained 114 loci total among the studied individuals. The average value of the effective number of alleles, Nei's genetic diversity, and Shannon's information index were 1.54, 0.32, and 0.124, respectively. A similar investigation was performed by Jiang et al. (2012) by working on *T. chinensis*. They produced mean gene diversity and Shannon's information index of 0.23 and 0.36, respectively. However, a higher magnitude of genetic variability is present in *T. chinensis* compared to that observed in *T. dubia* populations. This may indicate that various *Tamarix* species differ in crossing ability and gene flow possibly under the influence of matting populations and their geographical distance.

The Mantel test indicates that the population's genetic distance increases with an increase in geographical distance ($r = 0.21$, $p < 0.0001$).

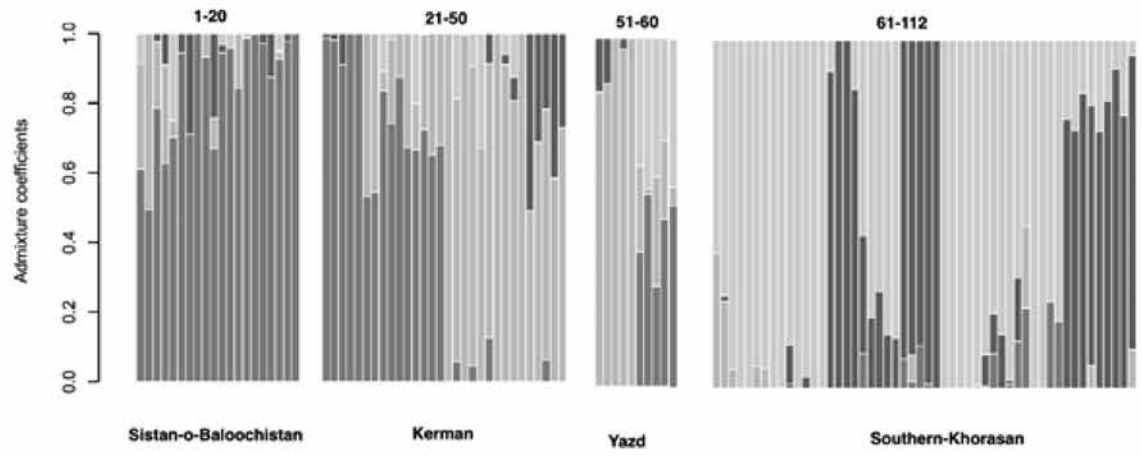

Fig. 4. Admixture plot of *Tamarix dubia* populations showing some degree gene flow among populations in Sistan-o-Baluchistan and Kerman provinces, as well as Kerman and Yazd provinces. A distinct genetic structure is present in trees of Southern-Khorasan province

Therefore, the primary reason for the low gene flow may be the geographical distance between the studied populations. Moreover, low genetic variability observed in these populations may be due to intra-population pollination as the neighboring populations are far distance. The number of *Tamarix* accessions is limited in each geographical population which in turn may cause genetic drift and low genetic diversity.

Based on the assignment test and gene flow results (Fig. 4), it seems that a high degree of admixture occurs in populations of Sistan and Baluchistan, Kerman, and Yazd. The accessions in these provinces may have a good source of genetic diversity for future breeding and conservation programs as they are forming the corridor of gene flow. However, we should not forget the conservation of relatively more isolated trees located in southern Khorasan province, as these trees also contain local adaptive genes and acquire more conservation action.

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