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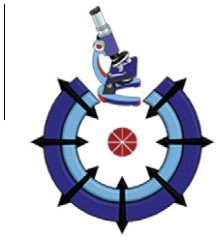

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ARTICLE

Molecular polymorphisms in Palestinian Figs (*Ficus carica* L.) as revealed by Random Amplified Polymorphic DNA (RAPD)

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KEYWORDS

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Abstract The genetic diversity of 13 local Palestinian fig genotypes was investigated using RAPD markers. Among the 30 tested primers, 28 revealed various banding patterns and 2 generated no polymorphic bands. In addition, 13 primers (46.4%) produced good amplification products with high intensity and pattern stability. A total of 94 DNA fragments (loci), separated by electrophoresis on agarose gel were detected, ranging in size from 190 to 1300 bp. Of these fragments, 72 (76.6%) were polymorphic and 22 (23.4%) were monomorphic. A minimum of three and a maximum of eight DNA fragments were obtained using (OPH-02 and OPT-10) as well as (OPA-13, OPA-18 and OPY-07) primers respectively. The maximum percentage of polymorphic markers was 100.0 (Z-5, Z-12, and OPT-10) and the minimum was 60.0 (OPH-02). Primers OPY-07 and OPA-13 revealed high collective resolving power (Rp) values with 4.640 and 4.760 respectively and therefore, they were the most useful RAPD primers to assess the genetic diversity in the Palestinian figs. Genetic distance matrix showed an average distance range from 0.186 to 0.559 with a mean of 0.373. Thus, the cultivars tested in this study were characterized by large divergence at the DNA level. To our knowledge, this is the first report using RAPD marker to assess genetic diversity of Palestinian figs.

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1. Introduction

The fig (*Ficus carica* L.) is one of the oldest known fruit tree species cultivated by man. Due to its nutritional value and health benefit, its importance is likely to continue worldwide [12]. Compared with other common fruits and beverages, the

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figs are an important source of minerals, vitamins, and amino acids [21]; it contains the highest concentrations of polyphenols [29]; and it includes relatively high amounts of crude fiber [30].

The Mediterranean region and especially the Middle East countries have been the most important center of fig growth from time immemorial [4]. The discovery of carbonized figs in an early Neolithic site in the Jordan Valley, dating back 11,400–11,200 years ago, suggests that figs were first domesticated during the early Neolithic Revolution preceding cereal domestication [19]. From there, fig cultivation spread to the neighboring western Asia and other Middle-East regions, and subsequently into the rest of the World countries [6].

The long domestication history with numerous cultivars and further exchange and spread into other growing regions of the world has resulted in ambiguity in the description and nomenclature of fig cultivars.

In Palestine (one of the original countries of fig cultivation), the long history of fig growth has determined a complex picture in which fig landraces and cultivars are either misidentified or called by different names in different areas. Therefore, it is crucial for discrimination between these landraces both for conservation of plant genetic resources and improvement purposes [26,25].

In general, three genetic markers are used for characterization and identification of different fruit species. These markers are: morphological or visible markers, biochemical variants or isozymes, and molecular markers.

Several authors have reported the inaccuracy and insufficiency of using morphological markers for identifying fig cultivars [27,5]. Indeed, characterization based on morphological criteria is often variable across years and locations due to the plasticity and susceptibility of these traits to genotype-environmental interactions [6].

Biochemical markers like isozymes have also been used for varietal recognition, nevertheless their use is limited due to their low variation in a given species and small number of loci that can be analyzed by conventional staining methods [22]. Furthermore, in some cases, isozyme polymorphism is influenced by environmental conditions [1].

To overcome these difficulties, new genetic markers based on DNA polymorphisms have been developed for genetic diversity analysis and cultivars identification between and within fruit species [16]. In figs, assessment of genetic relatedness and diversity has been investigated by using RFLP, AFLP, SSR, ISSR, and RAPD methods [18,17,11,13,9,23,2,10,26,1]. Compared with other molecular techniques, RAPD is a simple, fast, efficient, and inexpensive method. Further, it does not require prior knowledge of the sequences of the markers and can produce abundant polymorphic fragments [20,1,3]. Therefore, RAPD has become a powerful and accurate tool for analyzing the genetic relatedness and diversity in figs.

To date, characterization of Palestinian figs has not been accomplished neither by morpho-biochemical methods nor by molecular markers. Therefore, the present study is the first attempt to characterize and detect similarities among some fig cultivars grown in the southern region of Palestine using RAPD fingerprinting.

2. Materials and methods

2.1. Plant materials

This study was carried out during the growing season of 2010. A total of 13 fig accessions were surveyed throughout the southern region of West-Bank, Palestine (Table 1).

2.2. DNA extraction

Genomic DNA was extracted from fresh leaves of single adult trees using DNeasy Plant Mini Kit (Qiagen Inc.).

2.3. RAPD primers and PCR reactions

A total of 30 RAPD primers (Sigma-Aldrich) were used for the amplification of random DNA banding patterns (Table 2).

PCR reactions were repeated twice and carried out in a 25 ml volume mixture containing: 5 μl of a total DNA (30 ng), 2 μl primer (5 μM), 2 μl dNTPs (200 mM) (Fermentas), 2.5 μl Taq buffer (10X), 2 μl magnesium chloride (25 mM) and 1.5 U of Taq DNA polymerase (Hy Labs). Consequently, DNA was amplified by PCR on a Peltier Thermal Cycler-200 (MJ Research. Inc, Watertown, MA) and the PCR program was: 1 cycle, 94 °C (3 min); 35 cycles, 94 °C (1 min), 35 °C (1 min), 72 (1;30 min) 1 cycle, 72 °C (5 min), and then cooling down to 4 °C.

Amplified products (25 μl) were mixed with 5 μl of orange gel loading buffer and analyzed by electrophoresis in 2% agarose gels (Hy Labs) in 1X TAE buffer at 4 volt/cm for 4 h as

Table 1 Local Palestinian figs studied in relation to their collection site.

Acces. no.	Cultivar name	Fruit colour	Horticultural classifications	Collection site
1	Swadi	Black–purple	Uniferous	Al-Aroub
2	Smari	Black–purple	Uniferous	Al-Aroub
3	Eswadi	Black–purple	Uniferous	Hebron
4	Dafori	Green–yellow	Biferous	Dura
5	Biadi	Green–yellow	Uniferous	Al-Aroub
6	Hmadi	Green–purple	Uniferous	Yatta
7	Mwazi	Green–purple	Biferous	Al-Aroub
8	Khdari	Green–yellow	Uniferous	Al-Aroub
9	Ruzzi	Green–purple	Biferous	Beit Kaheel
10	Shhami	Brown–green	Uniferous	Dura
11	Kbari	Green–yellow	Uniferous	Dura
12	Ghzali	Green–yellow	Uniferous	Dura
13	Sfari	Green–yellow	Uniferous	Dura

Table 2 List of the selected RAPD primers, resolving power, and the degree of the polymorphism obtained among 13 local Palestinian figs.

#	Primer code	Sequence 5′–3′	RAPD total bands	Polymorphic bands	Monomorphic bands	Resolving power (Rp)	Percentage of polymorphic markers	Primer case*	Reference
1.	Z-5	TCCCATGCTG	5	5	0	2.760	100	Included	[15]
2.	Z-6	GTCCCGTTCA	6	5	1	2.000	84	Included	[15]
3.	Z-8	GGGTGGGTAA	8	7	1	2.320	88	Included	[15]
4.	Z-11	CTCAGTCGCA	5	4	1	2.340	80	Included	[15]
5.	Z-13	GACTAAGCCC	6	5	1	1.534	84	Included	[15]
6.	Z-12	TCAACGGGAC	5	5	0	2.460	100	Included	[15]
7.	OPA-11	CAATCGCCGT	8	5	3	2.760	63	Included	[18]
8.	OPA-13	CAGCACCCAC	12	8	4	4.760	67	Included	[3]
9.	OPA-18	AGGTGACCGT	12	8	4	3.042	67	Included	[3]
10.	OPY-07	AGAGCCGTCA	11	8	3	4.640	73	Included	[3]
11.	OPH-02	TCGGACGTGA	5	3	2	1.540	60	Included	[27]
12.	OPH-19	CTGACCAGCC	8	6	2	2.920	75	Included	[3]
13.	OPT-10	CCTTCGGAAG	3	3	0	1.360	100	Included	[27]
14.	Z-17	CCTTCCCACT	6	2	4	0.600	34	Excluded	[15]
15.	Z-18	AGGGTCTGTG	8	2	6	1.060	25	Excluded	[15]
16.	Z-19	GTGCGAGCAA	8	3	5	1.520	38	Excluded	[15]
17.	OPA-01	CAGGCCCTTC	10	5	5	3.240	50	Excluded	[27]
18.	OPA-02	TGCCGAGCTG	9	4	5	0.228	45	Excluded	[27]
19.	OPA-05	AGGGGTCTTG	6	2	4	0.920	34	Excluded	[27]
20.	OPA-12	TCGGCGATAG	2	1	1	0.160	50	Excluded	[3]
21.	OPY-04	GGTCGCAATG	6	1	5	0.460	17	Excluded	[3]
22.	OPY-11	AGACGATGGG	6	3	3	1.680	50	Excluded	[3]
23.	OPY-15	AGTCGCCCTT	8	2	6	0.760	25	Excluded	[3]
24.	OPX-11	GGAGCCTCAG	8	4	4	1.240	50	Excluded	[3]
25.	OPH-17	CACTCTCCTC	6	3	3	0.780	50	Excluded	[3]
26.	OPH-18	GAATCGGCCA	4	2	2	0.760	50	Excluded	[3]
27.	OPW-17	GTCCTGGGTT	6	2	4	0.460	34	Excluded	[3]
28.	OPT-20	GACCAATGCC	4	1	3	0.600	25	Excluded	[27]
29.	OPA-16	AGCCAGCGAA	4	0	4	0.000	00	Excluded	[3]
30.	Z-20	ACTTTGGCGG	3	0	3	0.000	00	Excluded	[15]
	Mean		7.2	5.6		2.648	77		
	Total		94	72	22	34.436			

* Primers presented $\leqslant$ 50 polymorphisms (either presented no bands or presented low, weak, and not clear complex amplification products), were excluded.

well as detected by staining with ethidium bromide (Sigma). A 100 bp DNA ladder was used as standard marker (Fermentas). Consequently, amplicons were visualized with UV transilluminator (ImageMaster®VDS)

2.4. Data Analysis

For each primer, the total number of bands and the polymorphic bands were calculated. The ability of the most informative primers to differentiate between cultivars was assessed by the estimation of their resolving power (Rp) [24]. The Rp has been described to correlate strongly with the ability to distinguish between cultivars according to the following formula: $Rp = \sum Ib$, where $Ib = 1-(2\times|0.5-p|)$ where p is the proportion of the 13 cultivars possessing the I band [14].

Banding profiles data were scored as present (1) or absent (0) for each sample. Besides, RAPD bands were transformed into a binary matrix. After that, a genetic distance matrix was estimated based on Jaccard's similarity coefficient using the multilocus fingerprinting data sets containing missing data (FAMD) software version 1.108 beta. Consequently, cluster analysis was made using the un-weighted pair-group method with arithmetic averages (UPGMA) [28] and the Tree view software (Win32, version 1.6.6).

3. Result and discussion

3.1. Genetic polymorphism and RAPD patterns

In this study, we investigate 30 primers for their potential to evaluate 13 unidentified commercial common fig genotypes (Table 1). As an initial step, 28 primers revealed various banding patterns and 2 primers generated no polymorphic bands. 13 primers (46.4%) produced good amplification products with high intensity and pattern stability (Table 2), and therefore were used in this study. Consequently, the remaining 15 primers exhibited low, weak, and not clear complex amplification products, and therefore were excluded.

A total of 94 DNA fragments (loci), separated by electrophoresis on agarose gel, were detected (Table 2), ranging in size from 190 to 1300 bp. Of these fragments, 72 (76.6%) were polymorphic and 22 (23.4%) were monomorphic. Comparable to those in the cited literature, our result is one of the highest percentage of polymorphisms ratio among fig

cultivars grown in Mediterranean countries which ranged between 39–81% using RAPD markers (39% in 12 RAPD primers: [18]; 67% in 7 RAPD primers: Papadopoulou et al. [23]; 72% in 6 RAPD primers: Salhi-Hannachi et al. [27]; 67% in 7 RAPD primers: Papadopoulou et al. [23]; 70% in 13 RAPD primers: Akbulut et al. [3]; and 77% in 6 RAPD primers: Sadder and Ateyyeh [26]; 81% in 7 RAPD primers: De Masi et al. (2005)).

Figs. 2–7 are examples of RAPD banding patterns generated in 13 fig cultivars using Z-13, OPY-07, OPA-18, OPH-02, OPA-19, and OPA-13 primers respectively.

Interestingly, the high degree of polymorphism obtained, particularly the more divergent cultivars, indicating a promising potential for selection and availability as a genetic source [7].

Our results also revealed an average of 7.2 loci per primer which is in agreement with Khadari et al. [18] and Galderisi et al. [13] who demonstrated that a low number of amplicons per primer was sufficient to produce useful fingerprints for cultivar and clone discrimination. Therefore, we may confidently assume that the RAPD technique can solve one of the major problems associated with varietal identification in Palestinian figs.

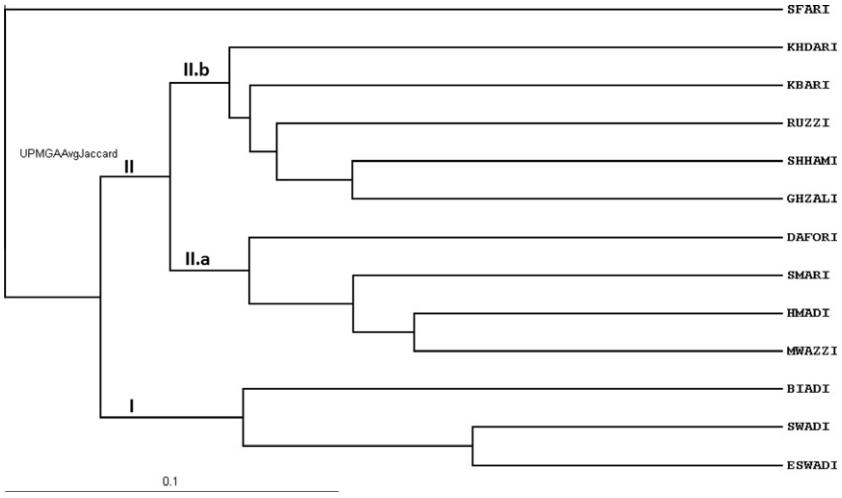

Figure 1 Dendrogram of 13 local Palestinian figs constructed by UPGMA based on RAPD banding patterns.

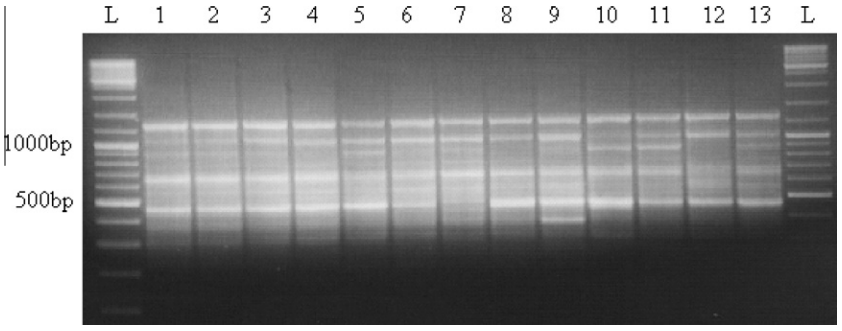

Figure 2 Example of RAPD banding patterns generated in Palestinian fig cultivars using Z-13 primer. L: 1 Kb ladder, 1: Biadi, 2: Swadi, 3: Eswadi, 4: Shhami, 5: Ruzzi, 6: Ghzali, 7: Sfari, 8: Khdari, 9: Kbari, 10: Dafori, 11: Mwazi, 12: Smari, 13: Hmadi.

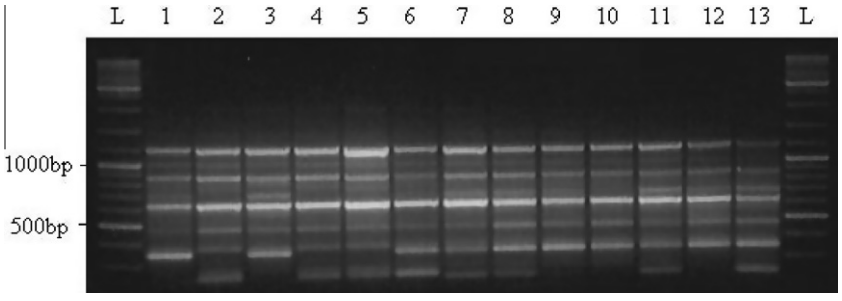

Figure 3 Example of RAPD banding patterns generated in Palestinian fig cultivars using OPY-7 primer. L: 1 Kb ladder, 1: Biadi, 2: Swadi, 3: Eswadi, 4: Shhami, 5: Ruzzi, 6: Ghzali, 7: Sfari, 8: Khdari, 9: Kbari, 10: Dafori, 11: Mwazi, 12: Smari, 13: Hmadi.

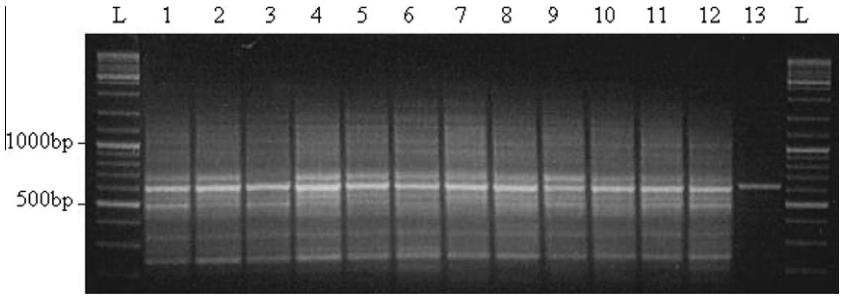

Figure 4 Example of RAPD banding patterns generated in Palestinian fig cultivars using OPA-18 primer. L: 1 Kb ladder, 1: Biadi, 2: Swadi, 3: Eswadi, 4: Shhami, 5: Ruzzi, 6: Ghzali, 7: Sfari, 8: Khdari, 9: Kbari, 10: Dafori, 11: Mwazi, 12: Smari, 13: Hmadi.

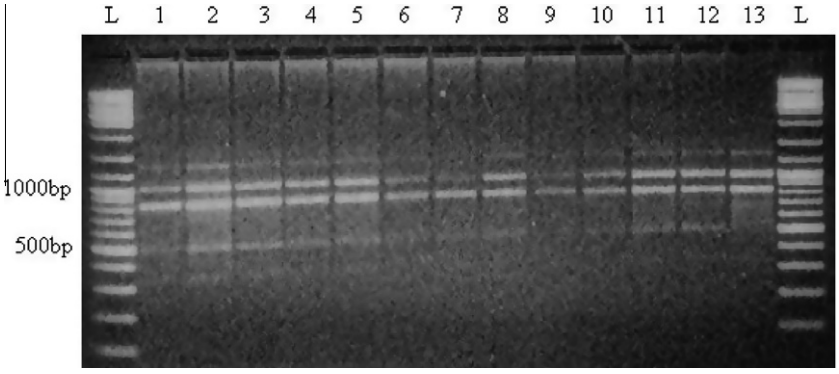

Figure 5 Example of RAPD banding patterns generated in Palestinian fig cultivars using OPH-02 primer. L: 1 Kb ladder, 1: Biadi, 2: Swadi, 3: Eswadi, 4: Shhami, 5: Ruzzi, 6: Ghzali, 7: Sfari, 8: Khdari, 9: Kbari, 10: Dafori, 11: Mwazi, 12: Smari, 13: Hmadi.

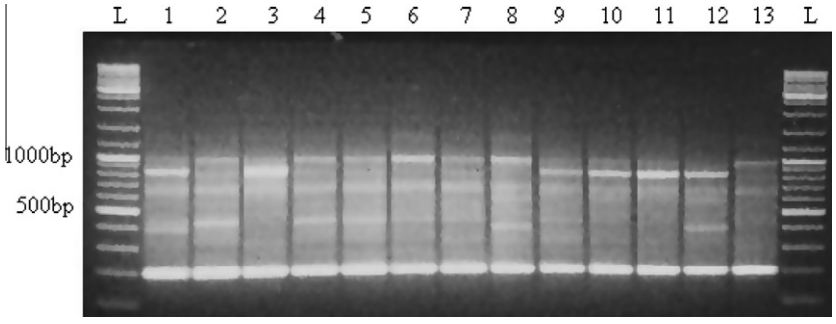

Figure 6 Example of RAPD banding patterns generated in Palestinian fig cultivars using OPA-19 primer. L: 1 Kb ladder, 1: Biadi, 2: Swadi, 3: Eswadi, 4: Shhami, 5: Ruzzi, 6: Ghzali, 7: Sfari, 8: Khdari, 9: Kbari, 10: Dafori, 11: Mwazi, 12: Smari, 13: Hmadi.

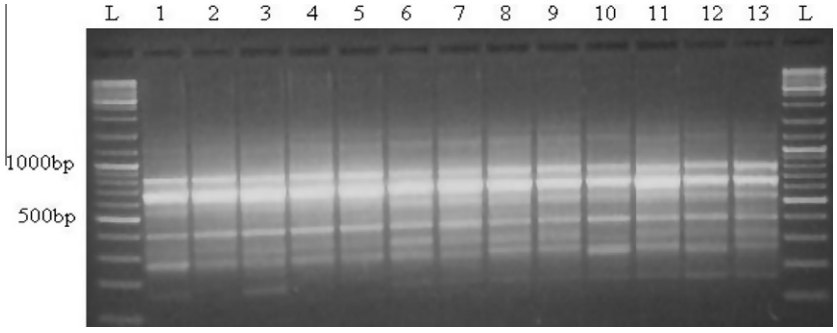

Figure 7 Example of RAPD banding patterns generated in Palestinian fig cultivars using OPA-13 primer. L: 1 Kb ladder, 1: Biadi, 2: Swadi, 3: Eswadi, 4: Shhami, 5: Ruzzi, 6: Ghzali, 7: Sfari, 8: Khdari, 9: Kbari, 10: Dafori, 11: Mwazi, 12: Smari, 13: Hmadi.

Table 3 Jaccard's distance index generated for the 13 local Palestinian figs' RAPD data.

	Swadi	Smari	Eswadi	Dafori	Biadi	Hmadi	Mwazi	Khdari	Ruzzi	Shhami	Kbari	Ghzali
Smari	0.257											
Eswadi	**0.186**	0.387										
Dafori	0.324	0.338	0.361									
Biadi	0.303	0.394	0.343	0.439								
Hmadi	0.311	0.250	0.368	0.319	0.507							
Mwazi	0.278	0.265	0.315	0.284	0.435	0.221						
Khdari	0.370	0.431	0.384	0.358	0.485	0.343	0.309					
Ruzzi	0.372	0.365	0.342	0.361	0.479	0.347	0.268	0.361				
Shhami	0.324	0.362	0.338	0.382	0.507	0.294	0.258	0.308	0.314			
Kbari	0.387	0.425	0.400	0.377	0.521	0.384	0.329	0.328	0.310	0.328		
Ghzali	0.347	0.384	0.338	0.357	0.500	0.271	0.286	0.309	0.292	0.258	0.329	
Sfari	0.480	0.479	0.452	0.433	**0.559**	0.414	0.406	0.409	0.362	0.359	0.449	0.281

A minimum of three and a maximum of eight DNA fragments were obtained using (OPH-02 and OPT-10) as well as (OPA-13, OPA-18 and OPY-07) primers respectively. The maximum percentage of polymorphic markers was 100.0 (Z-5, Z-12, and OPT-10) and the minimum was 60.0 (OPH-02).

3.2. Resolving power (Rp)

The tested primers exhibited relatively high collective Rp values of 34.436 and varied from 1.360 for the (OPT-10) primer to 4.640 and 4.760 for the (OPY-07 and OPA-13, respectively) with a mean of 2.648 (Table 2). This result extremely presented higher Rp value comparing with the most recent study reported for other Mediterranean fig by Salhi-Hannachi et al. [27]; Rp value = 21.771 in 6 RAPD primers. In addition, the primers OPY-07 and OPA-13 seemed to be the most useful RAPD primers to assess the genetic diversity since they revealed relatively high collective Rp rates.

Indeed, the higher polymorphism ratio (76.6%) and the relatively higher Rp values might suggest high genetic diversity in Palestinian fig population at the DNA level.

3.3. Genetic distances

The data matrix size analyzed was 1209 entries, 749 of which were for present loci (1) and 460 for absent loci (0). Accordingly, the Jaccard coefficient was calculated and presented in (Table 3).

The genetic distance matrix showed an average distance range from 0.186 to 0.559 with a mean of 0.373. Thus, the cultivars tested in this study are characterized by large divergence at the DNA level. Similar result was reported by [8].

The maximum genetic distance value of 0.559 was registered between Biadi and Sfari cultivars, suggesting high dissimilarities. Whereas, the lowest genetic distance of 0.186 (the highest similarities of 0.814) was exhibited between the Swadi and Eswadi cultivars, suggesting their close relatedness.

Among all tested cultivars, Biadi tends to show the highest genetic distance values from others. However, the remaining cultivars exhibited somewhat intermediate levels of genetic similarity.

3.4. Dendrogram of genetic relationship (similarity matrix and cluster analysis)

The average genetic relatedness among the genotypes is illustrated in Fig. 1. RAPD UPGMA dendrogram analysis divided the cultivars studied into two major clusters. The first one (I) is made up of three cultivars: Swadi and Eswadi, related to Biadi. All the remaining accessions are ranged in the second cluster (II) that revealed two sub-clusters. The first labeled (IIa) is made up of four cultivars: Mwazi, Hmadi and Smari which are related to Dafori. The second sub-cluster (IIb) is composed of five cultivars: (Ghzali and Shhami), in addition to Ruzzi, Kbari, and Khdari which are distantly related. However, Sfari cultivar is separated and identified as a distant genotype.

4. Conclusion

This study showed that there is an important genetic diversity at the DNA level among the fig genotypes sampled from the southern region of Palestine. Therefore, we conclude that RAPD analysis is an efficient technique for fingerprinting Palestinian fig genotypes. In fact, we obtained a very high percentage of polymorphisms (76.6%), indicating higher genetic diversity among the tested cultivars, which could be potentially incorporated into any further breeding programs and germplasm conservation.

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