Molecular analysis of genetic diversity among different grape accessions using DNA markers

Arwa Mujahed and Rezq Basheer-Salimia*

Department of Plant Production and Protection, College of Agriculture, Hebron University, Hebron, West-Bank, Palestine.

Received: April 15, 2019; **accepted:** June 25, 2019.

The genetic diversity of 36 local grape cultivars was investigated using RAPD markers. Among the 25 tested primers, 21 produced reasonable amplification products with high intensity and pattern stability, while the remaining 4 exhibited ambiguous, light, and non-clear complex amplification products. A total of 186 DNA fragments (loci) separated by electrophoresis on agarose gels were detected ranging in size from 150 to 1,400 bp. Of these fragments, 124 (62.5%) were polymorphic and 62 (37.5%) were monomorphic. The results also revealed an average of 7.7 loci per primer. A minimum of 2 and a maximum of 15 DNA fragments were obtained using OPG-8, OPG-15, OPR-12, and OPG-13 primers, in which the later primer is the most powerful primer and the maximum percentage of polymorphic markers was 100.0 with OPG-11 primer. The genetic distance matrix showed an average distance range from 0.07 to 0.50 with a mean of 0.29. The maximum genetic distance value of 0.50 (50%) was exhibited between Romi.Aswad.Habe.Tawele and Jandali genotypes, whereas the lowest genetic distance of 0.07 (93% similarity) was presented between Jandali.Tawel.Mofarad and Jandali-Kurawi.Mlzlz genotypes. Furthermore, UPGMA dendrogram is generally clustered the grape genotypes into two major clusters including divergent relationship. Based on these results, the cultivars tested in this study could be characterized by large divergence at the DNA level assuming that our region has a very rich and varied clonal grape genetic structure.

Keywords: grape; genetic diversity; RAPD; UPGMA and Jaccard index.

***Corresponding author:** Rezq Basheer-Salimia, Department of Plant Production and Protection, College of Agriculture, Hebron University, P.O. Box 40, Hebron, West-bank, Palestine. Phone: +972 2 2220995 ext. 165; Fax: +972 2 2229303. Email: rezqbasheer@hebron.edu.

Introduction

Grapevine (*Vitis vinifera* L.) is a crop of major economic and social importance worldwide used mainly for the production of wine and table fruit. According to the International Organization of Vine and Wine [1], 7.5 million ha is the global area under vines, and it is increasing dramatically by about 2-3% per year. The history of grapevine cultivation parallels the history of civilization along the Mediterranean basin including Palestine. Currently, grapevines in Palestine are the second important fruit crop after olive in terms of both areas covered and economic returns [2]. Due to the unique geographical and ecological environment for growing high quality table grapes, its growing and production are still restricted to the southern part of West-Bank especially Hebron and Bethlehem areas [3, 4].

Because of different biotic and a biotic cause, many of our cultivars have been subjected either to genetic deterioration and/or to disappearance leading thereby to lose a great pool of grape genetic materials that might include some interesting and promising traits such as drought and insect resistance. Therefore, determination of genetic variability and proper cultivar identification in grapevine would be of major importance in improvement programs

and in germplasm characterization and conservation to control genetic erosion. During the last two decades, many molecular markers have been implemented in several countries for cultivar identification, recognition of synonyms, and to establish genetic diversity and relatedness [5], in which amplified fragment length polymorphism (AFLP), anchored simple sequence repeats (ASSR), expressed sequence tags (EST), inter-simple sequence repeat (ISSR), random amplified microsatellite polymorphisms (RAMP), random amplified microsatellites (RAM), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), sequence characterized amplified regions (SCAR), simple sequence repeats (SSR), and single nucleotide polymorphism (SNP) are the most common techniques. In fact, DNA-based markers provided a wealth of polymorphisms, enabling the identification of cultivars and the construction of saturated genetic maps in many higher plants [6]. Among these markers, random amplified polymorphic DNA (RAPD) is one of the most widespread markers applied for several fruit trees due to its ease of use, speed, and low cost [7]. Moreover, it is used to estimate genetic distance between populations and to characterize germplasm banks or collections [7-13].

In grapes, numerous molecular studies have been conducted toward characterization of grape species in different countries [14-16]. However, few studies have been found in the literature on Palestinian grapes. The main goals of the present research were to determine the number of genetically different grapevine cultivars that were actually collected in Palestine using DNA-based RAPD technique, to infer possible cases of synonymy and homonymy, and to evaluate the genetic relationships of the characterized cultivars.

Materials and Methods

Plant materials

Healthy grape leaves were collected from the middle-region of the newly growing shoots from 36 assumed cultivars (>50 years) throughout the southern region of West-Bank (Hebron and Bethlehem districts), Palestine (Figure 1), and were stored in liquid nitrogen then at -80°C.

Figure 1. Map of West-Bank, Palestine showing the grapevine collection sites.

DNA extraction and purification

Healthy, young leaves of each assumed cultivar were ground to fine powder under the liquid nitrogen by using pestle and mortar. The DNAs were extracted by using QIAGEN DNeasy Plant Mini Kit (QIAGEN, Germantown, MD, USA). The DNA was stored at -20°C for future experiments.

Estimation of DNA quantification

DNA quality and quantity were tested on 0.8% agarose gel electrophoresis using Lambda DNA as a standard. Using spectrophotometer, other measurements were also done for DNA concentration and purity. Accordingly, final concentration of DNA was adjusted to 50 ng/µl.

Table 1. List of the used RAPD primers.

No.	Primer name	Primer sequence (5`→3`)
1	OPG-02	GGCACTGAGG
2	OPG-03	GAGCCCTCCA
3	OPG-06	GTGCCTAACC
4	OPG-08	TCACGTCCAC
5	OPG-11	TGCCCGTCGT
6	OPG-12	CAGCTCACGA
7	OPG-15	ACTGGGACTC
8	OPG-17	ACGACCGACA
9	OPG-18	GGCTCATGTG
10	OPN-05	ACTGAACGCC
11	OPN-11	TCGCCGCAAA
12	OPN-13	AGCGTCACTC
13	OPN-16	AAGCGACCTG
14	OPN-20	GGTGCTCCGT
15	OPO-05	CCCAGTCACT
16	OPT-20	ACACACGCTG
17	OPW-08	GACTGCCTCT
18	OPR-12	ACAGGTGCGT
19	OPG-13	CTCTCCGCCA
20	OPG-05	CTGAGACGGA
21	OPS-05	TTTGGGGCCT
22	OPW-13	CACAGCGACA
23	OPX-01	CTGGGCACGA
24	OPE-17	CTACTGCCGT
25	OPD-14	CTTCCCCAAG

Randomly Amplified Polymorphic DNA (RAPD)/PCR reaction mixture and program

A total of twenty-five RAPD primers "10 mer" (Sigma-Aldrich, Saint Louis, MO, USA) were used for the amplification of random DNA banding patterns according to Karatas and Agaoglu technique [17] (Table 1). PCR reactions were carried out in a 25 µl mixture containing 5 µl of DNA (30 ng), 4 µl of primer (5 µM), 2 µl of dNTPs (200 mM), 2.5 µl of 10X PCR buffer, 2 µl of magnesium chloride (25 mM), and 1.5 U of Taq DNA polymerase (Hy laboratories Ltd., Park Tamar, Rehovot, Israel). The PCR was carried out on a Peltier Thermal Cycler-200 (MJ Research. Inc, Watertown, MA, USA) with the following program: 94°C for 3 min (1 cycle), 94°C for 1 min, 53°C for 1 min, 72°C for 1.5 min (35 cycles), and 72°C for 5 min (1 cycle). PCR products were examined by 2% agarose gels at 4 volt/cm for 4h and were scored by "1" for band presence and "0" for absence. A 100 bp DNA ladder was used as standard marker. The amplicons were visualized and photographed black and white on Polaroid type film with UV trans-illuminator.

RAPD Data analysis

Data matrix was utilized to generate genetic similarity data among genotypes. Jacquard's similarity coefficient formula was employed as the following:

$$s_{ij,Jaccard} = \frac{n_{11}}{n_{11} + n_{01} + n_{10}}$$

where n_{xy} is the number of characters that have state x in individual i and state y in individual j. Unweighted Pair Group Method using Arithmetic averages (UPGMA) [18] phenogram was then calculated from the Jaccard's similarity using Fingerprint Analysis with Missing Data (FAMD) software (version 1.108 beta) (Copyright 2002-2013. Philipp M. Schlüter). TreeView software (Version 1.6.6) (Informer Technologies, Los Angeles, CA, USA) was used to visualize the resulted trees. All trees presented in this study were mid-pointed.

Results

PCR results:

Among the 25 tested primers used to investigate the pattern of genetic variation among 36 accessions of grape grown at the southern region of West-Bank, Palestine, 21 primers produced reasonable amplification products with high intensity and pattern stability (Table 2). However, the remaining four primers (OPN-11, OPN-20, OPG-17, and OPN-13) exhibited ambiguous, light, and non-clear complex amplification products, and therefore was excluded from our analysis.

Table 2. Analysis of the polymorphism obtained with RAPD markers.

primers name	Total No. RAPD bands	Monomorphic band	Polymorphic bands	Approximate band size (bp)		Polymorphic (%)	Primer case
				Min	Max		
OPG-3	8	3	5	280	1,000	62.5	Included
OPO-5	4	2	2	300	600	50.0	Included
OPE-17	11	1	10	150	1,200	91.0	Included
OPG-12	10	1	9	150	900	90.0	Included
OPN-11	9	6	3	300	1,050	33.3	Excluded
OPG-13	15	5	10	100	1,200	67.0	Included
OPN-16	11	4	7	300	1,200	63.6	Included
OPX-1	11	4	7	200	1,150	63.6	Included
OPG-5	4	1	3	300	900	75.0	Included
OPS-5	11	2	9	250	1,400	82.0	Included
OPG-2	8	4	4	380	1,200	50.0	Included
OPW-13	12	6	6	210	1,050	50.0	Included
OPD-14	9	1	8	250	1,100	89.0	Included
OPG-6	9	1	8	250	1,000	89.0	Included
OPG-11	9	0	9	150	1,200	100.0	Included
OPG-18	9	2	7	300	1,200	78.0	Included
OPN-20	6	4	2	250	1,000	33.3	Excluded
OPG-8	2	1	1	350	1,300	50.0	Included
OPT-20	5	2	3	200	800	60.0	Included
OPG-15	2	1	1	400	800	50.0	Included
OPG-17	4	3	1	500	1,200	25.0	Excluded
OPN-5	5	2	3	500	1,300	60.0	Included
OPW-8	5	2	3	350	800	60.0	Included
OPR-12	2	1	1	500	1,000	50.0	Included
OPN-13	5	3	2	350	1,000	40.0	Excluded
Total	**186**	**62**	**124**			**68.1**	

A total of 186 DNA fragments (loci) separated by electrophoresis on agarose gels were detected (Table 2) ranging in size from 150 to 1,400 bp. Of these fragments, 124 (62.5%) were polymorphic and 62 (37.5%) were monomorphic. Our results also revealed an average of 7.7 loci per primer. A minimum of 2 and a maximum of 15 DNA fragments were obtained using OPG-8, OPG-15, OPR-12, and OPG-13 primers, respectively (Table 3). Therefore, the OPG-13 primer is considered as the most powerful primer (Table 2, Figure 2). The maximum percentage of polymorphic markers was 100% with OPG-11 primer.

Genetic distances

The data matrix size analyzed included 6,696 entries with 4,443 for present loci (1) and 2,253 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 0.07 to 0.50 with a mean of 0.29. The maximum genetic distance value of 0.50 (50%) was exhibited between Romi.Aswad.Habe.Tawele and Jandali genotypes, whereas the lowest genetic distance of 0.07 (93% similarity) was exhibited between Jandali.Tawel.Mofarad and Jandali-Kurawi.Mlzlz genotypes.

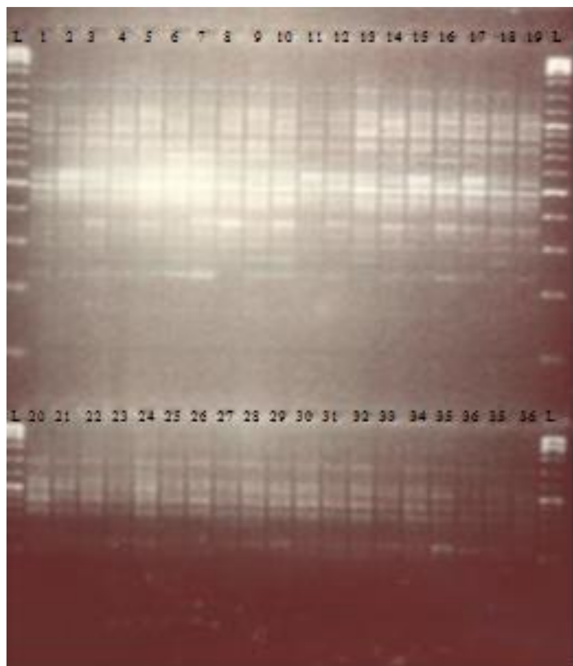

Figure 2. RAPD banding patterns generated in Palestinian grapevine cultivars using OPG-13 primer (100 bp ladder).

UPGMA analysis

UPGMA dendrogram clustered the grape genotypes into two major clusters (Figure 3). The cluster I consists of 18 genotypes and is divided into three main sub-clusters namely Ia, Ib, and Ic, which all are related to an isolated genotype (Id, Romi.Aswad.Habe.Tawela) as a distinctive cultivar. The sub-cluster Ia included two small groups (1) Jandali.Kurawi.Mlzlz and Jandali .Tawel.Mafrod, both are related to Jandali.Shabh.Dabuki; (2) Hamadani.Kadem and Marawi.Shabh.Beruti, both are related to Dabuki.Aswad.Baladi, in which all three genotypes are related to Halawani.Habe.Kabera. The sub-cluster Ib included highly related Darawishi and Mtartash genotypes. Sub-cluster Ic is composed of genotypes (Baluti.Abaid and Zaini.Habe.Tawela) and (Aswad.Baladi and Zaini.Baladi.Dabuki.Baladi), in which all related to Sulti.Khdari genotype. The minor group is composed of Marawi.Habe.Tawela and Hamadani, related to Fhaisi. The cluster II also composed of 18 genotypes and was grouped as IIa, IIb, IIc, and IId. Group IIa consists of 11 genotypes with the following relationships: Shami.Aswad, Shami.Mtartash.Mlwan, Hamadani.Ma´tr.Faranci, Marawi.Hamadani. Adi, and Romi.Aswad in addition to Dabuki and Zaini (which are closely related), Shami, Betuni, Baluti, and Bairuti. Group IIb is composed of Miskat.El.Eskandaria and Jandali (both are highly related) as well as Malikat.Libnan, Shukhi, and Halawani. Group IIc and grope IId are each composed of only one genotype Halawani.Baladi.Valantiki and Mtartash.Fhaisi, respectively and are related to the other 16 grape genotypes.

Discussion

Classical ampelographic and other morphometric methods are useful for cultivar identification but are not enough dependable for grape genotypes identification [19, 20] since they are affected by environment. In fact, the same cultivar may have different names and varied cultivars may have the same name. During the last decade, DNA molecular markers have been used intensively to characterize a wide range of plant species [2, 4, 21] including grapevines across the world. However, rare studies were done in Palestine.

In the present study, the level of polymorphism among 36 grapevine (*Vitis venifera* L.) accessions commonly grown in Palestine was estimated by using RAPD markers technique. The presence of the different patterns generated by RAPD primers shows variance between the grapevine accessions from the genetic point of view using twenty-five primers selected from the literature and based on their high ability to produce polymorphic RAPD markers. However, four primers (OPN-11, OPN-20, OPG-17, and OPN-13) were excluded from the analysis since they produced weak, unclear, and unreadable patterns in addition to their low polymorphisms (less than 50%). Other researchers reported that some primers seemed to be more efficient than others in producing stable and reproducible DNA fingerprints [22]. Indeed, primer selection is essential for discrimination analysis. Obviously,

Table 3. Jaccard´s distance index generated for the 36 local Palestinian grapevines' RAPD data.

#	Cultivar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	Mar.Hamadani.Adi	0.00																																			
2	Shami.Mtr.Mlwn	0.15	0.00																																		
3	Halawani.Bald..Valantiki	0.29	0.29	0.00																																	
4	Hamadani.Mtr.Faranci	0.13	0.15	0.20	0.00																																
5	Shami.Aswad	0.16	0.08	0.25	0.11	0.00																															
6	Romi.Aswad	0.24	0.23	0.29	0.21	0.16	0.00																														
7	Shukhi	0.35	0.30	0.32	0.27	0.27	0.24	0.00																													
8	Malikat.Lebanon	0.35	0.31	0.31	0.27	0.27	0.26	0.17	0.00																												
9	Jandali	0.29	0.32	0.28	0.26	0.26	0.24	0.29	0.27	0.00																											
10	Miskat.Eskandaria	0.32	0.31	0.31	0.25	0.26	0.26	0.29	0.23	0.22	0.00																										
11	Mtartash.Fhaisi	0.31	0.23	0.36	0.29	0.26	0.29	0.32	0.31	0.35	0.31	0.00																									
12	Halawani	0.32	0.32	0.29	0.29	0.30	0.25	0.24	0.27	0.27	0.25	0.27	0.00																								
13	Zaini	0.24	0.25	0.30	0.24	0.27	0.25	0.30	0.30	0.31	0.27	0.27	0.25	0.00																							
14	Betuni	0.21	0.23	0.30	0.21	0.22	0.28	0.29	0.29	0.28	0.26	0.26	0.22	0.20	0.00																						
15	Shami	0.23	0.16	0.31	0.23	0.19	0.25	0.30	0.32	0.30	0.32	0.26	0.27	0.22	0.14	0.00																					
16	Bairuti	0.26	0.25	0.25	0.20	0.21	0.28	0.31	0.31	0.32	0.27	0.28	0.28	0.29	0.19	0.20	0.00																				
17	Dabuki	0.24	0.26	0.29	0.22	0.27	0.25	0.33	0.32	0.34	0.29	0.31	0.27	0.18	0.24	0.22	0.23	0.00																			
18	Baluti	0.26	0.26	0.32	0.26	0.27	0.25	0.29	0.28	0.29	0.25	0.31	0.20	0.27	0.15	0.19	0.23	0.20	0.00																		
19	Salti.Khdari	0.38	0.38	0.43	0.37	0.38	0.41	0.37	0.37	0.46	0.40	0.39	0.39	0.35	0.37	0.38	0.41	0.36	0.39	0.00																	
20	Fhaisi	0.37	0.33	0.42	0.35	0.32	0.40	0.37	0.35	0.44	0.39	0.32	0.40	0.37	0.35	0.34	0.35	0.36	0.36	0.19	0.00																
21	Hamadani	0.35	0.38	0.47	0.36	0.39	0.43	0.41	0.39	0.46	0.42	0.42	0.41	0.40	0.35	0.39	0.37	0.37	0.36	0.18	0.20	0.00															
22	Darawishi	0.41	0.38	0.40	0.35	0.36	0.38	0.35	0.38	0.40	0.41	0.40	0.37	0.40	0.39	0.40	0.37	0.41	0.38	0.25	0.26	0.27	0.00														
23	Mtartash	0.36	0.36	0.40	0.34	0.32	0.35	0.40	0.40	0.41	0.39	0.39	0.36	0.37	0.31	0.35	0.35	0.39	0.36	0.27	0.23	0.26	0.14	0.00													
24	Marawi.Hba.Twela	0.25	0.28	0.39	0.25	0.28	0.35	0.38	0.37	0.38	0.36	0.41	0.39	0.34	0.32	0.34	0.33	0.33	0.35	0.22	0.20	0.18	0.24	0.21	0.00												
25	Zaini.Bal.Dabk.Bal	0.42	0.41	0.43	0.38	0.41	0.42	0.41	0.37	0.45	0.37	0.37	0.40	0.39	0.36	0.42	0.37	0.40	0.39	0.19	0.25	0.22	0.22	0.26	0.22	0.00											
26	Jandali.Twel.Mfarad	0.42	0.42	0.42	0.37	0.39	0.42	0.39	0.35	0.36	0.37	0.38	0.39	0.43	0.37	0.43	0.37	0.44	0.38	0.25	0.22	0.23	0.24	0.24	0.22	0.20	0.00										
27	Jandali.Kurawi.Mlzlz	0.39	0.41	0.40	0.35	0.38	0.39	0.39	0.35	0.39	0.37	0.37	0.40	0.43	0.38	0.44	0.37	0.43	0.38	0.25	0.24	0.23	0.22	0.22	0.21	0.18	0.07	0.00									
28	Aswad.Baladi	0.39	0.39	0.43	0.34	0.38	0.40	0.37	0.36	0.41	0.36	0.38	0.36	0.38	0.34	0.41	0.38	0.37	0.34	0.18	0.26	0.21	0.16	0.24	0.21	0.14	0.20	0.16	0.00								
29	Zaini.Hba.Twela	0.39	0.39	0.40	0.35	0.39	0.38	0.38	0.37	0.46	0.39	0.37	0.37	0.33	0.35	0.39	0.39	0.33	0.38	0.20	0.29	0.28	0.24	0.28	0.26	0.15	0.28	0.22	0.12	0.00							
30	Marawi.Shabh.Beruti	0.38	0.42	0.46	0.38	0.43	0.42	0.43	0.42	0.47	0.43	0.44	0.40	0.39	0.38	0.41	0.41	0.37	0.39	0.29	0.33	0.21	0.28	0.31	0.26	0.26	0.28	0.22	0.19	0.14	0.00						
31	Baluti.Abaid	0.40	0.39	0.42	0.37	0.38	0.40	0.40	0.35	0.47	0.39	0.40	0.41	0.37	0.36	0.38	0.38	0.36	0.38	0.17	0.25	0.24	0.27	0.29	0.25	0.17	0.25	0.21	0.15	0.08	0.17	0.00					
32	Dabuki.Aswad.Baladi	0.40	0.40	0.42	0.34	0.39	0.41	0.43	0.40	0.44	0.39	0.39	0.40	0.35	0.37	0.41	0.39	0.39	0.39	0.28	0.28	0.29	0.29	0.30	0.25	0.25	0.28	0.23	0.17	0.17	0.19	0.18	0.00				
33	Halawani.Hba.Kabera	0.39	0.39	0.36	0.34	0.37	0.39	0.42	0.41	0.46	0.43	0.40	0.42	0.43	0.39	0.42	0.35	0.41	0.43	0.30	0.31	0.26	0.26	0.27	0.27	0.23	0.27	0.22	0.22	0.22	0.22	0.23	0.22	0.00			
34	Jandali.Shabh.Daboki	0.45	0.45	0.43	0.41	0.43	0.45	0.41	0.42	0.42	0.45	0.45	0.46	0.47	0.42	0.45	0.37	0.46	0.43	0.32	0.33	0.24	0.26	0.31	0.29	0.28	0.20	0.15	0.24	0.26	0.20	0.23	0.29	0.21	0.00		
35	Hamadani.Kdem	0.37	0.40	0.46	0.39	0.41	0.42	0.42	0.41	0.46	0.43	0.43	0.41	0.41	0.37	0.41	0.40	0.38	0.35	0.26	0.28	0.15	0.25	0.28	0.23	0.23	0.25	0.19	0.15	0.21	0.08	0.18	0.20	0.20	0.18	0.00	
36	Romi.Aswad.Hba.Tawela	0.48	0.46	0.43	0.45	0.45	0.47	0.47	0.45	0.50	0.46	0.44	0.46	0.48	0.44	0.45	0.39	0.43	0.44	0.36	0.36	0.36	0.34	0.36	0.38	0.32	0.35	0.32	0.31	0.30	0.32	0.27	0.36	0.26	0.28	0.29	0.00

the more bands scored and plants studied, the higher the statistical significance of the calculation will be. About 100 bands should be enough to obtain statistically significant results [23]. In our study, among 186 bands obtained from 21 primers; 124 were polymorphic which presents high level of DNA polymorphisms (68.1%). In fact, the polymorphism presented at our study is comparably similar even higher to those exhibited by Karatas and Agaoglu who obtained 112 polymorphic bands with 65.49% [17]. Continuously, maximum number of polymorphic bands obtained in our study was 15 comparing to only 7 polymorphic band created by the previous study [17]. This implies high efficiency of our examined primers.

The values of the genetic distances ranged from 0.07 for the most closely related cultivars (Jandali.Tawel.Mafrod and Jandali.Kurawi.Mlzlz) to 0.50 for the most distantly related cultivars (Romi.Aswad.Habe.Tawela and Jandali). Therefore, we might assume that Jandali.Tawel-Mafrod and Jandali.Kurawi.Mlzlz are identical accessions with different names. The same trend goes also with Marawi.Shabh.Bairuti and Hamadani.Kadem, Zaini.Habe.Tawela and Baluti.Abiad, and Shami.Aswad and Shami.Mtartash.Mlwn by 0.08 distance. Our assumption is in agreement with the results of Francesca et al. [5] who reported that the genetic distance between the identical accessions was equal to 0. For the remaining genotypes, the genetic distance matrix showed a high level of divergence at the DNA level. Several authors also mentioned the existence of homonyms and synonyms in grapevine varieties that have been cultivated for centuries [24]. Actually, this confusion is mostly based on environmental and agronomical characteristics. In fact, ecological similarities and parallelism within the population composed of varieties adapted to a certain geographical region are important factors which should be taken into consideration [25, 26].

On the other hand, other researchers agree with the existing difficulties to detect intra-varietal polymorphism in grapevines [27, 28]. According to Ulanovsky et al. [25], the genetic intra-varietal variability has been attributed into two main factors: (1) a probable polyclonal origin of the varieties and (2) an accumulation of somatic mutations over the centuries.

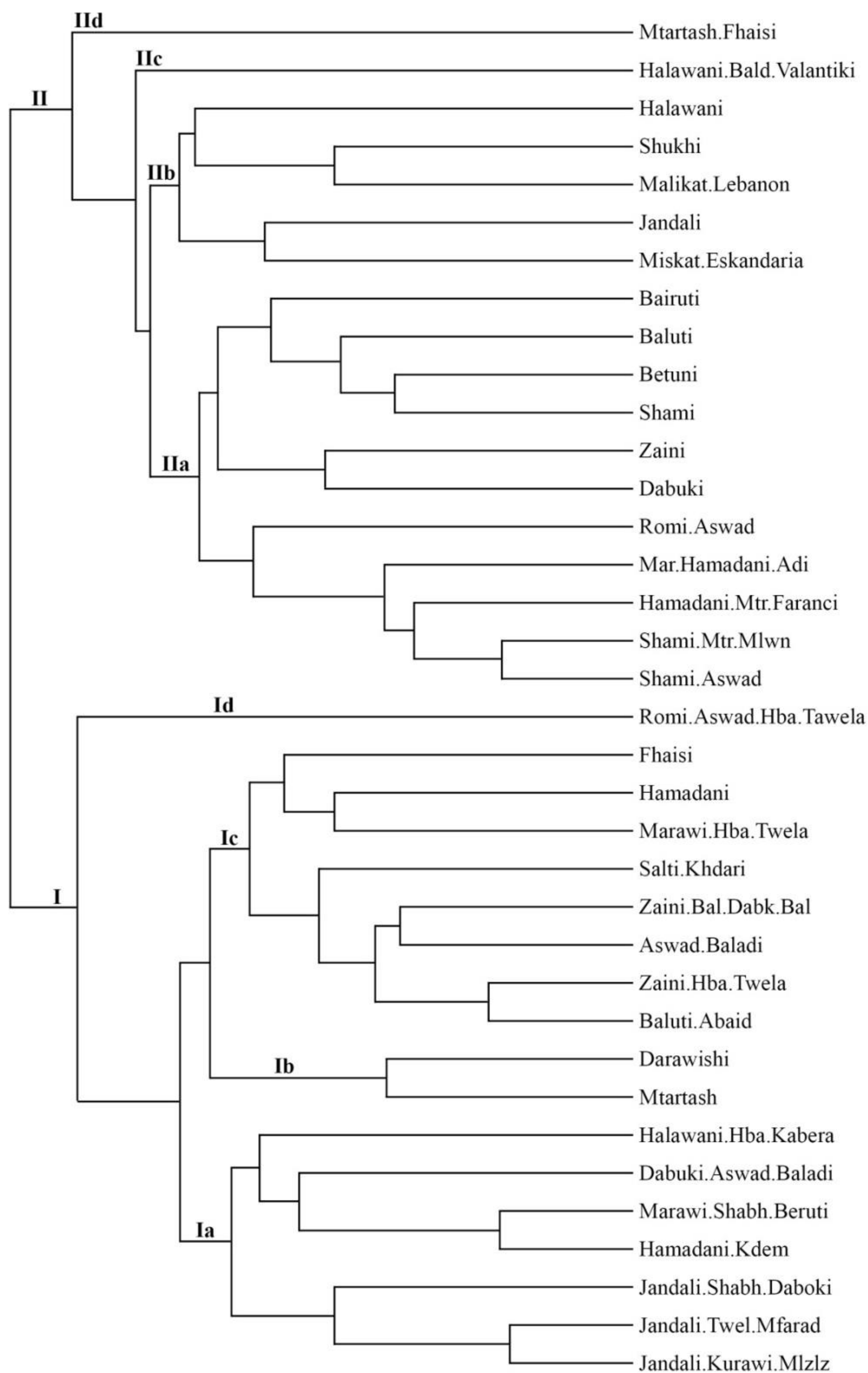

Figure 3. Dendrogram of 36 local Palestinian grapes constructed by UPGMA based on RAPD banding patterns.

The size of amplified fragments varied from 150 bp to 1,400 bp. This interval was narrower than the results obtained by previous studies [14, 29], which reported fragments between 400 bp and 2,000 bp and 200 bp and 2,500 bp for different grape cultivars in America and Canada, respectively. Indeed, the narrower amplified fragments exhibited by our cultivars might relate

to the smaller and restricted cultivated areas (Hebron and Bethlehem regions, about 2,000 Km^2) in which our grapevines were grown comparing to the very large studied areas of America and Canada.

The average of 7.7 amplicons (loci) per primer presented in our study (Table 2) was sufficient to produce useful fingerprints for many fruit crop genotypes [30-32]. Therefore, we may confidently assume that the RAPD technique can solve one of the major problems associated with varietal identification in Palestinian grapes. Based on the average genetic relatedness among the 36 examined Palestinian grape assumed genotypes, RAPD UPGMA dendrogram analysis divided the cultivars into two major clusters in which each cluster was made up of eighteen cultivars. The most closed cultivars were Jandali.Kurawi.Mlzlz and Jandali.Tawel.Mfrod (93%), Hamadani.Kadem and Marawi.Shabh. Bairuti (92%), Baluti.Abiad and Zaini.Habe. Tawela (92%), and Shami.Aswad and Shami. Mtartash.mlwn (92%). However, the most distant ones were Romi.Rswad.Habe.Tawela and Jandali cultivars (50%). Interestingly, Romi. Aswad.Habe.Tawela as well as Mtartash.Fhaisi cultivars respectively were separated and identified as distant genotypes. The results of this study may emerge that our region has a very rich and varied clonal grape genetic structure.

References

1. OIV 2017. International Statistical Report on World Vitiviniculture Situation. Pp 1-20.
2. Basheer-Salimia R, Lorenzi S, Batarseh F, Moreno-Sanz P, Emanuelli F, Stella Grando M. 2014. Molecular identification and genetic relationships of Palestinian grapevine cultivars. Mol. Biotech. 56(6): 546-556.
3. Sultan S. 2005. Grapevines, establishing, planting, training, pruning, servicing. Pp.459-464. (In Arabic).
4. Basheer-Salimia R, Camilli B, Scacchi S, Noli E, Awad M. 2015. Assessment of genetic diversity in lentils (Lens culinaris Medik.) based on SNPs. Genet. Mol. Res. 14(2): 5870-5878.
5. Francesca P, Pamfil D, Raica P, Petricele V, Sisea C, Vas E, Botos B, Bodea M, Botu, M. 2010. Assessment of the Genetic Variability among Some Juglans Cultivars from the Romanian National Collection at S.C.D. P. Valcea Using RAPD markers. Rom Biotech Letters. 15 (2): 41-49
6. Grando MS, Frisinghelli C. 1998. Grape microsatellite markers: sizing of DNA alleles and genotype analysis of some grapevine cultivars. Vitis. 37: 79-82.
7. da-Costa AF, Teodoro PE, Bhering LL, Tardin FD, Daher RF, Campos WF, Viana AP, Pereira MG. 2017. Molecular analysis of genetic diversity among vine accessions using DNA markers. Genet. Mol. Res. 16(2): 1-9.
8. Han J, Wang WY, Leng XP, Guo L, Yu ML, Jiang WB, Ma RJ. 2014. Efficient identification of ornamental peach cultivars using RAPD markers with a manual cultivar identification diagram strategy. Genet. Mol. Res. 13(1): 32-42.
9. Mendes RF, Araujo-Neto RB, Nascimento MP, Lima PS. 2014. RAPD analysis of the genetic diversity among accessions of Fabaceous forages (Poincianella spp) from the Caatinga. Genet. Mol. Res. 13(3): 5832-5839.
10. Asad HA, Meah MB, Begum SN, Khalil MI, Rafii MY, Latif MA. 2015. Study of genetic variation of eggplant cultivars by using RAPD-PCR molecular markers and the relationship with Phomopsis blight disease reaction. Genet. Mol. Res. 14(4): 17007-17018.
11. Santos M, Damasceno-Silva K, Carvalhaes M, Lima P. 2015. Genetic variation detected by RAPD markers in natural populations of babassu palm (Attalea speciosa Mart.). Genet. Mol. Res. 14(2): 6124-6135.
12. Sharaf-Eldin MA, Al-Tamimi A, Alam P, Elkholy SF, Jordan JR. 2015. Genetic relatedness of artichoke (Cynara scolymus L.) hybrids using random amplified polymorphic DNA (RAPD) fingerprinting. Genet. Mol. Res. 14(4): 18431-18439.
13. Costa L, Reiniger LRS, Heinzmann BM, Amaral LP, Serrote CML. 2015. Study of the genetic diversity and structure of a natural population of Nectandra megapotamica (Spreng.) Mez. using RAPD markers Genet. Mol. Res. 14(4): 18407-18413.
14. Pollefeys P, Bousquet J. 2003. Molecular Genetic Diversity of the French-American Grapevine Hybrids Cultivated in North America. Genome. 46: 1037-1048.
15. This P, Lacombem T, Thomas M. 2006. Historical origins and genetic diversity of wine grapes. Trends in Genetics. 22(9): 511-519.
16. Laucou V, Lacombe T, Dechesne F, Siret R, Bruno JP, Dessup M, Dessup T, Ortigosa P, Parra P, Roux C, Santoni S, Varès D, Péros JP, Boursiquot JM, This P. 2011. High throughput analysis of grape genetic diversity as a tool for germplasm collection management. Theor. Appl. Genet. 122(6): 1233–1245.
17. Karatas H, Agaoglu YS. 2010. RAPD Analysis of Selected Local Turkish Grape Cultivars (Vitis vinifera). Genet. Mol. Res. 9(4): 1980-1986.
18. Schluter PM, Harris SA. 2006. Analysis of multi-locus fingerprinting data sets containing missing data. Mol. Ecol.6: 569-572
19. Swanepoel JJ. De Villiers CE. 1987. A numerical taxonomic classification of Vitis spp and cultivars based on leaf characteristics. S. Afr. J. Enol. Vitic. 8: 31-35.

20. Sabir A, Tangolar S, Buyukalaca S, Kafkas S. 2009. Ampelographic and Molecular Diversity among Grapevine (Vitis spp.) Cultivars. Czech Journal of Genetic and Plant Breeding. 45(4): 160-168.
21. Herrera R, Cares V, Wilkınson MJ, Caligari PDS. 2002. Characterization of genetic variation between Vitis vinifera cultivars from central Chile using RAPD and inter simple sequence repeat markers. Euphytica. 124: 139-145.
22. This P, Jung A, Boccacci P, Borrego J, Botta R, Costantini L, Crespan M, Dang G. S, Eisenheld C, Ferreira-Monteiro F, Grando S, Ibanez J, Lacombe T, Laucou V, Magalhaes RCP, Meredith N, Milani E, Peterlunger F, Regner L, Zulini E, Maul E. 2004. Development of a common set of standard varieties and standardized method of scoring microsatellites markers for the analysis of grapevine genetic resources. Theor. Appl. Genet. 109: 1448-1458.
23. Kocsis M, Jaromi L, Putnoky P, Kozma P, Borhidi A. 2005. Genetic diversity among twelve grape cultivars indigenous to the Carpathian Basin revealed by RAPD markers. Vitis. 44(2): 1-5.
24. Borrego J, Andres MT, Gomez JL, Ibanez J. 2002. Genetic study of Malyasia and Torrontes groups through molecular markers. Am. J. Enol. Vitic. 53: 125-130.
25. Ulanovsky S, Gogorcena Y, Martínez de Toda F, Ortiz JM. 2002. Use of molecular markers in detection of synonymies and homonymies in grapevines (Vitis vinifera L.). Scientia Horticulturae. 92: 241-254
26. Agaoglu YS, Karatas H, Degirmenci D. 2006. Molecular characterization of some local (İskilip-Çorum) anatolian grape cultivars (Vitis vinifera L.). 9th International Conference on Grapevine Genetics and Breeding, Udine, Italia. Acta Horticul. 827: 207-210.
27. Gogorcena Y, Arulsekar S, Dandekar AM, Parfitt DE. 1993. Molecular markers for grape characterization. Vitis. 32: 183-185.
28. Sefc KM, Lopes MS, Lefort F, Botta R, Roubelakis-Angelakis KA, Ibanez J, Peji´c. I, Wagner HW, Gloss J, Steinkellner H. 2000. Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars. Theor. Appl. Genet. 100: 498-505.
29. Dalbó MA, Ye GN, Weeden NF, Steinkellner H, Sefc KM, Reisch BI. 2000. A gene controlling sex in grapevine placed on a molecular marker-based genetic map. Genome. 43: 333–340.
30. Khadari B, Lashermes P, Kjellberg F. 1995. RAPD identification and genetic characterization of fig (Ficus carica L.) genotypes. J. Genet. Breeding 49:77-86.
31. Galderisi U, Cipollaro M, Di Bernardo G, De Masi L, Galano G, Cascino A. 1999. Identification of Hazelnut (Corylus avellana) cultivars by RAPD analysis. Plant Cell. Rep. 18: 652-655.
32. Basheer-Salimia R, Awad M, Ward J. 2012. Assessments of Biodiversity Based on Molecular Markers and Morphological Traits among West-Bank, Palestine Fig Genotypes (Ficus carica L.). American Journal of Plant Sciences. 3: 1241-1251.
33. Bowers J, Boursiquot JM, This P, Chu K, Johansson H, Meredith C. 1999. Historical Genetics: The Parentage of Chardonnay, Gamay, and Other Wine Grapes of Northeastern France. Sci. 285: 1562-1565.