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Genetic Diversity of the Palestinian Fig (*Ficus carica* L.) Collection by Pomological Traits and RAPD Markers

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Abstract

Analysis of differentiation (genetic diversity and related relationships) among 22 landrace (*Ficus carica* L. *sativa*) and 2 wild form (*F. carica* L. *caprificus*) accessions of fig growing under the same environmental conditions in the Palestinian Fig Collection, Til, Nablus, Palestine, using PCR-based Random Amplified Polymorphic DNA (RAPD) and pomological markers, revealed considerable genetic diversity. The phenotypic analysis shows that pomological traits were permitted to evaluate morphological variability of fig landraces. The Jaccard similarity coefficient between landraces was determined by cluster analysis using the UPGMA method. Based on the genetic relationships among genotypes as illustrated by the dendrograms, generated from pomological and RAPD data by UPGMA clustering method, the following 12 genotypes: Qaisi, Mwazi, Barqawi, Inaqi, Swadi, Kharobi, Hmadibiadi, Sfari, Khdari, Biadi, Qrawi, and Slati, may be considered as distinct landraces. The remaining genotypes may be considered as synonymous (4) (Hmadi and Hmari, and Ajloni and Adloni), or closely related (6) landraces (Zraqi and Ghzali, Blati and Neami, and Qraee and Khurtmani). The wild fig forms clustered together and may be considered as distinct genotypes. Clustering patterns obtained from the combined (pomological and RAPD) markers had higher discriminatory power to discriminate fig landraces than using either pomological or RAPD markers alone. These results proved the importance of both pomological and RAPD markers to elucidate in part denomination problems and relationships among cultivars. Wide phenotypic and molecular diversity found in fig germplasm indicates a considerable potential for improving this crop.

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Keywords

Fig; DNA Markers; Genetic Diversity; RAPD; Pomological Traits; Palestine

1. Introduction

Fig (*Ficus carica* L., Moraceae), one of the most ancient cultivated fruit trees in the Mediterranean region which is the most important fig growing center worldwide, has recently drawn much attention because of its medicinal and nutritional values [1] [2].

In Palestine, locally adapted fig landraces and their wild forms, can be found growing all over the country with high level of phenotypic diversity in fruit color, size, shape, and flavor [3]. The fig germplasm consists of numerous landraces mainly selected by farmers for their fruit qualities and maintained in orchards. They are widely spread through different eco-geographical areas of the country and are threatened by genetic erosion due to biotic and abiotic stresses. Local fig landraces are available with different local names which were mainly given based on skin ground color, internal color, and maturity date [4]. The most famous local names are Khdari, Hmadi, Biadi, Khurtmani, Inaqi, Swadi, Mwazi, and Kharoubi. The discrimination between these landraces is, therefore, important for purposes of crop improvement and plant genetic resources conservation [5].

Prospecting and collecting actions have been initiated in Palestine and led to the identification of more than 50 ecotypes [3]. Initial classification of the *Ficus carica* landraces was carried out morphologically based on tree, leaf, and fruit characteristics. With support from The Global Environmental Facility (GEF) some of these are *ex situ* maintained in the Palestinian Fig Collection at the BERC-Til Botanic Gardens, Biodiversity and Environmental Research Center (BERC), Til, Nablus. This *ex situ* field genebank comprises the main local fig landraces (24 landraces, 2 trees per landrace) in the country. The trees are of similar age and growing under same environmental and cultivation conditions. The collection has been maintained and managed by researchers from BERC since its establishment in 2002.

Phenotypic and DNA-based markers have been used for the identification and characterization of fig genotypes [4] [6]-[10]. However, morphological characters are influenced by plant age, phenological stage, cultivation conditions, and environmental conditions and are therefore prone to phenotypic modifications, in addition to the high plasticity for many morphological traits [10]. Thus morphological characters can often yield unclear results [11]. On the other hand, the use of DNA-based markers (RFLP, AFLP, SSR, ISSR, and RAPD methods) [6]-[8] [12]-[19] has proved to be a powerful tool to assess genetic diversity and genotype identity in figs. In contrast to morphological markers, molecular markers, are stable and are not confounded by the environment effects [8].

Compared with other molecular techniques, the random amplified polymorphic DNA (RAPD) has considerable advantages because it is a simple, fast, efficient, and inexpensive method. Further, RAPD does not require prior knowledge of the sequences of the markers and can produce abundant polymorphic fragments [19] [20]. Therefore, RAPD has become a powerful and accurate method for analyzing the genetic biodiversity and relatedness in figs [6] [7] [11] [12] [18] [21].

In Palestine, assessments of biodiversity among nine fig genotypes growing in different parts of the northern West Bank, have been based on morpho-pomological and RAPD markers [11]. The molecular results in this study appeared contradictory to the phenotypic descriptors in several fig genotypes. This was attributed to phenotypic modifications caused by the prevailing weather conditions in the different areas where fig is grown. Other factors could have yielded such results including plant age, phenological stage, and cultivation conditions.

This paper aims to explore the diversity encountered in 24 fig genotypes (10-year-old) growing under the same environmental and edaphic conditions at the Palestinian Fig Collection in BERC-Til Botanic Gardens, Til, Nablus using pomological descriptors and RAPD markers.

2. Materials and Methods

2.1. Plant Material

A total of 24 fig accessions (22 common fig genotypes and 2 caprifigs) preserved in the Palestinian Fig Collec-

tion established in 2002 in the BERC-Til Botanic Gardens (altitude: 2008 Ft., latitude: 32˚1′43.42″ N; longitude: 35˚12′15.79″ E) Til, Nablus, Palestine, were sampled for this study (**Table 1**). The surveyed genotypes (10-year-old trees, 2 trees per cultivar) correspond to the main cultivated fig cultivars in Palestine. The climate is semi-arid Mediterranean climate with mild winter and hot summer. Annual average high temperature is around 21.5˚C (with hottest months being July and August being 28.9˚C), and annual average low temperature is around 10.87˚C (with coldest month is January with the average low temperature around 3.9˚C). Average annual rainfall is about 600 mm. All fig trees are cultivated under rain-fed conditions.

2.2. Molecular Analysis

2.2.1. DNA Extraction

Fresh young leaves were collected from fig accessions and directly ground under liquid nitrogen. Genomic DNA was extracted from the ground leaves of single adult trees using a modified Dellaporta method as described by Lin *et al.* [22]. After purification, the DNA concentration was estimated spectrophotometrically using Gene5 Take 3 Module (www.biotek.com). DNA integrity was performed by agarose gel electrophoresis [23].

2.2.2. RAPD primers and PCR reactions

A total of 25 RAPD primers were used for the amplification of random DNA banding patterns (**Table 2**). PCR reactions were repeated twice and carried out in a 25 µl volume mixture containing: 30 ng of a genomic DNA, 0.25 mM dNTPs, 0.25 mM $MgCl_2$, 2.5 µM primer (company, city), 0.5 U of *Taq* DNA Polymerase and 1 × enzyme buffer. Consequently, PCR reactions were performed in Gene Amp PCR System 9700 Thermal Cycler (Applied Biosystems). The amplification program was as follows: an initial denaturing step at 94˚C for 5 min, followed by 45 cycles of 94˚C for 0.5 min, 35˚C for 1 min and 72˚C for 1 min, and a final extension at 72˚C for

Table 1. Palestinian *Ficus carica* L. ecotypes (preserved in a collection established in the BERC Botanic Gardens in Til, Nablus) with their localities of origin.

Accession name (Abbrev.)	Accession no.	Botanical Variety	Locality Origin	Fruit skin Color Group
Adloni (AD)	BERC-BG-*F.carica-ADL*	Common Type	Nablus	Green-Purple
Ajloni (AJ)	BERC-BG-*F.carica- AJL*	Common Type	Nablus	Green-Yellow
Barqawi (BR)	BERC-BG-*F.carica-BAR*	Common Type	Jenin	Green-Yellow
Biadi (B)	BERC-BG-*F.carica-BIA*	Common Type	Til, Nablus	Green-Yellow
Blati (BL)	BERC-BG-*F.carica-BLA*	Common Type	Nablus	Green-Yellow
Ghzali (GH)	BERC-BG-*F.carica-GHZ*	Common Type	Ramalla	Suede
Hmadi-Biadi (HB)	BERC-BG-*F.carica-HM-B*	Common Type	Nablus	Green-Yellow
Hmadi (HD)	BERC-BG-*F.carica- HMA*	Common Type	Nablus	Green-Purple
Hmari (HM)	BERC-BG-*F.carica-HMR*	Common Type	Nablus	Green-Purple
I'naqi (IN)	BERC-BG-*F.carica-INA*	Common Type	Til, Nablus	Green-Purple
Kharobi (KHR)	BERC-BG-*F.carica-KHR*	Common Type	Ramalla	Black-Violet
Khdari (KHD)	BERC-BG-*F.carica-KHD*	Common Type	Nablus	Green-Yellow
Khortmani (KH)	BERC-BG-*F.carica-KHO*	Common Type	Nablus	Green-Purple
Mwazi (MW)	BERC-BG-*F.carica-MWA*	Common Type	Nablus	Green-Yellow
Neami (N)	BERC-BG-*F.carica-NEA*	Common Type	Jenin	Green-Yellow
Qaisi (QA)	BERC-BG-*F.carica-QAI*	Common Type	Salfit	Black-Violet
Qraee (QR)	BERC-BG-*F.carica-QRA*	Common Type	Jenin	Green-Yellow
Qrawi (QRW)	BERC-BG-*F.carica-QRW*	Common Type	Jenin	Green-Yellow
Sfari (SF)	BERC-BG-*F.carica-SFA*	Common Type	Salfit	Green-Yellow
Slati (SL)	BERC-BG-*F.carica-SLA*	Common Type	Salfit	Green-Yellow
Swadi (SW)	BERC-BG-*F.carica-SWA*	Common Type	Nablus	Black-Violet
Zraqi (Z)	BERC-BG-*F.carica-ZRA*	Common Type	Salfit	Black-Violet
Wild type1 (WT1)	BERC-BG-*F.carica-WT1*	Caprifig Type	Nablus	-
Wild type 2 (WT2)	BERC-BG-*F.carica-WT2*	Caprifig Type	Nablus	-

Table 2. List of selected RAPD primers, resolving power, Polymorphic information content and the degree of the polymorphism obtained among 24 local Palestinian fig varieties.

Primer code	Primer sequence	RAPD total bands	Monomorphic fragments	Polymorphic fragments	Resolving power (RP)	Polymorphic information content (PIC)	% of polymorphic marker
OPA14	TCTGTGCTGG	4	0	4	0	0.64	100
OPA07	GAAACGGGTG	6	0	6	0	0.68	100
OPA08	GTGACGTAGG	5	0	5	0	0.69	100
OPA16	AGCCAGCGAA	8	0	8	0	0.77	100
OPA03	AGTCAGCCAC	8	1	7	0.17	0.46	87.5
OPA20	GTTGCGATCC	2	1	1	0.25	0.09	50
OPH16	TCTCAGCTGG	1	1	0	0.42	0	0
OPA13	CAGCACCCAC	2	2	0	0.5	0.38	0
OPH02	TCGGACGTGA	9	1	8	0.5	0.71	88.89
OPH05	AGTCGTCCCC	6	2	4	0.75	0.65	66.67
OPA05	AGGGGTCTTG	8	0	8	0.83	0.83	100
OPA10	GTGATCGCAG	6	0	6	0.83	0.60	100
OPA19	CAAACGTCGG	4	2	2	0.92	0.45	50
OPA12	TCGGCGATAG	5	0	5	1	0.53	100
OPA15	TTCCGAACCC	3	2	1	1	0.58	33.33
OPA18	AGGTGACCGT	4	1	3	1.25	0.57	75
OPH12	ACGCGCATGT	11	3	8	1.33	0.87	72.73
OPA01	CAGGCCCTTC	9	1	8	1.42	0.82	88.89
OPA02	TGCCGAGCTG	7	2	5	1.42	0.75	71.43
OPA17	GACCGCTTGT	5	1	4	1.75	0.56	80
OPA09	GGGTAACGCC	7	2	5	1.83	0.78	71.43
OPA04	AATCGGGCTG	8	1	7	1.83	0.80	87.5
OPT20	GACCAATGCC	8	3	5	2.42	0.79	62.5
OPA11	CAATCGCCGT	10	2	8	3.08	0.86	80
OPH08	GAAACACCCC	6	1	5	3.5	0.79	83.33
Average		**6.08**	**1.17**	**4.92**	**0.98**	**0.62**	**73.58**
Total		**152**	**29**	**123**	**27**	**9.74**	

5 min. Amplified PCR products were resolved on 1% agarose gel stained with ethidium bromide (0.5 mg⁄ml), and the generated bands were visualized with UV transilluminator (Tl-2000 Ultraviolet Translinker, UVP, USA) and digitally photographed (Nikon).

2.2.3. Data Analysis of RAPD Markers

Only bands that were bright and reproducible were scored for analysis. Amplification products were scored as either present (1) or absent (0) for each sample. Besides, RAPD bands were transformed into a binary matrix. A genetic distance matrix was then estimated based on Jaccard's similarity coefficient using the multilocus fingerprinting data sets containing missing data FAMD software version 1.108. Similarity coefficient is defined as: [Similarity coefficient = (number of bands in common)/number of bands not in common + number of bands in common)] [7]. Consequently, cluster analysis was made using the un-weighted pair group method with arithmetic averages (UPGMA) [24].

To compare the efficiency of RAPD primers in identifying different fig genotypes the total number of bands and the polymorphic bands were calculated for each primer and the discriminatory power of RAPD marker was evaluated by 2 parameters. The polymorphic information content (PIC) and resolving power (R_P) for each RAPD marker. PIC has been known to provide an estimate of the discriminatory power of a locus or loci. It was calculated by taking into account not only the number of alleles that are expressed but also relative frequencies of those alleles. Calculations were made using the following formula as proposed by Roldan-Ruiz *et al.* [25]:

PIC = 1 − ∑fi ², where fi is the frequency of the marker bands present. The ability of the most informative primers to differentiate between cultivars was assessed by the estimation of their resolving power (Rp) [26]. The Rp of the 25 primers was calculated as Rp = ∑Ib where, Ib (band informativeness) takes the value of 1 − (2 × |0.5 − p|) where p is the proportion of genotypes containing the band.

2.3. Pomological Traits Analysis

2.3.1. Plant Material and Descriptors

A total of 21 quantitative and qualitative pomological traits were determined for the 22 common fig genotypes according to the fig descriptors prepared by IPGRI & CIHEAM [27], and Ajlane & Ferchichi [28], with some modifications (**Table 3** and **Table 4**).

2.3.2. Data Analysis of Pomological Traits

Each descriptor was scored as 1 for presence and 0 for absence. Accordingly, the relatedness among genotypes was estimated based on Jaccard's similarity coefficient using SPSS version 16.0. Consequently, cluster analysis was made using the (UPGMA) method [24].

2.4. Mantel's Test

Matrices (pomological and RAPD) correspondence test was performed and the significance of the correlation was performed using Mantel's t test to measure the degree of relationship between similarity index matrices produced by the two-marker systems [29] based on 1000 random permutations. These computations were performed using XLSTAT 2008, Version 7.03 (http://www.xlstat.com).

2.5. Data Analysis of Combined RAPD Markers and Pomological Descriptors

A binary matrix was obtained for the combined RAPD bands and pomological descriptors data, as outlined above. A genetic distance matrix was then estimated based on similarity coefficient. Clustering dendrogram was constructed by the UPGMA) method.

3. Results and Discussion

3.1. Molecular Results

3.1.1. Genetic Polymorphism and RAPD Patterns

Twenty five primers were investigated for their potential to evaluate 24 fig genotypes (**Table 2**). All primers revealed various banding patterns; two primers generated no polymorphic bands. A total of 152 DNA fragments (loci), separated by electrophoresis on agarose gel, were detected (**Table 2**), ranging in size from 300 to 2000 bp. Of these fragments, 123 (80.921%) were polymorphic and 29 (19.079%) were monomorphic. Compared to those results cited in the literature, our result is one of the highest percentage of polymorphisms ratio among cultivars grown in the Mediterranean countries which ranged between 39% - 81% using RAPD markers [7] [11] [12] [16] [21] [30] [31]. The high degree of polymorphism (about 81%) obtained among Palestinian fig cultivars suggests high genetic diversity in Palestinian fig population at the DNA level, and indicates a promising potential for selection and availability as a genetic source [32].

Our results also revealed an average of 6.08 loci per primer. However, this low number was according to Khadari *et al.* [6] and Galderisi *et al.* [14] adequate to generate useful fingerprints for fig cultivars and clone discrimination and therefore can help with varietal identification in Palestine.

A minimum of one and a maximum of 12 DNA fragments were obtained using (OPH16) and (OPH 12) primers, respectively. The maximum percentage of polymorphic markers was 100.0% in seven primers and the minimum was 0.00% in (OPA03, OPH16) primers.

3.1.2. Resolving Power (Rp) and Polymorphic Information Content (PIC)

The 25 primers exhibited variation with regard to their PIC and Rp values (**Table 2**). The PIC value of the primers ranged from 0.00 (OPH16) to 0.865 (OPH12) with an average of 0.615 per primer. The collective Rp value of the examined primers showed relatively high value of 27 in which the RAPD primers OPH08, OPA11, OPT20, OPA04 and OPA09 possess high Rp values of 3.5, 3.08, 2.41, 1.833 and 1.833 respectively, and

Table 3. Pomological descriptors determined in some fig (*Ficus carica* L.) genotypes grown in the Palestinian Fig Collection, West Bank- Palestine.

Fruit Descriptors	Abbrev.	Unit	Explanation	Abbrev.	Method/Reference
Beginning of Maturation	BM	Notification	Very early < 20 July	VE	IPGRI and CIHEAM, 2003
			Early 20 - 31 July	E	
			Mid-Season 1 - 15 Aug.	MS	
			Late 15 - 31 Aug.	L	
			Very late > Aug.	VL	
Fully Maturity	FM	Notification	Very early end of July	VE	IPGRI and CIHEAM, 2003
			Early 1 - 10 August	E	
			Mid-Season 11 - 31 Aug	MS	
			Late 1 - 30 Sep.	L	
			Very late > 1 Oct.	VL	
Harvesting Period	HP	Notification	Very Short < 15 day	VS	IPGRI and CIHEAM, 2003
			Short 15 - 20 days	S	
			Medium 21 - 40 days	M	
			Long 41 - 60 days	LG	
			Very long > 60	VLG	
Fruit External Color	EC	Notification	Green-purple	GP	This study
			Green-yellow	GY	
			Brown green	BG	
			Black purple	BP	
			Suede	SD	
			Yellow	Y	
Skin Cracks	SC	Notification	Cracked skin	CR	IPGRI and CIHEAM, 2003
			Scarce	SC	
			Minute	MN	
Fruit Shape	FS	Notification	Ovoid	OV	IPGRI and CIHEAM, 2003
			Globose	GL	
			Pyriformed	PY	
			Oblate	OP	
			Globose-Oblate	GLO	
			Pyriform-Oblong	PYO	
Fruit Weight	FW	G	Large 40 - 60	LR	In this study
			Medium 20 - 39	M	
			Small < 20	SM	
			Medium-Large	MLR	
Fruit Firmness	FF	Notification	Soft < 16	SF	A digital hand-held firmness meter fitted with a 5 mm probe (HPE-II: Qualitest; www.worldoftest.com
			Medium 16-20	M	
			Firm > 20	F	
Fruit Length	FL	Mm	Short 29 - 46	S	IPGRI and CIHEAM, 2003
			Medium 46 - 54	M	
			Long 54 - 75	LG	
			Very long > 75	VLG	
Fruit Width	FWth	Mm	Small 28 - 38	SM	IPGRI & CIHEAM, 2003
			Medium 38 - 49	M	
			Large 50 - 60	LR	
			Very Large > 60	VLAG	
Fruit Neck Length	NL	Mm	Absent	A	IPGRI & CIHEAM, 2003

Continued

		Short < 5	S	
		Medium 5 - 15	M	
		Long > 15	LG	
NW	Mm	Small < 8	SM	This study
		Medium 8 - 10	M	
		Large > 10	LR	
SL	Mm	Short < 4	S	This study
		Medium 4 - 8	M	
		Long > 8	LG	
SL	Mm	Small < 4	SM	This study
		Medium 4 - 5	M	
		Long > 5	LG	
		Medium-Long	MLG	
OT	Notification	Closed	CL	IPGRI & CIHEAM, 2003
		Semi-Open	SO	
		Open	O	
OW	Mm	Small < 1	SM	IPGRI & CIHEAM, 2003
		Medium 1 - 3	M	
		Large 4 - 5	LR	
		Very large > 5	VLR	
SP	Notification	Easy	ES	IPGRI & CIHEAM, 2003
		Medium	M	
		Difficult	DF	
IC	Notification	Amber	AM	This study
		Dark red	DR	
		Honey	HO	
		Honey - brownish	HOB	
		Light pink	LPK	
		Pink	PK	
		Pink-honey	PKH	
		Red	RD	
		Rosy	RO	
		Rosy-pink	ROP	
		Rosy-red	ROR	
FT	Mm	Small < 25	SM	This study
		Medium 25 - 35	M	
		Large > 35	LR	
PT	Notification	Fine	FN	IPGRI & CIHEAM, 2003
		Medium	M	
		Coarse	CS	
PF	Notification	Neutral	NT	IPGRI & CIHEAM, 2003
		Little flavor	LF	
		Aromatic	AR	
		Strong	ST	

therefore were able to distinguish more number of genotypes. Primers having high Rp along with high PIC values are more suitable for analysis of genetic diversity [33]. In the present study the primers OPH08 and OPA11 had high Rp and high PIC values. Hence these two primers seemed to be the most useful primers to assess the genetic diversity in fig cultivars.

Table 4. Pomological descriptors of some fig genotypes grown in the Palestinian Fig Collection, BERC-Til BGs, Nablus, Palestine.

Pomological descriptors**	Landrace*																				
	GH	**KHR**	**QA**	**SW**	**Z**	**BR**	**AJ**	**AD**	**QR**	**BL**	**SL**	**HB**	**KHD**	**MW**	**N**	**QR**	**SF**	**B**	**HD**	**HM**	**IN**	**KH**
Beginning of ripening	MS	L	L	L	L	E	L	L	L	E	E	E	E	MS	E	E	E	MS	E	E	L	E
Full ripening	MS	L	L	L	L	E	L	L	L	E	E	E	E	MS	E	E	E	MS	E	E	L	E
Harvest period	M	M	M	M	M	M	LG	LG	M	M	M	M	M	M	M	M	M	M	M	M	VLG	M
External color	SD	BP	BP	BP	BP	GP	GY	GY	GY	GY	GY	GY	GY	GY	GY	GY	Y	GY	GP	GP	BG	BG
Skin cracks	SC	CR	CR	MN	CR	CR	SC	SC	SC	CR	CR	CR	CR	MN	CR	SC	CR	SC	CR	CR	CR	CR
Fruit shape	OV	GL	GL	OV	OP	GLO	PYO	PYO	OV	PYO	PY	OV	OV	PY	GLO	PYO	PY	OV	GLO	GLO	PYO	PYO
Fruit weight	M	M	M	M	M	M	SM	SM	SM	LR	M	M	M	M	M	MLR	SM	M	M	M	MLR	M
Fruit firmness	F	F	M	F	F	SF	F	F	F	SF	SF	SF	M	F	SF	SF	M	SF	F	F	M	SF
Fruit length	S	S	M	S	S	M	M	M	M	LR	LR	S	S	M	M	LG	M	M	M	M	M	M
Fruit width	M	SM	M	M	M	M	S	S	S	LR	LR	SM	M	SM	SM	LR	M	M	M	M	M	SM
Neck length	M	S	S	S	S	S	M	M	M	M	M	S	S	S	S	M	M	M	S	S	LG	M
Neck width	M	SM	M	M	M	M	SM	SM	SM	M	M	M	SM	SM	M	M	M	M	M	M	M	LR
Stalk length	M	S	LG	M	S	M	M	M	M	MLG	LG	M	M	M	M	M	M	M	S	S	LG	S
Stalk width	LG	M	M	LG	M	M	SM	SM	SM	M	M	M	LG	LG	M	M	M	LG	M	M	M	M
Ostiole type	O	O	CL	O	O	O	CL	CL	CL	O	O	CL	O	CL	O	CL	O	O	O	O	O	O
Ostiole width	VLR	M	M	VLR	M	LR	M	M	M	M	M	SM	VLR	VLR	SM	M	M	VLR	LR	LR	SMM	M
Skin peeling	DF	ES	ES	DF	DF	ES	ES	ES	ES	ES	ES	ES	ES	ES	ES	ES	ES	ES	ES	ES	ES	ES
Internal color	RO	DR	RO	PK	PK	PKH	RD	RD	RD	PKH	PK	ROP	AM	AM	PKH	LPK	HO	ROR	ROP	ROP	HOB	HOB
Flesh thickness	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Pulp texture	FN	CS	FN	CS	FN	FN	FN	FN	FN	FN	CS	FN	FN	FN	FN	FN	CS	FN	FN	FN	CS	FN
Pulp flavor	AR	AR	AR	NT	AR	AR	AR	AR	AR	NT	NT	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR

*Landrace abbreviations as in **Table 1**; Pomological descriptors abbreviations as in **Table 3**.

3.1.3. Dendrogram of Genetic Relationship (Similarity Matrix and Cluster Analysis)

The data matrix size analyzed was 2701 entries, 901 (33.3%) of which were for present loci (1) and 1800 (66.6%) for absent loci (0). Accordingly, the Jaccard's coefficient was calculated and presented in (**Table 5**). The genetic similarity matrix showed an average similarity mean of 0.375. In an earlier RAPD study, Basheer-salimia *et al.* [11] observed genetic diversity values ranging from 0.238 - 0.477 with a mean of 0.358 among 9 different local Palestinian fig genotypes. The selected RAPD markers in this study revealed a wider range of genetic diversity than that observed by Basheer-salimia *et al.* [11]. The maximum similarity values of 0.750, and 0.679 were registered between the two wild type accessions and between Ghzali and Zraqi respectively; suggesting low dissimilarities and close relatedness. Whereas, the lowest similarity value of 0.00 (the highest dissimilarity of 1.0) was exhibited between Ajloni and Adloni varieties, and Biadi variety. Among all tested cultivars, Biadi tends to show the lowest genetic similarity values from others. However, the remaining cultivars exhibited somewhat intermediate levels of genetic similarity.

The genetic relationships among the genotypes are illustrated by a dendrogram, generated by UPGMA clustering method (**Figure 1**). The dendrogram was divided into two main clusters (I, II), and four single branches (Khdari, Kharobi, Slati and Biadi) as supported by their low similarities with other cultivars. The first cluster (I) was divided into four groups (Ia, Ib, Ic, Id). The first group (Ia) was composed of two sister groups (wild type accessions, and Zraqi and Ghzali), and four one-member subgroups (Qraee, Qrawi, Barqawi and Khurtmani). The two wild type accessions clustered together and exhibited the highest similarity value (0.750), followed by Zraqi and Ghzali (0.679) which share many pomological traits such as fruit size, flesh thickness, neck width and others. The second group (Ib) was composed of the two green-yellow fruit skin colored Blati, and Neami cultivars, and the Qaisi cultivar which branched separately. The third group (Ic) was composed of Sfari and Swadi cultivars which although have different fruit skin color of yellow and black-violet, respectively, they share the presence of open ostiole, pulp texture, flesh thickness, and fruit and neck width. The fourth group (Id)

Table 5. Jaccard's similarity measure generated for 24 local Palestinian figs based on RAPD data.

Landrace*	B	SL	IN	QR	KH	HB	HD	Z	KHD	KHR	HM	QA	AJ	BL	MW	N	GH	SF	BR	QRW	SW	AD	WT1	WT2
B	1.000																							
SL	0.104	1.000																						
IN	0.108	0.200	1.000																					
QR	0.048	0.234	0.218	1.000																				
KH	0.068	0.176	0.297	0.490	1.000																			
HB	**0.136**	0.189	0.417	0.382	0.390	1.000																		
HD	0.075	0.207	0.184	0.361	0.362	0.218	1.000																	
Z	0.066	0.238	0.294	0.468	0.469	0.339	0.519	1.000																
KHD	0.073	0.140	0.154	0.321	0.244	0.256	0.196	0.190	1.000															
KHR	0.081	**0.128**	0.206	0.158	0.119	0.136	0.357	0.300	0.158	1.000														
HM	0.100	0.239	0.184	0.250	0.308	0.256	0.452	0.278	0.231	0.189	1.000													
QA	0.077	0.232	0.357	0.302	0.313	0.294	0.404	0.421	0.224	0.273	0.277	1.000												
AJ	**0.000**	0.189	0.280	0.163	0.267	0.323	0.200	0.244	0.094	0.069	0.296	0.237	1.000											
BL	0.113	0.241	0.250	0.288	0.245	0.190	0.310	0.448	0.189	0.341	0.189	0.442	0.136	1.000										
MW	0.080	0.241	0.250	0.311	0.326	0.255	0.392	0.317	0.184	0.200	0.318	0.321	0.216	0.352	1.000									
N	0.094	**0.268**	0.283	0.400	0.327	0.308	0.442	0.456	0.265	0.318	0.319	0.423	0.195	0.571	0.385	1.000								
GH	0.067	0.242	0.226	0.500	0.365	0.276	0.473	0.679	0.214	0.280	0.283	0.404	0.196	0.456	0.322	0.547	1.000							
SF	0.048	0.140	0.184	0.296	0.342	0.200	0.298	0.380	0.297	0.257	0.333	0.277	0.167	0.235	0.208	0.240	0.333	1.000						
BR	0.060	0.179	0.256	0.339	0.364	0.260	0.346	0.472	0.140	0.205	0.295	0.380	0.189	0.286	0.218	0.291	0.400	0.326	1.000					
QRW	0.097	0.209	0.255	0.492	0.339	0.322	0.371	0.476	0.263	0.308	0.241	0.313	0.157	0.338	0.281	0.344	0.415	0.263	0.421	1.000				
SW	0.042	0.145	0.275	0.310	0.326	0.250	0.340	0.364	0.256	0.250	0.286	0.404	0.206	0.327	0.333	0.259	0.298	0.421	0.340	0.345	1.000			
AD	0.000	0.250	0.194	0.333	0.256	0.300	0.204	0.367	0.150	0.135	0.211	0.261	0.375	0.220	0.167	0.250	0.347	0.243	0.222	0.296	0.333	1.000		
WT1	**0.052**	0.175	0.216	0.554	0.417	0.340	0.345	0.509	0.300	0.196	0.226	0.328	0.182	0.356	0.210	0.386	0.491	0.300	0.396	0.534	0.315	0.340	1.000	
WT2	**0.057**	0.190	0.239	0.547	0.465	0.435	0.327	0.588	0.277	0.217	0.250	0.309	0.237	0.339	0.228	0.423	0.538	0.364	0.500	0.474	0.347	0.381	0.750	1.00

*Abbreviations as in **Table 1.**

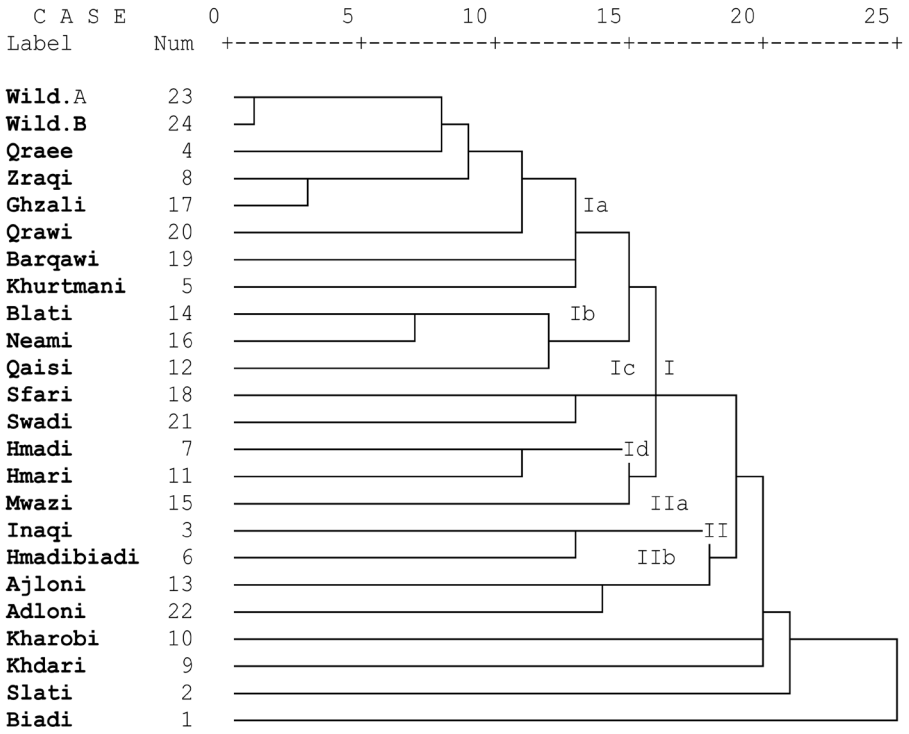

Figure 1. Dendrogram of 24 local Palestinian figs constructed by UPGMA based on RAPD binding patterns..

comprised a sister group of two cultivars with short fruit neck, green-purple external fruit color Hmadi and Hmari, and a separate branch containing Mwazi cultivar (with green-yellow skin color). The second cluster (II) was divided into two sister groups: the first is Hmadibiadi and Inaqi, and the second Ajloni and Adloni which also share all pomological traits.

Based on the genetic relationships among genotypes as illustrated by the dendrogram, the following genotypes Biadi, Kharobi, Khdari, Mwazi, Qaisi, Qraee, Qrawi, Slati, Barqawi and Khurtmani may be consi- dered as distinct landraces. It is noteworthy that all these cultivars are suitable for drying. The remaining genotypes, including the two wild form accessions which clustered together are closely related accessions.

3.2. Pomological Descriptors

3.2.1. Dendrogram of Pomological Relationship (Similarity Matrix and Cluster Analysis)

The data matrix size analyzed was 1782 entries, 457 (25.65%) of which were for present character (1) and 1325 (74.35%) for absent character (0). Accordingly, the Jaccard's coefficient was calculated and presented in **Table 6**. The genetic similarity matrix showed an average similarity range from 0.105 to 1.000 with a mean of 0.552. Thus, the cultivars tested in this study are characterized by large divergence at the morphological characteristics level. The maximum similarity values of 1.00 and 0.91 were registered between Ajloni and Adloni and between Hmadi and Hmari landraces, respectively, suggesting their close relatedness. Whereas, the lowest similarity value of 0.0105 (the highest dissimilarity of 0.895) was exhibited between Swadi and Khurtmani varieties. Among all tested cultivars, Swadi tends to show the lowest similarity values from the majority of the others.

Pomological analysis based on different characters showed high polymorphism with 22 fig cultivars. The dendrogram based on Jaccard's similarity index clustered cultivars into three major clusters (I, II, III) (**Figure 2**). The first cluster (I) was divided into two groups (Ia,Ib) the first group (Ia) contains three cultivars: Adloni, Ajloni with Qrawi in a separate branch. Ajloni and Adloni shared similar pomological traits, e.g., fruit internal color, oblong pyriform shape, small weight, medium length, short width, closed ostiole, and others. They also exhibited 1.00 similarity values which indicate that they should be considered synonyms. The second group (Ib) consisted of three cultivars: Biadi, and Ghzali in a sister group, and Mwazi branching separately. The second

Table 6. Jaccard's similarity measure generated for 22 local Palestinian figs based on pomological data.

Landrace*	B	SL	IN	QR	KH	HB	HD	Z	KHD	KHR	HM	QA	AJ	BL	MW	N	GH	SF	BR	QRW	SW	AD
B	1.000																					
SL	0.323	1.000																				
IN	0.206	0.212	1.000																			
QR	0.323	0.538	0.176	1.000																		
KH	0.313	0.414	0.281	0.414	1.000																	
HB	0.400	0.519	0.206	0.519	0.400	1.000																
HD	0.313	0.367	0.281	0.281	0.448	0.500	1.000															
Z	0.235	0.281	0.281	0.206	0.313	0.400	0.448	1.000														
KHD	0.448	0.323	0.206	0.281	0.313	0.556	0.400	0.313	1.000													
KHR	0.167	0.206	0.281	0.171	0.313	0.313	0.313	0.448	0.313	1.000												
HM	0.355	0.323	0.242	0.242	0.400	0.448	0.909	0.400	0.448	0.273	1.000											
QA	0.273	0.367	0.414	0.281	0.313	0.448	0.400	0.500	0.313	0.400	0.355	1.000										
AJ	0.273	0.206	0.206	0.323	0.273	0.235	0.167	0.200	0.200	0.273	0.167	0.273	1.000									
BL	0.250	0.500	0.182	0.500	0.481	0.379	0.290	0.212	0.290	0.212	0.250	0.212	0.212	1.000								
MW	0.448	0.281	0.108	0.242	0.235	0.313	0.273	0.200	0.448	0.273	0.313	0.273	0.355	0.143	1.000							
N	0.400	0.464	0.242	0.414	0.500	0.556	0.556	0.313	0.448	0.313	0.500	0.355	0.235	0.429	0.355	1.000						
GH	0.593	0.200	0.135	0.200	0.194	0.303	0.303	0.344	0.387	0.194	0.344	0.229	0.194	0.139	0.344	0.229	1.000					
SF	0.313	0.367	0.367	0.367	0.400	0.400	0.400	0.273	0.355	0.273	0.355	0.355	0.235	0.333	0.200	0.400	0.229	1.000				
BR	0.367	0.379	0.290	0.333	0.414	0.519	0.708	0.323	0.414	0.242	0.640	0.367	0.171	0.345	0.242	0.708	0.273	0.414	1.000			
QRW	0.355	0.242	0.171	0.323	0.273	0.313	0.200	0.235	0.273	0.313	0.200	0.313	0.826	0.212	0.400	0.273	0.265	0.273	0.171	1.000		
SW	0.313	0.139	0.206	0.108	0.105	0.273	0.235	0.500	0.355	0.313	0.273	0.273	0.135	0.111	0.273	0.200	0.433	0.200	0.206	0.200	1.000	
AD	0.273	0.206	0.206	0.323	0.273	0.235	0.167	0.200	0.200	0.273	0.167	0.273	1.000	0.212	0.355	0.235	0.194	0.235	0.171	0.826	0.135	1.000

*Abbreviations as in **Table 1.**

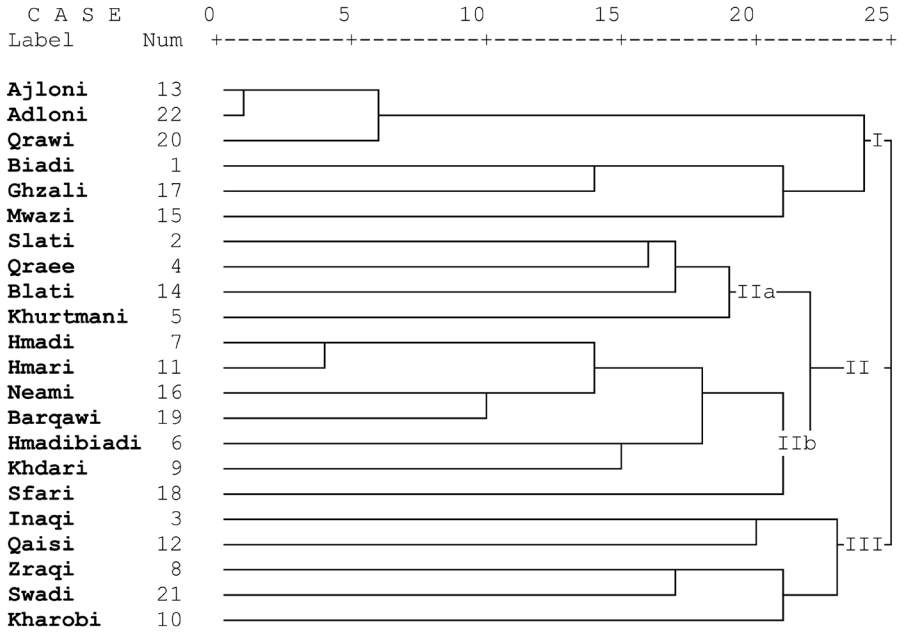

Figure 2. Dendrogram of 22 local Palestinian figs constructed by Jaccard's based on pomological traits.

cluster (II) was also divided into two groups. The first group (IIa) was made up of four cultivars: Qraee, and Slati in a sister group, and Blati and Khurtmani branching separately. The second group (IIb) was composed of seven cultivars: three sister groups Hmari and Hmadi; Neami and Barqawi; and Hmadibiadi and Khdari; and Sfari branching separately. The third cluster (III) was composed of five cultivars: two sister groups (Inaqi and Qaisi, and Zraqi, and Swadi) with Kharobi branching separately.

3.3. Correlation between Genetic Matrices

The Mantel test (two-tailed test), comparing RAPD matrix with phenotypic parameters did not indicate a significant correlation within the diversity of local fig landraces. Positive, although not significant correlation, was found between the two kinds of matrices [r(AB) = 0.039, *p* (two-tailed) = 0.542, alpha = 0.05; the *p*-value has been calculated using the distribution of r(AB) estimated from 10000 permutations] (**Figure 3**). As the computed p-value is greater than the significance level alpha = 0.05, one cannot reject the null hypothesis H_0 (The matrices are not correlated). The risk to reject the null hypothesis H_0 while it is true is 54.17%. In fact, the clustering pattern obtained by phenotypic traits and RAPD markers showed somewhat close clustering in some fig landraces, but on the other hand also showed some disparity (in some fig landraces) [16] (**Table 7**).

3.4. Combined Pomological Traits and RAPD

The present study is the first attempt to characterize and detect similarities among fig accessions preserved in the Palestinian fig *ex situ* field genebank, using combined (pomological and RAPD) markers. The data matrix size analyzed was 4483 entries, 1358 (30.29%) of which were for present loci (1) and 3125 (69.70%) for absent loci (0). Accordingly, the Jaccard's coefficient was calculated and presented in (**Table 8**). The genetic similarity matrix showed an average similarity range from 0.091 to 0.625 with a mean of 0.358. Thus, the maximum similarity values of 0.625 and 0.609 were registered between Hmari and Hmadi and between Ajloni and Adloni cultivars, respectively; suggesting low dissimilarities and their close relatedness. Whereas, the lowest similarity value of 0.091 (the highest dissimilarity of 0.909) was exhibited between Slati and Swadi. Among all tested cultivars, Slati tends to show the lowest similarity values from others.

The dendrogram based on Jaccard's similarity index clustered cultivars into two major clusters (I, II) in addition to another six genotypes (Hmadibiadi, Sfari, Kharrobi, Khdari, Biadi, and Slati) that did not cluster and branched separately (**Figure 4**).

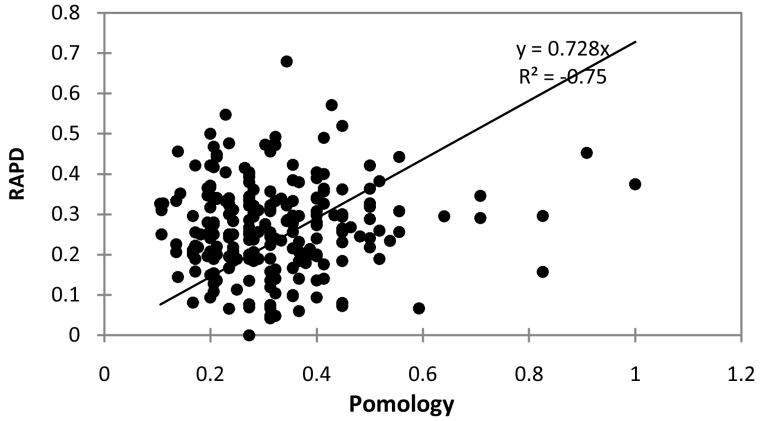

Figure 3. Product moment correlations (r) from the normalized Mantel statistics for compa- risons of different proximity matrices using random amplified polymorphic DNA (RAPD) bands, and pomological traits from 22 fig genotypes

Table 7. Clustering patterns in the dendrograms of 22 local Palestinian figs constructed by UPGMA based on RAPD, pomological, and combined (RAPD and pomological) markers.

	RAPD		Pomological markers		(RAPD + Pomological) markers	
Landrace*	**Non-cluster groups**	**Sister groups**	**Non-cluster groups**	**Sister groups**	**Non-cluster groups**	**Sister groups**
AD		AD + AJ		AD + AJ		AD + AJ
AJ		AD + AJ		AD + AJ		AD + AJ
BR		BR + KH		BR + N	BR	
B	B			B + GH	B	
BL		BL + N	BL			BL + N
GH		GH + Z		GH + B		GH + Z
HB		IN + HB		HB + KHD	HB	
HD		HD + HM		HD + HM		HD + HM
HM;		HD + HM		HD + HM		HD + HM
IN		IN + HB		IN + QA	IN	
KHR	KHR		KHR		KHR	
KHD	KHD		KHD	KHD + HB	KHD	
KH		KH + BR	KH			KH + QR
MW	MW		MW		MW	
N		N + BL		N + BR		N + BL
QA	QA			QA + IN	QA	
QR	QR			QR + SL		QR + KH
QRW	QR		QRW		QRW	
SF		SF + SW	SF		SF	
SL	SL			SL + QR	SL	
SW				SW + Z	SW	
Z				SW + Z		Z + GH

*Abbreviations as in **Table 1**.

The first cluster (I) was divided into two groups (Ia, Ib). Subgroup (Ia) was composed of nine genotypes (Hmadi, Hmari, Zraqi and Ghzali, Neami, Blat, Qaisi, Mwazi, Barqawi) of which Barqawi and Mwazi branched separately. Mwazi was the most divergent from the other cultivars in this group. Subgroup (Ib) was composed of three genotypes (Khurtmani, Qraee, Inaqi) of which Inaqi branched separately. The second cluster (II) was composed of four genotypes: Adloni, Ajloni, Qrawi, with Swadi branching separately.

Table 8. Jaccard's similarity measure generated for 24 local Palestinian figs based on combined RAPD and pomological data.

Landrace*	B	SL	IN	QR	KH	HB	HD	Z	KHD	KHR	HM	QA	AJ	BL	MW	N	GH	SF	BR	QRW	SW	AD
B	1.000																					
SL	0.231	1.000																				
IN	0.188	0.103	1.000																			
QR	0.256	0.233	0.414	1.000																		
KH	0.235	0.176	0.412	0.440	1.000																	
HB	0.208	0.250	0.214	0.368	0.365	1.000																
HD	0.264	0.169	0.413	0.410	0.380	0.340	1.000															
Z	0.204	0.136	0.415	0.426	0.410	0.316	0.589	1.000														
KHD	0.215	0.182	0.293	0.258	0.227	0.244	0.290	0.234	1.000													
KHR	0.138	0.141	0.281	0.194	0.267	0.229	0.393	0.396	0.250	1.000												
HM	0.276	0.179	0.303	0.281	0.348	0.317	0.625	0.385	0.276	0.247	1.000											
QA	0.239	0.167	0.382	0.324	0.320	0.304	0.511	0.495	0.281	0.405	0.378	1.000										
AJ	0.265	0.133	0.269	0.333	0.298	0.260	0.240	0.258	0.163	0.173	0.278	0.270	1.000									
BL	0.209	0.211	0.295	0.398	0.337	0.278	0.427	0.414	0.269	0.314	0.322	0.424	0.213	1.000								
MW	0.250	0.145	0.269	0.310	0.280	0.261	0.468	0.396	0.308	0.390	0.379	0.451	0.298	0.380	1.000							
N	0.250	0.183	0.413	0.424	0.408	0.326	0.609	0.452	0.344	0.393	0.429	0.511	0.240	0.522	0.453	1.000						
GH	0.344	0.108	0.378	0.387	0.297	0.245	0.525	0.539	0.311	0.300	0.374	0.423	0.263	0.402	0.440	0.481	1.000					
SF	0.267	0.203	0.296	0.315	0.269	0.277	0.320	0.260	0.253	0.224	0.341	0.312	0.222	0.315	0.242	0.280	0.241	1.000				
BR	0.227	0.154	0.400	0.411	0.351	0.352	0.500	0.412	0.258	0.219	0.430	0.364	0.258	0.368	0.284	0.439	0.374	0.315	1.000			
QRW	0.229	0.111	0.442	0.426	0.382	0.290	0.385	0.413	0.282	0.309	0.304	0.343	0.435	0.333	0.382	0.411	0.414	0.297	0.385	1.000		
SW	0.214	0.091	0.327	0.280	0.225	0.256	0.350	0.423	0.275	0.261	0.315	0.372	0.262	0.292	0.330	0.311	0.382	0.307	0.376	0.423	1.000	
AD	0.256	0.127	0.327	0.393	0.287	0.253	0.297	0.327	0.231	0.230	0.215	0.330	0.594	0.290	0.301	0.297	0.317	0.261	0.393	0.558	0.341	1.000

*Abbreviations as in **Table 1**.

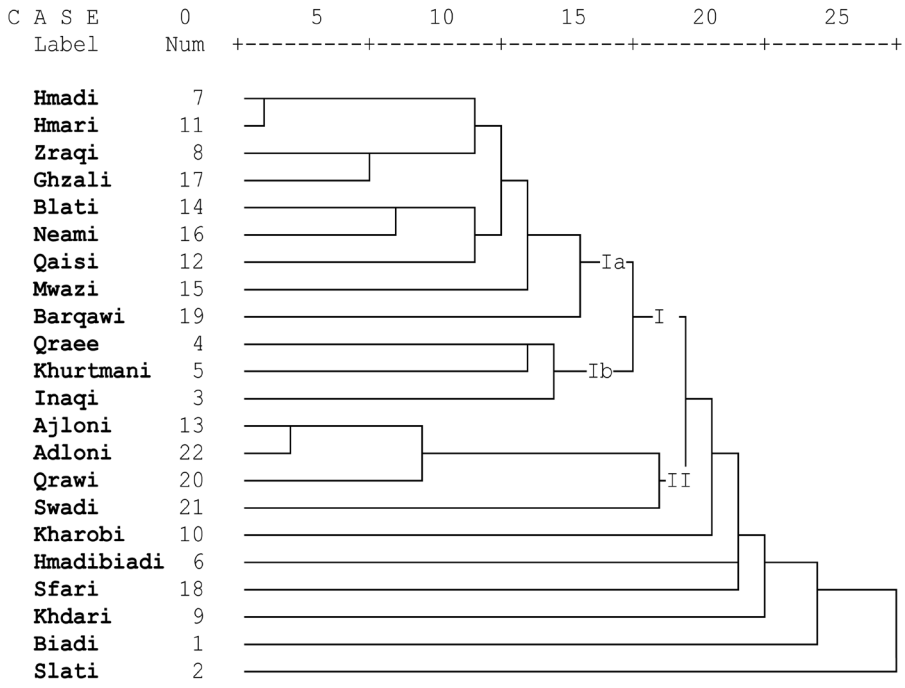

Figure 4. Dendrogram of 22 local Palestinian figs constructed by UPGMA based on combined (RAPD and pomological) markers.

Based on the genetic relationships among genotypes as illustrated by the dendrogram, generated from pomological and RAPD data by UPGMA clustering method, the following 12 genotypes: Qaisi, Mwazi, Barqawi, Inaqi, Swadi, Kharobi, Hmadibiadi, Sfari, Khdari, Biadi, Qrawi, and Slati, may be considered as distinct cultivars. The remaining genotypes may be considered as synonymous (4 landraces) (Hmadi and Hmari, and Ajloni and Adloni), or closely related landraces (6 accessions) Zraqi and Ghzali, Blati and Neami, and Qraee and Khurtmani).

It is interesting to note that clustering patterns obtained from the combined (pomological and RAPD) data had higher discriminatory power to discriminate fig landraces (16/22, 72.7%) than using either pomological (7/22, 31.8%) or RAPD (8/22, 36.4%) markers alone. Fig landraces are common in Palestine and their denomination is complicated because of morphological similarity. The main problem comes from denominating landraces based on common phenotypic traits. Discriminating of homonymous and synonymous cases in fig has also been reported by Khadari *et al.* [12], Papadopoulou *et al.* [16] and Basheer-salimia *et al.* [11] using RAPD markers or phenotypic markers [11]. In the current study, positive correlation was evidenced between morphological descriptors and RAPD markers. Thus, the combined analysis of (pomological and molecular markers) has shown to be a valuable tool for assessing the genetic diversity in figs.

4. Conclusion

Both pomological and RAPD markers are useful for elucidating in part denomination problems and relationships among fig cultivars. The limitations of the use of phenotypic-based genetic markers for assessing genetic variations in fig landraces can be minimized by the use of *ex situ* field fig genebanks where the trees are grown under the same environmental and edaphic conditions. However, the combined (pomological and RAPD) markers yield higher discriminatory power to discriminate fig landraces than using either pomological or RAPD markers alone. Wide phenotypic and molecular diversity found in the fig germplasm indicates a considerable potential for improving this crop.

Acknowledgements

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