Scientia Horticulturae 176 (2014) 282–289

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Characterization of Jordanian olive cultivars (*Olea europaea* L.) using RAPD and ISSR molecular markers

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ARTICLE INFO

Article history:
Received 25 July 2013
Received in revised form 4 July 2014
Accepted 7 July 2014

Keywords:
Olea europaea L.
Genetic diversity
RAPD
ISSRs
Varietal identification.

ABSTRACT

Thirteen Jordanian olive cultivars (*Olea europaea* L.) were characterized using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSRs) markers. Using 15 RAPD and 14 ISSR primers, 156 and 85 reproducible markers were obtained, respectively. The percentage of polymorphism was 55 for RAPD and 58 for ISSR. Of the total polymorphisms identified, eight RAPD and three ISSR markers were cultivar-specific. Thirty-nine RAPD markers were able to distinguish 10 cultivars and 12 ISSR markers were able to distinguish 6 cultivars. Both markers (RAPD and ISSRs) were able to discriminate between all cultivars, indicating that when used in tandem, they represent powerful tools for olive varietal identification, enabling an accurate characterization of all cultivars. Such information may prove useful in the selection of optimal varietals and help promote continued progress in olive breeding strategies.

1. Introduction

The olive tree, *Olea europaea* L., is adapted to the Mediterranean climate and produces table olives and olive oil, both of which are important commodities in world markets. The cultivation of olive trees started in the Mediterranean basin some 6000 years ago. The prevailing theory suggests that their domestication began in the eastern Mediterranean (Zohary and Spiegel-Roy, 1975; Loukas and Krimbas, 1983). A joint report by Jordanian and French archeologists indicated that an ancient village (Hadeib Al-Reeh, in the Rum area of southern Jordan) could be the oldest site of olive tree cultivation in the world. Ash analysis from three village fireplaces revealed cultivation of olive trees dating back to the Chalcolithic period (ca. 5400 BC) (Al-Shdiefat et al., 2012).

The most common local olive cultivars grown in Jordan are 'Nabali Baladi,' 'Nabali Muhassan,' and 'Rasei.' However, several clones of these cultivars are widespread in Jordan and have therefore been given various common names, depending on the region in which they were grown. 'Nabali Baladi' is one of the oldest olive cultivars in the Middle East, originating on the banks of the Jordan River. It is also the predominant type of olive cultivar found in Jordan, used for both table olives and for oil. Its subcategory is 'Nabali Muhassan,' and both types of olives are strong and take root easily. The olives are usually plump with a soft texture and have an oil content of between 28 and 33%. 'Rasei' is an improved cultivar of 'Nabali' used mainly for oil production. It has an oil content that ranges between 15 and 28% and is known for its mixture of sweet and pungent flavors, with a hint of apple (http://www.zaitt.com/about-zaitt).

We are faced with a problem of nomenclature that is mainly due to the frequent use of synonyms. In the last three decades, a number of olive cultivars have been introduced from other countries, with olive plantations developing in several provinces. Additionally, the renewal of olive orchards using certified olive cultivars or farmers practicing grafting, in which cultivars are combined, benefit from having a single genotype. This has resulted in a wide range of olive genetic diversity that is not being properly utilized, ultimately causing confusion for both farmers and researchers (Ayoub et al., 2009). Therefore, the identification of genetic diversity within local varieties is essential for correct acquisition and maintenance, with genetic resources creating an opportunity to improve breeding features. Furthermore, this identification can decrease the future risk of genetic erosion and improve conservation strategies. Therefore, it is of great importance to evaluate and characterize the existing

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http://dx.doi.org/10.1016/j.scienta.2014.07.012
0304-4238/

Table 1
Cultivar common name, geographical region of production, end use, and oil content.

	Cultivar	Geographical region of production	End use	Oil content
1	Nabali Baladi	Widespread in rain-fed areas of Jordan	Both (table and oil)	High
2	Rasei	Widespread in rain-fed and irrigated areas	Both (table and oil)	Medium
3	Nabali Muhassan	Widespread in rain-fed and irrigated areas	Both (table and oil)	Medium
4	Nasouhi Jaba	Few plantations	Table	Low
5	Souri	Few plantations	Both (table and oil)	High
6	Shami	Few plantations	Table	Low
7	Kfari Romi	Irbid	Oil	High
8	Kfari Baladi	Irbid	Oil	High
9	Kanabisi	Jerash	Oil	High
10	Arabi Altafila	Tafeleh	Table	Medium
11	Ketat	Tafeleh	Table	Low
12	Bathni	Tafeleh	Table	Medium
13	Rosai	Tafeleh	Table	Low

genetic diversity of Jordanian olive germplasms, which still have a well-preserved genetic patrimony despite the differences of environments from which they have been cultivated.

The identification of olive tree cultivars has been performed using methods based on morphological, agronomical, or biochemical traits (Cantini et al., 1999; Barranco et al., 2000; Barranco and Rallo, 2000).

Molecular fingerprinting techniques, including random amplified polymorphic DNA (RAPD) (Wiesman et al., 1998; Sanz-Cortes et al., 2001; Belaj et al., 2001, 2002a,b, 2003a,b,c, 2004; Gemas et al., 2004; Ergulen et al., 2002; Khadari et al., 2003; Hassawi and Hadeib, 2004; Hagidimitriou et al., 2005; Hadeib and Hassawi, 2006; Ganino et al., 2007; Muzzalupo et al., 2007; Cordeiro et al., 2008; Gomes et al., 2008; Martins-Lopes et al., 2007, 2008, 2009; Muzzalupo and Perri, 2009) and inter-simple sequence repeats (ISSRs) (Vargas and Kadereit, 2001; Pasqualone et al., 2001; Gemas et al., 2004; Terzopoulos et al., 2005; Essadki et al., 2006; Martins-Lopes et al., 2007, 2008, 2009; Gomes et al., 2008, 2009; Hegazi et al., 2012; Noormohammadi et al., 2012), have been used to analyze the genetic variability of olive cultivars. The combined use of RAPD and ISSR markers allows for a high level of genomic coverage as RAPD markers are potentially associated with functionally important loci (Penner, 1996) and ISSR markers amplify hypervariable non-coding regions (Esselman et al., 1999). RAPD and ISSR are multi-locus profiling techniques able to distinguish genotypes below the species level, such as cultivars and clones, and have been used in numerous diversity studies (Karp et al., 1997).

Studies characterizing Jordanian olive germplasms using molecular markers are limited. Rawashdseh (2003) used RAPD markers and detected genetic variation in mutants of Nabali olive cultivars. Hassawi and Hadeib (2004) and Hadeib and Hassawi (2006) utilized RAPD markers for genetic analyses and found variability in Jordanian olive cultivars, all of which were clearly identified using six RAPD primers. Moreover, AFLP markers confirmed the existence of genetic variability among Romanian olive trees and varieties grown in Jordan (Rawashdeh et al., 2009). In this study, the most common olive cultivars are investigated using two molecular markers, with ISSR used for the first time to assess genetic diversity. This study aims to evaluate the genetic diversity of olive germplasms cultivated in Jordan utilizing two types of molecular markers, RAPD and ISSR.

2. Materials and methods

2.1. Plant material

Plant samples were collected from an olive gene bank located in the "Al-Mushager" region, belonging to the National Center for Agricultural Research and Extension (NCARE). The cultivar common names, origin, geographical region of cultivation, end use, and oil content are presented in Table 1 and Fig. 1.

2.2. DNA extraction

Leaves were washed several times with distilled water and ground in liquid nitrogen to a fine powder with a chilled mortar and pestle. Genomic DNA was extracted using the CTAB method of Doyle and Doyle (1987) with minor modifications. The quantity and quality of DNA were determined by spectrophotometer and 1% agarose gel electrophoresis, respectively.

2.3. RAPD fingerprinting

RAPD amplification was performed with 15 primers (Alpha DNA) in a 25 μL reaction containing 1× buffer, 3 mM $MgCl_2$, 0.3 mM each dNTP, 0.3 μM each primer, 0.3 U of Taq DNA polymerase and 40 ng of template DNA. Amplification was performed in a PTC-100 programmable thermal cycler (MJ Research Inc., USA) programmed for an initial 5 min denaturation at 94 °C, 45 cycles of 1 min denaturation at 94 °C, 1 min annealing at 38 °C, and a 2 min extension at 72 °C followed by a final extension for 10 min at 72 °C according to Williams et al. (1990). Reproducibility of 60 RAPD and 30 ISSR primers were tested for their ability to prime PCR amplification of three selected cultivars with independent amplifications from three independent DNA isolations per cultivar. For the choice of RAPD/ISSR primers, unambiguous and qualitative (present or absent) fragments that gave repeatable patterns two or three times with the same cultivar were considered. Primers that amplified consistently reproducible polymorphisms were selected and used to analyze all 13 olive cultivars.

2.4. ISSR fingerprinting

The same DNA samples analyzed by RAPD were also analyzed using ISSR markers. ISSR analysis was performed according to Bornet and Branchard (2001) using 14 primers. Amplification was performed in a 25 μL reaction mixture containing 40 ng of genomic DNA, 0.3 μM each primer, 1× Taq DNA polymerase reaction buffer, 1.5 U of Taq DNA polymerase and 0.2 mM each dNTP. Amplifications were performed in a PTC-100 thermal cycler (MJ Research Inc., USA) programmed for an initial 5 min denaturation step at 94 °C, followed by 45 cycles of 30 s denaturing at 94 °C, 45 s annealing at 52 °C, and 90 s extension at 72 °C. The program was completed with a final extension at 72 °C for 5 min. Amplifications were performed at least twice, and only reproducible products were taken into account for further data analysis. Amplified products of RAPD and ISSR were separated on 1.5% agarose gels in 1× TBE buffer. Fragment size was estimated using a 100 base pairs (bp) molecular size ladder (Fermentas, Germany) and detected by staining with

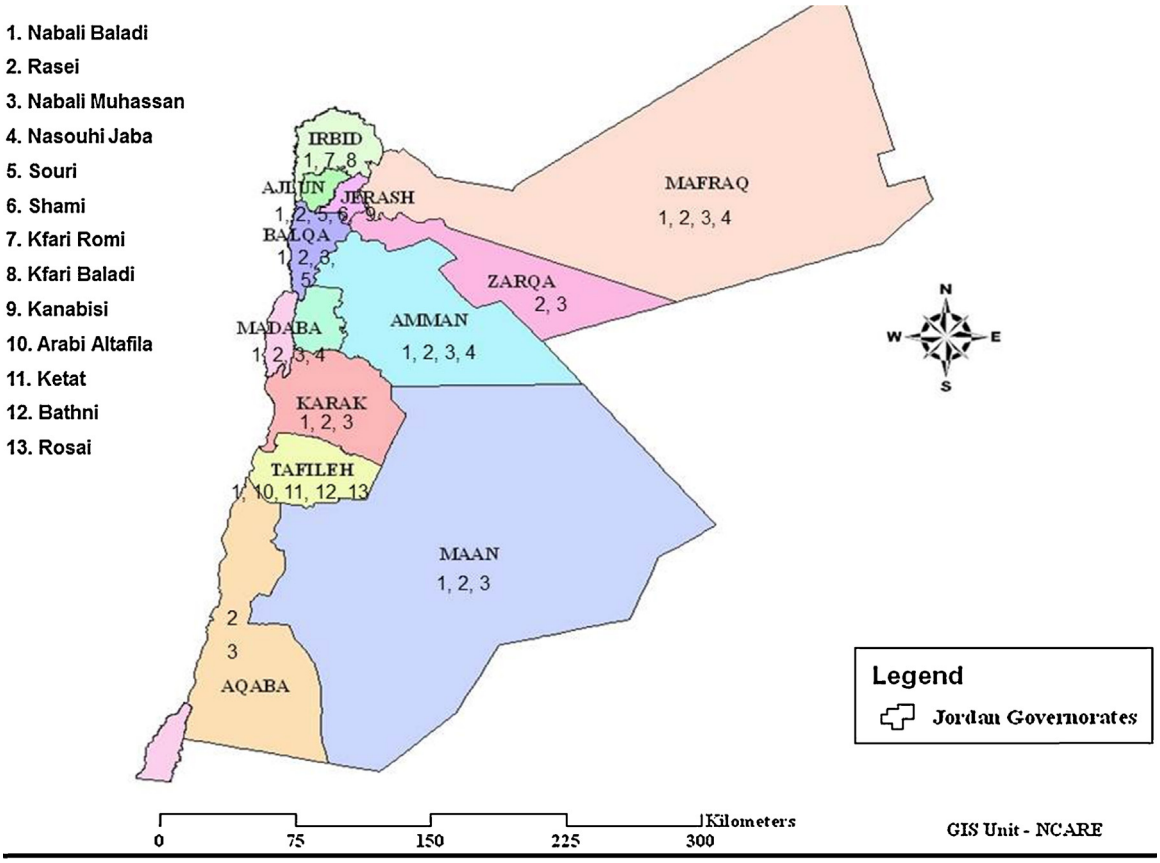

Fig. 1. Map of Jordan showing the distribution of olive cultivars.

ethidium bromide (10 mg/mL) according to Sambrook et al. (1989). The PCR products were then visualized using a UV-transilluminator and photographed with an AlphaImager gel documentation system.

2.5. Data analysis

The DNA profiles were scored visually from gel photographs. Clear and reproducible amplified bands were chosen in the analyses. Primer efficiency was calculated by dividing the number of markers produced for each primer by the total number of markers obtained. Discrimination power was calculated by dividing the number of polymorphic markers amplified for each primer by the total number of polymorphic bands obtained (Khierallah et al., 2011). The presence of a band was designated as (1); the absence of a band was recorded as (0). The data obtained by scoring the RAPD and ISSR profiles both individually as well as collectively 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).

3. Results

3.1. RAPD analysis

Fifteen primers were able to generate 156 markers (86 of which were polymorphic), with 55.53% polymorphism and an average of 5.7 polymorphic markers generated per primer. The highest numbers of markers obtained was 13, using primers OPO06, OPO10, and OPX18; the lowest was 7, obtained by use of the primers OPA01 and OPE02, an average of 10.4 markers per primer. The size of the smallest marker was 100 bp and was generated by the primer OPX18, while the largest was obtained using primers OPO06 and OPX18 (1100 bp). The highest polymorphism percentage was shown by the primer OPC08 and the lowest by the primer OPX18. The primer OPA01 amplified three polymorphic markers, showing the lowest discrimination power (3%), whereas different primers showed a discrimination power of 9% (Table 2).

Eight of the 39 polymorphic markers were cultivar-specific, and the remaining 31 markers were observed in all but one sample. These 39 markers were able to distinguish 10 cultivars (Table 3).

The matrix of RAPD markers allowed the distinction of the 13 Jordanian olive cultivars and was used to generate a dendrogram (Fig. 2). The UPGMA cluster analysis of the cultivars based on RAPD data showed a similarity of 0.80, which represented 50% of the distance from the maximum similarity of 0.96 to the minimum of 0.64. Cutting the dendrogram at this similarity value resulted in two clusters, with 'Kanabisi' failing to form clusters. The main cluster included the cultivars 'Nabali Baladi,' 'Nabali Muhassan,' 'Shami,' 'Kfari Romi,' 'Kfari Baladi,' 'Bathni,' 'Rasei,' 'Rosai,' 'Nasouhi Jaba,' and 'Souri;' the second cluster grouped the 'Arabi Altafila' and 'Ketat' cultivars. Cutting the dendrogram at a similarity value of 0.88, which represented 75% of the distance and was chosen because it appeared to give the best clustering balance possible, resulted in a major cluster comprising the cultivars 'Nabali Baladi,' 'Nabali Muhassan,' 'Shami,' 'Kfari Romi' and 'Kfari Baladi.' Here, the cultivars are 88% similar, with eight isolated branches (in the descending order of similarity) representing one cultivar each. Extreme relationships were observed, with proximal similarity detected between 'Nabali Baladi' and 'Nabali Muhassan' and distal similarity noted for 'Kanabisi' in relation to the other cultivars. At 92% genetic similarity, all cultivars with the exception of

Table 2
RAPD and ISSR primers names and sequence, total number of markers and bands, %primer efficiency, polymorphic bands, unique bands, % polymorphism, and % discrimination power.

Primer	Sequence 5′–3′	Total # of marker	% Primer efficiency	Polymorphic markers	Unique bands	% Polymorphism	%Discrimination power
RAPD marker							
OPA01	CAGGCCCTTC	7	4	3		43	3
OPC08	CCCAAGGTCC	10	6	8		80	9
OPC13	GGTGCGGGAA	9	6	5		56	6
OPE02	TGGACCGGTG	7	4	4		57	5
OPE16	AAGCCTCGTC	9	6	4		44	5
OPO03	CTGTTGCTAC	10	6	7	1	70	8
OPO04	AAGTCCGCTC	9	6	4		44	5
OPO05	CCCAGTCACT	12	8	6		50	7
OPO06	CCACGGGAAG	13	8	6	2	46	7
OPO07	CAGCACTGAC	12	8	6		50	7
OPO10	TCAGAGCGCC	13	8	7	1	54	8
OPO13	GTCAGAGTCC	11	7	8	1	73	9
OPX03	TGGCGCAGTG	11	7	8	2	73	9
OPX18	GACTAGGTGG	13	8	3	1	23	3
OPX19	TGGCAAGGCA	10	6	7		70	8
	Total	156	–	86	8	–	–
	Average	10.4	6.53	5.73	1.33	55.53	6.60
ISSR marker							
UBC 807	(AG)8 T	9	11	8		89	16
UBC 809	(AG)8G	5	6	3		60	6
UBC 810	(GA)8 T	5	6	1		20	2
UBC 811	(GA)8 C	4	5	3		75	6
UBC 817	(CA)8A	5	6	4		80	8
UBC 823	(TC)8 C	5	6	4		80	8
UBC 825	(AC)8 T	7	8	5		71	10
UBC 826	(AC)8 C	4	5	0		0	0
UBC 846	(CA)8RT	9	11	5	1	56	10
UBC 850	(GT)8TYC	5	6	3		60	6
UBC 855	(AC)8YT	5	6	1		20	2
UBC 856	(GGAGA)3	7	8	5	1	71	10
UBC 880	(GGAGA)3	7	8	1		14	2
UBC 889	DBD(AC)7	8	9	6	1	75	12
	Total	85		49	3		
	Average	6.10	7.21	3.50	1.0	55.07	7.00

Y = (CT); R = (AG); D = (AGT); B = (CGT).

'Nabali Baladi' and 'Nabali Muhassan' were separated and formed individual branches.

3.2. ISSR analysis

Eighty-five scored markers were obtained using 14 primers; 49 markers were polymorphic with 55.07% polymorphism and an average of 3.5 polymorphic markers generated per primer. The primers UBC 807 and UBC 846 produced the highest number of markers (9) and primers UBC 811 and UBC 826 produced the smallest (4), with an average of 6.1 markers per primer. The molecular size of the scored band ranged from 120 to 1000 bp for the primer UBC 889. The primer UBC 807 showed 8 polymorphic markers while UBC 826 showed a monomorphic pattern. The highest discrimination power was observed using the primer UBC 807, while the primer UBC 826 failed to discriminate between the cultivars (Table 2).

Three markers out of 49 were cultivar-specific, with nine markers present in all samples but one. These 12 markers were able to distinguish a total of six cultivars (Table 3).

The genetic similarity values based on Jaccard's coefficient ranged from 0.7 between 'Rosai' and 'Rasei' to 0.89 between 'Nabali

Table 3
Cultivar common name, unique bands, and unique missing bands generated with RAPD and ISSR markers.

	Cultivar	Unique bands	Unique missing bands
1	Nabali Baladi		
2	Rasei	OPO10-780, OPO13-180	
3	Nabali Muhassan		
4	Nasouhi Jaba	UBC846-450, OPX18-390	UBC823-350, UBC825-520, OPC13-300, OPO05-450
5	Souri	OPOX03-250	OPX03-500
6	Shami		UBC807-550, OPX19-520
7	Kfari Romi		OPO07-710
8	Kfari Baladi		
9	Kanabisi	OPO06-380, OPO06-200, OPX03390	OPC08-750, OPC08-500, OPC08-280, OPE02-450, OPE02-400, OPE02-310, OPO05-500, OPO05-300, OPO05-260, OPO10-490, OPO10-460, OPO10-400, OPX03-400, OPX03-380, OPX19-970, OPO13-650, OPO13-400, OPO13-350, OPO07-400, OPO07-290
10	Arabi Altafila		OPO03-400, UBC811-500
11	Ketat	UBC889-600, UBC856-670, OPO03-220	UBC856-330, UBC823-300, OPC08-800, OPC13-1000
12	Bathni		UBC889-850, UBC810-300, UBC823-450, OPA01-440
13	Rosai		OPO03-500, OPO04-550

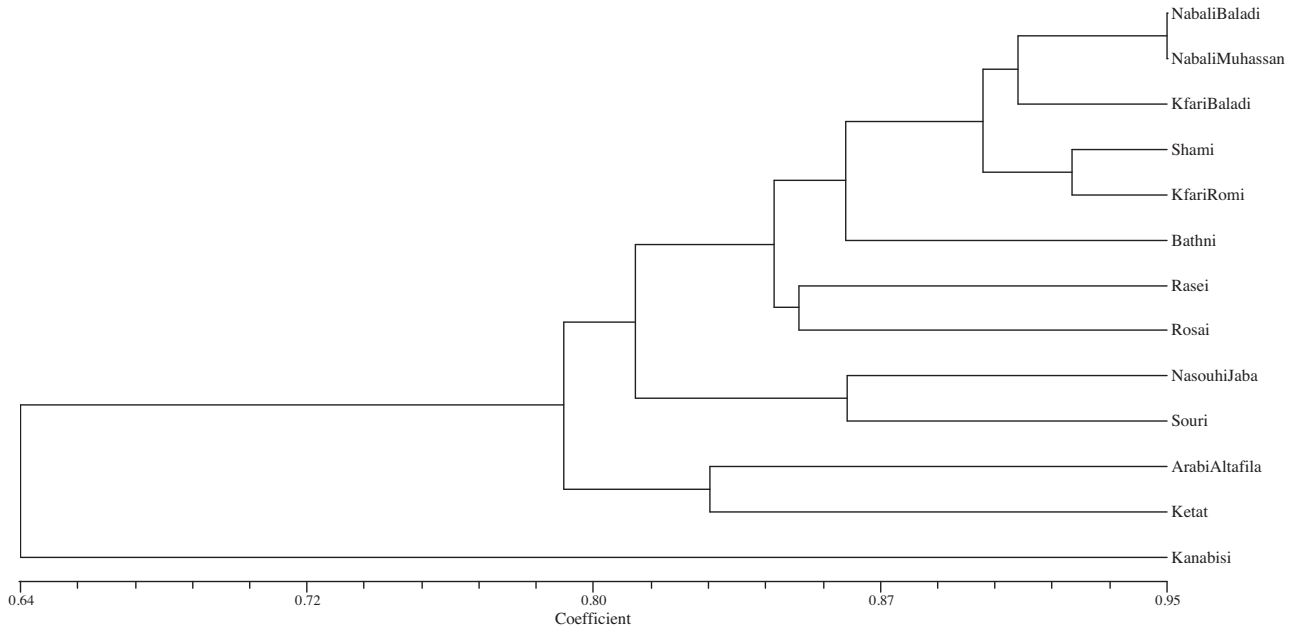

Fig. 2. Dendogram of 13 olive cultivars generated by UPGMA cluster analysis of the genetic similarity values using RAPD data.

Muhassan' and 'Souri'. The cultivars are clustered into two subgroups, one of which contains two cultivars, and the other contains the remainder, divided further into an additional two subgroups (Fig. 3). The UPGMA cluster analysis of the cultivars based on ISSR data illustrated that cutting the dendrogram at a similarity of 0.80, representing 50% of the distance from the maximum similarity of 0.89 to the minimum of 0.70, resulted in three clusters, with a total of five cultivars that failed to form clusters. The first main cluster comprises the cultivars 'Nabali Baladi,' 'Nabali Muhassan,' 'Souri,' and 'Kanabisi,' and the second cluster grouped 'Shami' and 'Kfari Romi.' The third cluster grouped 'Arabi Altafila' and 'Rosai'.

At a similarity of 0.85, which represents 75% of the distance from the maximum similarity of 0.89 to the minimum of 0.70, a major cluster comprises the cultivars 'Nabali Baladi,' 'Nabali Muhassan,' and 'Souri,' where the cultivars are 85% similar, with 10 isolated branches (in descending order of similarity), each representing one cultivar. Extreme relationships were observed by proximal similarity detected between 'Nabali Muhassan' and 'Souri' and distal similarity observed for 'Rasei' in relation to the other cultivars. At 86% genetic similarity, all cultivars, with the exception of 'Nabali Muhassan' and 'Souri,' were separated and formed individual branches.

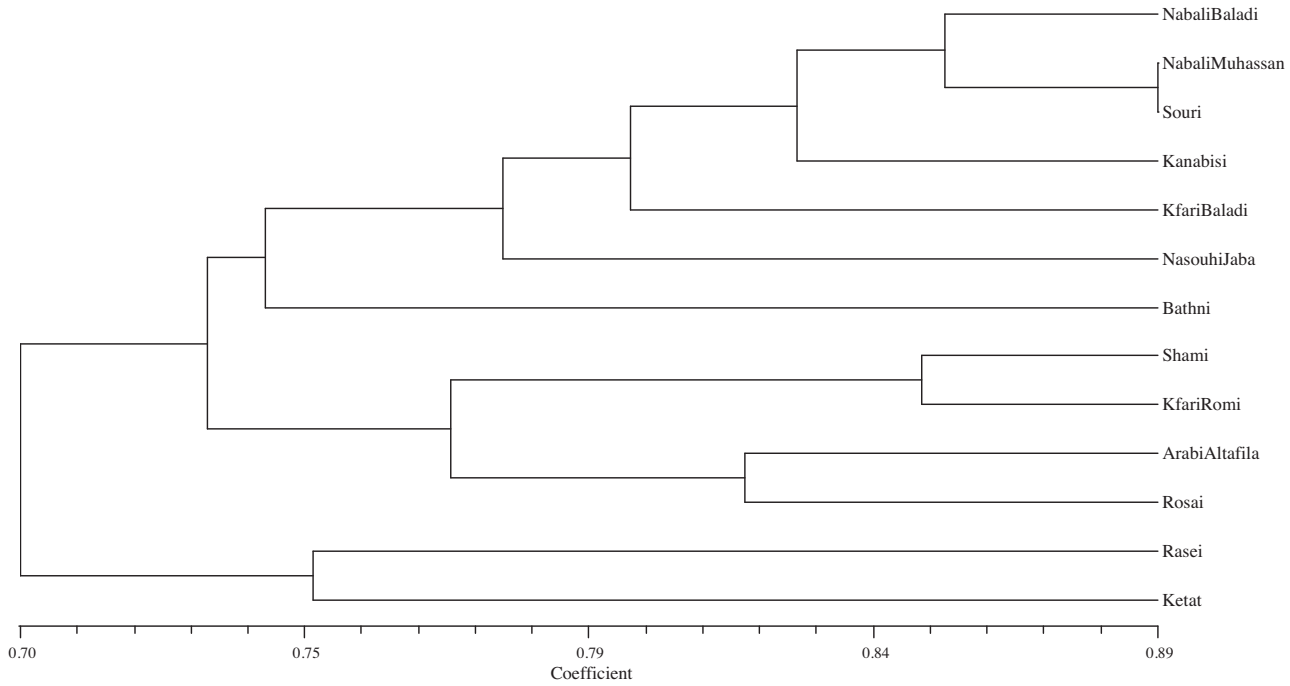

Fig. 3. Dendogram of 13 olive cultivars generated by UPGMA cluster analysis of the genetic similarity values using ISSR data.

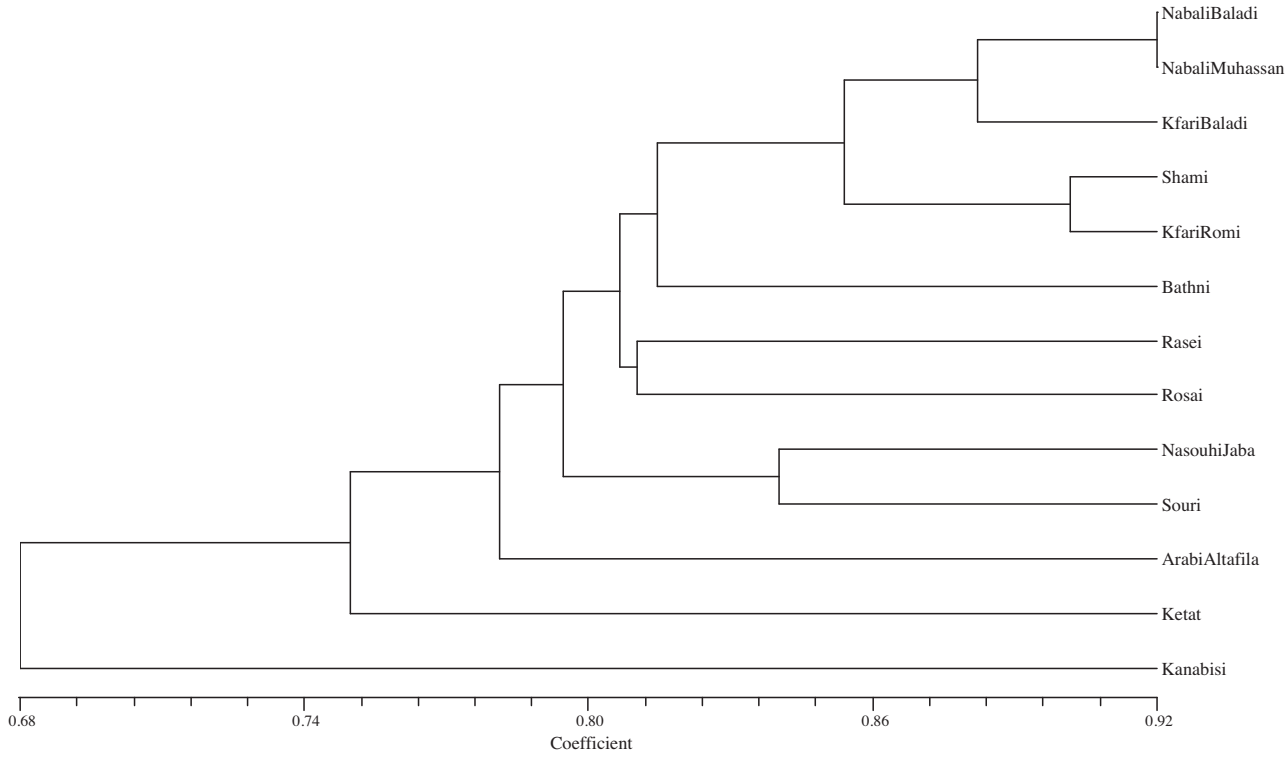

Fig. 4. Dendogram of 13 olive cultivars generated by UPGMA cluster analysis of the genetic similarity values using RAPD and ISSR data.

3.3. Combined analysis

The combined data obtained by RAPD and ISSR (241 markers) was used for the construction of a dendrogram using Jaccard's coefficient to show the relationship between the cultivars. The highest value of genetic similarity (0.92) was between 'Nabali Baladi' and 'Nabali Muhassan.' The lowest similarity (0.68) was recorded between 'Ketat' and 'Kanabisi'. Fig. 4 shows the dendrogram constructed based on Jaccard similarity index.

The UPGMA cluster analysis of the cultivars based on combined RAPD and ISSR data illustrated that cutting the dendrogram at a similarity of 0.80, representing 50% of the distance from a maximum similarity of 0.92 to the minimum of 0.68, resulted in two clusters, with three cultivars failing to form clusters. The first main cluster comprises the cultivars 'Nabali Baladi,' 'Nabali Muhassan,' 'Kfari Baladi,' 'Shami,' 'Kfari Romi,' 'Bathni,' 'Rasei,' and 'Rosai'. The second cluster grouped 'Nasouhi Jaba' and 'Souri' at a 0.86 similarity value, representing 75% of the distance. The highest similarity value detected was between 'Nabali Baladi' and 'Nabali Muhassan;' the lowest similarity value observed was for 'Kanabisi,' in relation to the other cultivars and was considered the most diverse cultivar. At 90% genetic similarity, all cultivars, with the exception of 'Nabali Baladi' and 'Nabali Muhassan,' were separated and formed individual branches.

A comparison between the three dendrograms shows close relationships between cultivars: 'Shami' and 'Kfari Romi' are clustered together in the three analyses; 'Nabali Baladi' and 'Nabali Muhassan,' 'Rasei' and 'Rosai,' and 'Nasouhi Jaba' and 'Souri' are clustered together in RAPD and combined dendrograms; the cultivars 'Nabali Baladi,' 'Nabali Muhassan,' 'Kfari Baladi,' 'Shami,' and 'Kfari Romi' are clustered in small groups within the dendrograms of RAPD and combined markers; finally, the 'Kanabisi' cultivar was the most distant in the dendrograms of RAPD and combined analysis. All cultivars were identified by both RAPD and ISSR markers and both of them indicated good discrimination power.

4. Discussion

Recently, the genetic resources of olive trees from different regions were described using PCR-based markers; in all cases, however, Jordanian cultivars were underrepresented. In this study, two molecular marker systems were used to study the genetic relatedness and diversity among the most common Jordanian olive cultivars. Differences in the rate of polymorphism yielded by the primers could be explained by differences in the primer sets used, the germplasms analyzed, and in the criteria (faint band/clear band) used for marker selection. The polymorphism percentage obtained in this study is consistent with the results from previous studies conducted on olive cultivars using RAPD/ISSR molecular markers in Jordan (Hassawi and Hadeib, 2004; Hadeib and Hassawi, 2006), Israel and the West bank (Weisman et al., 1998), Syria and other Mediterranean regions (Besnard et al., 2001; Belaj et al., 2003a,c), Cyprus (Banilas et al., 2003), Greece (Hagidimitriou et al., 2005), Morocco and western countries of the Mediterranean Basin (Essadki et al., 2006), Egypt (Hegazi et al., 2012), Iran (Noormohammadi et al., 2012), Portugal (Gemas et al., 2004; Martins-Lopes et al., 2007; Cordeiro et al., 2008), Italy (Ganino et al., 2007) and Spain (Sanz-Cortes et al., 2001; Caraffa et al., 2002; Belaj et al., 2004; Gomes et al., 2009).

The high polymorphism generated by these markers indicates that the olive tree is a highly polymorphic species. The high diversity found between olive cultivars is probably due to a diverse germplasmic origin that resulted in a predominant allogamous species with a high degree of outcrossing (Zohary and Spiegel-Roy, 1975; Bartolini et al., 1998). Additionally, the complexity of the olive genome (23 pairs of chromosomes, which is believed to have been originated by allopolyploidy (Zohary and Spiegel-Roy, 1975) resulted in new cultivars found throughout the Mediterranean amid low breeding pressures (Besnard et al., 2001a; Contento et al., 2002; Belaj et al., 2003c; Martins-Lopes et al., 2007). The dendrograms generated by RAPD and ISSR markers grouped the cultivars

differently. This can be explained by two factors: differences in the number of markers (156 and 85 by RAPD and ISSR, respectively) generated by the two techniques, and the fact that ISSR primers target-specific genome regions, whereas RAPD primers amplify arbitrary regions (Martins-Lopes et al., 2007). There is no clear structure with the geographical cultivation of the cultivars that has been observed in this study. Nevertheless, with the UPGMA method, some evidence of relationships according to their geographical origin and/or diffusion was observed. For instance, RAPD markers grouped 'Nabali Baladi,' 'Nabali Muhassan', 'Kfari Baladi,' and 'Kfari Romi', which are mainly cultivated in the Irbid area of northern Jordan, into one cluster, whereas 'Arabi Altafila' and 'Ketat,' which are cultivated in the Tafeleh area of southern Jordan, were grouped into another. These results were in agreement with several studies (Besnard et al., 2001a; Caraffa et al., 2002; Khadari et al., 2003; Martins-Lopes et al., 2007) that found no clear correlation between olive genotypes and the geographical origin. However, a good correlation between the banding patterns of olive cultivars and their geographical origin was obtained in other studies using RAPD markers conducted in both Jordan (Hassawi and Hadeib, 2004) and the Mediterranean (Belaj et al., 2001, 2003a, 2003b, 2004; Sanz-Cortes et al., 2001), which agreed with both the hypotheses of autochthonal origin as well as the limited diffusion of olive cultivars from their zones of cultivation (Belaj, 2001; Besnard et al., 2001). Furthermore, some associations between cultivars and their end use were also observed. For example, except for 'Shami', clustering of cultivars containing high-to-medium percentage oil content ('Nabali Baladi,' 'Nabali Muhassan,' 'Kfari Baladi,' and 'Kfari Romi') was observed using RAPD markers. Similarly, 'Nabali Baladi,' 'Nabali Muhassan,' and 'Souri' cultivars, which are used for both table and oil production, were clustered according to ISSR markers. This suggests that the end use was a criterion for local selection in Jordanian olive cultivars. In Jordan, Hadeib and Hassawi (2006) found a relationship between the genetic identities of cultivars studied from one region and the fruit size and oil contents from the other.

In other studies, fruit characteristics were reflected in some relationships independent of ecological adaptation (Besnard et al., 2001; Gemas et al., 2004; Hagidimitriou et al., 2005; Cordeiro et al., 2008). However, Martins-Lopes et al. (2007) were unable to find clear clustering in terms of olive end-use (oil, table, or both), explained by differences in cultivars, markers, and primers used in their analysis. Sanz-Cortes et al. (2001) also found no apparent clustering of forty olive cultivars from Valencia, Spain by fruit size or other morphological traits.

In RAPD and combined analyses, the highest value of genetic diversity was observed between 'Kanabisi' and the rest of cultivars due to its great differences from others, with respect to numerous morphological characteristics. The 'Ketat' and 'Arabi Altafila' cultivars are grouped into one subgroup by RAPD analysis; these cultivars are cultivated in Tafeleh and their fruits are mainly used as table olives. 'Nabali Baladi' and 'Nabali Muhassan' appear to be related; these cultivars are widely cultivated in rain-fed areas of Jordan, with fruits being used for both oil and table, having a medium-to-high range of oil content.

In this study, the use of 15 RAPD and 14 ISSR primers allowed for the screening of 48 (36 RAPD and 12 ISSR) markers specific to different cultivars. These markers can be converted into SCAR markers for individual cultivar identification analysis (Hernandez et al., 2001; Bautista et al., 2003; Martins-Lopes et al., 2007). The high discrimination capacity of these markers, as determined by unique RAPD/ISSR markers and unique banding patterns generated by different primer combinations, will be useful for management of a germplasm bank, thus providing the nursery industry with a useful tool for certification of their plant material (Belaj et al., 2001).

This study confirmed that both RAPD and ISSR markers are powerful tools for olive varietal identification, enabling an accurate characterization of all cultivars examined. Such information may prove useful in the selection of optimal varietals and help promote continued progress in olive breeding strategies.

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