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Genetic Relatedness among Wild and Cultivated Almond Genotypes Using Randomly Amplified Polymorphic DNA (RAPD) Markers in Jordan

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Abstract

Randomly amplified polymorphic DNA (RAPD) technique was used to study the genetic relatedness between 16 almond cultivars (*Prunus. Dulcis)* grown at field gene bank (Al-M'shaqer Research Station), wild genotypes and farmers' orchards genotypes in Jordan. Five primers showed polymorphic bands were used for construction dendrogram and similarity matrix. Similarity values among the studied genotypes were ranged from 0.000 to 0.500. High similarity values were obtained between the two sweet almond cultivars Fuksii (0.500) and Tuono (0.480). RAPD analysis confirmed the existence of genetic variation among tested almond cultivars. Oja cultivar was the most distant compared to the rest of the cultivars, even, wild types and sweet genotypes were grouped separately. Using a minimum number of samples with the most polymorphic RAPD primers was a key for having rapid results of genetic relationships among almond genotypes.

الملخص

استخدمت تقنيةRAPD لدراسة الارتباط الوراثي بين 16 صنف لوز المزروع في الحقل الوراثي (محطة المشقر) والأنواع البرية والمزروعة في حقول المزارعين. خمسة بادئات أظهرت باندات متشعبة استخدمت لبناء الشجرة العنقودية والتشابه.قيم التشابه بين الأنواع المدروسة كانت بين صفر- 50%. أعلى قيم التشابه كانت بين نوعين من صنف اللوز فوسكي (50%) وتونو 48% . إن التحليل RAPD اثبت وجود اختلافات وراثية بين الأنواع المدروسة. صنف عوجا كان أكثر بعدا وراثيا مقارنة الأصناف الأخرى وكانت الأنواع البرية والأنواع الحلوة تجمعت في مجموعات مفصولة عن بعضهم. إن استخدام عدد قليل من العينات مع أكثر البادئات المتشعبة تعبر المفتاح للحصول على نتائج للعلاقات الوراثية بين أنواع اللوز.

Keywords: Almond; Jordan; Polymorphism; *Prunus; Dulcis;* RAPD; Wild.

1. Introduction

Almond [*Prunus dulcis* (Mill.) D. A. Webb; syn. *P. amygdalus* Batsch] which belongs to the subgenus *Amygdalus* (Rosaceae, subfamily Prunoideae) (Martínez – Gómez *et al.*, 2007), is characterized by its adaptability to arid and semi arid regions. Almonds have high nutritive value, high lipid content with concentrated energy sources and can be used in many food products. (Ahrens *et al*., 2005; Martínez –Gómez *et al*., 2007), and it is widely grown at the Mediterranean area including Jordan, Syria, Turkey and Iraq. (Martins et al. 2004; Martínez –Gómez et al., 2007).

In Jordan, wild almonds (with small nuts, hard-shelled and bitter kernels) were grown at the high mountains. Kester *et al.* (1991) reported that the native almond species predominantly have bitter kernels because of high levels of the glucoside amygdalin, and the forest department at agriculture ministry of Jordan was adopted the planting this type in order to become the source of seeds as well to cover the lands and protect the soil from erosion. Moreover, it is used as a rootstock for sweet almond and peach due to it's high adaptability to drought and disease resistance. Although, large numbers of almond native genotypes in Jordan show attractive characteristics such as color type, but most of the sweet cultivars were introduced from other countries. Twenty four cultivars were planted in Jordan, (during 1996), at Al-M'shaqer Research Station in the field gene bank for research purposes (Al-Hmoud *et al*., 2006).

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Traditionally, the identification and characterization of almond cultivars was based on morphological traits. However, such traits were not always available for analysis because of it's highly environmental effect, and it may only be visible in adult materials (Martínez–Gómez *et al*., 2007). In Lebanon, Talhouk *et al.* (2000) studied the phenotypic diversity and morphological characterization of *Amygdalus* species and found high genetic diversity of *Amygdalus communis* L., *Amygdalus korshinskyi* Hand.-Mazz., and *Amygdalus orientalis* Duh.

Molecular DNA markers have succeeded in distinguishing among accessions, clarifying synonyms, identifying mislabeled cultivars, establishing genetic similarities or geographical origins and giving hints about the process of domestication (Wünsch and Hormaza, 2002). Morphological and biochemical studies were unable to emphasis the relatedness due to their exposure to environmental influences. Therefore, the molecular marker techniques have a great potential for studying the genetic variation and relatedness among targeted cultivars. Among those markers, RAPD has the ability to detect the genetic variation among numerous of plants and animals species. The main advantages of RAPD are: adequate for a primer screening of a large number of samples, rapid scanning of the genome, higher band-sharing, greater number of loci per assay and less laborious test (Bara´nek et al., 2006 and Vidal *et al*., 1999). Wünsch and Hormaza, (2002) mentioned that RAPD markers were used by several laboratories to identify genotypes of different temperate fruit tree species.

MirAli and Nabulsi, (2003) and Shiran et al. (2007) used RAPD technique to study the genetic relatedness among Syrian and Iranian almond cultivars, respectively. Moreover, Gouta *et al*. (2007) used RAPD technique and reported that the similarity values among 58 almond cultivars were ranged from 0.45 to 0.94. RAPD markers have been used to characterize *Prunus* rootstocks from different species (Casas *et al*., 1999) such as studying the genetic relationship among grapevine varieties (Vidal *et al*., 1999) and determining the diversity level among 24 Iranian pomegranate genotypes (Sarkhosh *et al*., 2006).

Since no molecular information is available regarding almond cultivars grown in Jordan. This study aimed at determining the genetic relationships among 16 almond cultivars grown in the field gene bank at Al-M'shaqer Research Station, farmers' orchards genotypes and wild type genotypes using RAPD technique. The results of this study will be used for monitoring, management and conservation of almond types in the future.

2. Materials and Methods

2.1. Plant Material

Fresh leaves were collected from 16 almond cultivars grown at the field gene bank of Al-M'shaqer Research Station in Jordan, 23 genotype of farmers' orchards, 4 unknown and 12 wild types (Table 1).

2.2. DNA Isolation

DNA was obtained after grounding the stored leaves (4ºC) of almond samples in liquid nitrogen (-196ºC), the quality and quantity of genomic DNA were detected on agarose gel. Total cellular DNA was extracted, using procedure as described by (Doyle and Doyle, 1987), with minor modifications of chemical concentrations. Approximately 18 to 20 mg of fresh and stored leaves were ground and mixed with 750 µl of freshly and preheated 2x CTAB solution with 0.8g PVPP in 2ml tubes then placed at 65ºC for 30 min. The mixture was incorporated with 750 µl of chlorophorm/isoamyl alcohol (24:1), vortexed for few seconds, and then centrifuged at 14,000g for 20 min. The supernatant was placed in 2ml tubes with 600ml isopropanol, and then shaked until the thread of DNA was appeared, then centrifuged for 20 min at 14000g. The solution is poured in tubes and left to dry, then 600 µl of cooled 70% ethanol was added to the solution and placed in the refrigerator (–20ºC) overnight. Next day, ethanol was poured in the dried tubes and 100µl of TE was added and the whole mixture was placed at 65ºC for 30min. Four microlitters of RNase (10mg/ml) were added per tube and left for 45min at 37ºC. DNA quantity was measured using S2100 UV/VIS DIODE-Array-Spectrophotometer, machine Version 1.7.

2.3. PCR Amplification

PCR reaction was performed as described by Williams *et al.* (1990) with 10-mer ologonucleotides synthesized by Opern technologies (Almeda, Calif.). The final volume of 25µl containing, 10x buffer, 20ng of total genomic DNA, 10 mM dNTPs, 100 µM of primers, 1.5Mm MgCl2 and 1U of *Taq* polymerase. Amplification was carried out in thermocycler (MJ Research model PCT-100), one cycle of 1 min at 94°C followed by 44 cycles, each consisting of a denaturation step for 1min at 94°C, followed by an annealing step for 1min at 36°C and an extension step for 2 min at 72°C, followed by a further extension step for 5 min at 72°C. After the final cycle the samples were cooled at 4°C. Samples of 10 µl were analyzed by electrophoresis on 1.4% agarose gel and the amplified products were detected after staining by ethidium bromide.

The RAPD-PCR product was detected in 1.4% agarose mixed with 0.5X TBE. Forty 10-mer primers, corresponding to kits A, B, C, N and Z by Operon Biotechnologies, were initially applied to the whole group of genotypes. Each primer was applied twice. Only repeatable fragments with strong and medium intensity were evaluated.

2.4. Data Analysis

RAPD polymorphic bands were scored as present (1) or absent (0) and the estimation of similarity among all accessions were calculated according to Ne and Li, (1979). The matrix of similarity was analyzed by the Unweighted Pair-Group Method (UPGMA) and the dendrogram was obtained using SPSS, V. (11.0), software to estimate genetic similarities with the Jaccard's coefficient.

3. Results

From 40 initially applied primers, only 6 showed reproducible fragments with easily recordable fingerprints. When screening all the 16 cultivars, 4 unknown genotypes, 16 sweet genotypes, 12 wild and (3 Awajee, one awajee,

Table 1. List of almond cultivars, wild genotypes and frames orchard genotypes grown in Jordan.

Type/ name	Source	Type/ name	Source
1-Wild	Wadi Shouiab /Sult/ farmer orchard	29-Wild	Alaal- Irbid / farmer orchard
2-Wild	Wadi Shouiab /Sult/ farmer orchard	30-Awajee	Samarwsan/ farmer orchard
3-Sweet*	Wadi Shouiab /Sult/ open fields	31-Sweet	Samarwsan/ farmer orchard
4-Sweet	Wadi Shouiab /Sult/ open fields	32-Sweet	Samarwsan/ farmer orchard
5-Sweet	Wadi Shouiab /Sult/ open fields	33-Sweet	Samarwsan/ farmer orchard
6-Sweet	Wadi Shouiab /Sult/ open fields	34-Sweet	Samarwsan/ farmer orchard
7-Sweet	Wadi Shouiab /Sult/ open fields	35-Sweet	Samarwsan/ farmer orchard
8-Flakee	Ajloun/ farmer orchard	36-Sweet	Samarwsan/ farmer orchard
9-Wild	Ajloun/ farmer orchard	37-Sweet	Samarwsan/ farmer orchard
10-Wild	Ajloun/ farmer orchard	38-Sweet	Samarwsan/ farmer orchard
11-Wild	Ajloun/ farmer orchard	39-Sweet	Samarwsan/ farmer orchard
12-Wild	Ajloun/ farmer orchard	40-Hamah	Field gene bank Al-M'shaqer Research Station /NCARE
13-Unknown	Sakhrah / Ajloun/ farmer orchard	41-Primorski	Field gene bank Al-M'shaqer Research Station /NCARE
14-Unknown	Sakhrah / Ajloun/ farmer orchard	42-Douma 1	Field gene bank Al-M'shaqer Research Station /NCARE
15-Unknown	Sakhrah / Ajloun/ farmer orchard	43-Chellaston	Field gene bank Al-M'shaqer Research Station /NCARE
16-Unknown	Jerash/ Balilah/forest reserve	44-Douma 3	Field gene bank Al-M'shaqer Research Station /NCARE
17-Wild	Jerash/ Balilah/ forest reserve	45-Ne Plus Ultra	Field gene bank Al-M'shaqer Research Station /NCARE
18-Wild	Jerash/ Balilah/ forest reserve	46-SF121	Field gene bank Al-M'shaqer Research Station /NCARE
19-Wild	Jerash/ Balilah/ forest reserve	47- Shami fark	Field gene bank Al-M'shaqer Research Station /NCARE
20-Wild	Jerash/ Balilah/ forest reserve	48- Princesses	Field gene bank Al-M'shaqer Research Station /NCARE
21-Wild	Jerash/ Balilah /forest reserve	49-Ardshwar	Field gene bank Al-M'shaqer Research Station /NCARE
22-Shami	Koufrawn/ farmer orchard	50- Ardoma	Field gene bank Al-M'shaqer Research Station /NCARE
23-Awjah	Koufrawn/ farmer orchard	51-Dafadii	Field gene bank Al-M'shaqer Research Station /NCARE
24-Fark	koufrawn/ farmer orchard	52-Texas	Field gene bank Al-M'shaqer Research Station /NCARE
25-Awajee	Alaal- Irbid/ farmer orchard	53-Fuksii	Field gene bank Al-M'shaqer Research Station /NCARE
26-Awajee	Alaal- Irbid/ farmer orchard	54-Tuono	Field gene bank Al-M'shaqer Research Station /NCARE
27-Sweet	Alaal- Irbid/ farmer orchard	55-Oja	Field gene bank Al-M'shaqer Research Station /NCARE
28-Sweet	Alaal- Irbid/ farmer orchard	----------	----------

* local name

Table 2. Statistical reading of RAPD analysis.

Primer name	Total bands/primer	Number of polymorphic bands	% of polymorphism	Max./ Min. band per primer
OPA05	119	35	29	3/10
OPA17	133	32	24	1/8
OPA20	293	45	15	2/9
OPN14	174	25	14	1/7
OPN16	261	39	15	1/9
Total band	980	Average : 35.2	Mean: 19.4	

one Flakii, one Fark and one Shami cultivars, from farmers orchids) five primers (12.5% of primers were polymorphic) shown polymorphism in the whole group analysis. Total number of bands, number of polymorphic bands and percent of polymorphism and maximum/minimum number of bands per primer were depicted by Table 2. A total of 980 RAPD fragments (Table 2) and 176 bands of them were polymorphic in the whole genotypes group and generating 14.2% polymorphism. Among the used primers, OPA05, OPA17, OPA20, OPN14 and OPN16 with recognized fingerprints showed the best suitability for identification, management, conservation and genetic study of almond species. Representative amplification patterns resultant from primer OPA20 and OPN16 shows at Figure 1 and Figure 2.

The number of bands detected by each primer depends on primer, sequence and the extent of variation in specific genotype (Shiran *et al.*, 2007). Therefore, the number of bands varied in different genotypes. In this study, number of bands varied from one to ten bands with an average of 8 bands.

High levels of similarity ranged between 0.500 to 0.000 (Table 3). All almond cultivars were the most distant from

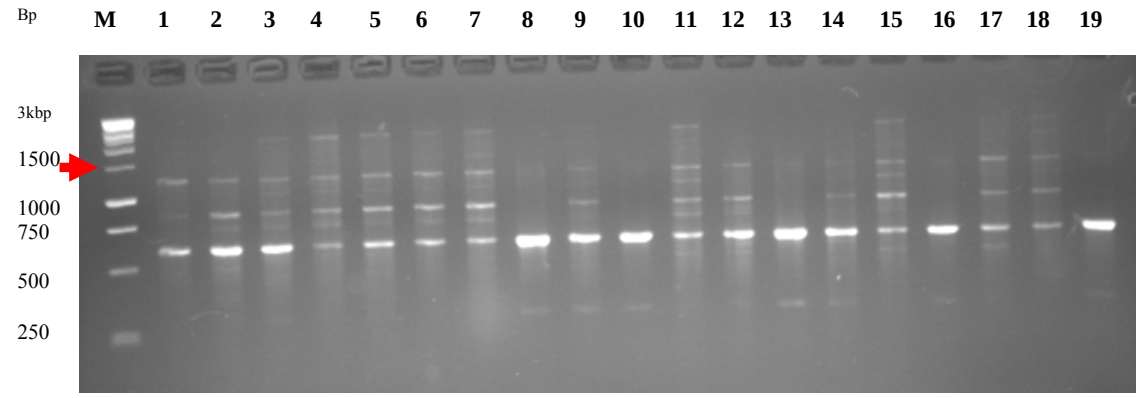

Figure 1. PCR-RAPD profiles generated from different genotypes of almond using primer **OPA20**. Lane 1: Wild ; lane 2: Wild ; lane 3: Sweet ; lane 4: Sweet; lane 5: Sweet; lane 6 : Sweet; lane 7: Sweet; lane 8: Flakee; lane 9: Wild; lane10: Wild; lane11: Wild; lane12: Wild; lane 13: Unknown; lane 14: Unknown; lane 15: Wild; lane 16: Wild ; lane17: Wild; lane 18: Wild; lane 19: Wild; Lane M: 1kb ladder (Promega).

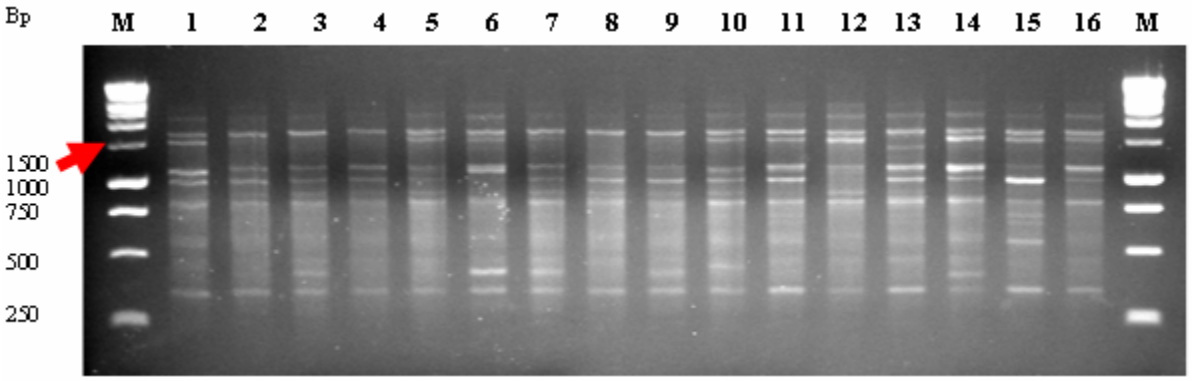

Figure 2. Agarose gel electrophoresis of DNA fragments amplified from different cultivars of almond using primer **OPN16**. Lane 1: Hamah; lane 2: Primorski; lane 3: Douma 1; lane 4: Chellaston; lane 5: Douma 3; lane 6: Ne Plus Ultra; lane 7: F121; lane 8: Shami fark; lane 9: Princesses; lane 10: Ardshwar; lane 11: Ardoma; lane 12: Dafadii; lane 13: Texas; lane 14: Fuksii; lane 15: Tuono; lane 16: Oja; Lane M: 1kb ladder (Promega).

Table 3. Highest and lowest similarity values among selected almond genotypes grown in Jordan.

Series No.	Type	Similarity value	Series No.	Type	Similarity value	Series No.	Type	Similarity value
1Wild	5 sweet 26 awajee 11 sweet	0.267 0.150 0.158	12Wild	15unknown 28sweet	0.304 0.280	23Awajee	25Awajee 27sweet 29wild 31sweet 7sweet	0.452 0.343 0.360 0.276 0.303
2- Wild	21 wild 34 sweet	0.278 0.206	13Unknown	16unknown 30awajee	0.412 0.222	24Fark	26Awajee 29wild 31sweet	0.419 0.379 0.280
3- Sweet	4 sweet 5 sweet 6 sweet 15unknown	0.333 0.269 0.280 0.292	14Unknown	17wild 18wild	0.429 0.200	25Awajee	29wild 33sweet 31sweet 45Ne Plus Ultra	0.438 0.429 0.31 0.000
4- Sweet	7sweet 33sweet 32sweet 29wild 5sweet	0.433 0.258 0.250 0.250 0.242	15Unknown	18wild 48Princesses 8 Flakee	0.292 0.000 0.241	26Awajee	27sweet 28sweet 29wild 24Fark	0.368 0.353 0.316 0.419
5 Sweet	11 sweet 37 sweet 33 sweet	0.222 0.189 0.200	16Unknown	31sweet 40Hamah 41Roma 44Douma3 45NePlusUltra 48Princesses 49Ardshwar	0.200 0.000 0.000 0.000 0.000 0.000 0.000	27Sweet	26Awajee 23Awajee 28sweet 27sweet	0.368 0.347 0.263 0.222
6 Sweet	7 sweet 12 wild 15 unknown	0.393 0.292 0.250	17Wild	14unknown 13unknown 18wild 43Chellaston	0.429 0.412 0.391 0.000	28 Sweet	24Fark 23awajee 26awajee 28sweet	0.379 0.364 0.353 0.200

				44Douma 3	0.000		31sweet	0.172
7 Sweet	33 sweet 23 Oja 25 awajee	0.310 0.303 0.257	18 Wild	19wild 40Hamah 43Chellaston 44Douma 3 17wild	0.333 0.000 0.000 0.000 0.391	29Wild	25awajee 31sweet 33sweet 43Chellas-ton	0.438 0.310 0.250 0.000
8 Flakee	16unknown 13unknown	0.333 0.238	19Wild	21wild 46SF121 51Dafadii 18wild	0.240 0.000 0.000 0.333	30Awajee	31sweet 10wild 36sweet 41primorski	0.333 0.286 0.227 0.000
9Wild	17wild 19wild 30Awajee	0.296 0.000 0.250	20 Wild	21wild 32sweet	0.154 0.135	31Sweet	30awajee 32sweet 43Chellas-ton 45NePlus Utra	0.333 0.320 0.000 0.000
10Wild	50Ardoma 30Awajee 31sweet 16unknown	0.000 0.286 0.208 0.208	21Wild	31sweet 27sweet	0.241 0.211	32Sweet	31sweet 33sweet 35sweet 45NePlus Ultra	0.320 0.296 0.292 0.000
11 Wild	17wild 26awajee	0.265 0.238	22Shami	24Fark 26Awajee	0.236 0.278	33Sweet	25awajee 7sweet 34sweet 35sweet 51Dafadii	0.429 0.310 0.269 0.154 0.000
Series No.	Type	Similarity value	Series No.	Type	Similarity value	Series No.	Type	Similarity value
34Sweet	35sweet 40Hamah 41Roma 43Chellst-on 45Ne Plus Ultra	0.318 0.000 0.000 0.000 0.000	42Douma 1	43Chellast. 44Douma3 47Shami 40Hamah	0.452 0.400 0.303 0.345	50Ardoma	51Dafadii 34sweet 53Fuksii 10 wild 13unknown	0.263 0.257 0.237 0.000 0.000
35Sweet	36sweet 40Hamh 41Rroma 45Ne Plus Ultra 47Shami 48Princesses 53Fuksii	0.227 0.000 0.000 0.000 0.000 0.000 0.000	43Chellaston	44Douma3 45Ne Plus 55Oja 12wild 17wild	0.286 0.229 0.225 0.000 0.000	51Dafadii	53Fuksii 52Dafadii 54Tuono	0.306 0.297 0.294
36Sweet	30awajee 41Roma 43Chellaston 44Douma3 50Ardoma 54Tuono	0.227 0.000 0.000 0.000 0.000 0.000	44Douma3	45NePlus Ultra 47ShamiFark 13 unknown 17 wild	0.333 0.265 0.000 0.000 0.000	52Texas	53Fuskii 54Tuono 55Oja 38sweet	0.424 0.375 0.324 0.000
37Sweet	38sweet 41Primorski 12wild	0.500 0.167 0.000	45NePlusUltr-a	46SF121 47ShamiFark 55Oja	0.250 0.242 0.257	53Fuskii	54Tuono 55Oja	0.483 0.297
38Sweet	43Chellaston 40Hamah 52Texas 12wild	0.189 0.000 0.000 0.000	46SF121	48Princesses 54Tuono 50Ardoma 47Shamifark	0.345 0.367 0.286 0.345	54Tuono	55Oja 10wild	0.250 0.000
39Sweet	42Douma 44Douma3 40Hamah 43Chellaston 3sweet 22Shami	0.324 0.286 0.273 0.263 0.000 0.000	47ShamiFurk	48Princesses 49Ardshwar 52Texas	0.345 0.227 0.215	55Oja	52Texas 53Fuskii 54Tuono	0.324 0.297 0.250
40Hamah	41Primorski 44Douma3 45Ne Plus Ultra 34sweet 12wild 17wild	0.462 0.393 0.345 0.000 0.000 0.000 0.000	48Princesses	51Dafadii 53Fuksii 52Texas 50Adoma 13unknown	0.333 0.323 0.313 0.236 0.000	--------	---------	---------
41Primorski	42Rouma 3 45Ne Plus Ultra 43Douma3 12 wild 16 unknown 22 Shami 34 sweet	0.414 0.345 0.294 0.000 0.000 0.000 0.000	49Ardshwar	50Ardoma 55Oja 52Texas	0.225 0.220 0.195	----------	----------	--------

the rest in the dendrogram. The highest average similarity index value among all varieties (0.48) was observed between Fuksii and Tuono. Most sweet genotypes showed high similarity values with each other (Table 3), with value more than 0.172. On the other hand, among 5 unknown genotypes, only two of them showed high similarity with sweet genotype (0.292 and 0.250) which could be confirmed that those are sweet genotypes, two registered 0.238 and 0.333 similarity values with Flakee variety, one unknown had 0.208 similarity with wild type. Sweet genotype showed high similarity values with Awajee genotype (0.429, 0.368, 0.347, 0.364, 0.353 and 0.438). Fark genotype had 0.419 similarity with Shami, Awajee showed 0.419 similarity with Awajee.

For almond cultivars, Hamah showed 0.462 similarities with Primorski and 0.000 similarity with sweet and wild genotypes, Primorski had 0.414 similarity with Douma 3 and 0.000 similarity value with Shami and sweet genotype. Douma 3 had 0.452 and 0.400 similarity with Chellaston and Douma 3, respectively, and 0.000 similarity with wild. Douma 3 has 0.333 similarity with Ne Plus Ultra. SF 121 had 0.367 with Touno. Shami Fark had 0.345 similarity with Princesses whereas, Princesses with Dafadii has 0.333 similarity value. Ardoma had 0.263 similarity with Dafadii whereas Dafadii had 0.306 similarity with Fuskii, but the former had 0.483 similarity with Fuskii. Oja had 0.324 similarity with Texas. However, there is no relationship between almond cultivars, wild and unknown genotypes. Based on the dendrogram, Oja was the most distant followed by one sweet genotype. Moreover, a dendrogram can be divided arbitrarily into three main clusters.

The first cluster contained, 4 unknown genotypes, 16 Sweet genotype, 11 wild, 2 Awajee, one Oja, one Flakii, one Fark, one Shami cultivar. This was divided into three sub-clusters, the first sub-cluster contained the most related genotypes (sweet-sweet genotype) with 0.500 similarity (Figure 3 and Table 3) two unknown, Flakii, Awajee, Sweet, Sweet, wild Sweet and Sweet). The second sub-cluster includes two unknown genotype, 5 wild genotypes and one sweet genotype. The third sub-cluster has 4 wild genotypes, one unknown, 10 sweet genotypes and the following cultivars including Oja, 2 Awajee, Fark and Shami.

The second major cluster contained only one wild genotype. The third major cluster contains 16 cultivars together with the most related cultivars (Tolono and Fuchsia). The third major cluster was divided into three sub-clusters, the first one contained Fuksii, Tuono, Texas, SF121, Princesses, Dafadii and Ardoma. While the second one includes Shami Fark and Redshawer and the third one contained Roma, Chellaston, Duma 3, Hamah, Primorski, Ne Plus Ultra, and Oja in addition to one sweet genotype.

4. Discussion

Knowledge of the genetic diversity and relationships among cultivated species of *Prunus* is important to recognize it is gene pool in order to identify pitfalls in germplasm collections, and to develop effective conservation and management strategies (Aradhya *et al*., 2004). Present study showed a high level of genetic variation existed in Jordanian almond gene pool as indicated by the wide range of similarity index values generated by using RAPD markers between cultivars and genotypes. These findings agreed with results obtained by Shiran et al. (2007). Presence of genetic variability among tested genotypes lead to the suitability of RAPD for determining genetic relationship either within cultivar or with other genotypes. The relatedness of the studied cultivars was efficient and good established through the use of RAPD markers.

Shiran *et al.* (2007) and Martinez-Gomez *et al*. (2003) used RAPD technique for almond and concluded that RAPD technique has the ability to discriminate between Sefied and Monagha almond cultivars. In the current study, wild types were clustered separately from other cultivars which mean that wild types were not ancestor of cultivated almonds and could be belong to other sources (rootstock); this result was supported by results obtained by Shiran *et al*. (2007). The lower similarity values and more divergence dendrogram branch points of wild species and cultivars demonstrate the greater genetic variability of these plant materials.

Wild almond species plays a socio-economical and ecological role as well they have been used for different purposes by native people including direct consume, grazing of livestock or oil extraction (Zeinalabedini *et al*., 2007). In this study, wild species which is usually considered as the origin of almond cultivars was found separately placed in one main cluster away from cultivars. Same results was observed by Shiran *et al*. (2007) who mentioned that the two wild species of almond (*P. orientalis* and *P. scoparia* were clustered out of the rest of cultivars and genotypes. The presence of wild crop relatives and the continuous flow of genes among and within plant species determined the rise of new genetic variability (Chessa and Nieddu, 2005). Considering the similarity matrixes values it is concluded that two varieties of Oja and Awajee were introduced from Syria are similar based on the similarity value of 0.452 (Table 3 and Figure 3). Because sweet genotypes were sub-grouped with awajee, it is concluded that all sweet genotypes could be Awajee with wild ancestor. Present study showed that almond genotypes or cultivars with similarity values less than 0.200 can be considered as genetically differs. However, Shiran *et al*. (2007) reported that the accessions of almond showing at least 20% genetic differences from each other may be regarded as distinct cultivars. The results obtained by RAPD indicated that this technique was efficient in detecting genetic similarities in almond. However, the results obtained from this study help in management, collection, monitoring and conservation of Jordanian almonds. Using techniques such as simple sequence repeats (SSR) and amplified fragment length polymorphic DNA (AFLP) in further investigation.

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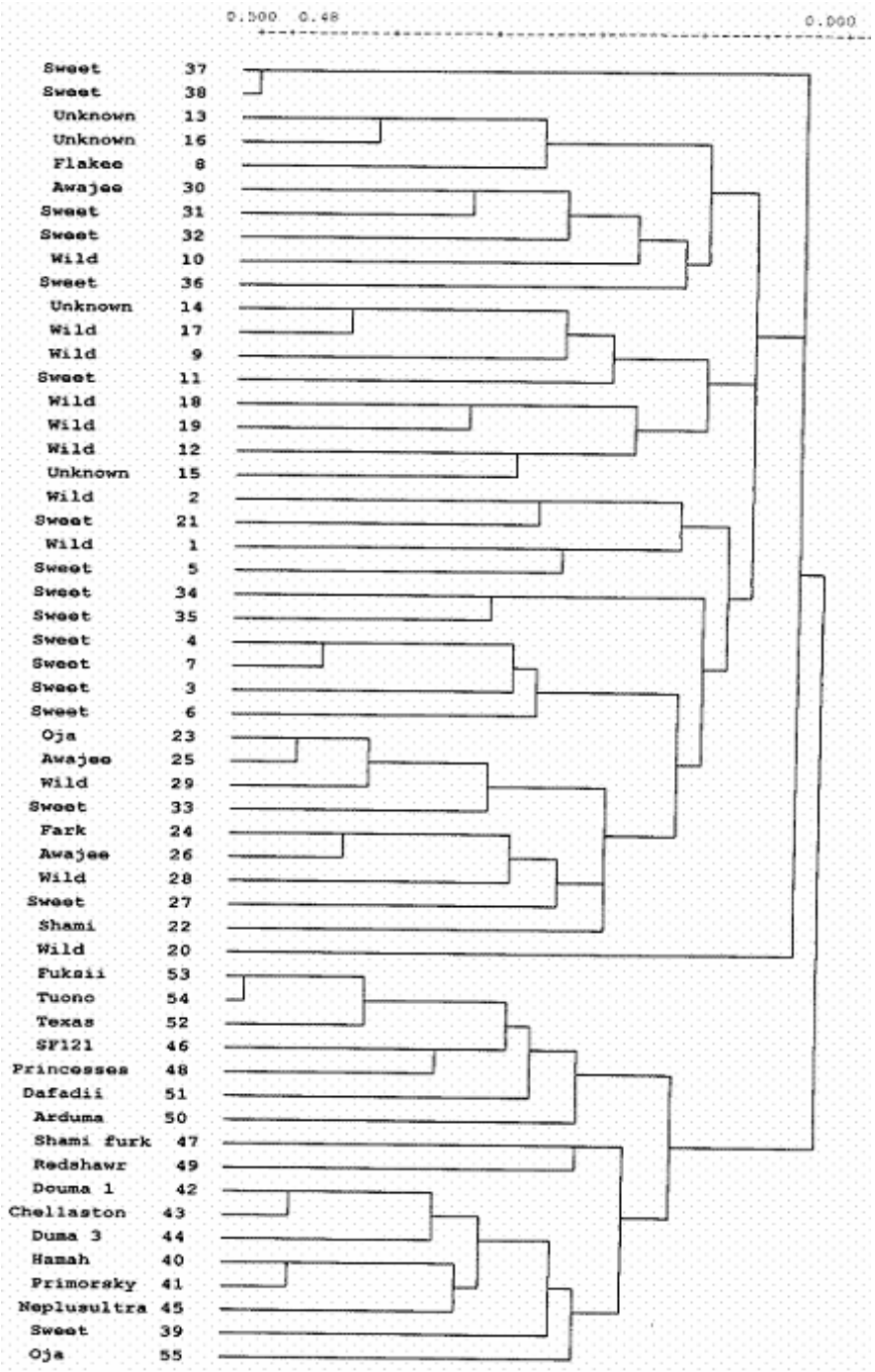

Figure 3. A dendrogram among wild type genotype, farmers orchid genotypes and cultivars of almond by using five RAPD primers.

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