

MOLECULAR AND AGRO-MORPHOLOGY BIODIVERSITY OF WILD *PISTACIA* IN JORDAN

 Wesam Al Khateeb^{1*},  Mohanad Aljarrah¹,  Mohammad Brake²,
 Muhammad Alu'datt³,  Mohammed Najib Saidi⁴, Ahmad El-Oqlah¹

¹Department of Biological Sciences, Faculty of Science, Yarmouk University, Irbid, Jordan

²Department of Chemistry and Biological Sciences, Faculty of Science, Jerash University, Jerash, Jordan

³Department of Food Science and Nutrition, College of Life Sciences, Kuwait University, Kuwait

⁴Biotechnology and Plant Improvement Laboratory, Centre of Biotechnology of Sfax, P.O. Box 1177, Road Sidi Mansour 6 km, Sfax 3018, Tunisia

Abstract. The genus *Pistacia* is a member of the Anacardiaceae family which includes eleven species. This genus is very important at economic, medicinal and environmental levels. The aim of this research is to study the genetic diversity of wild *Pistacia* species in Jordan. Forty samples from four wild *Pistacia* species; *Pistacia atlantica* Desf., *Pistacia terebinthus* L. subsp. *palaestina* Boiss., *Pistacia khinjuk* Stocks and *Pistacia lentiscus* L. collected from different geographical regions in Jordan were included in the study. Genetic diversity among the four species was investigated using twelve agro-morphological traits and three types of molecular markers. Both analysis; allowed discrimination between samples belonging to different species. The dendrograms generated by the two data sets reveal that *Pistacia terebinthus* L. subsp. *palaestina* Boiss and *Pistacia atlantica* Desf were the closest species and both separated from the remaining ones. The results of this study may be useful in developing national strategies for conservation of wild *Pistacia* species specially for *Pistacia khinjuk*, the use of *Pistacia* trees in forestation and land restoration in dry and semi-arid areas and as source of candidate genes for plant breeding program due to ability of *Pistacia* to grow in harsh and marginal environments.

Keywords: *Pistacia*, biodiversity, Jordan, molecular markers, morphological markers.

Corresponding Author: Wesam Al Khateeb, Department of Biological Sciences, Faculty of Science, Yarmouk University, Irbid, Jordan, Tel.: +962797172687, e-mail: wesamkh@yu.edu.jo

Received: 29 August 2025;

Accepted: 3 December 2025;

Published: 6 April 2026.

1. Introduction

The family, Anacardiaceae, comprises about 81 genera and 800 species. The best-known group of this family is the genus *Pistacia*. Species of the genus *Pistacia* are dioecious trees and are widely distributed in the Mediterranean region, Middle Eastern areas (Ahmed *et al.*, 2021). *Pistacia* genus is believed to have originated in Central Asia 80 million years ago (El Zerey-Belaskri, 2019). The genus *Pistacia* comprised of 11 species, with the *P. vera* L. is the most economically important due to its fruits and the only cultivated one.

Pistacia is a genus of flowering plants which is widespread in Jordan and is represented by four species, namely *P. atlantica* Desf., *P. terebinthus* L. subsp. *palaestina* Boiss., *P. khinjuk* Stocks and *P. lentiscus* L. (Taifour & El-Oqlah, 2014). *P. atlantica* Desf., is a semi-evergreen, monoecious tree with a length of 5-15 m and

grayish-white branches (Awwad *et al.*, 2017). In Jordan, *P. atlantica* Desf. trees grow in Wadi Butum, Azraq and Irbid (Mahjoub *et al.*, 2018). *P. terebinthus* L. subsp. *palaestina* Boiss. (known in Arabic as “butom”) is a dioeciously, deciduous tree or shrub, between 3 and 15 m long and with edible bluish fruits. *P. terebinthus* L. subsp. *palaestina* Boiss. trees have a wide geographic range and a huge population size mainly on the hills and mountains of Jordan and Palestine and in association with *Quercus calliprinus* Webb. trees (Flamini *et al.*, 2004). *P. khinjuk* Stocks is an evergreen, dioeciously tree or shrub used as an ornamental plant and for shade and as a windbreaker. Moreover, it is used commercially as rootstock due to drought tolerance and adaptability to poor soils (Tilkat *et al.*, 2005). It is considered an important plant in various regions of Turkey, Iran, Syria, northern Iraq and southern Jordan (Abolghasemi *et al.*, 2018). In Jordan, *P. khinjuk* Stocks trees are found in Petra and they are threatened by habitat loss and have a restricted distribution. *P. lentiscus* L. (Mastic tree) is an evergreen, dioeciously small tree or shrub (Elloumi *et al.*, 2022), native to the Mediterranean areas, especially in regions that are characterized by nutrient and water scarcity (Ak & Parlakçı, 2009). In Jordan, *P. lentiscus* L. tree is distributed in Jerash, Ajloun, Kufuranje and Dibeen and it shares habitat with olives and carob trees. These species are threatened in Jordan due to excessive logging, extensive grazing and urban expansion, there are fewer than 50 individual trees recorded. *P. lentiscus* L. leaf extracts are rich in essential chemical compounds, particularly terpenoids and polyphenols compounds, which provide an essential antioxidant power to the plant (Hadini *et al.*, 2022). Extracts and different plant parts of the four species of the genus *Pistacia* were used in Jordanian traditional medicine (Awwad *et al.*, 2017; 2020; Akbar, 2020).

Morphological traits (Ghrab *et al.*, 2012) and molecular markers including random amplified polymorphic DNA (RAPD) (Karimi *et al.*, 2012; Al-Saghir & Porter, 2006; Iranjo *et al.*, 2015), inter-simple sequence repeats (ISSRs) (Kebour *et al.*, 2012; Bouta *et al.*, 2022; Baghizadeh & Dehghan, 2018), inter-retrotransposon amplified polymorphism (IRAP) (Zhao *et al.*, 2022; Sorkheh *et al.*, 2016; Serttaş & Özcan, 2018) and long terminal repeat (LTR) (Kırdök & Çiftçi, 2016) have been used to study the genetic diversity in *Pistacia* species.

The objective of this study is to assess the genetic diversity within and between four species of the genus *Pistacia* grown in Jordan using 12 agro-morphological traits and different class of molecular markers like RAPD, ISSR, IRAP and LTR.

2. Materials and methods

2.1. Plant materials

For this study, a total of 40 samples belonging to four different Jordanian *Pistacia* species from five main sites in Jordan were collected (Figure 1) (Table 1). Collected samples were transported properly to the laboratory and held at -80°C until analysis. Following the guidelines for identification set forth by Taifour and El-Oqlah (2014), samples were cautiously identified.

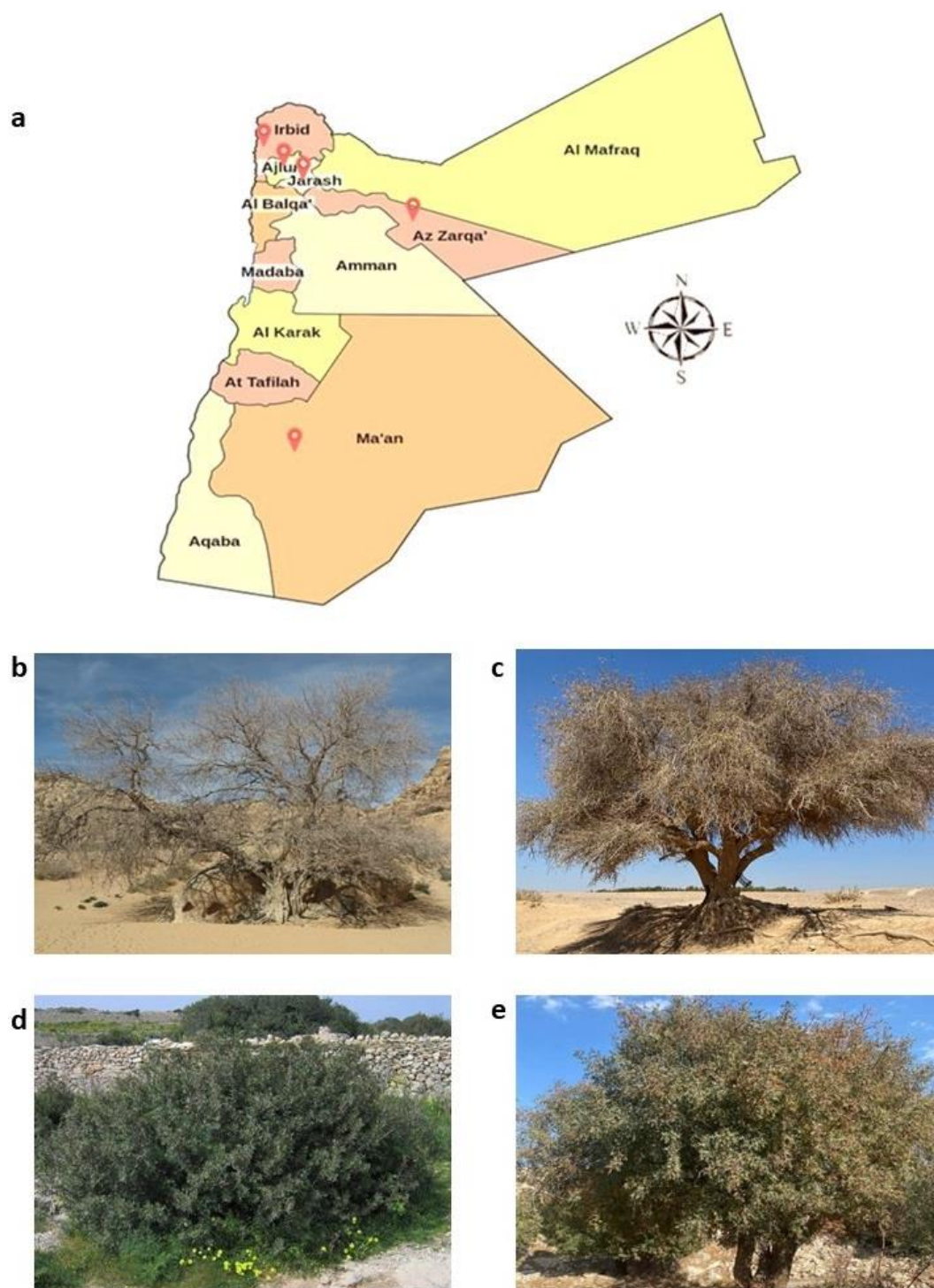


Figure 1. a) Map of Jordan showing sampling sites of four Jordanian *Pistacia* species distributed over various geographical regions. b) *Pistacia khinjuk* tree located in Petra, Jordan. c) *Pistacia atlantica* tree located in Wadi Albutom, Azraq, Jordan. d) *Pistacia lentiscus* shrub located in Khsheibeh, Jerash, Jordan. e) *Pistacia terebinthus* L. subsp. *palaestina* Boiss. tree located in Al- Mazar al Shamali, Irbid, Jordan

Table 1. Localities of *Pistacia* species samples

Accession number	Species	Province	Location	Longitude	Latitude
1	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Irbid	Al- Mazar al Shamali	N 32.4322544	E 35.8004599
2	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Irbid	Al- Mazar al Shamali	N 32.45284	E 35.80842
3	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Irbid	Al- Mazar al Shamali	N 32.4361472	E 35.8058136
4	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Irbid	Al- Mazar al Shamali	N 32.4340087	E 35.8039494
5	<i>Pistacia atlantica</i> Desf.	Jerash	Dibbeen Forest Reserve	N 32.24199	E 35.82953
6	<i>Pistacia atlantica</i> Desf.	Jerash	Dibbeen Forest Reserve	N 32.24108	E 35.82053
7	<i>Pistacia atlantica</i> Desf.	Jerash	Dibbeen Forest Reserve	N 32.2445	E 35.82963
8	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Irbid	Al- Hawi	N 32.4479	E 35.7242
9	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Irbid	Al- Hawi	N 32.45061	E 35.72253
10	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Irbid	Al- Hawi	N 32.45278	E 35.72468
11	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Irbid	Zoubia	N 32.4433270	E 35.7696720
12	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Irbid	Zoubia	N 32.4384028	E 35.7742390
13	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Irbid	Zoubia	N 32.4465124	E 35.7570391
14	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Irbid	Zoubia	N 32.4466630	E 35.7671240
15	<i>Pistacia lentiscus</i> L.	Jerash	Khsheibeh	N 32.227600	E 35.716175
16	<i>Pistacia lentiscus</i> L.	Jerash	Khsheibeh	N 32.247185	E 35.739098
17	<i>Pistacia lentiscus</i> L.	Jerash	Khsheibeh	N 32.24179	E 35.73609
18	<i>Pistacia atlantica</i> Desf.	Maan	Shobak	N 30.41565	E 35.50921
19	<i>Pistacia atlantica</i> Desf.	Maan	Shobak	N 30.41388	E 35.50879
20	<i>Pistacia atlantica</i> Desf.	Maan	Shobak	N 30.41516	E 35.50692
21	<i>Pistacia atlantica</i> Desf.	Maan	Shobak	N 30.41755	E 35.50808
22	<i>Pistacia atlantica</i> Desf.	Maan	Shobak	N 30.42034	E 35.51188
23	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Jerash	Khsheibeh	N 32.2294690	E 35.7308770
24	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Jerash	Khsheibeh	N 32.2474370	E 35.7438550
25	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Jerash	Khsheibeh	N 32.2463070	E 35.7479740
26	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Ajloun	Ras Munif	N 32.41022	E 35.81699
27	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Ajloun	Ras Munif	N 32.40895	E 35.81742
28	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Irbid	Rhaba	N 32.429017	E 35.7929633
29	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Irbid	Rhaba	N 32.4357084	E 35.7863794
30	<i>Pistacia terebinthus</i> L. subsp. <i>palaestina</i> Boiss.	Irbid	Rhaba	N 32.4348939	E 35.7865374
31	<i>Pistacia atlantica</i> Desf.	Zarqa	Wadi Albutom	N 31.7995720	E 36.5893210
32	<i>Pistacia atlantica</i> Desf.	Zarqa	Wadi Albutom	N 31.8035052	E 36.5810458
33	<i>Pistacia atlantica</i> Desf.	Zarqa	Wadi Albutom	N 31.80068	E 36.58119
34	<i>Pistacia atlantica</i> Desf.	Zarqa	Wadi Albutom	N 31.80336	E 36.5783
35	<i>Pistacia atlantica</i> Desf.	Maan	Wadi Musa	N 30.38155	E 35.47595
36	<i>Pistacia atlantica</i> Desf.	Maan	Wadi Musa	N 30.38123	E 35.48005
37	<i>Pistacia atlantica</i> Desf.	Maan	Wadi Musa	N 30.38229	E 35.47194
38	<i>Pistacia Khinjuk</i> Stocks.	Maan	Petra back road	N 30.35274	E 35.45434
39	<i>Pistacia Khinjuk</i> Stocks.	Maan	Petra back road	N 30.34845	E 35.45555
40	<i>Pistacia Khinjuk</i> Stocks.	Maan	Petra back road	N 30.35741	E 35.45542

2.2. Morphological analysis

Based on the *Pistacia* descriptor developed by the International Plant Genetic Resources Institute (1998), twelve morphological characters were determined namely: Leaf length, width, leaflet length, width, leaflets number, absence or presence of terminal leaflets, leaf rachis wing, relative size of terminal leaf, leaf persistency and nut length, width and nut penicle length.

2.3. DNA extraction

Total genomic DNA was extracted from fresh leaves for all samples using the CTAB method developed by Doyle and Doyle (1987). The quantity and quality of DNA were determined by spectrophotometer and 1% agarose gel electrophoresis, respectively. Extracted DNA were kept at -20°C until use.

2.4. Markers fingerprinting

To study the diversity of Jordanian *Pistacia* species, four molecular markers were used. Nine Random Amplified Polymorphic DNA (RAPD) primers, 12 Inter-Simple Sequence Repeat (ISSR) primers, 5 Inter-Retrotransposon Amplified Polymorphism (IRAP) primers and 3 Long Terminal Repeat (LTR) primers were used in this study (Table 2). Polymerase Chain Reactions (PCR) reactions were performed in a 25 µL reaction containing 1 µL of genomic DNA (30ng/µL), 0.2 µM of the primer, 1× Taq polymerase reaction buffer, 1.5 unit of Taq DNA polymerase and 0.2 mM of each dNTP. Amplifications were performed using Genepro model TC-E-96G thermal cycler programmed for an initial 5 min denaturation at 94°C followed by 40 cycles of 45 seconds of denaturation at 94°C, 1 min annealing at appropriate temperature for each primer (Table 2) and a 2 min extension at 72°C followed by a final extension for 10 min at 72°C. The amplified products of all markers were separated on 1% agarose gel 1× TBE buffer. Fragment size was estimated using 100 base pair (bp) DNA marker (Fermentas, Germany). PCR fragments were stained by ethidium bromide (10 mg/ml) according to Sambrook et al. (1989) and then visualized using a UV-transilluminator and photographed with an AlphaImager gel documentation system.

Table 2. ISSR primers name, sequence, total number of markers, percentage of primer efficiency, polymorphic marker, unique bands, percentage of polymorphism, percentage of discrimination power and PIC

Primer	Sequence 5'–3'	Annealing temperature	Total # of markers	%Primer efficiency	Polymorphic markers	Unique bands	%Polymorphism	%Discrimination power	PIC
OPA-10	5'GTGATCGCAG 3'	32	11	3	10	0	91	3	0.35
OPB-04	5'GGAGTGGAGT 3'	32	15	4	15	0	100	4	0.34
OPE-15	5'ACGCACAACC 3'	32	15	4	15	0	100	4	0.39
OPJ-12	5'GTCCGTGGT 3'	42	15	4	14	0	93	4	0.36
OPL-19	5'GAGTGGTGAC 3'	44	11	3	11	0	100	3	0.34
OPF-04	5'GGTGATCAGG 3'	45	8	2	7	0	88	2	0.28
OPD-16	5'AGGGCGTAA 3'	48	13	4	13	1	100	4	0.30
OPC-20	5'ACTTCGCCAC 3'	43	16	4	16	0	100	4	0.29
OPA-12	5'TCGGCGATAG 3'	45	10	3	10	0	100	3	0.28
UBC-822	5'TCTCTCTCTCTCTCA 3'	52.8	7	2	7	2	100	2	0.19
UBC-888	5'GCTAGTGCTCACACACA 3'	52.8	16	4	16	0	100	4	0.37
UBC- A	5'GAGAGAGAGAGAGAGACT 3'	54	17	5	17	0	100	5	0.35
UBC-855	5'CACACACACACACACCT 3'	58.3	15	4	15	0	100	4	0.36
UBC-810	5'GAGAGAGAGAGAGAGAT 3'	52	12	3	12	2	100	3	0.23
UBC-823	5'TCTCTCTCTCTCTCC 3'	52	12	3	12	1	100	3	0.28
UBC-856	5'ACACACACACACACAYA 3'	52.8	18	5	18	0	100	5	0.30
UBC-807	5'AGAGAGAGAGAGAGAGT 3'	52	7	2	7	0	100	2	0.39
UBC841	5'GAGAGAGAGAGAGAGAYC 3'	48	11	3	10	1	91	3	0.22
UBC-825	5'ACACACACACACT 3'	44	15	4	15	0	100	4	0.40
UBC-864	5'ATGATGATGATGATGATG 3'	48	11	3	11	0	100	3	0.37
UBC-834	5'AGAGAGAGAGAGAGACYT 3'	55	13	4	13	0	100	4	0.30
IRAP-4334	5'CCATGGCGAGCAGATGTGCT 3'	51.7	16	4	16	1	100	4	0.28
IRAP-4370	5'ATGCCGTATCTCAGCATCC 3'	51	20	5	20	1	100	6	0.33
IRAP-4375	5'ATCGCTCCGGGTGCTAACAC 3'	52.6	15	4	15	1	100	4	0.21
ATHILA 4-6	5'AAATGCATGCTCAAACATGC 3'	51.7	6	2	6	1	100	2	0.24
NIKITA	5'CGCTCCAGCGGTACTGCC 3'	55	18	5	18	0	100	5	0.38
LTR-4	5'GACATTTTGGGA 3'	56	12	3	11	0	92	3	0.36
LTR2467	5'ACGGTTACGGCGGTGTTCTCTTCCA 3'	48	7	2	7	0	100	2	0.32
LTR2452	5'TCCTGGTAACACTATGGATACGAC 3'	48	7	2	6	0	86	2	0.26
Total			369		363	11			
Average			12.7	3.4	12.5		97.96	3.48	0.31

Note: Y=CT

2.5. Data analysis

To determine the relative importance of the quantitative morphological characters on the variability between the samples included in the study, Pearson correlation coefficients were performed (Benesty *et al.*, 2009). The DNA profiles were scored visually from gel photographs. Clear and reproducible amplified bands were chosen in the analyses. Amplification bands were converted into a binary matrix for all samples, where a (1) indicates the presence of a DNA band at a specific position on gels and a (0) indicates the absence. Primer efficiency and discrimination power were calculated according to Brake *et al.* (2014). The data obtained by scoring the molecular marker profiles were subjected to the calculation of a similarity matrix using Jaccard's coefficients. The similarity values were used for cluster analyses. Sequential agglomerative hierarchical nonoverlapping (SAHN) clustering was applied using the unweighted pair group method with arithmetic averages (UPGMA). Dendrograms were plotted using NTSYSpc 2.02 software (Rohlf, 1998). GenAlEx version 6.501 (Peakall & Smouse, 2012) was used to calculate genetic diversity indicators including the number of different alleles (N_a), number of effective alleles (N_e), Shannon diversity index (I), expected heterozygosity (H_e). GenAlEx version 6.501 was used also to calculate Principal Coordinates Analysis (PCoA).

3. Results

3.1. Morphological analysis

Firstly, we have employed morphological analysis to distinguish between the analysis samples. The recorded morphological traits were subjected to statistical analysis. Results (Table 3) showed significant differences ($P < 0.05$) among all *Pistacia* species for the measured quantitative traits suggesting morphological variability between the samples studied. Moreover, descriptive statistics of agro-morphological traits of four *Pistacia* species (Table 3, Table 4) showed that the high level of variation was observed for nut penicle length (diversity index=37.00) while the low level of variation (diversity index=19.69) was observed for nut width.

Table 3. Descriptive statistics of *Pistacia* leaf length, width, leaflet length, width, number of leaflets, nut length, width and nut penicle length in four wild species

	Mean	Std.error	Std.dev	Range	Max	Min	Shannon-Wiener
Leaf length	12.77	0.281	3.555	17.3	19.6	2.8	1.461
Leaf width	9.740	0.162	2.055	11.2	15.5	4.3	1.236
Leaflet length	4.861	0.084	1.063	6.2	8.5	2.3	1.658
Leaflet width	1.935	0.039	0.498	2.0	3.0	1.0	1.271
Number of	8.361	0.189	2.273	10.0	14.0	4.0	1.302
Nut length	0.657	0.011	0.133	0.6	1.0	0.4	1.458
Nut width	0.594	0.010	0.117	0.4	0.8	0.4	1.367
Nut penicle	11.551	0.367	4.275	20.8	22	1.2	1.592

Furthermore, correlation analysis was used as an indicator to facilitate the selection of promising ecotypes that combine different important agro-morphological traits. Pearson coefficients determination revealed significant correlation between

different quantitative traits evaluated. Besides, to assess association between traits in the four studied species, we calculate the correlation coefficients between all samples studied. Results showed leaflet length and leaf width had the highest positive correlation ($r = 0.909$), followed by leaf width and leaf length with a correlation coefficient of 0.724 (Table 5).

Table 4. Descriptive statistics of four wild species of *Pistacia*

Character	Species	Mean	Std.error	Std.dev	Range	Max	Min	Diversity index
Leaf length	<i>P. lentiscus</i>	3.566	0.29	1.033	3.7	6	2.3	28.96
	<i>P. khinjuk</i>	12.2	0.532	1.854	7.9	15.3	7.4	15.196
	<i>P. terebinthus</i>	14.18	0.269	2.345	9.7	19.6	9.9	16.53
	<i>P. atlantica</i>	12.94	0.324	2.515	10.3	19.4	9.1	19.43
Leaf width	<i>P. lentiscus</i>	5.316	0.178	0.619	2.1	6.4	4.3	11.64
	<i>P. khinjuk</i>	11.316	0.564	1.954	6.5	15.5	9.0	17.267
	<i>P. terebinthus</i>	10.24	0.184	1.604	7.5	15	7.5	15.66
	<i>P. atlantica</i>	9.675	0.204	1.584	8.1	14.3	6.2	16.37
Leaflet length	<i>P. lentiscus</i>	2.625	0.083	0.286	0.9	3.2	2.3	10.89
	<i>P. khinjuk</i>	6.058	0.335	1.161	3.6	8.5	4.9	19.164
	<i>P. terebinthus</i>	5.110	0.093	0.814	4.0	7.8	3.8	15.92
	<i>P. atlantica</i>	4.755	0.094	0.7344	3.5	7.0	3.5	15.44
Leaflet width	<i>P. lentiscus</i>	1.225	0.037	0.129	0.4	1.4	1	10.53
	<i>P. khinjuk</i>	2.1	0.089	0.310	0.9	2.6	1.7	14.761
	<i>P. terebinthus</i>	2.184	0.047	0.414	1.6	3.0	1.4	18.95
	<i>P. atlantica</i>	1.73	0.057	0.446	1.9	3.0	1.1	25.78
Number of leaflets	<i>P. lentiscus</i>	5.333	0.31	1.073	3	7	4	20.12
	<i>P. khinjuk</i>	7.166	0.270	0.937	3	9	6	13.075
	<i>P. terebinthus</i>	9.578	0.24	2.09	9	14.0	5	21.82
	<i>P. atlantica</i>	7.409	0.239	1.589	6	11.0	5.0	21.44
Nut length	<i>P. lentiscus</i>	0.658	0.038	0.131	0.3	0.8	0.5	19.91
	<i>P. khinjuk</i>	0.508	0.019	0.066	0.2	0.6	0.4	12.99
	<i>P. terebinthus</i>	0.623	0.012	0.100	0.5	0.9	0.4	16.05
	<i>P. atlantica</i>	0.753	0.020	0.131	0.5	1	0.5	17.42
Nut width	<i>P. lentiscus</i>	0.533	0.014	0.049	0.1	0.6	0.5	9.19
	<i>P. khinjuk</i>	0.508	0.022	0.079	0.2	0.6	0.4	15.55
	<i>P. terebinthus</i>	0.572	0.014	0.118	0.4	0.8	0.4	20.6
	<i>P. atlantica</i>	0.672	0.014	0.095	0.3	0.8	0.5	14.13
Nut penicle length	<i>P. lentiscus</i>	2.675	0.247	0.856	3.1	4.3	1.2	32
	<i>P. khinjuk</i>	10.633	0.671	2.32	7.1	14.6	7.5	21.818
	<i>P. terebinthus</i>	13.038	0.393	3.24	14.8	22	7.2	24.85
	<i>P. atlantica</i>	11.934	0.559	3.668	13.4	20.5	7.1	30.73

Table 5. Correlation values between the character pairs in all *Pistacia* species

	Leaf	Leaflet	Leaflet	Number of	Nut	Nut	Nut penicle
Leaf length	0.724	0.674	0.576	0.390	0.0569	0.148	0.629
Leaf width		0.909	0.692	0.291	0.0104	0.054	0.513
Leaflet			0.680	0.284	-0.074	0.039	0.454
Leaflet width				0.257	-0.119	-0.111	0.507
Number of					-0.144	-0.086	0.279
Nut Length						0.5334	0.110
Nut width							0.101

Similarity between studied samples was assessed based on morphological traits. The highest similarity coefficient value ($r = 0.916$) was observed between samples 15 and 16 (*P. lentiscus*). On the other hand, the lowest similarity coefficient value was found between several samples for example between samples 3 and 5 (*P. terebinthus* L. subsp. *palaestina* and *P. atlantica*). Furthermore, based on similarity matrix,

dendrogram based on morphological data was constructed. Cluster analysis grouped the samples studied into four main groups (Figure 2). The first group contained samples 15, 16 and 17. The second group contained samples 38, 39 and 40. The third group contained samples 5, 33, 18, 19, 31, 20, 37, 21, 22, 32, 36, 7, 35, 6 and 34. The fourth group contained 1, 8, 9, 30, 12, 28, 3, 13, 24, 25, 4, 10, 23, 11, 27, 14, 29, 2 and 26. The dendrogram shows also that samples of each species grouped in a single clade (Figure 2).

3.2. Molecular marker analysis

Genetic diversity and relatedness among *Pistacia* species samples were examined also using molecular markers. Three hundred sixty-nine scored markers were obtained using 29 primers; 363 markers of which were polymorphic (97.96%) and with an average of 12.5 polymorphic markers per primer (Table 2). Primer IRAP-4370 produced the highest number of markers (20) and primer ATHILA 4-6 produced the lowest (6), with an average of 12.7 markers per primer. The highest discrimination power was observed using primer IRAP-4370, while primers UBC-822, UBC-807, OPF-04, ATHILA 4-6, LTR2452 and LTR2467 showed the lowest discrimination power. The lowest and the highest polymorphic information content (PIC) values were 0.19 and 0.40, for primers UBC-822 and UBC-825, respectively. The mean PIC value was 0.31.

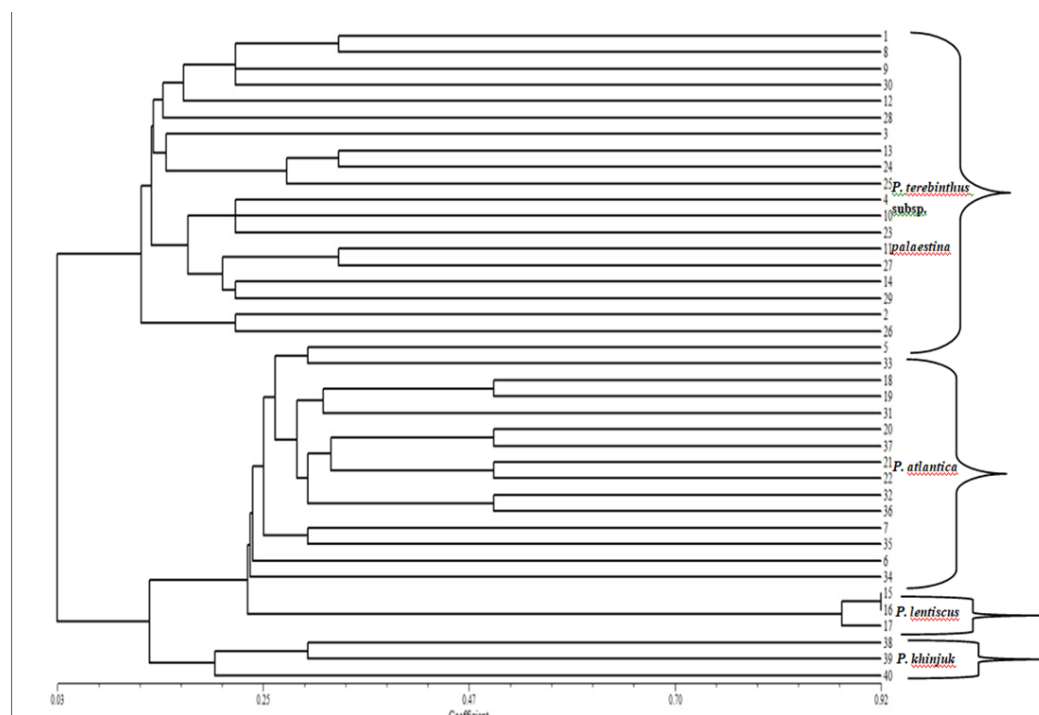
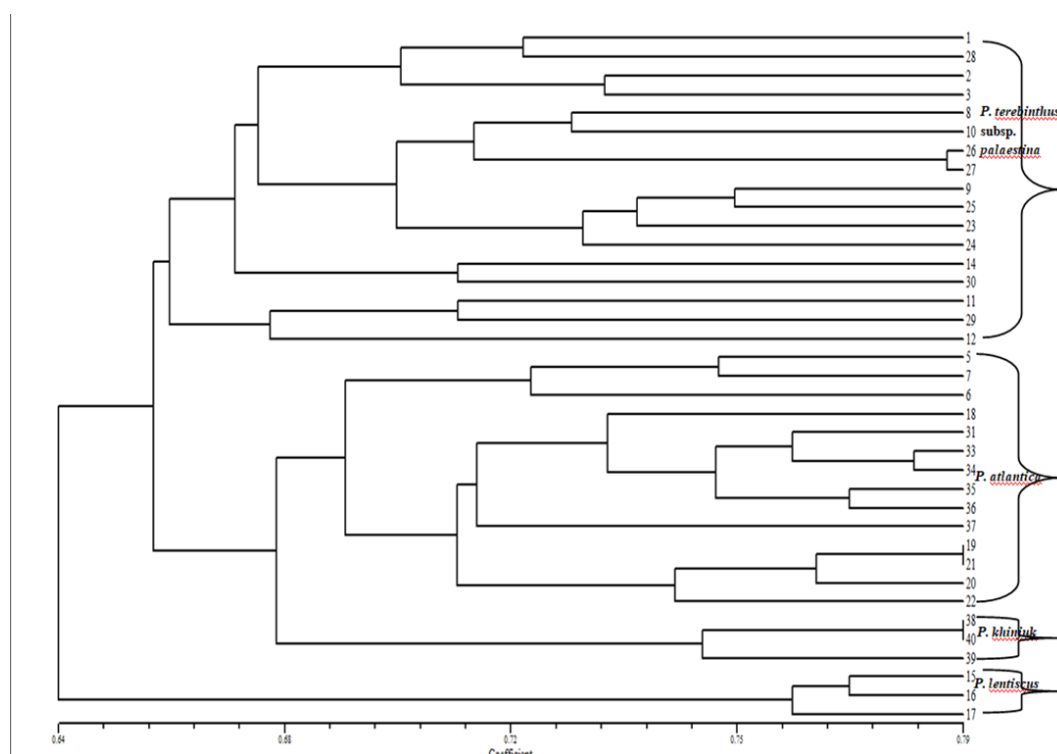


Figure 2. Dendrogram of collected *Pistacia* samples, generated by UPGMA cluster analysis method, based on morphological traits

Table 6. Unique and unique missing bands generated with molecular markers

Samples	Species	Unique bands	Unique missing bands
6	<i>Pistacia atlantica</i> Desf.	OPD-16	
20			OPC-20
22		IRAP-4375	
31			OPF-04
34		UBC-810	
35		UBC822	
37		UBC841	
2	<i>Pistacia terebinthus</i> L. subsp. <i>Palaestina</i>	IRAP-4370	
10		UBC-810	
9		UBC-823	
26		UBC-822 , ATHILA 4-6	
30		IRAP-4334	

Thirteen markers out of 363 were species-specific, with two markers present in all samples except one. These 13 markers were able to distinguish *P. atlantica* and *P. terebinthus* L. subsp. *palaestina* (Table 6).

**Figure 3.** Dendrogram of collected *Pistacia* samples, generated by UPGMA cluster analysis method, based on data of molecular markers

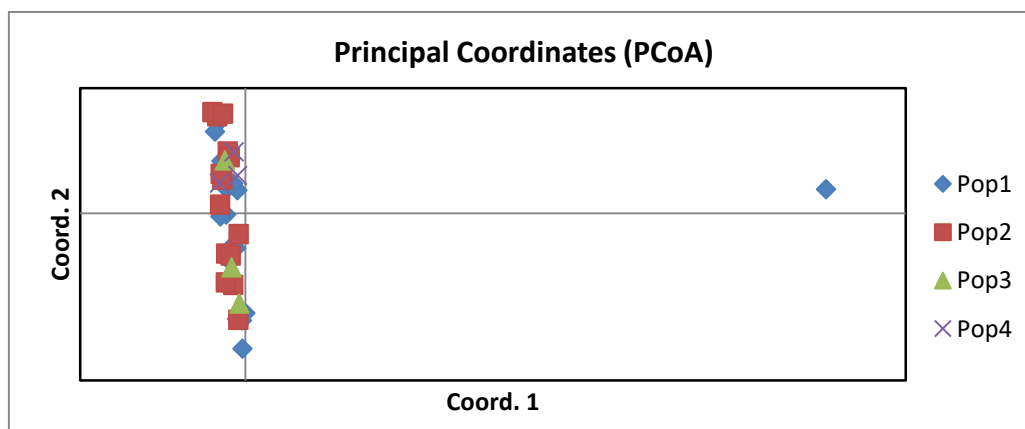


Figure 4. Distribution of principal coordinates of *P. terebinthus* (blue rhombus), *P. atlantica* (red square), *P. lentiscus* (green triangle) and *P. khinjuk* (black ex) respectively

Genetic similarity matrix and dendrogram based on scored markers were then elaborated. The highest similarity coefficient value was 0.791 was observed between samples 38 and 40 (*P. khinjuk*). While the lowest similarity coefficient value of 0.569 was obtained between samples 16 and 39 (*P. lentiscus* and *P. khinjuk*). The dendrogram showed that the samples are clustered into two main groups, the first one contains three samples 15, 16 and 17 and the second one further divided further into two subgroups. The first subgroup contained samples 12, 29, 11, 30, 14, 24, 23, 25, 9, 27, 26, 10, 8, 3, 2, 28 and 1. The second subgroup contained samples 38, 40 and 39 which belong to *P. khinjuk* and samples 5, 7, 6, 18, 31, 33, 34, 35, 36, 37, 19, 21, 20 and 22 which belong to *P. atlantica* as it is illustrated in Figure 3. The genetic diversity parameters for the four *Pistacia* species were calculated (Table 7). The highest mean value (1.896) of Na was in *P. terebinthus*, while the lowest was in *P. lentiscus*. The mean of Ne was *P. terebinthus* (1.369) > *P. atlantica* (1.339) > *P. khinjuk* (1.324) > *P. lentiscus* (1.282). *P. lentiscus* shows the lowest mean values of I and He, while *P. terebinthus* shows the highest. The four species of *Pistacia* are not separated according to PCoA analysis. The first and second coordinates represent 18% of the genetic variance (Figure 4).

Table 7. Genetic diversity parameters among *P. terebinthus*, *P. atlantica*, *P. lentiscus* and *P. khinjuk*

Species	Na	Ne	I	He
<i>P. terebinthus</i>	1.896	1.369	0.383	0.239
<i>P. atlantica</i>	1.784	1.339	0.357	0.222
<i>P. lentiscus</i>	1.024	1.282	0.250	0.167
<i>P. khinjuk</i>	1.149	1.324	0.286	0.192

4. Discussion

Given the importance of the genus *Pistacia* from different aspects such as: economic, medicine and environmental aspects, although, there are many research studied the diversity of this genus worldwide, there are no detailed study on Jordanian *Pistacia* at morphological and genetic level. In this study we use combination of morphological and molecular markers to clarify the phylogenetic relationship of *Pistacia* species grown in Jordan.

Eight quantitative and four qualitative morphological variables successfully distinguished *Pistacia* plants collected from the thirteen sites, with a significant difference among species means, indicating high variability existing among *Pistacia* species. Our finding is consistent with the findings of Kafkas et al. (2002) who find high diversity between three different wild *Pistacia* species using a total of 30 morphological characters. Furthermore, Barazani et al. (2003) analyze the genetic variability of Mediterranean *Pistacia lentiscus* genotypes using molecular, chemical and morphological characters. High polymorphism in morphological parameters was found among accessions, with no significant differences in relation to geographical origin or to gender.

Pearson Correlation analysis revealed strong correlations between leaf width and leaflet length ($r=0.909$) and between leaf width and leaf length ($r=0.724$) in the four *Pistacia* species, this indicates that these traits are helpful to *Pistacia* breeders because it is associated with longest leaf length and longest leaflet length. On the other hand, the highest level of variation was recorded for nut penicle length followed by leaf length and number of leaflets among the four *Pistacia* species; this indicates a high level of diversity among the samples for these traits. So, the selection on the basis of these traits can be useful.

The dendrogram generated using morphological traits was able to separate samples according to species and it is composed of four groups, where samples of each of the four species are grouped together. The dendrogram show no clear relationship between morphological characteristics and geographical distribution of the collected samples.

The same samples analyzed by morphological characters were used to be analyzed using molecular markers. Three main classes of molecular markers were used. The results reveal a high percent of polymorphism (97.96%), which is consistent with results from previous analyses for *Pistacia* species using ISSR markers in Algeria, Turkey and Algeria and Italy (Kebour et al., 2012; Serttaş & Özcan, 2018; Labdelli et al., 2022; Bouta et al., 2022) and by using RABD markers in South of Syria (Alhajjar et al., 2011). Kebour et al. (2012) have used 6 ISSR primers to study genetic relationships among 10 pistachio accessions collected from Algeria and observed 54.04% of polymorphism percentage and 6.6 of an average resolving power value of markers was recorded. The genetic similarity matrix constructed using molecular data reveals that the highest genetic similarity matrix value is between samples belonging to the same species while the lowest is between samples from two different species.

Like the dendrogram generated using morphological traits, the dendrogram constructed using molecular data was able to separate samples into four groups where samples from the same species are grouped together. The dendrogram shows no clear clustering according to the collection site of *Pistacia*. This results are consistent with the results of previous studies and explained that the accessions from different regions might have a similar genetic background and those from the same origin might also have a different genetic background (Pazouki et al., 2010; Iranjo et al., 2015).

The high polymorphism generated by these markers indicates that the *Pistacia* tree is a highly polymorphic species. The high diversity could be explained due to a diverse germplasmic origin that resulted in a predominant allogamous species with a high degree of out-crossing. Out-crossing decreases intraspecific diversity while increasing interspecific diversity.

Acknowledgments

Authors would like to thank the deanship of research at Yarmouk University for funding this project.

References

- Abolghasemi, A., Shojaaddini, M., Tajabadipour, A. & Sefidkon, F. (2018). Composition of Pistacia khinjuk (Anacardiaceae) leaf essential oil and its insecticidal activity on common Pistachio Psyllid, *Agonoscena pistaciae* (Hem., Psylloidea). *Journal of Essential Oil Bearing Plants*, 21(3), 796-802. <https://doi.org/10.1080/0972060X.2018.1498397>
- Ahmed, Z.B., Yousfi, M., Viaene, J., Dejaegher, B., Demeyer, K. & Vander Heyden, Y. (2021). Four Pistacia atlantica subspecies (atlantica, cabulica, kurdica and mutica): A review of their botany, ethnobotany, phytochemistry and pharmacology. *Journal of Ethnopharmacology*, 265, 113329. <https://doi.org/10.1016/j.jep.2020.113329>
- Ak, B.E., Parlakci, H. (2009). Pistacia lentiscus in the Mediterranean region in Turkey. *Acta Horti*, 818, 77-82. <https://doi.org/10.17660/ActaHortic.2009.818.10>
- Akbar, S. (2020). *Handbook of 200 Medicinal Plants: A Comprehensive Review of Their Traditional Medical Uses and Scientific Justifications*. Springer.
- Alhajjar, N.M., Muzher, B.M. & Hamed, F. (2011). Morphological and Molecular characterization of Pistacia atlantica Desf. in the South of Syria/Swaida Province/. *Jordan Journal of Agricultural Sciences*, 7(3).
- Al-Saghir, M.G., Porter, D.M. (2006). Random amplified polymorphic DNA (RAPD) study of Pistacia species (Anacardiaceae). *Asian Journal of Plant Sciences*, 5, 1002-1006. <https://doi.org/10.3923/ajps.2006.1002.1006>
- Awwad, O., Abu-Dahab, R., Abaza, I.F., Alabbassi, R., Majdalawi, L. & Afifi, F.U. (2017). Effect of the galling aphid of Baizongia pistaciae L. on composition and biological activities of essential oils of Pistacia atlantica Desf. growing wild in Jordan. *Journal of Essential Oil Bearing Plants*, 20(3), 791-800. <https://doi.org/10.1080/0972060X.2017.1341343>
- Awwad, O., Alabbassi, R., Abaza, I.F., Coperchini, F., Rotondi, M., Chiovato, L. & Afifi, F.U. (2020). Effect of Pistacia palaestina Boiss. essential oil on colorectal cancer cells: Inhibition of proliferation and migration. *Journal of Essential Oil Bearing Plants*, 23(1), 26-37. <https://doi.org/10.1080/0972060X.2020.1729244>
- Baghizadeh, A., Dehghan, E. (2018). Efficacy of SCoT and ISSR markers in assessment of genetic diversity in some Iranian pistachio (Pistacia vera L.) cultivars. *Pistachio and Health Journal*, 1(1), 37-43. <https://doi.org/10.22123/PHJ.2017.54299>
- Barazani, O., Dudai, N. & Golan-Goldhirsh, A. (2003). Comparison of Mediterranean Pistacia lentiscus genotypes by random amplified polymorphic DNA, chemical and morphological analyses. *Journal of Chemical Ecology*, 29(8), 1939-1952. <https://doi.org/10.1023/A:1024862614345>
- Benesty, J., Chen, J., Huang, Y. & Cohen, I. (2009). Pearson correlation coefficient. In *Noise Reduction in Speech Processing*, 1-4. Berlin, Heidelberg: Springer Berlin Heidelberg. http://dx.doi.org/10.1007/978-3-642-00296-0_5
- Bouta, W., Bouda, S., Ait Bella, Y., Khachtib, Y., El Hansali, M., El Rasafi, T. & Haddioui, A. (2022). Assessment of genetic diversity of Moroccan 'Pistacia lentiscus' L. populations using ISSR markers. *Australian Journal of Crop Science*, 16(3), 365-371.
- Brake, M., Migdadi, H., Al-Gharaibeh, M., Ayoub, S., Haddad, N. & El Oqlah, A. (2014). Characterization of Jordanian olive cultivars (Olea europaea L.) using RAPD and ISSR molecular markers. *Scientia Horticulturae*, 176, 282-289.
- Doyle, J.J., Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19, 11-15.
- El Zerey-Belaskri, A. (2019). Taxonomic and botanical retrospective review of Pistacia atlantica

- Desf.(Anacardiaceae). *Arabian Journal of Medicinal and Aromatic Plants (AJMAP)*, 5, 47-77.
- Elloumi, W., Maalej, A., Ortiz, S., Michel, S., Chamkha, M., Boutefnouchet, S. & Sayadi, S. (2022). Pistacia lentiscus L. distilled leaves as a potential cosmeceutical ingredient: Phytochemical characterization, transdermal diffusion and anti-elastase and anti-tyrosinase activities. *Molecules*, 27(3), 855. <https://doi.org/10.3390/molecules27030855>
- Farshadfar, M. (2021). Morphometric analysis and genetic diversity in Pistacia species populations using sequence related amplified polymorphism. *Genetika*, 53(3), 1193-1205.
- Flamini, G., Bader, A., Cioni, P. L., Katbeh-Bader, A. & Morelli, I. (2004). Composition of the essential oil of leaves, galls and ripe and unripe fruits of Jordanian Pistacia palaestina Boiss. *Journal of Agricultural and Food Chemistry*, 52(3), 572-576.
- Ghrab, M., Zribi, F., Ben Mimoun, M. & Rhouma, A. (2012). Morphological investigation of genetic diversity of pistachio (*Pistacia vera*) germplasm in arid land of Tunisia. *Plant Ecology and Evolution*, 145(3), 363-372. <https://doi.org/10.5091/plecevo.2012.614>
- Hadini, A., Azdimousa, A., Khoulati, A., El Bekkaye, K. & Saalaoui, E. (2022). Valorization of Moroccan pistacia lentiscus L. Leaves: Phytochemical and in vitro antioxidant activity evaluation compared to different altitudes. *The Scientific World Journal*, 1, 6367663. <https://doi.org/10.1155/2022/6367663>
- IPGRI (1997). Descriptors for Pistachio (*Pistacia vera* L.). International Plant Genetic Resources Institute, Rome, Italy.
- Iranjo, P., NabatiAhmadi, D., Sorkheh, K., Memeari, H.R. & Ercisli, S. (2016). Genetic diversity and phylogenetic relationships between and within wild Pistacia species populations and implications for its conservation. *Journal of Forestry Research*, 27(3), 685-697. <https://doi.org/10.1007/s11676-015-0098-9>
- Kafkas, S., Kafkas, E. & Perl-Treves, R. (2002). Morphological diversity and a germplasm survey of three wild Pistacia species in Turkey. *Genetic Resources and Crop Evolution*, 49(3), 261-270. <https://doi.org/10.1023/A:1015563412096>
- Karimi, H.R., Abadi, M.H.H. & Kohbanani, A.M. (2012). Genetic diversity of Pistacia khinjuk Stocks. using RAPD markers and leaf morphological characters. *Plant Systematics and Evolution*, 298(5), 963-968. <https://doi.org/10.1007/s00606-012-0605-9>
- Kebour, D., Boutekrabt, A. & Mefti, M. (2012). Using ISSR markers to study genetic polymorphism of pistachio (*Pistacia vera* L.) in Algeria. *African Journal of Biotechnology*, 11(29), 47-53.
- Kırdök, E., Çiftçi, Y.Ö. (2016). Retrotransposon marker systems as a tool to analyze molecular diversity of Mediterranean Pistacia species. *International Journal of Agriculture and Biology*, 18(3), 601-606. <https://doi.org/10.17957/IJAB/15.0132>
- Labdelli, A., De La Herrán, R., Resentini, F., Trainotti, L., Tahirine, M. & Merah, O. (2022). Evaluation of genetic variability among three Pistacia species using Internal Transcribed Spacer 1 (ITS1) marker. *Diversity*, 14(12), 1051. <https://doi.org/10.3390/d14121051>
- Mahjoub, F., Rezayat, K.A., Yousefi, M., Mohebbi, M. & Salari, R. (2018). Pistacia atlantica Desf. A review of its traditional uses, phytochemicals and pharmacology. *Journal of Medicine and Life*, 11(3), 180. <https://doi.org/10.25122/jml-2017-0055>
- Pazouki, L., Mardi, M., Shanjani, P.S., Hagidimitriou, M., Pirseyedi, S.M., Naghavi, M.R., ... & Khayam Nekoui, S.M. (2010). Genetic diversity and relationships among Pistacia species and cultivars. *Conservation Genetics*, 11(1), 311-318. <https://doi.org/10.1007/s10592-009-9812-5>
- Peakall, R.O.D., Smouse, P.E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics*, 28(19), 2537-2539.
- Rohlf, M. (1998). *NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System, Version 2.2*. Department of Ecology and Evolution, State University of New York, New York.
- Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd edition. Cold Spring Harbor, New York.

- Serttaş, P.T., Özcan, T. (2018). Intraspecific variations studied by ISSR and IRAP markers in mastic tree (*Pistacia lentiscus* L.) from Turkey. *Trakya University Journal of Natural Sciences*, 19(2), 147-157. <https://doi.org/10.23902/trkjnat.433329>
- Sorkheh, K., Amirbakhtiar, N. & Ercisli, S. (2016). Retracted article: Potential start Codon targeted (SCoT) and inter-retrotransposon amplified polymorphism (IRAP) markers for evaluation of genetic diversity and conservation of wild *Pistacia* Species Population. *Biochemical Genetics*, 54(4), 368-387. <https://doi.org/10.1007/s10528-016-9725-1>
- Taifour, H., El-Oqlah, A. (2014). *Jordan Plant Red List*, 1 edition. Jordan, Royal Botanic Garden.
- Tilkat, E., Işıkan, Ç. & Onay, A. (2005). In vitro propagation of khinjuk pistachio (*Pistacia khinjuk* Stocks) through seedling apical shoot tip culture. *Propagation of Ornamental Plants*, 5(3), 124-128.
- Zhao, Q., Guo, Z., Gao, M., Wang, W., Dou, L. & Rashid, S.H. (2022). Evaluation of genetic diversity and gene-pool of pistacia khinjuk stocks based on retrotransposon-based markers. *Caryologia*, 75(2), 119-127. <https://doi.org/10.36253/caryologia-1540>