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Genetic Relationships among Some *Pistacia* Species (Anacardiaceae) in Syria

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Abstract: Genetic diversity and relationships among species and subspecies of *Pistacia atlantica* and *P. terebinthus* growing in Syria have been investigated using RAPD and ISSR techniques. Samples of *Pistacia spp.* genotypes were collected from seven different locations in Syria. The collected genotypes were morphologically identified based on descriptors available in the literature. When RAPD technique was used, an average of 74.1% of the amplified fragments was polymorphic whereas an average of 77.74% was polymorphic when ISSR technique was used. Cluster analysis of the data divided the studied genotypes into two main groups. *P. vera* and the two genotypes collected from southern Syria (both belong to *P. Atlantica*) came in one group, while the other four genotypes collected from the coastal mountains (Wadi Alqale, Nahr Alkhawabi and Al-boudi) and southwest of the country (Bet Jin) comprised the second group. These four genotypes, all identified as *P. terebinthus* were subsequently separated into two subgroups (subspecies) which were identified as *P. terebinthus subsp. terebinthus* and *P. terebinthus subsp. palaestina*. Our molecular analysis has agreed with our morphological analysis with regard to the distinction between these two subspecies.

Key words: Biodiversity · *Pistacia* · RAPD · ISSR · Syria

Abbreviations:

RAPD: Random amplified polymorphic DNA	ISSR: Inter–simple sequence repeat.
DU: *P. vera* (Dubaya)	NA: *P. terebinthus subsp. terebinthus* (Nahr Alkhawabi)
JA: *P. atlantica (Jabbata Alkhashab)*	BO: *P. terebinthus subsp. palaestina* (Alboudi)
BA:*P. atlantica* (Bir Ajam)	BJ: *P. terebinthus subsp. palaestina* (bet Jin)
WA: *P. terebinthus subsp. terebinthus* (Wadi Alqale')	

INTRODUCTION

Pistacia is genus belonging to the Anacardiaceae family which contains 11 species [1]. The genus includes *Pistacia vera* L., the cultured pistachio, which has edible nuts and considerable commercial importance [2]. The other *Pistacia* species grow in the wild and their seedlings are used mainly as rootstocks [3] and their nuts are used for local consumption, oil and soap production [4]. Based on morphology, Zohary [1] divided *Pistacia* species into four sections which included *Lentiscella Zoh*. *Eu-Lentiscus Zoh*, *Butmela Zoh*. and *Eu-Terebinthus*. *Eu-Terebinthus* contains *P. vera L., P. khinjuk Stocks, P. terebinthus L., P. palaestina Bois. and P. chinensis Bunge. P. saportae* is now recognized as an inter-specific hybrid [5].

Pistacia species are very valuable trees for the Mediterranean and North African countries due to their

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tolerance to drought where they can be grown in regions with low annual precipitation [6].

Syria is an important center of origin and biodiversity of some wild *Pistacia* species, such as *P. atlantica Desf*, *P. palaestina Boiss, P. khinjuk Stocks* and *P. terebinthus L.* [7, 8, 9]. Additionally, many varieties of *Pistacia vera* are cultivated in Syria [10, 11].

Genetic diversity among individuals or populations can be assessed using morphological and molecular markers. Morphological markers are influenced by environmental conditions and developmental stage which limits their use in genetic diversity studies. On the other hand, DNA molecular markers are independent of environmental conditions or developmental stage and show high level of polymorphism.

Several types of molecular markers, including random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) and inter–simple sequence repeat (ISSR) and amplified fragment length polymorphism (AFLP) have been successfully used for germplasm identification and genetic diversity studies [12-20].

A number of genetic studies have been conducted worldwide on biodiversity of *Pistacia*, using isozymes [21, 22], RAPD [23-30], SSR [31, 32, 33], ISSR [34-37], RFLP of chloroplast DNA (cpDNA) [38, 39], AFLP [27, 40], SAMPL [41] and SRAP [42].

In Syria, a few studies have been conducted on genetic diversity among *Pistacia* species. Some of these studies have been done on *Pistacia vera* cultivars using RAPD [43] and AFLP [44]. While Al hajjar *et al.* [45] studied genetic diversity of *Pistacia atlantica* genotypes using RAPD technique.

The aim of this study was to examine genetic diversity and relationships among species and subspecies of *P. atlantica* and *P. terebinthus* collected from several areas in Syria using RAPD and ISSR markers.

MATERIALS AND METHODS

Plant Materials: Samples of *Pistacia* spp. genotypes were collected from seven different locations in Syria (Table 1). Samples were also collected from trees of *P. vera*, the cultivated *Pistacia* species, for comparison studies. Young leaves from these samples were well washed in distilled water, frozen in liquid nitrogen and stored at -80°C for later DNA extraction. Small branches with leaves and fruits were also collected for species identification.

Study of the Morphological Traits: The collected genotypes were identified based on Mouterde [9], Yaltirik [46, 47] *Pistacia* descriptors IPGRI [48] and Ozuslu [49]. The following characteristics were evaluated: Length, width, thickness and shape of the fruits, length, width and texture of the leaves, leaf rachis wing, length, width, length/width ratio and size of terminal leaflet (if any) compared with lateral ones, number of pairs of leaflets, leaflets shape and their apex shape, petiole shape.

DNA Extraction: Genomic DNA was extracted from leaf tissue following the CTAB method of [24] with some modifications. Young leaf tissue (100 mg) was grinded into a smooth powder in liquid nitrogen, then transferred into 2 ml eppendorf tube and mixed with 900 µl of CTAB extraction buffer (100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% PVP, 0.2% mercaptoethanol, 0.1% $NaHSO_3$). The tube was incubated at 65°C for 45 min, then mixed with an equal amount of chloroform-isoamyl alcohol (24:1) and centrifuged for 5 min at 13000 rpm.

The upper aqueous part was restored into 2 ml eppendorf tube and mixed with 2/3 volume of iced-cold isopropanol. Tubes are gently inverted several times and centrifuged at 13000 rpm for 5 min. The precipitated nucleic acid was washed with 1 ml of 10mM ammonium acetate in 76% ethanol for a few minutes, dried for 10-15 min at room temperature and re-suspended in 100 ml TE buffer (10 mM Tris-HCl, 1 mM EDTA). RNAase (10 mg/ml) was used to get rid of the RNA. The quality and quantity of DNA samples were determined using 1% agarose gel electrophoresis in TBE buffer. and the concentration were established by spectrophotometry measurement. DNA samples were diluted to 20 ng/µl and kept at -20°C.

RAPD-PCR Amplification: Twenty-five primers listed in (Table 2) were tested to amplify the isolated DNA. The PCR reaction was performed in a total volume of 25 µl reaction mixture containing: 4 µl of genomic DNA (20 ng), 1.5 µl of 100 pmol of primer, 1 µl of mix 10 mM dntps, 1 µl of $MgCl_2$ (50 mM), 2.5 µl of reaction buffer (10X), 0.3 µl of Taq DNA Polymerase (5 unit/µl), 14.7 µl of HPLC purified water.

Amplifications were carried out in a gradient thermal cycler (Techne TC-512, Cambridge, England) with the following RAPD program: 10 min of initial denaturing at 94°C, 40 cycles of three steps: 45 sec of denaturing at 94°C, 1 min of annealing at 36°C and 1 min 30 sec of extension at 72°C, followed by a final extension for 10 min at 72°C.

ISSR-PCR Amplification: Fifty primers were tested, 32 of them were used in the study (Table 3). Amplification reaction was done in a 20 µl volume containing: 1.7 µl of

Table 1: Locations, geographical areas with altitude and annual rainfall of *Pistacia* spp. samples collected for the study

Sample number	Assigned Sample code	Location	Geographical area	Altitude (m)	Rainfall (mm)
1	DU	Dubaya	Southwest	970	400
2	JA	Jabbata Alkhashab	South	1100	700
3	BA	Bir Ajam	South	950	750
4	WA	Wadi Alqale'	Coastal mountains	600	900
5	NA	Nahr Alkhawabi	Coastal mountains	450	1100
6	BO	Al-boudi	Coastal mountains	700	1350
7	BJ	Bet Jin	Southwest	1200	800

Table 2: Primers used for the RAPD analysis and number of DNA polymorphic bands produced.

Primer	5`- 3` Sequence	Total number of bands	Number of polymorphic bands	Polymorphic bands (%)
OPA-01	CAGGCCCTTC	2	0	0
OPA-03	AGTCAGCCAC	12	6	50
OPA-04	AATCGGGCTG	9	4	44.4
OPA-09	GGGTAACGCC	4	0	0
OPA-19	CAAACGTCGG	12	10	83.3
OPB-13	TTCCCCCGCT	15	14	93.3
OPB-15	GGAGGGTGTT	13	10	76.9
OPB-16	TTTGCCCGGA	3	0	0
OPB-17	AGGGAACGAG	18	16	88.8
OPB-19	ACCCCCGAAG	8	8	100
OPC-13	AAGCCTCGTC	10	7	70
OPC-14	TGCGTGCTTG	11	8	72.7
OPC-20	ACTTCGCCAC	11	9	81.8
OPE-05	TCAGGGAGGT	11	4	36.3
OPE-10	CACCAGGTGA	11	10	90.9
OPF-09	CCAAGCTTCC	11	10	90.9
OPF-11	TTGGTACCCC	14	14	100
OPF-18	TTCCCGGGTT	10	5	50
OPK-08	GAACACTGGG	13	10	76.9
OPL-08	AGCAGGTGGA	16	12	75
OPL-15	AAGAGAGGGG	10	4	40
OPL-18	ACCACCCACC	13	8	61.5
OPO-02	ACGTAGCGTC	11	9	81.8
OPY-10	CAAACGTGGG	17	17	100
OPZ-13	GACTAAGCCC	13	11	84.6
Total		278	206	74.1

genomic DNA (20 ng), 2 µl of 100 pmol of primer, 0.8 µl of mix 10 mM dntps, 0.8 µl of $MgCl_2$ (50 mM), 2 µl of reaction buffer (10X), 0.2 µl of Taq DNA Polymerase (5 unit/µl), 12.5 µl of HPLC purified water.

PCR reaction was performed with the previous thermal cycler. ISSR program included an initial denaturing at 94°C for 10 min, followed by 35 cycles of 45 sec denaturing at 94°C, 45 sec annealing at desired temperatures (50-52-54-56°C) and 2 min extension at 72°C, followed by a final extension for 10 min at 72°C.

RAPD and ISSR amplification products were separated by electrophoresis in 1.8% agarose gel in 1X TBE, stained with Ethidium Bromide, along with 100 bp DNA ladder Plus (Fermentas Co. cat No SM0321) as size marker.

PCR products were digitally photographed under UV light in a transilluminator documentation system (UVP, USA).

Data Analysis: The amplified bands were scored as 1 (present) and 0 (absent), RAPD and ISSR data were clustered and dendrograms based on similarity matrices were generated using the paired group method. Bootstrap values were obtained using PAST program (1.94b version), available free online [50].

Table 3: Primers used for the ISSR analysis and number of DNA polymorphic bands produced.

Primer	5`- 3` Sequence	Annealing Temperatures (°C)	Total number of bands	Number of polymorphic bands	Polymorphic bands (%)
ISSR-2	5`- $(GA)_9$C - 3`	52	8	2	25
ISSR-4	5`- $(CAC)_7$G - 3`	50	4	2	50
ISSR-5	5`- GT$(CAC)_7$ - 3`	50	12	3	25
ISSR-6	5`- $(GTG)_7$C - 3`	52	9	0	0
ISSR-7	5`- $(CA)_{10}$G - 3`	52	1	1	100
ISSR-10	5`- BDB$(TCC)_5$ - 3`	54	8	3	37.5
ISSR-13	5`- $(AG)_8$G - 3`	50	12	7	58.3
ISSR-14	5`- $(GA)_8$T - 3`	54	14	11	78.5
ISSR-15	5`- $(GA)_8$C - 3`	52	16	10	62.5
ISSR-16	5`- $(GA)_8$A - 3`	54	17	17	100
ISSR-17	5`- $(CT)_8$A - 3`	52	13	11	84.6
ISSR-18	5`- $(CT)_8$G - 3`	50	11	10	90.9
ISSR-19	5`- $(CT)_8$T - 3`	54	15	14	93.3
ISSR-22	5`- $(GT)_8$A - 3`	56	5	5	100
ISSR-28	5`- $(AC)_8$T - 3`	52	11	11	100
ISSR-29	5`- $(AC)_8$C - 3`	50	15	11	73.3
ISSR-30	5`- $(AC)_8$G - 3`	52	12	12	100
ISSR-32	5`- $(TG)_8$G - 3`	54	14	13	92.8
ISSR-34	5`- $(GA)_8$YT - 3`	50	12	7	58.3
ISSR-37	5`- $(CA)_8$RT - 3`	50	13	13	100
ISSR-38	5`- $(CA)_8$RC - 3`	50	5	4	80
ISSR-39	5`- $(GT)_8$YA - 3`	52	9	7	77.7
ISSR-41	5`- $(TC)_8$RT - 3`	50	12	11	91.6
ISSR-42	5`- $(AC)_8$YG - 3`	52	10	9	90
ISSR-43	5`- $(AC)_8$YA - 3`	52	17	15	88.2
ISSR-44	5`- $(AC)_8$YT - 3`	52	8	8	100
ISSR-46	5`- $(TG)_8$RC - 3`	52	11	11	100
ISSR-47	5`- $(ACC)_6$ - 3`	56	10	7	70
ISSR-48	5`- $(ATG)_8$ - 3`	54	11	10	90.9
ISSR-49	5`- $(CTC)_6$ - 3`	56	9	6	66.6
ISSR-50	5`- $(GAA)_6$ - 3`	50	16	14	87.5
ISSR-52	5`- $(TCC)_5$RY - 3`	56	6	4	66.6
Total			346	269	77.7

(R = purines: G or A; Y = pyrimidines: C or T) (B= non A; D= non C)

RESULTS

Morphological Characterization: A total of 20 accessions of each genotype were collected. Leaf and nut characteristics and measurements were taken according to the descriptors; some of them are presented in (Table 4).

PCR Reaction: Out of the 25 RAPD and 50 ISSR Primers, only 25 RAPD and 32 ISSR primers produced visual DNA fragments. Smear or no amplified products were produced by the other primers.

RAPD: A total of 278 fragments were amplified and 206 (74.1%) of them were polymorphic. Only three primers (OPA-01, OPA-09 and OPB-16) did not produce any polymorphic amplification fragments for any of the 7 *Pistacia* genotypes. The number of polymorphic fragments varied from 4 (OPA-04, OPE-05, OPL-15) to 17 (OPY-10) with an average of 8.2 per primer (Table 2). Polymorphism ranged from 0% for primers OPA-01, OPA-09, OPB-16 and 100% for primers OPB-19, OPF-11 and OPY-10. The size of the amplified products ranged from 150 bp to 3000 bp. (Fig. 1) shows an example of amplification products with the primers (OPY-10 and OPC-13).

According to the dendrogram resulting from cluster analysis of the data and bootstrap analysis with 1000 replicates of the same data (Fig. 2), the studied genotypes were separated into two main groups. The first group included two clusters, one of them contained DU (*P. vera*) and the other cluster included JA and BA which belong to *P. atlantica*, while the second group comprised WA, NA, BO and BJ which in turn belong to *P. terebinthus* species. The dendrogram grouped BO and BJ in one cluster that made a sub-cluster with NA which in turn assembled in a further cluster with WA.

Table 4: Leaves characterization of *Pistacia* genotypes.

No.	Location	Species name	Leaf length Cm	Leaf width Cm	Leaflet number	Leaf rachis wing	Terminal leaflet	Leaflets shape	Leaflets Apex shape
1	Dubaya	*P. vera*	9.7	7.3	3-5	present	present	Round ovate	Obtuse
2	Jabbata Alkhashab	*P. atlantica*	7.4	4.3	7-9	present	present	Ovate oblong	Retuse
3	Bir Ajam	*P. atlantica*	9.4	7.8	5-9	present	present	Narrow elliptic	Acute
4	Wadi Alqale'	*P. terebinthus subsp. terebinthus*	8.8	8.1	8-12	absent	Generally absent	lanceolate	Acuminate
5	Nahr Alkhawabi	*P. terebinthus subsp. terebinthus*	9.5	7.7	8-12	absent	Generally absent	lanceolate	cospidate
6	Al-boudi	*P. terebinthus subsp. palaestina*	8.9	8.5	8-14	absent	absent	Narrow elliptic	cospidate
7	Bet Jin	*P. terebinthus Subsp. palaestina*	10.5	9.2	8-11	absent	Generally absent	elliptic	cospidate

Table 5: Genetic similarity among studied *Pistacia* genotypes based on Jaccard coefficient from RAPD data

	DU	JA	BA	WA	NA	BO	BJ
DU	1						
JA	0.50233	1					
BA	0.51852	0.735	1				
WA	0.43172	0.47639	0.54425	1			
NA	0.41333	0.46522	0.48696	0.76563	1		
BO	0.37281	0.44348	0.46522	0.72165	0.77717	1	
BJ	0.39726	0.43805	0.45374	0.73016	0.75824	0.82081	1

Table 6: Genetic similarity among studied *Pistacia* genotype based on Jaccard coefficient from ISSR data.

	DU	JA	BA	WA	NA	BO	BJ
DU	1						
JA	0.46119	1					
BA	0.47881	0.68812	1				
WA	0.37849	0.39095	0.42248	1			
NA	0.37549	0.35458	0.41379	0.7346	1		
BO	0.39669	0.39241	0.41339	0.64815	0.69668	1	
BJ	0.40816	0.38115	0.4023	0.71226	0.71362	0.70673	1

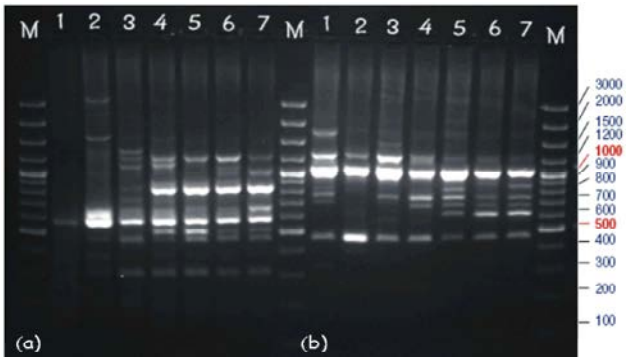

Fig. 1: Genomic DNA amplification pattern in seven *Pistacia* genotypes, with two RAPD primers (a: OPY-10,b: OPC-13). (M= size marker)

Estimates of genetic relationships were obtained from the markers data using Jaccard's similarity coefficient (Table 5). According to the results, genetic similarity ranged from 0.37 (low similarity) between DU (*P. vera*) and BO up to 0.82 (high similarity) between BO and BJ.

ISSR: The 32 selected ISSR primers produced various numbers of DNA fragments, depending on their simple sequence repeat motifs.

In total, 346 fragments were produced out of which, 269 fragments (77.74%) were polymorphic. Only one primer (ISSR-6) did not produce any polymorphic fragments for any genotype. The size of the amplified products ranged from 170 bp to 3000 bp. The range of polymorphism was between (0%) for ISSR-6 primer up to (100%) for the primers ISSR-7, 16, 22, 28, 30, 37, 44 and 46. The number of polymorphic fragments varied from 1 (ISSR-7) to 17 (ISSR-16) with an average of 8.4 per primer (Table 3). An example of amplification products with two primers (a: ISSR-50 and b: ISSR-15) is shown in (Fig. 3).

The dendrogram was constructed on the basis of Jaccard's similarity matrix, followed by paired group method based on clustering analysis. (Fig. 4) shows that the genotypes were grouped in two major groups.

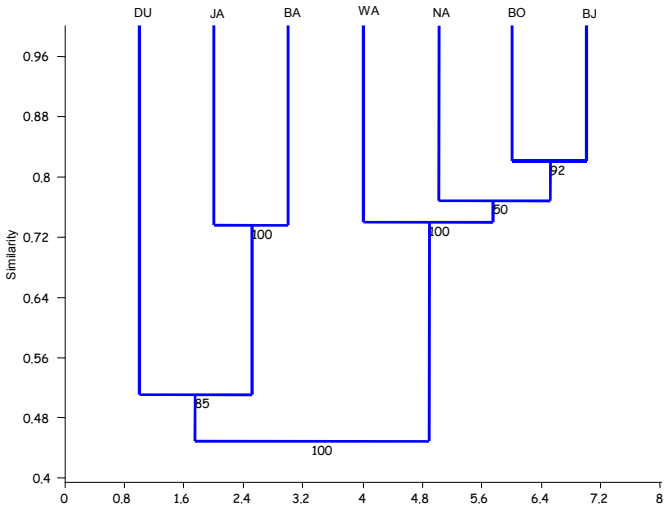

Fig. 2: Dendrogram of seven *Pistacia* genotype generated by the paired group method using the Jaccard similarity matrix, based on RAPD marker analysis. The numbers on the tree branches indicate the percentage of replicates included in a given node calculated out of 1000 bootstrap replicates

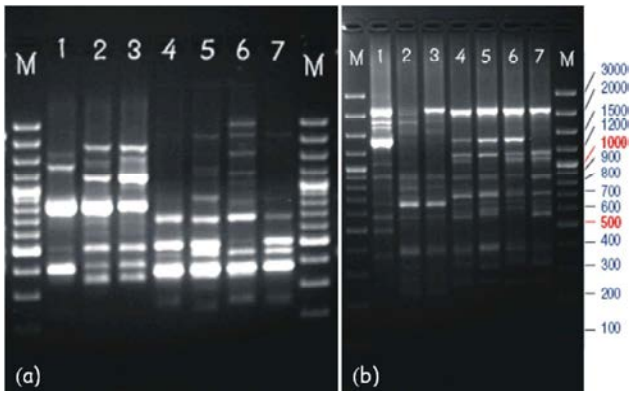

Fig. 3: DNA amplification products of seven Pistacia genotype with two ISSR primers: (a: 50, b: 15). (M= size marker)

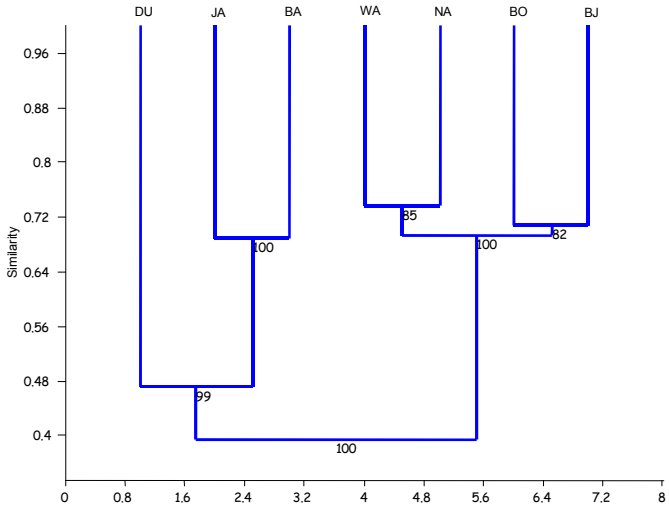

Fig. 4: Dendrogram of seven *Pistacia* genotype generated by the paired group method using the Jaccard similarity matrix, based on ISSR marker analysis. The numbers on the tree branches indicate the percentage of replicates included in a given node calculated out of 1000 bootstrap replicates

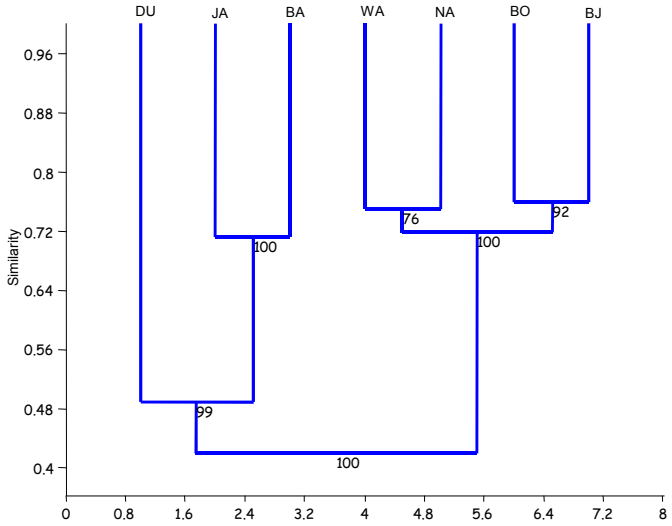

Fig. 5: Dendrogram of seven *Pistacia* genotype generated by the paired group method, based on combined data of RAPD and ISSR. The numbers on the tree branches indicate the percentage of replicates included in a given node calculated out of 1000 bootstrap replicates

The first group included DU and a sub-cluster that contained JA and BA, while the second group included the other four genotypes. WA and NA were in the same cluster, whereas BO and BJ belonged to a different cluster. The two clusters were close to each other in a larger group. The range of genetic similarity was from 0.35 (low similarity) between JA (*P. atlantica*) and NA (*P. terebinthus*) up to 0.73 (high similarity) between WA and NA (Table 6).

Combined (RAPD and ISSR) Data: The data obtained from both markers were combined, a total of 624 DNA fragments were scored, of which 475 (76.1%) were polymorphic with an average of 8.33 polymorphic fragments per primer. The genetic relationships among studied genotypes through RAPD and ISSR markers, showed similar dendrograms as obtained from each single technique with some exceptions. However, the general dendrogram constructed using Jaccard's similarity coefficient values of combined molecular data (RAPD + ISSR) revealed a better representation of the relationship than from individual markers. The genotypes were grouped into two main clusters (Fig. 5). The first cluster separated DU from JA and BA, but it showed a close relationship between them (*P. vera and P. atlantica*) with a very high bootstrap value (99). While the second cluster was divided into two sub-clusters, one of them included WA and NA, with 76 bootstrap value, whereas the other one included BO and BJ, with 92 bootstrap value. The last four genotypes clearly belong to the same species (*P. terebinthus*) but as we expected they belong to two different sub species according to our morphological and genetic analyses.

DISCUTION

This study comprises the first comparative study between two *Pistacia* species (*P. atlantica* and *P. terebinthus*) and their possible subspecies growing in Syria based on RAPD and ISSR markers. Genetic analysis in this study has confirmed the outcomes of morphological and taxonomic analyses done on the collected samples.

The clustering of genotypes within groups was similar in both techniques with some exceptions, in RAPD dendrogram, NA was closer to BO and BJ, WA was the most distant one between the four genotypes, while in ISSR dendrogram, WA and NA were gathered in one cluster which belongs to a larger cluster (this was also confirmed with Genetic similarity values). A possible reason for this difference is the lower number of primers used in RAPD. According to Souframanien and Gopalakrishna [51], the difference in the resolution of RAPD and ISSR is that the two marker techniques target different regions of the genome. These differences can be attributed to marker sampling errors and/or the level of polymorphism detected. This enforces the significance of the number of loci and their coverage of the whole genome for acquiring trustworthy estimates of genetic relationships among cultivars [52].

BO and BJ came in one cluster in all three dendrograms (RAPD, ISSR, RAPD-ISSR) with high bootstrap value, despite the fact that both samples were collected from two different locations (BO from Alboudi and BJ from Bet Jin).

The percentage of polymorphism revealed by ISSR (77.7%) is higher than revealed with RAPD (74.1%). This result is in agreement with findings of Parveen *et al.* [53] who detected genetic polymorphism in *Tilletia indica* using RAPD and ISSR and compared between them, but in disagreement with Tagizad *et al.* [54] who found that RAPD markers were marginally more informative than ISSR in the assessment of genetic diversity in Iranian pistachio cultivars. On the other hand, the combination of ISSR-RAPD data gave similar results to ISSR markers alone and this consistent with the findings of Gupta *et al.* [55] who used ISSR and RAPD markers for genetic diversity study among different *Jatropha curcas* genotypes and Lai *et al.* [56] who studied genetic relationships between cultivated and wild tea using RAPD and ISSR.

The current results show that *P. vera* was closer to *P. atlantica* than *P. terebinthus* based on molecular data and this is consistent with Kafkas and Perl-Treves [57] who found that *P. terebinthus* is the most diverged one among the four studied species based on his RAPD data.

Moreover, the current results show that JA and BA belong to *P. atlantica*, where similarity values between them were for RAPD=0.73, ISSR=0.68 and for RAPD-ISSR=0.71, but the three dendrograms showed the two genotypes in one cluster with high bootstrap value (100). As some morphological differences were observed between them this could lead to the conclusion that they are two different subspecies, but this needs further analysis to prove. The current genetic analysis confirmed their close relationship since JA samples were collected from areas (Jabbata Alkhashab and Bir Ajam), which are less than 20 Km apart.

Previous studies differed in classifying *P. palaestina* and *P. terebinthus*. Engler [58] who was the first to classify *Pistacia* species, in fact, considered *P. palaestina* as a variety of *P. terebinthus*. However, *P. palaestina* was regarded by Zohary [1] as a separate species due to the 2 major distinguishing characteristics. *P. palaestina* has mainly paripinnate leaves and acuminate leaflets, while *P. terebinthus* has imparipinnate leaves and obtuse or acute leaflets. Conversly, Yaltirik [46, 47] retained *P. palaestina* as a variety of *P.terebinthus*. and depicted the two subspecies within *P. terebinthus*. The first, *P. terebinthus subsp. terebinthus*, had imparipinnate leaves with the terminal leaflet of the median leaves often as large as the lateral ones and obtuse or ovate-oblong lateral leaflets. The second is *P. terebinthus subsp. palaestina* with either paripinnate and/or imparipinnate leaves, the terminal leaflet of the median leaves always smaller than the laterals or reduced to a bristle and acuminate or oblong-lanceolate lateral leaflets. Consequently, *P. terebinthus* and *P. palaestina* are not considered independent species but only two intergrading geographic races or subspecies [59]. The botanical naming of the 2 subspecies according to Kafkas and Prel-Treves [3], therefore, must be: *P. terebinthus L. subsp. terebinthus* and *P. terebinthus L. subsp. Palaestina*. Kafkas and Prel-Treves [3] classified nine species by RAPD analysis and showed that *P. palaestina* was in fact a subspecies of *P. terebinthus* and Yi *et al.* [60] as well, stated, " *P. palaestina* may need to be merged in to *P. terebinthus*".

Close relationship between these two species were also suggested by AFLP and RAPD analyses [27, 29].

AL-Saghir *et al*. [61] established that *P. palaestina* and *P. terebinthus* are very closely related. He confirmed that they are the same species with 100% support using the neighbor joining analysis based on morphological data.

CONCLUSION

Based on the molecular data obtained by our study and the results of several previous studies, we can posit, that the four samples (WA, NA, BO and BJ) belong to one species (*P. terebinthus*), but separated into two subspecies (*P. terebinthus subsp. terebinthus* and *P. terebinthus subsp. palaestina*) that we have been able to distinguish morphologically.

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