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Genetic Diversity of *Pistacia* Species in Iraqi Kurdistan and Yazd, Iran: Insights from ISSR Markers

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Abstract

Objective

Ongoing climate change and anthropogenic pressures are accelerating the loss of genetic diversity in numerous plant species, including those within the *Pistacia* genus, which holds significant economic and ecological importance. Despite their critical roles as rootstocks and sources of traits such as drought and salinity tolerance, wild *Pistacia* populations in the western Zagros region of 0 and the arid zones of central Iran remain poorly characterized at the molecular level. This study aimed to investigate the genetic diversity and structure of *Pistacia* species across these two regions utilizing inter-simple sequence repeat (ISSR) markers.

Materials and methods

Leaf samples from 24 representative genotypes of *P. atlantica*, *P. khinjuk*, and *P. vera* were selected from an initial pool of 67 trees collected in Iraq and Iran. DNA was extracted using the CTAB protocol and amplified with 10 ISSR primers. Genetic variation was assessed through polymorphism statistics and diversity indices, including polymorphic information content (PIC), marker index (MI), resolving power (Rp), and effective multiplex ratio (EMR). Cluster analysis (UPGMA), principal coordinate analysis (PCoA), and analysis of molecular variance (AMOVA) were conducted to determine genetic structure and population differentiation.

Results

Out of 171 amplified bands, 163 (95.3%) were polymorphic, indicating a high level of genetic variability. The ISSR4 primer produced the highest number of polymorphic bands, while OW5 demonstrated the highest discriminative power (MI and Rp). AMOVA revealed that the majority of genetic variation (83%) was distributed within species. Notably, *P. khinjuk* exhibited the highest genetic diversity (He = 0.19; I = 0.30), followed by *P. atlantica*, while *P. vera* exhibited minimal variation. Neither the cluster analysis nor the PCoA revealed distinct geographic structuring, suggesting historical gene flow and potential anthropogenic influence.

Conclusions

These findings highlight significant intra-specific genetic diversity among wild *Pistacia* populations, particularly within *P. khinjuk*, underscoring their value as reservoirs of adaptive genetic traits. The absence of clear geographic clustering further supports the notion of long-term gene exchange across regions. This research provides a critical baseline for conservation efforts and cultivar development, especially in the context of increasing environmental stress. Future studies should incorporate co-dominant markers and environmental data to more effectively associate genetic variation with adaptive potential.

Keywords: Biodiversity, Genetic distance, Western Zagros, Wild pistachio

Paper Type: Research Paper.

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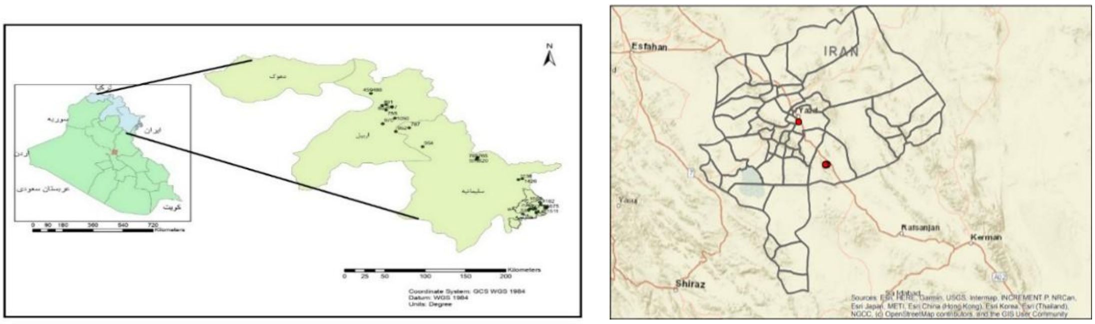
Introduction

Recently, ethnobotany studies in various countries have indicated that several species of the genus *Pistacia* (family Anacardiaceae) significantly affect the nutrition and economy of human societies (Rauf et al., 2017). The world production of pistachios has increased dramatically over the past few decades, from about 50 thousand tons in 1970 to 500 thousand tons in 2000 and more than one million tons in 2020 (FAOSTAT, 2022). According to researchers, pistachio originated from South Central Asia such as Northern Afghanistan and Northeastern Iran (Zeng et al., 2019). Then, different species of this genus were later distributed in the Mediterranean, Iraq, Syria, Pakistan, Afghanistan, Turkey, and North Africa in temperate to arid and semi-arid areas. Diverse ecological conditions in the growth and cultivation areas of this genus in Iran and genetic crossing between different genotypes due to its variable nature have caused a significant increase in its genetic diversity (Zeng et al., 2019). *Pistacia* species are widely distributed across the southern slopes of the Himalayas and extend to western Asia and the Mediterranean region. In Iran, these species are mainly found in mountainous areas and the central highlands (Vargas et al., 2008; Rauf et al., 2017). Four key species of pistachio: *P. vera* L., *P. khinjuk* Stocks., *P. eurycarpa* Yalt. (subsp. *kurdica*), and *P. atlantica* Desf (Karimi et al., 2009) are common in Iran. Among these *P. atlantica subsp. mutica* and *P. khinjuk* are primarily used as rootstocks for *P. vera* (Hosseinifard et al., 2010). However, ongoing climate change -particularly increasing drought frequency and rising temperatures- poses a serious threat to *Pistacia* populations. These stressors may reduce natural regeneration and increase population fragmentation. Ultimately, such processes can lead to erosion of genetic diversity and, in some cases, local population loss. On the other hand, studies conducted in Iraq have shown *P. atlantica* (specifically), *P. eurycarpa*, and *P. khinjuk* (AL-Saghir, 2010) as the essential species in this country. Due to artificial selection (such as overgrazing, fires, deforestation, desertification, and pollution caused by human activities), genetic drift, and climate changes, these pistachio species have been severely affected by genetic erosion and are at high risk of extinction (Said et al., 2011; Amara et al., 2017). However, very few genetic studies have been conducted on these species, especially in the vast western regions of the Zagros Mountains in Iraq (Kurdistan) (Golan-Goldhirsh et al., 2004; Karcı et al., 2022). Previous studies have indicated that chromosomal data and molecular markers, especially ISSR markers (Shahghobadi et al., 2019; Labdelli et al., 2020; Kostas et al., 2021; Mirahmadi et al., 2021), provide a valuable resource for plant breeders to gain a better and more accurate understanding of the phylogenetic relationships and classification of species in *Pistacia* genus (Stuessy, 2009). In addition, chromosomal data and molecular markers play a crucial role in developing new pistachio cultivars to enhance resistance to biotic stresses and tolerance to abiotic stresses such as drought, cold, heat, and general unfavorable climatic conditions (Shahghobadi et al., 2019). Thus, studying different species of the *Pistacia* genus, particularly its wild species at the molecular level, in the steppe regions of Iraqi Kurdistan and the arid and semi-arid central regions of Iran is critical due to their ongoing decline in distribution, as well as human activities leading to deforestation and desertification. Despite the ecological and economic importance of *Pistacia* species across the wild habitats of Iraq and Iran, few studies have explored their molecular genetic diversity-particularly in the western Zagros region of Kurdistan. Despite being rich in native *Pistacia* stands, this region (Iraqi Kurdistan) remains understudied. Moreover, limited comparative research has been conducted with populations from the arid central regions of Iran. Molecular markers are an important instrumentation for achieving precise estimates of the genetic diversity and population structure among plant taxa which can be used in crop improvement projects (Askari et al., 2011; Mohammadabadi, 2016). Among molecular markers, ISSR (inter simple sequence repeat) has been widely and precisely utilized to access the genetic variation of plant and animal germplasm, screening of mutant plants and animals, and recognizing closely related taxa (Ghasemi et al., 2010; Mohammadabadi et al., 2021). High reliability, speed, simplicity, cost-effectiveness, ability to determine genetic diversity between closely related individuals are among the most frequently reported advantages of this marker system (Zamani et al., 2011; Mohammadabadi, 2016). ISSR markers have been successfully utilized for genetic diversity analysis and identifying related species in Pistacia genus (Tagizad et al., 2010; Mahmoodnia Meimand et al., 2017). The objectives of the present study were to identify and compare the genetic diversity and population structure of *Pistacia* species using ISSR markers collected for the first time from across the Iraqi Kurdistan region and to examine their genetic relationship with species found in the protected arid and semi-arid regions of Yazd Province, Iran.

Materials and methods

Study area and plant sample: For the first time, this study has considered the wide distribution of *Pistacia* species, their threatened status, and the geography of the western Zagros region in Iraqi Kurdistan (Figure 1A), to include 29 samples for Sulaimaniayah, Erbil, and Halabja governorates. As representative of the arid and semi-arid climates of the country, leaf samples from 9 trees (Figure 1B) were collected randomly from the protected areas of Kalmand-Bahadoran in Yazd Province, Iran (Figure 1B). The populations of these species have been continuously monitored in these regions due to their risk of extinction. During sampling, young leaf samples were randomly collected from different parts of each tree for botanical studies, with details such as sample number, collector's name, collection date, exact location, GPS coordinates, physiographic features, proximity to industries, oil fields, water reservoirs, and other significant climatic or geographic characteristics labeled on the samples. For molecular analyses, the samples were placed in aluminum bags, preserved in liquid nitrogen, and stored in a -80°C freezer. The samples were first recorded based on Mouton’s 1967 studies, using 22 morphological indices (Yousif Kamal et al., 2022). The identification of the species and their botanical classification were confirmed by two expert botanists (Dr. Rubar Hussein Muhammed-Salih[1], Associate Professor at the Faculty of Agriculture, University of Sulaimani, Iraq, and Dr. Asghar Mosleh-Arani[2], Professor at the Faculty of Natural Resources, University of Yazd). Subsequently, 24 genotypes from the 38 frozen genotypes were selected for molecular diversity analysis using ISSR markers. Identifying the initial population of 24 selected genotypes for molecular analysis (marked with * in Table 1) were based on morphological diversity, geographic range and genetic proximity. Accordingly, six out of nine Iranian samples (P40–P48) were included, while the remaining three samples were excluded due to high overlap in morphological traits and sampling locations with the selected genotypes.

Genomic DNA extraction and genetic diversity analysis using ISSR markers: The genomic DNA was extracted from the collected leaf samples using the Cetyltrimethylammonium bromide (CTAB) Method (Doyle, 1991). Then, the quality and quantity of the DNA samples were examined using a Nanodrop spectrophotometer (Analytik Jena ScanDrop250) at 260 and 280 nm as well as through 0.8% agarose gel electrophoresis. Ten ISSR primers were utilized for PCR (Table 2).



A B

Figure 1. The location of studied *Pistacia* species in Iraqi Kurdistan (A) and Yazd province in Iran (B)

The PCR protocol included an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation (95°C for 45 seconds), annealing (50°C for 40 sec.), extension (72°C for 2 min.), and a final extension (72°C for 7 min.).

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[1] Https://Sites.Google.Com/A/Univsul.Edu.Iq/Rubar-Hussein/

[2] Https://Yazd.Ac.Ir/People/Mosleh

Table 1. Species, subspecies, and geographical description of the studied *Pistacia* species in Iraqi Kurdistan and Yazd of Iran

Region		No.	Code	Species/subsp.	Longitude (E)	Latitude (N)	Altitude (m)
Iraqi Kurdistan	Sulaymaniyah	1	P1*	*P.khinjuk*	36° 03’50”	44° 48’13”	954
		2	P2*	*P.atlantica* sub.kurdica	36° 20’02”	44° 38’72”	787
		3	P27*	*P.atlantica* sub.kurdica	35° 53’98”	45° 24’64”	925
		4	P28	*P.khinjuk*	35° 53’42”	45° 23’98”	820
		5	P29*	*P.khinjuk*	35° 53’00”	45° 23’84”	765
		6	P30*	*P.khinjuk*	35° 53’00”	45° 23’84”	765
		7	P32*	*P.atlantica* sub.mutica	35° 34’96”	45° 51’80”	1138
	Erbil	1	P3*	*P.atlantica* sub.atlantica	36° 27’82”	44° 28’96”	1050
		2	P4	*P.atlantica* sub.mutica	36° 35’22”	44° 23’46”	755
		3	P5	*P.khinjuk*	36° 37’65”	44° 27’45”	733
		4	P6	*P.khinjuk*	36° 39’53”	44° 23’51”	708
		5	P7	*P.khinjuk*	36° 39’52”	44° 23’46”	694
		6	P8	*P.atlantica* sub.kurdica	36° 39’54”	44° 23’48”	697
		7	P9*	*P.atlantica* sub.atlantica	36° 48’97”	44° 13’37”	459
		8	P10	*P.atlantica* sub.atlantica	36° 48’99”	44° 13’40”	480
		9	P11*	*P.atlantica* sub.mutica	36° 38’73”	44° 20’69”	891
		10	P12*	*P.atlantica* sub.mutica	36° 23’35”	44° 20’90”	970
		11	P13*	*P.atlantica* sub.mutica	36° 16’58”	44° 29’76”	952
	Halabja	1	P14*	*P.atlantica* sub.kurdica	35° 06’81”	46° 04’26”	1124
		2	P15*	*P.khinjuk*	35° 07’00”	46° 04’19”	1078
		3	P16	*P.atlantica* sub.mutica	35° 10’26”	45° 59’57”	754
		4	P18*	*P.atlantica* sub.mutica	35° 10’05”	46° 02’94”	1299
		5	P19*	*P.atlantica* sub.mutica	35° 09’74”	46° 02’19”	1055
		6	P20	*P.atlantica* sub.kurdica	35° 06’10”	45° 54’77”	539
		7	P21*	*P.atlantica* sub.mutica	35° 09’39”	45° 58’70”	823
		8	P22	*P.atlantica* sub.kurdica	35° 11’67”	46° 10’99”	1435
		9	P23*	*P.atlantica* sub.atlantica	35°10’65”	46° 10’55”	1442
		10	P24	*P.atlantica* sub.mutica	35° 11’14”	46° 09’72”	1511
		11	P25*	*P.atlantica* sub.kurdica	35° 12’53”	46° 09’58”	1675
Iran	Yazd	1	P40*	*P.atlantica* sub.mutica	31° 28’54”	54° 81’89”	1643
		2	P41	*P.atlantica* sub.mutica	31° 28’50”	54° 81’94”	1634
		3	P42*	*P.khinjuk*	31° 28’21”	54° 79’07”	1677
		4	P43	*P.khinjuk*	31° 28’25”	54° 79’07”	1725
		5	P44*	*P.atlantica* sub.mutica	31° 28’17”	54° 78’99”	1688
		6	P45*	*P.khinjuk*	31° 28’16”	54° 79’00”	1683
		7	P46*	*P.khinjuk*	31° 28’12”	54° 79’11”	1686
		8	P47	*P.khinjuk*	31° 28’11”	54° 79’05”	1721
		9	P48*	*P.vera*	31° 83’29”	54° 35’67”	1257

* The selected samples from a collection of 38 were chosen to investigate molecular diversity using ISSR markers.

The reactions were carried out in a total volume of 15µL, including 1.5 µL of 10 µM primer solution (to a final concentration of 1 µM), 7 µl of 1X ready-to-use mastermix (Amplicon, Denmark), 2 µL of 50 ng µL^{-1} DNA solution (final amount of 100 ng), and 4.5 µL of distilled water in a BioRad C1000 Touch thermal cycler. Then 5µL of the final product was run on a 1.5% agarose gel for 30 minutes at 80 volts. A 100 bp DNA size marker (Sinaclon, Iran) was utilized to estimate the band sizes. The resulted banding patterns were visualized and imaged by the Gel documentation system.

Table 2. ISSR primers used for genetic diversity analysis in *Pistacia* species

No.	Oligo name	Oligo sequence (5'→3')	T_m (°C)
1	ISCS11	CTCTCTCTCTCTCTCTT	47.6
2	ISSR6	DBDACACACACACACAC	47.6
3	ISSR4	GAGAGAGAGAGAGAGAT	47.3
4	OW5	GAGAGAGAGAGAGAYC	54.4
5	OW4	GAGAGAGAGAGAGAGAYA	48.2
6	ISSR3	AGAGAGAGAGAGAGAGT	48.7
7	ISSR7	AGAGAGAGAGAGAGAGRC	50.6
8	ISCS7	TCTCTCTCTCTCTCTCC	48
9	OW2	GAGAGAGAGAGAGAGGAC	53.7
10	ISCS23	HBDBGACCGACCGACCGACC	52

Genetic data analysis: The amplified bands were scored based on the dominant banding pattern for each of the ten primers, with a score of 0 (absence of a band) or 1 (presence of a band). The corresponding matrix was then prepared using Excel software. The polymorphic information content (PIC) index was calculated for each primer by the equation (1):

$$\mathrm{PIC} = 1 - \sum_{i=1}^{n} pi^2 \qquad (1)$$

Where considering n as the total number of alleles identified for a specific locus and pi as the frequency of allele i at each locus for all genotypes (Chesnokov & Artemyeva, 2015). The experiment utilized the number of polymorphic bands per unit assay (Powell et al., 1996; Nagaraju et al., 2001) to determine the effective multiplex ratio (EMR), marker index (MI) and resolving power (R_P), indices for each primer (Aouadi et al., 2019). The Effective Multiplex Ratio (EMR) was obtained through (2):

$$\mathrm{EMR} = np(np/n) \qquad (2)$$

where n is the total number of amplified bands and np indicates the number of polymorphic bands. The marker index (MI) was calculated using the equation (3):

$$\mathrm{MI} = \mathrm{PIC} * \mathrm{EMR} \qquad (3)$$

The resolving power was calculated using (4):

$$Rp = \sum I_b \text{ where } I_b = 1 - (2 \times |0.5 - p|) \qquad (4)$$

is an amplicon informativeness, p is a proportion of individuals with identified amplicon I (Chesnokov & Artemyeva 2015). Additionally, Nei's Gene Diversity (H), Shannon's Information Index (I), the Number of Observed Alleles (Na), and the Effective Number of Alleles (Ne) were computed using GenAleX V.6.4 software (Peakall & Smouse, 2006). The Jaccard similarity coefficient was utilized to evaluate the genetic similarity between the examined samples, and the UPGMA algorithm was implemented to group the samples according to the Jaccard similarity coefficient.

Results and discussion

In this study, polymorphic bands were observed for all ten ISSR primers used (Table 3). Out of the 171 scored bands, 163 were polymorphic. The highest number of polymorphic bands (22) was produced by primer ISSR4, while the lowest (7) was seen in primer ISCS23 (Figure 2). The average number of total bands produced and polymorphic bands were 17.1 and 16.3, respectively, with an average polymorphism percentage of 95.3%. Polymorphism ranged from 87.5% for primer ISCS23 to 100% for primers ISCS11, ISSR4, and ISCS7. The degree of polymorphism of the markers and the genetic diversity among genotypes was demonstrated by PIC values for the studied primers, ranging from 0.28 to 0.39, indicating the ability of these primers to reveal a high level of polymorphism in the evaluated genotypes. The highest PIC value (0.39) was observed in primer ISCS11. Additionally, the Marker Index (MI), which determines the primer's contribution to achieving the desired outcomes, and Resolving Power (Rp), which indicates a primer's ability to distinguish differences among a large number of genotypes (Venkatesan et al., 2021), were ranged from 2.03 to 6.5 for MI and 3.75 to 12.53 for Rp. Primer OW5 showed the highest values for both indices, whereas ISCS23 showed the lowest MI and Rp values (Table 3).

Table 3. The polymorphism indices, Resolving power, band informativeness and polymorphic content in the ISSR primers

Primer name	TNB	NPB	PP	PIC	MI	Rp	EMR
ISCS11	9	9	100	0.39	3.49	4	9
ISSR6	20	19	95	0.28	5.12	10	18.05
ISSR4	22	22	100	0.30	6.12	11.06	22
OW5	21	20	95.24	0.34	6.50	12.53	19.05
OW4	20	19	95	0.32	5.78	11.80	18.05
ISSR3	22	21	95.45	0.30	6.00	9.47	20.05
ISSR7	18	17	94.40	0.26	4.14	5.07	16.06
ISCS7	10	10	100	0.26	2.63	4.20	10
OW2	21	19	90.48	0.28	4.79	7.33	17.19
ISCS23	8	7	87.50	0.33	2.03	3.75	6.13
Total	171	163					
Mean	17.10	16.30	95.30	0.31	4.66	7.92	15.56

TNB: Total number of bands, NPB: Number of polymorphic bands, PP: Polymorphism percentage, PIC: Polymorphic information content, MI: Marker index, Rp: Resolving power, EMR: Effective multiplex ratio

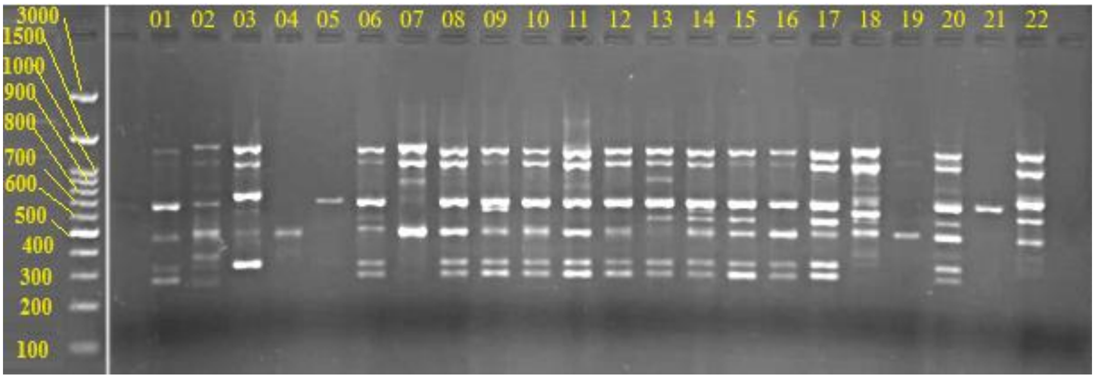

Figure 2. The ISSR4 primer banding pattern for the *Pistacia* genotypes was collected from the Kurdistan region of Iraq and the Kalmend Bahadaran protected area in Yazd, Iran. The numbers indicated above each lane correspond to the ISSR-PCR samples 1-22

The efficiency of the primers can be assessed by calculating the Effective Multiplex Ratio, where a higher value indicates more significant polymorphism produced by the primer. EMR for ISSR primers depends mainly on the number and ratio of polymorphic loci (Sinchan Adhikari et al., 2013). The high values of EMR and MI in ISSR markers reveal the effectiveness of the ISSR marker system in determining the molecular diversity and genetic relationships among plant species (Singh et al., 2014). In this study, primer ISSR4 showed the highest EMR value (22) among the primers, followed by ISSR3 (20.05) (Table 3). ISSR markers were selected to assess genetic diversity among the studied *Pistacia* species due to their high reproducibility, simplicity, and ability to detect high levels of polymorphism without prior sequence information. These features make ISSR markers particularly suitable for genetic studies in under-characterized wild populations (Gemmill & Grierson, 2020). In line with the present results, Mirahmadi et al. (2021) used ISSR markers to examine the genetic diversity of wild and cultivated *Pistacia* genotypes in Khorasan Razavi province, Iran. They found 74 bands overall, with 35 (47.30%) exhibiting polymorphism. Similarly, Bouta et al. (2022) used 13 ISSR markers to assess 11 *P. lentiscus* L. populations collected from various parts of Morocco. They reported that 110 of the 121 bands produced (90.90%) were polymorphic. The primers' average PIC (0.79) and Rp (4.89) values indicated significant genetic diversity among the studied populations. Another study selected 40 elite genotypes (20 male and 20 female) from an open-pollinated population of 800 genotypes to identify superior genotypes based on genetic diversity. The genetic distance between the genotypes was estimated using 20 dominant DNA markers (10 ISSR and 10 RAPD). From 16 (S.14) to 27 (UBC.886), microsatellite loci were found to be created using ISSR primers, totaling 227 polymorphic bands. Additionally, 213 polymorphic bands (96%) were obtained from the 10

RAPD primers. The R_P values obtained by ISSR markers ranged from 4.53 to 14.71 (Neishabouri et al., 2022). In the present study, the dendrogram obtained from ISSR markers by the AHC method and the Jaccard similarity coefficient were employed to categorize the genetic diversity of the genotypes into three groups (Figure 3). The first two groups contained the majority of the genotypes. The first set of genotypes included P2, P3, P13, P18, P14, P40, P44, P23, and P9. The second set of genotypes included P11, P25, P15, P42, P45, P48, P19, P30, P1, P32, and P12. Group three comprised genotypes P27, P46, P29, and P21. Although the 24 samples were collected from two different geographical regions (the Kurdistan region of Iraq and Yazd province in Iran), the dendrogram showed no clustering following geographical origin. Regardless of their collection site, all genotypes in group one were from the species *P. atlantica*. Similarly, in group two, genotypes from *P. atlantica*, *P. khinjuk*, and *P. vera* were grouped based on genetic similarity. In contrast, genotypes from *P. atlantica* and *P. khinjuk* were clustered in a separate group. The lack of a clustering pattern which is in line with geographic distribution of the studied samples may be caused by the historical gene flow across the habitats through animal-mediated seed dispersal or human cultivation practices that introduced genetic material across different regions (Spengler, 2020). Although previous studies on various species of the genus *Pistacia* and their geographical distribution have categorized plants based on their specific regions (Labdelli et al., 2020), a survey of *P. atlantica* in different parts of northwestern Algeria found that genotypes from specific geographical regions were distributed across different clusters when analyzed using SSR molecular markers based on climatic characteristics and leaf morphological characteristics. According to these researchers, genetic similarity occurs through the dispersal of pollen or seeds between geographically distant populations (El Zerey-Belaskri & Benhassaini, 2016; El Zerey-Belaskri et al., 2018). In the same study, Vendramin et al. (2007) did not report any species or geographical distribution grouping using microsatellite markers when analyzing 82 samples from various species of *Pistacia* genus. Although the highest genetic diversity was observed in *P. mutica*, the genetic distances within each cluster and species showed that *P. vera* had the lowest expected genetic distance. The researchers attributed this limited genetic distance to the restricted genetic pool of the samples studied. Karcı et al. (2022) analyzed 66 *Pistacia* genotypes originating from six countries and found that genetic relationships across samples from different regions (Turkey, Iran, Italy, Greece, Syria, and Tunisia) were similar.

In this study, Principal Coordinate Analysis (PCoA) was performed to better understand the genetic distances between the genotypes and visualize these distances in a multi-dimensional space reflecting genetic distances with minimal deviation. The results identified five components, with the first two accounting for most of the variation. These two components explained 23.3%

of the total variation (Table 4), with the first component alone accounting for 13.60%. The two-dimensional plot derived from the PCoA confirmed the clustering results, showing that genotypes grouped in the cluster analysis also appeared together in the PCoA (Figure 4).

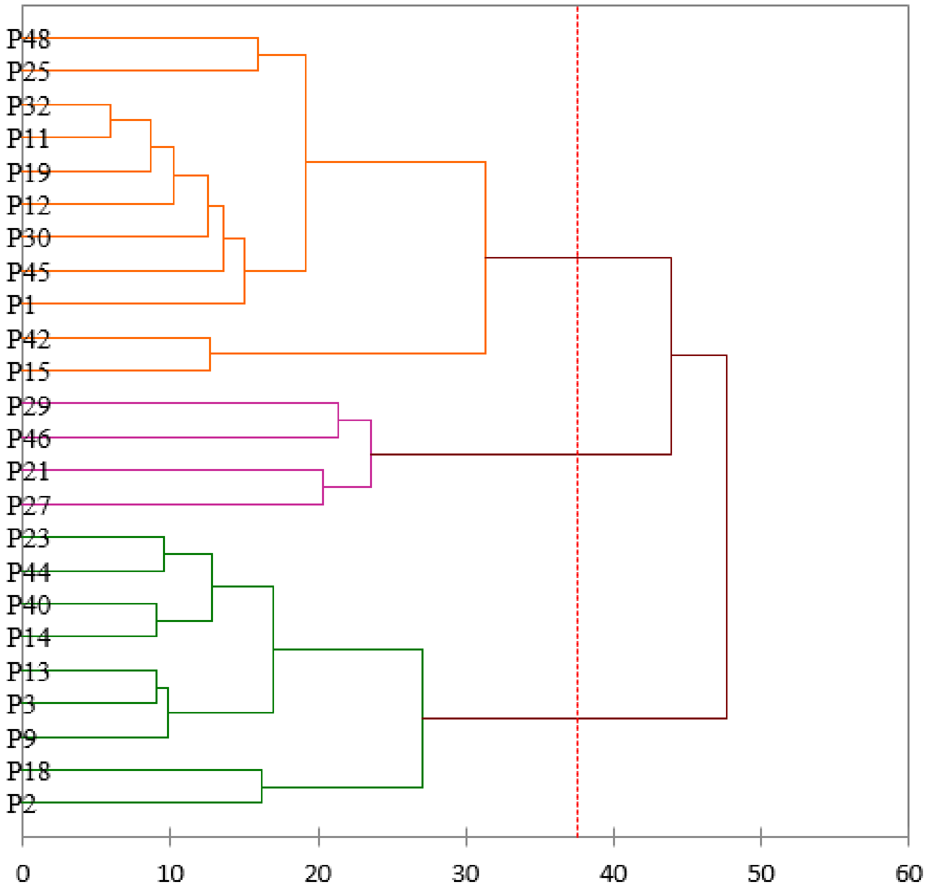

Figure 3. The dendrogram of the cluster analysis on *Pistacia* using ISSR markers and AHC algorithm based on the Jaccard similarity coefficient

Based on the Analysis of Molecular Variance (AMOVA), the genetic diversity observed in the evaluated set was partitioned into diversity within and among species. The results showed that a significant portion of genetic diversity (83%) was found within species. These findings indicate a high level of genetic diversity within the populations of various *Pistacia* species, providing a valuable resource for breeding and genetic studies. The high within-species diversity, especially in *P. khinjuk*, indicates a strong evolutionary potential and adaptability, suggesting its value as a drought-tolerant rootstock for future improvement program of the species (Habel et al., 2021). In line with the present study, a similar analysis of several *Pistacia* species attributed 83% of the total genetic diversity to within-population diversity, with the remaining 17% attributed to diversity among populations (TEKİN et al., 2020). Similarly, Labdelli et al. (2020) reported 55.7% diversity within populations of *P. atlantica* across different regions of Algeria.

Table 4. Special vectors of genetic data of 24 studied *Pistacia* samples

Genotype	F1	F2	F3	F4	F5
P11	0.27	0.11	0.10	0.04	0.02
P25	0.26	0.18	0.00	0.26	0.03
P15	0.06	0.02	0.38	0.01	0.08
P42	0.02	0.00	0.50	0.00	0.01
P45	0.04	0.01	0.10	0.16	0.01
P48	0.02	0.12	0.01	0.00	0.12
P19	0.02	0.03	0.00	0.01	0.04
P30	0.09	0.01	0.18	0.00	0.06
P2	0.07	0.02	0.03	0.09	0.20
P3	0.23	0.05	0.02	0.00	0.09
P13	0.23	0.10	0.03	0.00	0.03
P18	0.07	0.00	0.00	0.02	0.24
P14	0.39	0.00	0.06	0.00	0.00
P40	0.29	0.01	0.16	0.02	0.02
P44	0.22	0.06	0.02	0.00	0.09
P23	0.27	0.01	0.02	0.01	0.12
P9	0.38	0.01	0.01	0.01	0.05
P27	0.02	0.14	0.01	0.06	0.12
P46	0.05	0.39	0.02	0.00	0.04
P29	0.02	0.29	0.00	0.03	0.00
P21	0.01	0.24	0.06	0.13	0.04
P12	0.09	0.21	0.11	0.03	0.01
P1	0.12	0.00	0.09	0.26	0.06
P32	0.14	0.11	0.14	0.20	0.00

According to the results of the genetic diversity indices for the studied genotypes, the highest observed alleles (Na) were found in *P. atlantica* with 1.65, while the lowest was seen in *P. vera* (1.18). Effective alleles (Ne), representing alleles with equal frequency and good distribution, were highest in *P. khinjuk* at 1.29 and lowest in *P. vera* at 1.00. The diversity in *P. khinjuk*, based on Shannon's Information Index and Nei's Gene Diversity Index (He=0.19, I=0.30), was higher than in *P. atlantica* and *P. vera* (Table 5). Therefore, *P. khinjuk* was concluded to exhibit a greater diversity than *P. atlantica* and *P. vera*. Additionally, the percentage of polymorphic loci in *P. atlantica* (82.31%) was higher than that in *P. khinjuk* and *P. vera*. In this study, consistent with the clustering analysis results, the Shannon Information Index and Nei's Gene Diversity values for *P. vera* were reported close to zero. Studies on natural populations have indicated that although the percentage of polymorphic loci is an essential criterion for genetic diversity, variations in these values are observed (Soares et al., 2016). According to Nei (1987), the percentage of polymorphic loci is not the most essential criterion for genetic diversity; instead, the Gene

Diversity Index (He) is more validated. Shannon's Index can range from 0.0 to 1.0, with lower genetic diversity reflected by values closer to zero (da Silva et al., 2016). Generally, in natural conditions, Hardy-Weinberg equilibrium is not expected, as individuals likely combine new alleles through mating and lose alleles through genetic drift (de Salva et al., 2012).

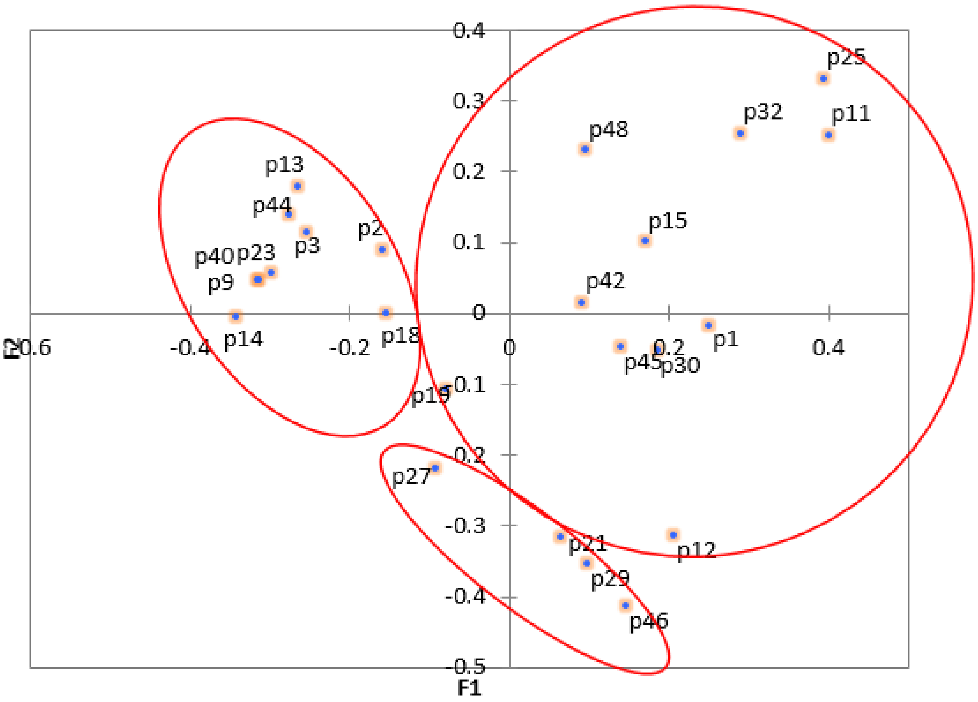

Figure 4. PCoA of 24 *Pistacia* samples identified with 10 ISSR markers

Table 5. Genetic diversity indices in 24 *Pistacia* genotypes revealed by ISSR markers

Population	Na	Ne	I	He
P.atlantica	1.65	1.26	0.28	0.17
P.khinjuk	1.41	1.29	0.30	0.19
P.vera	1.18	1.00	0.00	0.00

Conclusions: The indigenous genotypes, which have evolved extensively in response to the prevailing climatic conditions, can serve as valuable candidates for breeding programs, particularly as a genetic reservoir. The ISSR analysis reported significant genetic diversity in *P. atlantica* and *P. khinjuk* populations based on marker discriminating power indices (PIC, MI, and Rp) as well as population genetic diversity indices (Na, Ne, I, and He). The ISSR primers used in this research generated an average of 95.3% polymorphism, sufficient to differentiate among the three studied populations. Among ten evaluated ISSR markers, OW5 showed the highest values for PIC, MI, and Rp. Cluster analysis using the Jaccard similarity coefficient and PCoA analysis revealed consistent patterns in identifying genetic variation among populations from two distinct geographical regions (Kurdistan of Iraq and Yazd province in Iran). The populations were grouped into three clusters based solely on genetic patterns, irrespective of geographic location. Additionally, a substantial level of genetic diversity was observed within species, with *P. khinjuk* showing the highest values for He and I, indicating greater diversity. This study used ISSR markers to determine the genetic composition of *Pistacia* species collected across the Kurdistan region of Iraq and to assess the similarity of these species with species from protected areas in Yazd province, Iran, which are critical for the management and conservation of endangered species. Prioritizing genetically diverse populations are important step in any conservation program, especially those in northern Kurdistan and Yazd, to preserve adaptive alleles critical for persistence against current and future environmental changes. Moreover, this information is valuable for identifying the genetic reservoir for breeding, production, and genetic improvement of commercially important pistachio cultivars. While ISSR markers effectively revealed genetic structure of the populations here, future studies should incorporate other co-dominant markers such as SSRs and SNPs, and larger sample sizes for higher resolution. Indeed, integrating environmental and ecological data could help link genetic diversity to adaptive traits.

Author contributions

Rasty Yousif Kamal participated in fieldwork, laboratory analysis, data collection, and writing the initial draft of the manuscript. S. Ebrahim Seifati designed the overall study and was responsible for the methodology and formal analysis. Saeed Tarkesh Esfahani and Nariman Salih Ahmad contributed to molecular data analysis. Additionally, Nariman Salih Ahmad was involved in designing the sampling strategy in the Kurdistan Region of Iraq. All authors reviewed and approved the final version of the manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author, (S. Ebrahim Seifati), upon reasonable request.

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Ethical considerations

This study was conducted in accordance with ethical principles for scientific research. Plant sampling was carried out responsibly and in compliance with national environmental regulations.

Coordination was made with the Department of Environment of Yazd Province to ensure that sampling did not harm protected habitats or species. No human or animal subjects were involved in this research. The authors declare that all data were collected with academic integrity and transparency, and there is no conflict of interest among the authors.

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Conflict of interest

The authors declare no conflict of interest.

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دانشگاه شهید باهنر کرمان

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تنوع ژنتیکی گونه‌های *Pistacia* در کردستان عراق و یزد ایران: یافته‌هایی بر پایه نشانگرهای ISSR

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چکیده

هدف: گونه‌های وحشی *Pistacia* نقش مهمی در حفاظت تنوع زیستی و به‌نژادی ارقام مقاوم به تنش‌های محیطی نظیر خشکی و شوری دارند. با این حال، اطلاعات مولکولی موجود درباره تنوع ژنتیکی آن‌ها در نواحی کوهستانی کردستان عراق و مناطق خشک مرکزی ایران همچنان محدود است. هدف این پژوهش، بررسی ساختار و تنوع ژنتیکی گونه‌های *Pistacia* در این دو ناحیه با استفاده از نشانگرهای ISSR بود.

مواد و روش‌ها: در مجموع ۲۴ ژنوتیپ منتخب از گونه‌های *P. atlantica*، *P. khinjuk* و *P. vera* از میان ۶۷ نمونه جمع‌آوری‌شده در استان یزد ایران و اقلیم کردستان عراق انتخاب شدند. استخراج DNA با روش CTAB انجام گرفت و ۱۰ آغازگر ISSR برای تکثیر ژنومی به‌کار رفت. برای ارزیابی تنوع ژنتیکی، شاخص‌های آماری شامل درصد چندشکلی، محتوای اطلاعاتی

چندشکلی، شاخص نشانگر، قدرت تفکیک و نسبت مؤثر چندتایی محاسبه شد. تجزیه‌های خوشه‌ای، مختصات اصلی و واریانس مولکولی نیز به منظور بررسی ساختار جمعیتی انجام شد.

نتایج: از مجموع ۱۷۱ باند تکثیرشده، ۱۶۳ باند (۹۵/۳ درصد) چندشکل بودند. آغازگر ISSR4 بیشترین باند چندشکل را تولید کرد، در حالی که OW5 بالاترین مقادیر شاخص نشانگر و قدرت تفکیک را نشان داد. نتایج تجزیه واریانس مولکولی نشان داد که ۸۳ درصد از تنوع ژنتیکی درون گونه‌ها قرار دارد. بیشترین میزان تنوع ژنتیکی در *P. khinjuk* (I= ۰/۳۰ و He=۰/۱۹) مشاهده شد، در حالی که *P. vera* کمترین تنوع را داشت. تجزیه‌های خوشه‌ای و مختصات اصلی تطابقی با پراکنش جغرافیایی نداشتند که بیانگر جریان ژنی تاریخی یا تأثیر فعالیت‌های انسانی است.

نتیجه‌گیری: یافته‌ها نشان‌دهنده تنوع ژنتیکی درون‌گونه‌ای قابل توجه در جمعیت‌های وحشی *Pistacia* بود که اهمیت آنها را به‌عنوان منابع ژنی برای برنامه‌های اصلاحی و حفاظت تأیید می‌کند. همچنین به طور خاص در مورد *P. khinjuk*، پتانسیل بالایی برای بهره‌برداری در برنامه‌های به‌نژادی در شرایط تنش‌زا به نظر می‌رسد. به طور کلی، توصیه می‌شود در مطالعات آتی، از نشانگرهای هم‌بارز و داده‌های زیست‌محیطی به منظور پیوند دقیق‌تر میان تنوع ژنتیکی و صفات سازگارکننده استفاده شود.

کلمات کلیدی: تنوع زیستی، قرابت ژنتیکی، زاگرس غربی، بنه

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