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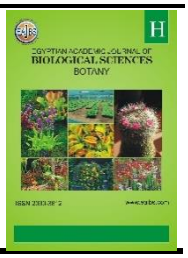

Determination of Genetic Variation and Fingerprinting of Some Genotypes of (*Prunus Armeniaca*) In Salah Al-Din -Iraq

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ABSTRACT

This research included studying the genetic markers for nineteen cultivars of apricot, comprising: Khudrawi1, Heshari, Sharika, Saboni, Labibi, Zaghini, Zingili, Herfi, Aisha, Tariq1, Tariq2, Al-Mostafa, Pigeon eggs, Al-Qaissi, Laozi, Khudrawi2, Khawki, Khairi and Orange Hashari. Hereditarily, it was employed one of the markers that depend on PCR, was Randomly Amplified Polymorphic DNA (RAPD), in order to detect the genetic variation among cultivars and to find genetic distance and cumulative analysis for nineteen samples. DNA was isolated from the plant leaves, then the RAPD reactions were employed by using sixteen primers; the products then were electrophoresed on a 1.5% agarose gel. It was noticeable that the primer which gave more bands is OP D-18, it gave 154 bands; its efficiency was 10.9, while its differentiation capacity was11.1. The variety rate of the cultivars by using this primer was 100%. On the other hand, the less producible primer was OP H-16, it produced only 29 bands and had less efficiency (2), while its differentiation capacity was 2.1. The variety rate of the cultivars by using OP H-16 was100%. It must be mentioned that the summation of the unique bands that appeared by using primers was 28 bands distributed among the studied cultivars. Khudrawi2 was butter luck, it owned 12 unique bands. It's clear that the genetic markers had a role in the morphological variation, isolation of apricots' cultivars and classifying them. The genetic tree which depended on RAPD primers showed that the most relative cultivars were Tariq1 and Al-Mustafa from Al-Alam; their relative rate was (0.9209), so they are regarded as the most relative variety. Whereas, the maximum genetic distance was 0.3220, which appeared between Zengili and Khudrawi2. The next was 0.3446 between Sharika and Khudhrawi2, then 0.3672 between Khudhrawi2 and Tariq2.

INTRODUCTION

Apricot (*Prunus armeniaca*): It is a type of tree, and according to the scientific classification established by scientists for this plant, it belongs to the known genus: peach; in Latin: (Prunus). This genus belongs to (Rosaceae) family. Among the stone fruits, the apricot tree is one of the deciduous fruit trees and reveals the highest genetic divergence, showing robust relation between the cultivars (Salazar 2022). In Iraq, there are many Local varieties (phenotypes) of *Prunus armeniaca*, including Khudrawi1, Heshari, Sharika,

Saboni, Labibi, Zaghini, Zingili, Herfi, Aisha, Tariq1, Tariq2, Mostafa, Pigeon eggs, Al-Qaissi, Laozi, Khudrawi2, Khawki, Khairi, Orange Hashari. Genetic diversity is a prerequisite to improving any type of agricultural crop.

To ensure long-term changes to the unpredictable ecological environment, genetic diversity is essential (Sreekanth *et al*., 2012). The evaluation of the genetic variability of crop germplasm is essential for selecting superior genotypes and preserving those resources at a high risk of being destroyed (Ouborg *et al*., 2006, shah *et al*., 2021).

Evaluating the range and distribution of genetic variation between types of crops and their relatives is very necessary for understanding the pattern of diversity and the evolutionary relationships between them, which help improve the plant in a more systematic way (Sheikh *et al*., 2021). It has been established that genetic diversity analysis during gross vegetative development necessitates a long and extensive interpretation. It's worth mentioning that genetic diversity is essential to ensure long-term changes to the irregular ecological environment. (Sreekanth *et al.*, 2012).

To evaluate the variability and genetic characterization of apricot and other stone fruits, numerous kinds of genetic markers have been employed, such as SSR, RAPD, AFLP, and RFLP (Ballester and de-Vicente, 1998; Hurtado *et al*., 2002; Hormaza, 2002; Wang *et al.*, 2011; Yilmaz *et al*., 2012; Shah *et al*., 2020). In apricot, RFLP and RAPD markers have been used to identify cultivars and to group them depending on their genetic similarity (Sheikh *et al*., 2021).

Molecular parameters played an important role in the genetic characterization and improvement of a large number of plant species. They also contributed to expanding our capabilities in estimating biodiversity and establishing trees that determine the degree of kinship of genetic "DNA" types and the relationships between them (Sheikh *et al*., 2021).

Random Amplified polymorphism of the DNA strand, which is characterized by its simplicity, Polymorphic DNA (RAPD), speed, its lack of requirement for a large amount of DNA, and its applicability to large genetic populations, in addition to the possibility of using in this technique (Universal) random or generic primers, to cover different regions of the genomes of individuals studied. Randomly amplified polymorphic DNA (RAPD) analysis has been used to study genetic relationships in a number of fruit trees. In most cases, data on genetic similarity obtained by RAPD analysis matched classifications based on morphological and agronomic traits (Mir *et al*., 2012).

The aim of the study research is determination the genetic diversity and fingerprint of some local verities of cultured (*Prunus armeniaca*) in Salah al-Din - Iraq.

MATERIALS AND METHODS

Sample Collection:

Samples were collected from the cultivated and exotic apricot plant *Prunus armeniaca* in Salah El-Din Governorate during the time period 4-20 to 6-1- and experienced farmers and breeders were used for diagnosis based on many quantitative characteristics and the general shape of the tree.

Genomic DNA Isolation:

Genomic DNA was extracted from recently arisen leaves of apricot genotypes using CTAB method. DNA was extracted from a single tree of each accession. Approximately 1 g of tissue samples from each kind of apricot were snap frozen in liquid nitrogen. DNA was purified and quantified spectrophotometrically (Mir *et al*., 2012).

RAPD-PCR Primers and Program:

The chosen primers in this study are illustrated in Table 1. The program of RAPD-PCR was mutual for all employed primers as followed: 94oC (4min) for initial denaturation,

93oC (45sec) denaturation, 38oC (45sec) annealing, 72oC (90sec) extension and 72oC (7 min) for final extension (Abdulrahman *et al*., 2020).

Table 1: The RAPD primers.

No.	Primer	Sequence'5 →→→ 3'	No.	Primer	Sequence'5 →→→ 3'
1.	OP A-01	CAGGCCCTTC	9.	OP B-20	GGACCTTAC
2.	OP A-06	GGTCCCTGAC	10.	OP C-16	CACACTCCAG
3.	OP B-04	GGACTGGAGT	11.	OP C-10	TGTCTGGGTG
4.	OP B-12	CCTTGACGCA	12.	OP D-03	GTCGCCGTCA
5.	OP B-14	TCCGCTCTGG	13.	OP D10	GGTCTACACC
6.	OP C-08	TGGACCGGTG	14.	OP D-18	GAGAGCAAC
7.	OP H-16	TCTCAGCTGG	15.	OP G-02	GGCACTGAGG
8.	OP J-04	CGGAACACGG	16.	OP G-08	TCACGTCCAC

DNA Molecular Size Estimation:

The molecular sizes of DNA are estimated by electrophoresis on an agarose gel, using a lambda DNA ladder with known molecular weight. (Ausubel *et al.*, 2003).

Statistical Analysis:

The results of the doubling operations of the primers used in the RAPD indicators were taken in tables based on the comparison of the presence or absence of DNA pieces for different samples, as it symbolizes the presence of a piece of DNA with the number (1) and its absence by the number (0). The genetic dimension coefficient, as well as the similarity coefficient between the studied species, were calculated using Nei's coefficient 72 (Nei and Li 1979), then a combinatory analysis was conducted and a genetic dimension diagram was drawn between the inputs using the method: The unweighted pair group method for the arithmetic average (UPGMA). (Sneath and Sokal 1973,). Statistical analyzes were carried out by computer using the program: Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc, Rohlf, 1993).

The conclusion of the results of this program was based on the equation (1972, Nei) to detect genetic similarity by creating a sequential table that includes the results of all the prefixes with all the models studied. The equation states: (Nij / Ni + Nj)] × GD = 1- 2)
Since Nij: represents the number of packets shared between the model j, i.

Ni: represents the number of beams in form i.

Nj: represents the number of packets in the form j.

RESULTS AND DISCUSSION

Among other DNA-based approaches, randomly amplified polymorphic DNA PCR, amplifications of DNA segments using short primers of random nucleotide sequence have been used to generate specific profiles or genomic fingerprints that are used to compare the genotypic diversity among organisms, for example, apricots' genome or whole apricots' tree communities (Guti´errez *et al.*, 2011). (RAPD)-PCR were applied by using 16 primers in order to measure the genetic diversity of apricots' varieties and evaluate whether they are related to each other or not. All employed primers gave amplified fragments, which sizes varied in size from 20- more than 2000 bp. It must be mentioned that the primer OP D-18 gave more bands than the other primers 154 bands, its efficiency was 10.9, while its recognizing capacity was 11.1 as is clear in Tables (2 and 3); so, it might be the best one among other employed primers because it was able to cover many spaces on the genome (Alasie, 2002). On the other hand; the primer OP H-16, which gave the least bands among other primers, its band was 29 only, its efficiency was 2 only and the recognition capacity

was 2.1; that might due to the limited locations on the genome which let the primer OP H - 16 to bind. The number of bands is essential to describe the variety and discriminate it among other apricots' types; they give us primary categorization for each group of apricots.

Most of the bands were polymorphic bands; only three primers gave monomorphic bands which were: OPA-06, OPC-08 and OPC-16; their bands were remarkable at the sizes: 700bp, 1700bp and 700bp respectively as it is shown in Table (3) and Figure (1). There were also many noticeable unique bands; that distinguish apricots' varieties from each other, especially Al-Qaissi and Khadrawi2, these two kinds had many unique bands and the primer OP D-18 resulted in two unique so clear shined bands, and their molecular weight is more than 1500bp. It was appeared also, that heavy bands (due to high copy number) for some varieties at determined molecular sizes with the primers: OP A-06, OP C-O8, OP C-10, OP D-10, OP D-18 and OP G-08. It is noticeable that khadrawi2 was so special cultivar due to having 12 unique bands by using these all primers.

Table 2: Is clarifying the unique bands, primer efficiency and recognizing capacity.

primer		P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16		
Band size		400-1200	20-1400	20-1300	210-1800	200-1200	250-1300	600-1200	300-1800	290-1300	400-900	220-1500	400-1500	600-1700	250-1650	250-2000	300-1600		
1	absence	5	9	7	4	5	6	2	9	6	3	11	10	5	7	12	8	-	30
2	unique	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	5	
	absence	2	10	6	4	3	5	2	9	6	3	11	8	5	8	1	8		
3	unique	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
	absence	3	7	6	4	3	6	3	7	5	2	10	7	8	6	9	7		
4	unique	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	absence	3	9	7	8	3	5	3	9	5	2	10	6	6	6	9	6		
5	unique	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	absence	3	9	7	5	4	6	3	10	5	3	10	7	8	7	8	7		
6	unique	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	absence	2	9	6	4	3	6	3	8	5	2	12	6	5	7	7	8		
7	unique	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	absence	2	9	6	3	0	6	3	8	4	1	10	7	4	6	7	5		
8	unique	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	absence	2	10	6	4	2	4	3	8	5	2	10	6	6	5				
9	unique	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
	absence	3	9	6	6	4	7	3	9	5	3	9	6	5	5	9	5		
10	unique	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	absence	2	2	8	6	4	3	4	2	9	4	3	8	6	6	5	11		
11	unique	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	absence	3	10	6	4	1	5	1	11	4	1	9	7	7	5	10	5		
12	unique	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	absence	3	9	6	6	2	4	2	9	4	3	8	8	6	4	11	6		
13	unique	1	1	1	-	-	1	1	-	-	1	-	1	-	-	-	-	6	Unique band suggested
	absence	4	9	7	3	7	5	1	11	4	3	9	9	6	6	12	7		
14	unique	-	1	-	-	-	-	-	2	-	-	-	-	-	-	-	-	3	
	absence	4	10	8	8	7	7	3	5	5	3	9	5	8	11	11	9		
15	unique	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	absence	3	10	6	3	8	5	3	9	5	2	9	7	4	7	10	7		
16	unique	-	4	4	-	-	-	-	-	1	-	4	1	-	-	-	-	12	
	absence	7	8	6	8	10	7	3	8	6	7	8	7	7	11	10	9		
17	unique	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	absence	2	9	6	5	6	4	1	8	5	2	10	7	5	6	6	3		
18	unique	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	absence	3	10	6	4	6	4	2	8	4	2	11	7	7	8	14	8		
19	unique	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	absence	2	9	6	3	3	6	3	8	5	1	11	9	7	6	14	6		
efficiency		5.3	6.4	4.8	4.3	9.2	5.7	2	7.5	8.5	4.6	6.7	9.3	6.7	10.9	5.9	8.6		
Recognition capacity		4.3	5.4	4.8	4.3	9.4	4.8	2.1	7.6	8.6	4.7	6.8	9.4	6.8	11.1	6	8.7		

RAPD responses are so sensitive and variable that if any component concentration or any condition of their action were altered; then, result achievement is non-reproducible without optimization (AL- Asie, 2002). In the analysis, Band density is a regarded distinguishing if the optimization was achieved, it explains the alteration of copy number in organisms then more description for the studied samples. Moreover, it could be trusted by a number of the produced bands. Their different molecular weight, shining intensity and presence or absence of them in different studied samples (Abdulrahman 2020; Altaiee, 2018). All these varieties are giving evidence of polymorphism and high genetic diversity among studied samples (Gaber *et al.*, 2018). Bands' presence caused to possession of the primer of the paired sites which allows it to bind with the studied genome or not; depending on that, varieties between samples appear and so, the importance of band presence is the same as its absence. The other rules in primer binding depend on the size of the studied genome and primer sequence, which differ in binding depending on alteration even in one nucleotide in the same primer or genome (AL-Asie, 2002).

Table 3: Is clarifying the polymorphism percent and monomorphic bands.

No.	Primer name	Location numbers	Monomorphic Location No.	Polymorphic Location No.	Bands summation	Monomorphic Bands no.	Polymorphic Bands No.	Unique bands number	Absence band number	Polymorphism Percent %
1	OP A-01	7	1	6	75	15	60	1	58	86%
2	OP A-06	15	1	14	91	16	75	6	173	93.33%
3	OP B-04	10	-	10	68	-	68	6	120	100%
4	OP B-12	8	-	8	61	-	61	-	90	100%
5	OP B-14	12	-	12	130	-	130	-	80	100%
6	OP C-08	11	1	10	81	16	65	1	102	90%
7	OP H-16	6	-	6	29	-	29	1	46	100%
8	OP J-04	14	-	14	106	-	106	3	163	100%
9	OP B-20	11	-	11	119	-	119	-	92	100%
10	OP C-16	6	2	4	65	36	29	1	48	66.7%
11	OP C-10	15	-	15	95	-	95	4	185	100%
12	OP D-03	14	-	14	131	-	131	2	122	100%
13	OP D10	11	-	11	95	-	95	-	115	100%
14	OP D-18	15	-	15	154	-	154	-	126	100%
15	OP G-02	14	2	12	83	32	51	5	180	86%
16	OP G-08	13	-	13	121	-	121	-	126	100%
17	Sum.	181	7	174	1504	115	1389	30	1826	\

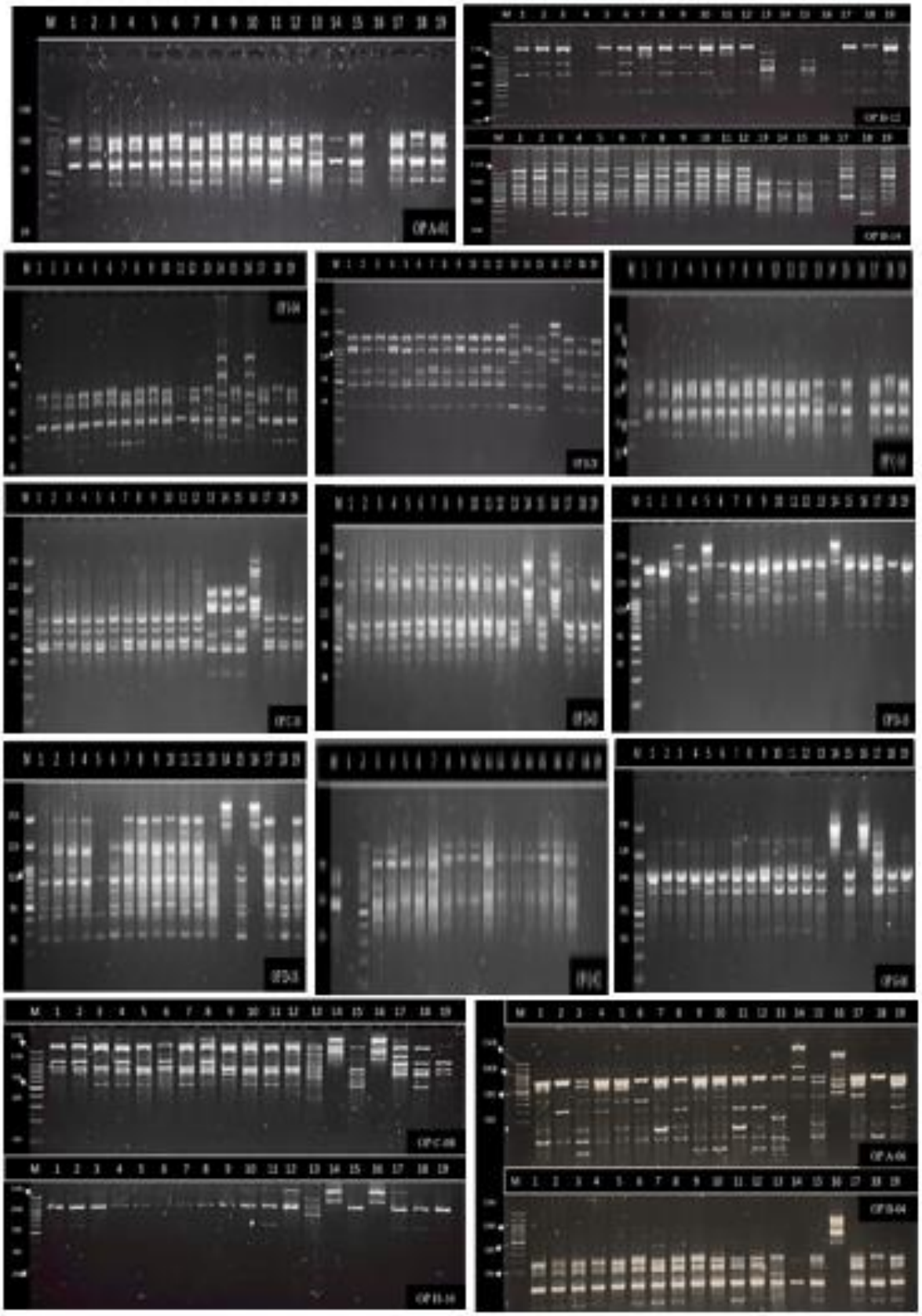

Fig.1: RAPD electrophoresis for 19 apricots samples on 1.5% agarose gel by using 16 primers.

Mutations (insertion, deletion and substitution) and recombination during cell division play role in genome variation. This variation may happen at primer binding sites, which causes alteration in the arrangement of the remaining nucleotides, then prevents primer attachment at its specialized site on the genome (Williams *et al*., 1990).

So, the absence of the main band in one sample without the other is regarded as an important marker to distinguish the genetic distance of this sample. Besides, the dissimilarity in molecular weight (band size) and the number of bands is also another rule that RAPD depends on to find genetic diversity. The variance in band size is due to the distance between two binding sites of the primer that will be polymerized in every cycle of the PCR (AL-Asie, 2002).

RAPD reaction results were achieved in valuing the genetic distance for the studied local apricot varieties by special application on the computer; they showed that the minimum genetic distance was 0.9040, which seemed between Tariq1 and Mustafa. The next closeness was 0.9040 between Herfi and Heshari; then 0.8983 between zengili and Tariq2. Whereas, the maximum genetic distance was 0.3220, which appeared between Zengili and Khudrawi2. The next was 0.3446 between Sharika and Khudhrawi2, then 0.3672 between Khudhrawi2 and Tariq2 as it's shown in table 2. According to the genetic distance data, a dendrogram for all varieties was primed; two main clusters appeared (A and B), which included other sub-clusters as it's presented in a chart (4-4). The first sub-cluster (B1) was divided into two groups the first one is also divided into branches whereas the second one included the variety Labibi (5) only. The second sub-cluster (B2) included: Pigeon eggs and Laozi. The second main cluster (A) included Al-Qaisi (14) and Khudhrawi2 (16) only as it is shown in Table (4) and Diagram (1).

It's not necessary to correlate the phenotype of these varieties with genotype because they might be affected by their environments (Cosmulescu *et al*., 2010) especially temperature, soil nature and nutrition that are added to the variety (Karatas, 2022); so it is must study the genomic content in order to improve it (Muzher, 2004).

Table 4: The closeness and distance rates between 19 apricots' variety

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	1.0000																		
2	0.8023	1.0000																	
3	0.8023	0.8079	1.0000																
4	0.8192	0.7910	0.8588	1.0000															
5	0.7684	0.7175	0.8305	0.8136	1.0000														
6	0.8023	0.8192	0.8305	0.8475	0.7740	1.0000													
7	0.7797	0.7853	0.8414	0.8588	0.7966	0.8305	1.0000												
8	0.8136	0.8305	0.8757	0.8701	0.8079	0.8757	0.8757	1.0000											
9	0.7910	0.7627	0.8305	0.8249	0.8418	0.7853	0.8079	0.8531	1.0000										
10	0.7740	0.7458	0.8136	0.8148	0.7910	0.7797	0.8362	0.8701	0.8814	1.0000									
11	0.7797	0.7514	0.8079	0.8362	0.7740	0.7740	0.8983	0.8644	0.7966	0.8588	1.0000								
12	0.7853	0.7797	0.8249	0.8418	0.7684	0.7910	0.8136	0.8701	0.8475	0.9209	0.8475	1.0000							
13	0.7232	0.6158	0.6497	0.6554	0.6384	0.6384	0.6158	0.6497	0.6836	0.6667	0.6497	0.6667	1.0000						
14	0.5367	0.5085	0.5311	0.5480	0.5989	0.5650	0.6330	0.5537	0.5537	0.5367	0.5198	0.5480	0.5537	1.0000					
15	0.7571	0.6949	0.7401	0.7345	0.7514	0.7627	0.7514	0.7740	0.7401	0.7119	0.7175	0.7119	0.7514	0.5537	1.0000				
16	0.4181	0.3898	0.3446	0.3729	0.4350	0.3898	0.3220	0.3672	0.4237	0.3842	0.3672	0.4068	0.4463	0.7006	0.4124	1.0000			
17	0.7853	0.8136	0.7910	0.7853	0.7797	0.8136	0.8136	0.8475	0.8249	0.8079	0.8023	0.7853	0.6215	0.5141	0.7345	0.3842	1.0000		
18	0.8757	0.7797	0.7910	0.7966	0.8023	0.8249	0.8023	0.8588	0.8023	0.7853	0.7910	0.7740	0.6780	0.5593	0.7797	0.3840	0.8192	1.0000	
19	0.8757	0.8249	0.8588	0.8531	0.7684	0.8362	0.8136	0.9040	0.8362	0.8531	0.8136	0.8418	0.7006	0.5706	0.7571	0.3729	0.8305	0.8757	1.0000

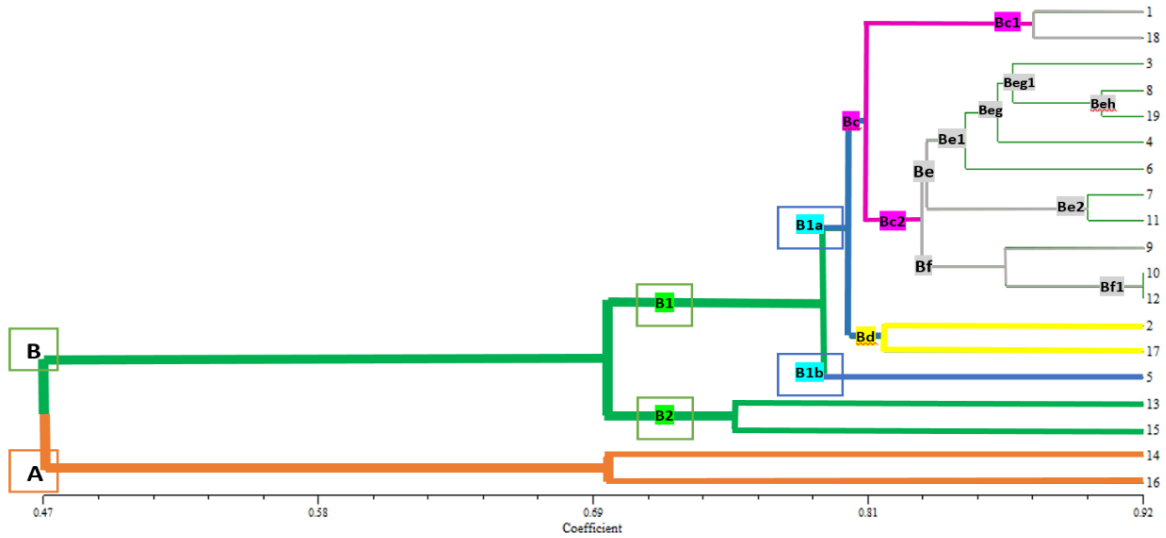

Diagram 1: Genetic relationship tree.

Conclusion

In a conclusion, the present study provides one of the first detailed data describing the genetic characteristics of local and cultured (*Prunus armeniaca*) in Salah Al-Din -Iraq. The results indicated considerable variation in most of the varieties like Zengili and Khudrawi2 whose genetic distance was 0.3220. On the other hand, there was a noticeable correlation between Tariq1 and Mustafa, with a minimum genetic distance of 0.9040. the best primer was OP D-18 due to its many bands (154 bands) that overdid the other primers; On the other hand, the primer OP H-16, which gave the least bands (29 bands only) among other primers, due to the limited locations on the genome which let the primer OP H -16 to bind.

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