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## **Molecular characterization of some apricot *Prunus armeniaca* L. and plum *Prunus domestica* L. cultivars grown in northern Iraq using RAPD markers**

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**Abstract**---The current research aimed to use the Random Amplification Polymorphic DNA (RAPD) markers based on the polymerase chain reaction (PCR) technique to find genetic variation between six cultivars of *Prunus armeniaca* L. and *Prunus domestica* L. planted in North Iraq. DNA was extracted from young plant leaves, Thirteen primers were used in RAPD reactions, and samples were migrated using electrophoresis using an agarose gel. The results showed that the concentration of the extracted DNA ranged between (25.8 - 66.3) µg/ml, and the results showed a discrepancy in the number and molecular size of the DNA bands for each cultivar, as the total number of bands reached (1038), of which 882 were polymorphic, and 156 monomorphic bands. The results showed that the primer (OPF-20) gave the highest number of bands, which amounted to (118), while the primer (OPA-19) gave the lowest number of bands, which amounted to (51), and the results recorded the highest value for the genetic dimension and it was between the two cultivars Showtime and Pissardi, which amounted to (0.887), while the two cultivars Priana and Sndyany recorded the lowest genetic dimension among the cultivars, reaching (0.065), and the synthesis analysis showed that the cultivars of the two species under study were divided into two main groups. The first group included all apricot cultivars, which were divided into two sub-groups, which in turn were divided into several sub-groups, and the second major group included all plum cultivars, which were divided into two sub-groups.

**Keywords**---apricot, plum, RAPD markers, molecular characteristics.

## Introduction

The apricot plant *Prunus armeniaca* L. and plum *Prunus domestica* L. belong to the genus *Prunus* and to the subfamily Prunoideae of the Rosaceae family, which is of great importance among stone fruits because its fruits are classified as important and desirable almond fruits [1]. The Rosaceae family is one of the large plant families, as it includes globally 122 genera and 3370 species, while in Iraq it includes about 19 genera and 50 wild and farmed species [2]. The plants of this family are spread all over the world, as they are found in abundance in East Asia America, and Europe [3]. In Iraq country, it is more abundant in the mountainous northern regions, and it is either in the form of trees, shrubs (standing or ascending) or herbs [4].

The two plants apricot *Prunus armeniaca* L. and plum *Prunus domestica* L. are located within the largest genus of the Rosaceae family is the genus *Prunus*, which includes several types of fruits of high economic and nutritional value because they contain proteins, fats, carbohydrates, fiber, many minerals, and salts, as well as many important vitamins, which also earns them to the medical benefit [3].

Despite the efforts made to use different methods to study the phenotypic characteristics of plant cultivars, which were considered one of the mainstays in the past and used to distinguish cultivars, these methods are affected by environmental conditions as well as the need for a long time, and also cytological studies are often unable to detect simple variances on the chromosome, so it is necessary to support these methods with modern biotechnology that works to characterize the inputs and determine the variance and genetic similarity between them, these methods include the use of DNA markers called molecular indicators, which are characterized by the stability of their results and are not affected by environmental factors, the type of tissue, the age stage of the plant and its large number, as well as its ability to detect changes in DNA, not only in the coded regions of the DNA, but also in noncoded regions that make up approximately 50-90% of the size of the genome of higher organisms, and this technique has been developed for use in plant breeding programs in addition to being considered to be markers to help speed up election and education processes [5].

Several studies have been conducted using molecular indicator technologies in the characterization of plants at the DNA level as an effective tool, as these studies have shown that molecular markers are valuable evidence in describing and evaluating genetic variation within plant species [6]. Random amplification polymorphic DNA (RAPD) is considered one of the most important direct applications of PCR, which is characterized by its speed, simplicity, low cost, need for small quantities of samples, and possible use of random or generic primers, as well as being uncomplicated and without radioisotopes, thus facilitating the possibility of use in many States as used in genetic identification, they are characterized by their ability to differentiate between different species or cultivars, or even strains that follow one class [7]. Following the evolution of apricot and

plum cultivation in recent years in Iraq, the Ministry of Agriculture was able to introduce new cultivars planted under a special program. Horticulture and forestry stations in the governorates and districts have been mandated to produce seedlings for cultivation [8]. This current research is aimed at diagnosing apricot and plum cultivars and determining their genetic dimension by relying on the technique of RAPD-PCR.

## **Materials and Methods**

### **Samples collection**

Surveys of the genus *Prunus* L. were conducted through field visits in various areas, including the Nineveh horticultural station and some northern areas of the Dohuk, Zakho, Zaweta, and Erbil horticulture station, and its fields, which were diagnosed by the Ministry of Agriculture and Cultivated in those areas that its ages ranged in from 4 to 10 years. Six cultivars of *Prunus armeniaca* L. were Sayeb, Canion, Castelbrite, Priana, Sndyany, Zaginia and six of plum *Prunus domestica* L. cultivars were Songold, Freedom, Showtime, Pissardi, Italica, and Sant Rosa were studied.

Plant leaves were collected in mid-April and young, newly developed leaves ranging in age from (3-4) weeks for each plant type under study, free of bacterial and insect disease injuries, and were thoroughly washed with distilled water, placed inside nylon bags and stored in the refrigerator at a temperature of 8°C until use.

### **DNA Extraction and Electrophoresis**

The extraction was carried out at the RNA lab in Nineveh province, where DNA was extracted from young soft leaves using a commercially equipped extraction kit by Korean Favorgen/Biotech CORP, 90 mg of leaves were taken from each plant cultivar, and grinded using a sterile ceramic mortar until a sticky liquid was obtained, the liquid was taken using micropipette and placed in plastic Eppendorf tubes separately from impurities, then the concentration and purity of DNA were estimated by nanodrop device, and then 5 microliters of extracted DNA were separated by electrophoresis using agarose gel at a concentration of 0.9%.

### **DNA Analysis of cultivars**

Thirteen primers were used and provided by the Korean company Macrogen as installed their sequences (nitrogen bases) as in Table 1. RAPD reactions were conducted as pointed out by Ercisly *et al.* [9]. On DNA samples isolated from apricot and plum cultivars studied, electrophoresis was performed on an agarose gel dyed with ethidium bromide for a duration (25-30) minute, then the gel was exposed to Transilluminator-UV ultraviolet radiation source and the volumes of the amplified DNA bands were estimated depending on the distance traveled by the bands within the agarose gel compared to the standard DNA ladder guide.

### Statistical analysis

Results shown on agarose gel when converted into tables were analyzed (binary character), where (1) was placed when the band was present and (0) when the band was absent, and the molecular size of the amplification products was estimated when compared with the ladder. In order to create the genetic relationship between the approved varieties, characterization Data was converted to similarity Data, and to analyze these data, genetic similarity values were estimated using the spacing and convergence factor by the Nei and Li method [10]. The Numerical Taxonomy and cultivar Analysis System have been used. The genetic dimension between the two classes has been calculated based on the number of bands shared between the two classes using all primers, thus creating the relationship between any of the two classes studied to include all of the cultivars, then the data of genetic similarity and genetic dimension were analyzed using synthesis analysis Dendrogram based on the computational mean method of unweighted marital groups (Unweighted pair group method for the arithmetic average) UPGMA [11], as the cultivars are distributed into groups, very similar cultivars remain in one group and similar groups are associated in larger groups with a lesser degree of similarity... Thus, using the same PAST statistical program as mentioned by Rohlf [12], the phylogenetic tree is identified, which shows the genetic relationship between the apricot and plum cultivars studied, and the percentage of promoter efficiency is calculated by converting the presence or absence of the band (0,1) through the following equation:

Primer efficiency = total number of primer bands/total number of all primers bands× 100.

The differential power of each indicator is determined by the following equation:

Differential power = number of variant bands of the primer/number of variant bands for all primers x 100.

### Results and Discussion

The results showed that the DNA concentration of the studied cultivars ranged between (25.8 - 66.3) µg/ml, while its purity ranged between (1.65 - 1.80).

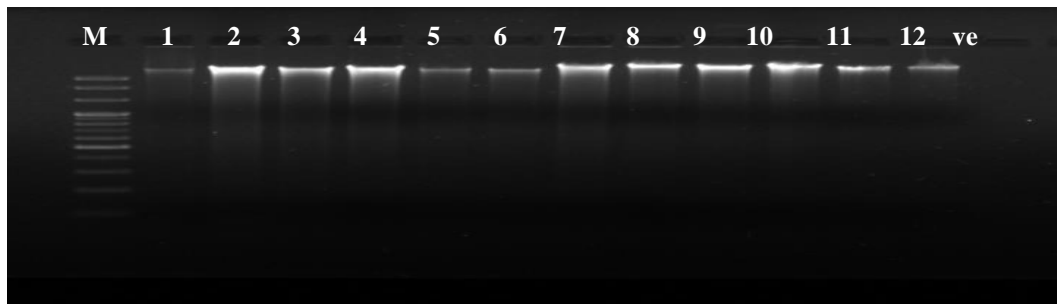


Figure (1): DNA isolated from studied apricot and plum cultivars.

1.Sayeb 2.Canion 3. Castelbrite 4.Priana 5. Sndyany 6. Zagina 7. Songold  
8.Freedom 9. Showtime 10. Pissardi 11. Italica 12.Sant rosa

The results of the reactions of RAPD technology for thirteen primers that migrated on the agarose gel showed a clear discrepancy in the number of amplified DNA bands and in their molecular sizes. As different types of bands were found, their total sum was (1038) bands, and the total number of sites produced by the primers amounted to (205). The average number of (15.76) bands for each primer and (13) of them are main sites at a rate of (1) for each primer and (192) polymorphic sites at a rate of (14.76) bands for each primer, whose molecular sizes ranged between (35 - 4000) bp and was characterized by OPF -20 with the highest number of sites and bands that were (21) sites that gave (118) bands, while the primer OPA-19 had the lowest yield of sites and bands that reached (10) sites and (51) bands. As shown in Table (1) and (2). And this is in agreement with [13].

The identification of cultivars at the level of DNA using RAPD indicators indicates the finding of the fingerprint for each of the two studied species, in which the amplified regions are distributed over the cultivars using a specific primer, that is, the number of bands and their molecular size, which is a characteristic of that cultivar without the rest of the cultivars. Finding the genetic fingerprint is an identity for that cultivar that can be used by plant breeders [8]. The results of this study were analyzed by depending on the genetic relationship in the presence or absence of the bands resulting from amplification of a certain region of the genome of the plants studied and on the molecular sizes of those bands that depended on the number and complementary regions of the primer sequences on the template DNA strand, the very light bands resulted were neglected, and this was confirmed by [14].

RAPD markers depend mainly on the number of bands shown by the gene of any model, which in turn represents the number of sites that the primer finds and binds to it, and the difference in the number, types and sizes of bands in the studied apricot and plum cultivars is due to the ability of the primer to find and determine its link sites that fit a specific size of the gene within the DNA, and this is evidence of the existence of genetic variation between cultivars based on the sequence of nitrogenous bases in the DNA, and the variation occurs as a result of induced or endogenous mutations that affect the distance between the sites that occur naturally during the evolutionary processes of living organisms, Additionally, deletion or replacement in the sites to which the primer is linked [15], This is consistent with the results of [16] for some cultivars of wild peach. From the products of the amplification of the primers, it was possible to distinguish four patterns of bands through which the varieties of the two species studied were diagnosed and the genetic relationships between them were found, as follows:

The first discriminative pattern: This pattern was characterized by the appearance of a unique band in a particular variety that distinguished it from the rest of the studied cultivars when the genetic DNA of the cultivars was amplified using the primers. The sum of the unique bands produced in this study was (32) unique bands, as shown in Table (2). These bands are among the important and approved diagnostic characteristics in distinguishing species and cultivars. The occurrence of a unique band for a particular cultivar indicates a mutation in a specific site that led to the identification of this site by the primer and the

emergence of the unique band, which is the focus of attention of researchers for DNA fingerprinting, because it shortens the possibilities for efforts with the least number of interactions[17].

The second discriminatory pattern: This pattern was characterized by the absence of the main band for a particular cultivar without the other studied cultivars. The total sum of the absent bands resulting from the primers in this study was (9), and their absence is considered evidence to distinguish that cultivar from others, as shown in Table (2). The absence of the main band from the cultivar alone may indicate the absence of a suitable binding site for the primer on the DNA band of the cultivar, or it indicates the occurrence of a mutation in the primer recognition site only for one cultivar, which led to the cancellation of the identification site and the disappearance of the band, and this is an indication of that variety and supports genetic variation In isolating cultivars and species [18].

The third discriminatory pattern: This pattern showed the production of correlation sites with different bands in their molecular size and in their number within the products of an amplified DNA strand of the two types studied, with the total number of polymorphic bands in this study (882). The OPA-12, OPA-19, OP-26-08, and AB10-10 primers showed a variation rate of (100%), these primers are of great importance to find the genetic variation, as the genetic variation law is the ratio of the number of polymorphic bands to the total number of bands of those primers [19].

The fourth discriminatory Pattern : This pattern was characterized by binding sites that showed main bands for the general characteristics of the types of apricot and plum species of the *Prunus* L. which amounted to (156) main band, the presence of main bands between the cultivars considered as an important diagnostic character for the cultivars of the two types studied, as the presence of these bands between the two types indicates the association of these cultivars with certain characteristics. Penner *et al* [20] also confirmed that main bands identify unknown or unclassified cultivars because they often represent a site shared by genus, species, or cultivars. The results of the four patterns were used to identify genetic relationships in terms of similarity, difference, and genetic dimension between *Prunus* L. apricot and plum cultivars.

Table (1) Random primers used for the cultivars under study with their sequence, molecular weight, primer efficiency and the distinguishing capacity of the primer

no.	primer prefix	Nitrogen base sequence	Molecular size	primer efficiency%	distinguishing capacity%
1	OPA – 1	CAGGCCCTTC	2846 – 248	8.77	8.96
2	OPA – 10	GTGATCGCAG	1563 – 200	6.65	5.10
3	OPA – 12	TCGGCGATAG	2285 – 228	7.22	8.50
4	OPA – 19	CAAACGTCGG	1285 – 35	4.91	5.78
5	OPA – 20	GTTGCGATCC	2100 – 225	10.50	11.00
6	OP – 26 – 08	TGGTAAAGGG	2000 - 300	6.94	8.16
7	OP – 26 – 15	GATCCAGTAC	1160 – 290	6.65	6.46
8	OPF – 08	GGGATATCGG	1700 – 246	9.92	8.96
9	AB7 – 14	GAACGAGGGT	4000 – 221	7.61	8.96

10	AB9 – 18	TGTCCTGCGT	1700 – 190	7.80	7.82
11	AB10 – 10	CCCGTCTACC	1400 – 125	6.64	6.46
12	OPF – 17	AACCCGGGAA	1920 – 327	5.01	4.54
13	OPF – 20	GGTCTAGAGG	2000 – 212	11.37	9.30
				100%	100%

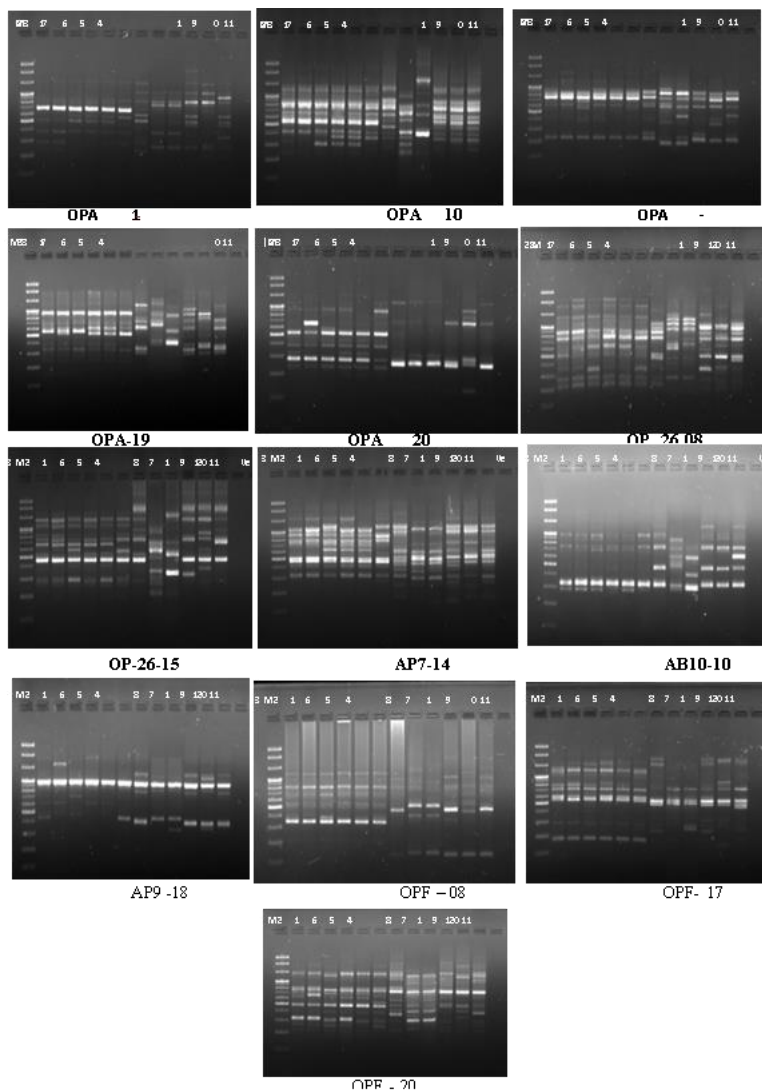


Figure (2) The products of the random amplification reactions of the primers used in the studied apricot, and plum cultivars

1.Sayeb 2.Canion 3. Castelbrite 4.Priana 5. Sndyany 6. Zaginia 7. Songold  
8.Freedom 9. Showtime 10. Pissardi 11. Italica 12.Sant rosa.

\* M represents the ladder volumetric guide path.

Table (2) Results of the primers used in RAPD reactions

No.	Primer prefix	No. of sites produced by primer	Main sites	polymer- phic sites	total bands	main bands	polymorphic bands	unique bands	absent bands	variation %
1	OPA-1	18	1	17	91	12	79	4	1	94.44
2	OPA-10	14	2	12	69	24	45	3	1	85.71
3	OPA-12	15	0	15	75	0	75	2	1	100
4	OPA-19	10	0	10	51	0	51	1	0	100
5	OPA-20	17	1	16	109	12	97	0	1	94.12
6	OP-26-08	15	0	15	72	0	72	2	0	100
7	OP-26-15	11	1	10	69	12	57	2	1	90.90
8	AB7-14	20	2	18	103	24	79	3	2	90.00
9	AB10-10	20	0	20	79	0	79	6	0	100
10	AP9-18	15	1	14	81	12	69	3	2	93.33
11	OPF-08	15	1	14	69	12	57	2	0	93.33
12	OPF-17	14	1	13	52	12	40	2	0	92.85
13	OPF-20	21	3	18	118	36	82	2	0	85.71
	Total	205	13	192	1038	156	882	32	9	93.94

The genetic similarity values of the apricot and plum cultivars studied using 13 random RAPD primers were observed in Table 3, with the highest genetic similarity between the Priana and Sndyany cultivars recorded at 0.937 and the lowest genetic similarity between the Showtime and Pissardi cultivars, The remaining cultivars ranged from 0.413 to 0.921. If the genetic material corresponds to any two cultivars studied, the genetic dimension between them must be equal to zero and the similarity ratio is 100%.

As result shown in Table 4, Priana and Sndyany recorded a lower genetic dimension between the cultivars, which amounted to 0.065, indicating that they had the greatest genetic similarity, depending on the primers used in this study. The highest genetic dimension was between Showtime and Pissardi, which was 0.887. This means that there is the lowest similarity in genetic material between these two cultivars, as they shared the lowest number of bands compared to other cultivars.

Table (3) Genetic similarity matrix between apricot and plum cultivars studied

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
P1	1											
P2	0.921	1										
P3	0.845	0.851	1									
P4	0.876	0.867	0.869	1								
P5	0.867	0.857	0.842	0.937	1							
P6	0.805	0.785	0.802	0.841	0.847	1						
P7	0.514	0.519	0.483	0.516	0.508	0.538	1					
P8	0.434	0.420	0.439	0.432	0.456	0.427	0.486	1				
P9	0.471	0.450	0.449	0.413	0.428	0.423	0.422	0.718	1			
P10	0.529	0.511	0.519	0.508	0.533	0.544	0.702	0.442	0.412	1		
P11	0.500	0.532	0.553	0.526	0.512	0.565	0.589	0.482	0.454	0.715	1	
P12	0.453	0.465	0.464	0.478	0.477	0.508	0.785	0.461	0.436	0.800	0.652	1

P1.Sayeb P2.Canion P3. Castelbrite P4.Priana P5. Sndyany P6. Zagina P7. Songold P8.Freedom P9. Showtime P10. Pissardi P 11. Italice P12.Sant rosa

Table (4) Represents the genetic dimension matrix between the apricot and plum cultivars studied

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
P1	0											
P2	0.082	0										
P3	0.168	0.161	0									
P4	0.132	0.143	0.140	0								
P5	0.143	0.154	0.172	0.065	0							
P6	0.217	0.242	0.221	0.173	0.166	0						
P7	0.665	0.655	0.727	0.661	0.677	0.619	0					
P8	0.835	0.867	0.823	0.839	0.785	0.851	0.721	0				
P9	0.753	0.798	0.801	0.884	0.848	0.860	0.863	0.331	0			
P10	0.637	0.671	0.656	0.677	0.629	0.609	0.353	0.816	0.887	0		
P11	0.693	0.631	0.592	0.642	0.669	0.571	0.529	0.730	0.789	0.335	0	
P12	0.792	0.765	0.768	0.738	0.740	0.677	0.242	0.774	0.830	0.223	0.428	0

P1.Sayeb P2.Canion P3. Castelbrite P4.Priana P5. Sndyany P6. Zagina P7. Songold P8.Freedom P9. Showtime P10. Pissardi P 11. Italice P12.Sant rosa

Based on the analysis of RPD-PCR evidence between twelve cultivars, and for the purpose of determining the genetic relationship between all them, the phylogenetic tree or the Dendrogram of the two species cultivars studied using the UPGMA method was made. The tree showed that the studied cultivars were divided into two main groups. The first group included all the *Prunus armeniaca* L. 4, 5, 3, 6, 2, and 1, and this group was divided into two subgroups, The first subgroup was singled out as Sayeeb. The second subgroup was divided into two groups, including Canion, Castelbrite, Priana, Sndyany and Zagina. As in figure (3). The second major group included all the *Prunus domestica* L., 7, 11, 10, 9, 12, 8. This group consisted of two subgroups, the first of which included Songold and Italice, Pissardi, while the second subgroup was split into two subgroups. The

first included Showtime and Sant Rosa, and the second included Freedom as shown in figure (3).

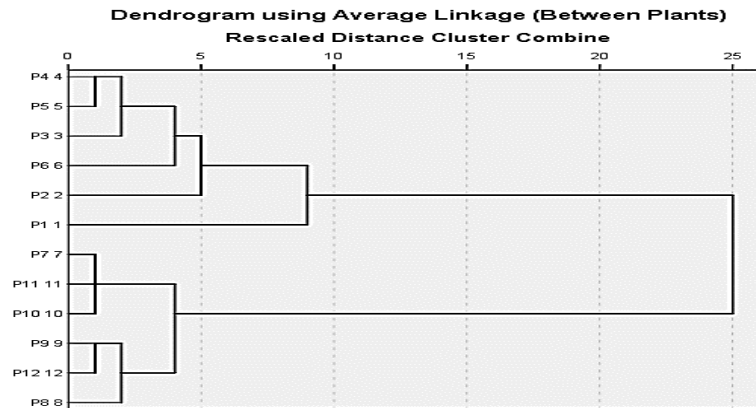


Figure (3) Dendrogram genetic variation tree for apricot and plum cultivars studied resulting from UPGMA analysis of RAPD data

The order of the cultivars depends on the genetic dimension values or Dendrogram based on the genetic range in which the two main groups are associated and therefore the presence of some of the cultivars in a given group indicates the extent to which the genetic makeup of those cultivars in that group is similar [21]. Through these findings, it is clear that there is a degree of similarity or genetic variation between the studied cultivars and this makes genetic markers based on DNA bands separated a good tool in the identification and diagnosis of species and cultivars, as are not affected by any environmental factor Because they are stable and present in all cells and at all stages of development, they can thus be relied upon in the study of plant diversity [22]. In the study of genetic diversity of the Genome of *Prunus* L. Using RAPD, Warburton and Bliss [23] identify genetic relationships between cultivated peach cultivars in the United States of America. Casas *et al.* [13] also observed when studying some species of the genus *Prunus* L. commercial and genetic improvement-selected of 41 genetic cultivars, That study used 80 primers in RAPD technology, 7 of which were selected to identify and classify all species of the *Prunus* L. genus. .Ercisli *et al.* [9] also referred to the efficacy of RAPD in comparison to the phenotypic indicators where (40) RAPD primers were used to study genetic kinship among 23 cultivars of apricots grown in Turkey. . In order to assess the genetic similarity of forty-three Indian apricot cultivars, Mir *et al.* [24] studied the genetic variation of apricot cultivars in genetic banks in India using RAPD genetic indicators. Martínez-Mora *et al.* [25] noted the use of genetic traits of several cultivars of apricots grown in Spain. Lisek *et al.* [26] were able to use RAPD technology extensively among the sweet cherry cultivars in Poland, where it showed great effectiveness in distinguishing between cultivars [16] also demonstrated the efficiency of RAPD technology in showing obvious differences between cultivars of *Prunus cerasia* spread overland in Syria, and conducted PCR on DNA strands with eighteen random primers and demonstrated high efficiency in detecting genetic differences between the studied cultivars. Ait bella *et al.* [27] studied the genetic relationship between plum cultivars and emphasized that RAPD technology is excellent in distinguishing between *Prunus* L species, and cultivars

## Conclusions

These results demonstrated a degree of similarity or genetic variation between the studied cultivars. This study demonstrated the efficiency of RAPD technology in distinguishing between apricot and plum cultivars in terms of determining the degree of kinship and genetic dimension between them which helped to learn the genetic diversity between apricot and plum cultivars studied. The study indicates that the two species have the greatest similarity in genetic material depending on the prefixes used in this study.

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