

## Using random amplification polymorphism (RAPD-PCR) markers to determine the genetic variability of some local and tissue cultured date palm *Phoenix dactylifera* L. cultivars.

<sup>1</sup> Saja Hassan Awda Al-Osmi , <sup>2</sup> Ahmed Dinar Khalaf Al-Asadi 

Department of Horticulture and Landscape Engineering, College of Agriculture and Marshlands , University of Thi-Qar

<sup>1</sup>Email: [saja.post.2022@utq.edu.iq](mailto:saja.post.2022@utq.edu.iq)

<sup>2</sup>Email: [ahmed-d@utq.edu.iq](mailto:ahmed-d@utq.edu.iq)

### Abstract

This study was conducted during the growing season (2024) in one of the private orchards in Nasiriyah district, the center of Dhi Qar Governorate, at longitude (46.06) and latitude (31.90) on some date palm cultivars produced by tissue culture and their counterparts grown by the offshoot method (traditional method). The aim of this study is to study the molecular characterization and determine the genetic fingerprint of the studied cultivars, which are (local Barhi and produced by tissue culture, local Balka and produced by tissue culture, local Sukkari and produced by tissue culture), in addition to the cultivars produced by tissue culture only (Khalas, Saqai and Majhool).

Study molecular characters by using RAPD technique for (11) primers, And the results were analyzed for the molecular indicators using Photocapt ready-made program package, and then tree cluster analysis was conducted to distinguish between the varieties under study and draw the cluster convergence tree between the cultivars.

The results showed that all the all primers used in the study (HB12, OPA12, OPB07, OPC09, OPC13, OPD10, OPD20, OPH-03, OPH-06, OPK18, and OPN-17) were efficient in determining the genetic fingerprint and finding genetic variation among the studied cultivars. The results showed that all primers gave a total number of bands (530 bands). The primer (OPH03) gave the highest number of bands (61 bands) and the number of different bands among them (51 bands), thus achieving a variation ratio of (83.60%), the highest efficiency ratio of (11.50%), and highest diagnostic power of (14.69%), while the primer (OPN17) gave lowest number of bands (31 bands), the number of different bands among them (17 bands), with contrast ratio of (54.83%), and gave lowest efficiency ratio of (5.84%).

The results of the tree cluster analysis of studied cultivars and all the primers showed that studied cultivars were distributed into two groups. The first group included the cultivars (Khalas, Majhool and Saqai produced by tissue culture), while the second group included the cultivars (local Sukkari and produced by tissue culture, Barhi produced by tissue culture and local, local Balka and produced by tissue culture). The largest genetic distance was recorded by Khalas produced by tissue culture cultivar, which reached (28.8%), and thus it is the most distant genetically from the rest of cultivars, while the cultivars of second group recorded great genetic convergence, as percentage of genetic similarity between local Barhi and produced by tissue culture cultivars reached (100%), and between the local Balka and produced by tissue culture cultivars reached (100%), while the percentage of genetic similarity between the local Sukkari and produced by tissue culture cultivars reached (97%).

**Keywords:** Random Doubling RAPD, Date Palm, Molecular Traits, Primers

## I. Introduction

The date palm *Phoenix dactylifera* L. dates back to the palm family Arecaceae which has about 200 genders and 4,000 species, and known palm 4000 years ago BC in Mesopotamia from which it spread to the Arabian peninsula and is one of the most common fruit trees in the world( Al-Bakr ,1972 ; Hussein and Kader ,2009 and Haider *et al.*, 2012).

Many strains have emerged that after some time have become good and widespread cultivars, calling for classification scientists to find a way in which they can be documented in order to avoid confusion (Haider *et al.*, 2012). Many labels have emerged that may belong to the same category, and it can give a single name to more than one category because the appearance traits are similar, and research and studies that lay the scientific foundations have gradually identified the distinctive traits of Markers indicators, a characteristic through which to distinguish and differentiate cultivars, It has a high degree of stability and gives the highest ratio of variation and variation, first of all the phenomenon indicators, hence the cellular, protein and enzymatic indicators of molecule (Abdul Wahid, 2011).

Molecular descriptions are the most important means of identifying genetic origins, helping to determine the relationship between species within the sex and thus becoming the most important elements of the division of organisms, and molecular analysis of genetic material, using DNA indicators that helped determine the genus of palms (Mathew *et al.*, 2014).

Genetic fingerprint is an effective method for identifying date palm cultivars, estimating genetic diversity, analysis of the tree of origin and evolution, and in recent years the DNA fingerprint index technique has become increasingly important to distinguish between closely related cultivars, Molecular indicators have proven to be a very powerful tool in the analysis of plant diversity because they are not only site-specific, but also possess a high degree of multiple shapes, meeting most requirements for accurate palm fingerprinting analysis (Khanam *et al.*, 2012).

The study of the genetic footprint of palm cultivars is of high research and applied importance, contributing to the documentation of the cultivars with a view to preserving them, identifying distinct strains within the cultivars and studying the genetic diversity of the cultivars, and giving accurate information in the genetic convergence of items that are formally difficult to distinguish (Ibrahim and Saleh, 2018). The study of the genetic variation between the different cultivars of date palm using the genetic footprint follows several techniques based on the genetic index which is a characteristic used to infer the existence of a particular location on the chromosome (Al-Najjar *et al.*, 2020). Among these indicators used to estimate genetic variation are Random Amplification Polymorphic DNA indices. (RAPD), based on the multiplier reaction where essential genetic material in an organism's body is used as genetic indicators called DNA Markers and in this method the Loci sites are doubled On the DNA bar using short random primers, these random sequences consist of about (10) Nitrogen bases as these starters find their complementary sites on the DNA strip (Khair Allah *et al.*, 2017). The diagnosis of DNA-level items using RAPD indicators means the creation of the genetic footprint of each item, which is how multiplier pieces of the items studied are distributed using a given starter genetic fingerprint ", that is, the number of these beams and their molecular sizes, which are distinctive to that cultivar , but not to the rest of the cultivars, and that the creation of a genetic fingerprint of the cultivar is the identity of that cultivar that can be used (Dumireih *et al.*, 2010). Therefore, this study aims to use RAPD technology to determine the genetic fingerprint and find the genetic similarity or divergence of the studied cultivars, especially the cultivars produced by tissue culture and their counterparts grown by off shoots separated from their mothers.



## II. Materials and methods

### Method of extracting DNA :

The genetic material was extracted from the leaves of the date palm cultivars (local Barhi and produced by tissue culture, local Balka and produced by tissue culture, local Sukkari and produced by tissue culture, Khalas, Majhool, and saqai produced by tissue culture). Select newly formed leaves close to the growing tip, which are white in colour and have a little green pigment, They were brought to the laboratory of the Center for Research of the Marshes of the Faculty of Agriculture at Dhi Qar University and sterilized by a piece of cotton containing (70%) ethyl alcohol to dispose of dust and pathogens for fear of contamination of the extract's DNA sample. The sample was then grinded by liquid nitrogen into a ceramic mortar and several times until it reached white fine powder. DNA was extracted in a CTAB manner, according to Doyle and Doyle (1990). Thereafter, the genome extracted on the gel of the acarose (% 2) was deported with the electrical relay device Electrophoresis to ensure the success of the extraction process, The purity of the genome produced was also estimated using the Nanodrop device along the wavelength (260-280 nm). The molecular weights of the packages were estimated and drawn via a special PhotoCapt computer program. (11) random primers prepared by the Korean company Microgen were used, consisting of ten random nucleotide bases, and their base sequence is as follows:

**Table (1) The primers and their sequences used in this study.**

No.	Primers	Sequences('5 - '3)
1	HB12	CACCACCACGC
2	OPA12	CAATCGCCGT
3	OPB07	GGTGACGCAG
4	OPC09	CTCACCGTCC
5	OPC13	AAGCCTCGTC
6	OPD10	GGTCTACACC
7	OPD20	ACCCGGTCAC
8	OPH – 03	AGACGTCCAC
9	OPH – 06	ACGCATCGCA
10	OPK18	CCTAGTCGAG
11	OPN-17	CATTGGGGAG

The following parameters were calculated by taking them from the gel image:

1- Primer efficiency is calculated as follows:

Primer efficiency (%) = (Number of bands produced by the resulting primer / (Total number of bands for all primers) × 100.

2- The percentage of variation is calculated as follows:

Percentage of variation (%) = (Number of different primer bands) / (Total number of primer bands) × 100.

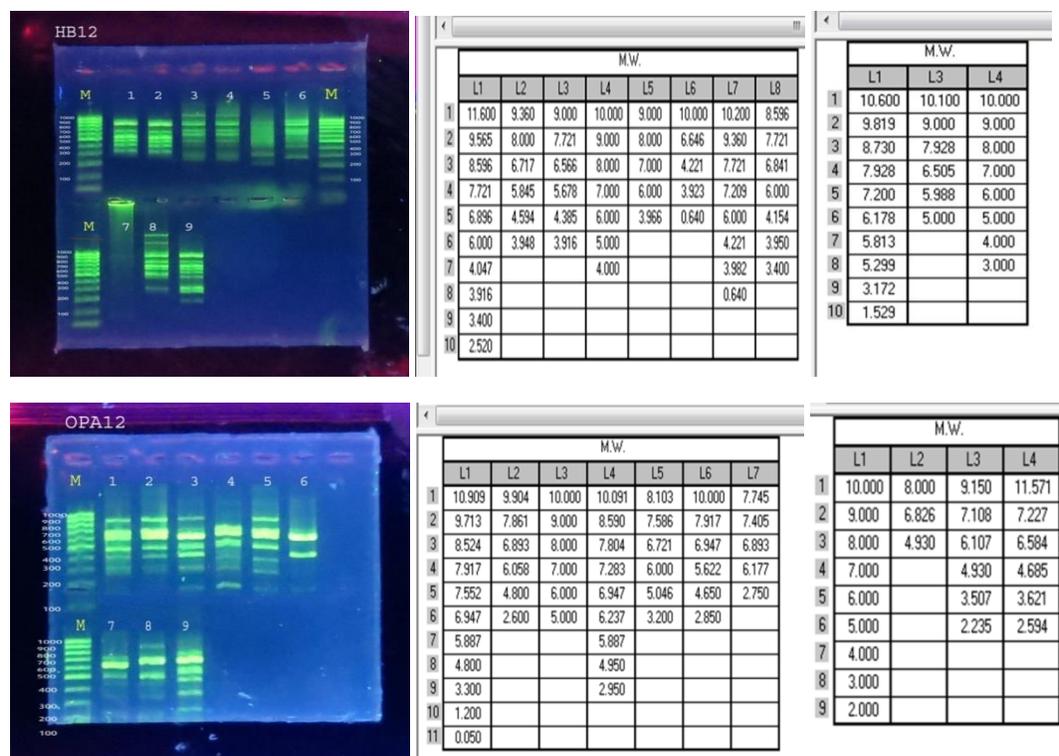
3- The discrimination power of the initiator is calculated as follows:

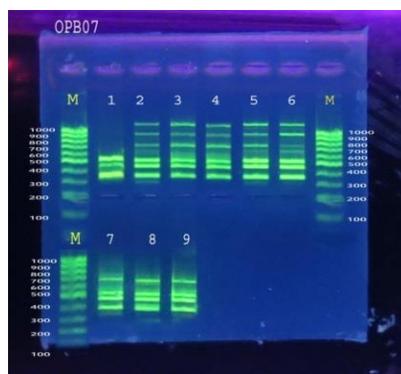
Discrimination power of the initiator (%) = (Number of different primer bands) / (Number of different bands produced from all primers) × 100 .

The results of the molecular indicators were analysed following the identification of bundles locations using the Photocap software bundles.

The magnitude of the molecular weights of the apparent beams was identified and the results were then taken and the cluster analysis Cluster Analysis was conducted to draw on them to distinguish between the cultivars under study, estimate the genetic dimension and draw the cluster convergence tree between the species.

### III. Results and discussion





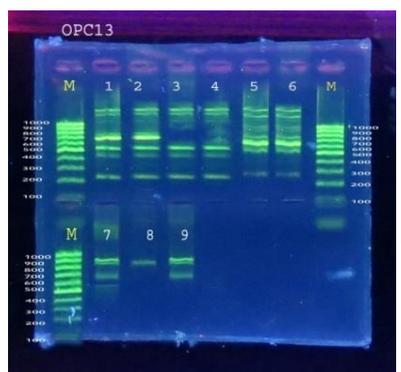
		M.W.							
		L1	L2	L3	L4	L5	L6	L7	L8
11		13.154	10.000	12.692	12.692	12.462	12.539	12.395	12.692
12		12.385	9.000	11.000	11.000	11.462	10.923	10.846	12.000
13		11.538	8.000	10.154	10.077	10.846	10.231	10.077	11.308
14		10.769	7.000	9.701	8.830	9.474	8.744	9.626	10.154
15		9.701	6.000	8.744	7.664	8.744	7.664	8.569	9.319
16		8.744	5.000	7.776	5.510	7.664	5.384	7.441	8.295
17		6.965		6.674		5.128		6.455	6.000
18		4.250		5.257				5.384	3.500
19		0.875							0.875
20									0.125

		M.W.			
		L1	L2	L3	L4
11		13.200	10.100	10.000	9.000
12		11.700	9.255	9.000	8.508
13		10.500	8.743	8.000	7.916
14		9.164	8.079	7.000	6.919
15		8.743	7.266	6.000	5.944
16		8.222	6.460		
17		6.126	6.042		
18		4.833			
19		3.722			
20		1.333			



		M.W.						
		L1	L2	L3	L4	L5	L6	L7
11		20.714	10.857	10.000	7.821	8.000	12.857	10.857
12		16.000	7.731	9.000	6.901	7.000	12.000	9.159
13		12.143	6.331	8.000	5.544	5.728	9.733	6.077
14		10.571	5.858	7.000	4.430	4.533	7.641	4.533
15		8.819	4.825	6.000			6.421	
16		7.369	4.000	5.000			5.604	
17		6.331		4.000			4.324	
18		5.544					3.800	
19		4.109						
20		2.100						

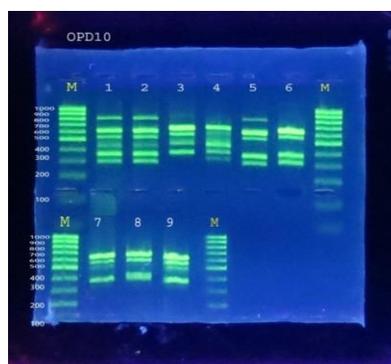
		M.W.			
		L1	L2	L3	L4
11		10.000	6.095	6.794	6.794
12		9.000	4.681	5.098	5.098
13		8.000	3.727	3.932	4.180
14		7.000	2.813	3.128	3.064
15		6.000	1.865	2.506	2.066
16		5.000	0.929	2.000	1.074
17		4.000			
18		3.000			
19		2.000			
20		1.000			
21					
22					



		M.W.							
		L1	L2	L3	L4	L5	L6	L7	L8
11		10.000	7.910	8.736	5.191	6.109	8.867	10.778	17.956
12		9.000	6.732	7.910	4.406	5.000	7.117	9.351	9.351
13		8.000	6.000	7.000	2.291	4.406	5.770	8.352	8.477
14		7.000	5.191	6.000		2.291	4.866	7.000	7.634
15		6.000	4.406	5.125			2.492	5.618	6.346
16		5.000	2.291	4.406				4.731	5.327
17		4.000		2.252				2.451	4.686
18		3.000						0.917	3.653
19		2.000							2.712
20		1.000							1.836
21									0.944
22									
23									
24									
25									
26									
27									

		M.W.			
		L1	L2	L3	L4
11		10.000	9.494	8.612	9.578
12		9.000	9.081		9.081
13		8.000	8.535		8.612
14		7.000	6.664		6.721
15		6.000			1.306
16		5.000			0.889
17		4.000			
18		3.000			
19		2.000			
20		1.000			





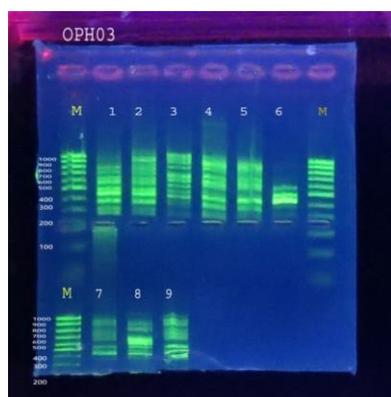
M.W.								
	L1	L2	L3	L4	L5	L6	L7	L8
11	11.038	10.000	10.000	8.522	9.636	9.910	7.810	11.038
12	10.423	9.000	9.457	7.323	8.711	8.807	6.891	10.577
13	9.965	8.000	8.081	6.227	7.561	7.440	4.873	10.038
14	9.348	7.000	7.000	5.000	6.076	6.781	3.739	9.673
15	7.873	6.000	5.962		5.249	4.701		8.339
16	6.781	5.000	4.958		4.484	3.957		7.000
17	5.572	4.000	3.957			3.609		6.189
18	4.177							6.000
19	2.261							4.830
20								3.478
21								1.391

M.W.				
	L1	L2	L3	L4
11	10.000	6.586	7.000	6.586
12	9.000	5.714	5.828	5.438
13	8.000	5.000	4.000	4.716
14	7.000	3.748		3.748
15	6.000			5.828
16	5.000			4.768
17	4.000			4.030
18	3.000			3.905
19	2.000			1.895
20	1.000			



M.W.								
	L1	L2	L3	L4	L5	L6	L7	L8
1	12.529	9.403	10.000	9.000	9.000	9.581	8.791	12.059
2	11.882	8.000	9.000	8.000	8.083	9.000	7.824	10.176
3	11.353	6.779	8.000	7.000	7.170	7.913	6.848	9.345
4	10.706		7.000	5.923	5.923	6.848	6.179	8.506
5	9.880		6.000			5.923	5.769	6.848
6	8.897							6.848
7	6.597							
8	5.846							
9	4.692							
10	3.038							

M.W.			
	L1	L2	L3
1	11.563	9.763	10.000
2	11.063	7.811	9.000
3	10.563	7.000	8.000
4	9.810		7.000
5	8.642		6.000
6	7.000		5.000
7	4.417		
8	2.250		
9			
10			



M.W.								
	L1	L2	L3	L4	L5	L6	L7	L8
1	9.927	9.927	9.964	9.902	10.633	10.000	9.072	9.927
2	9.792	9.477	9.821	9.792	10.122	9.000	8.472	9.792
3	9.644	9.266	9.605	9.585	9.714	8.000	7.900	9.644
4	9.477	8.913	9.173	9.295	9.477	7.000	5.947	9.403
5	9.295	8.517	8.826	9.037	9.236	6.000		9.037
6	8.913	7.900	8.381	8.606	8.472			8.241
7	8.049	6.787	7.642	8.194	8.049			7.483
8	7.215		7.215	5.895	7.215			6.471
9					6.628			6.052
10					6.000			4.895

M.W.			
	L1	L2	L3
1	10.000	9.421	9.000
2	9.000	7.812	8.345
3	8.000	6.686	6.357
4	7.000	5.597	5.497
5	6.000	4.343	4.343
6	5.000	2.368	2.505
7	4.000		
8	3.000		
9	2.000		



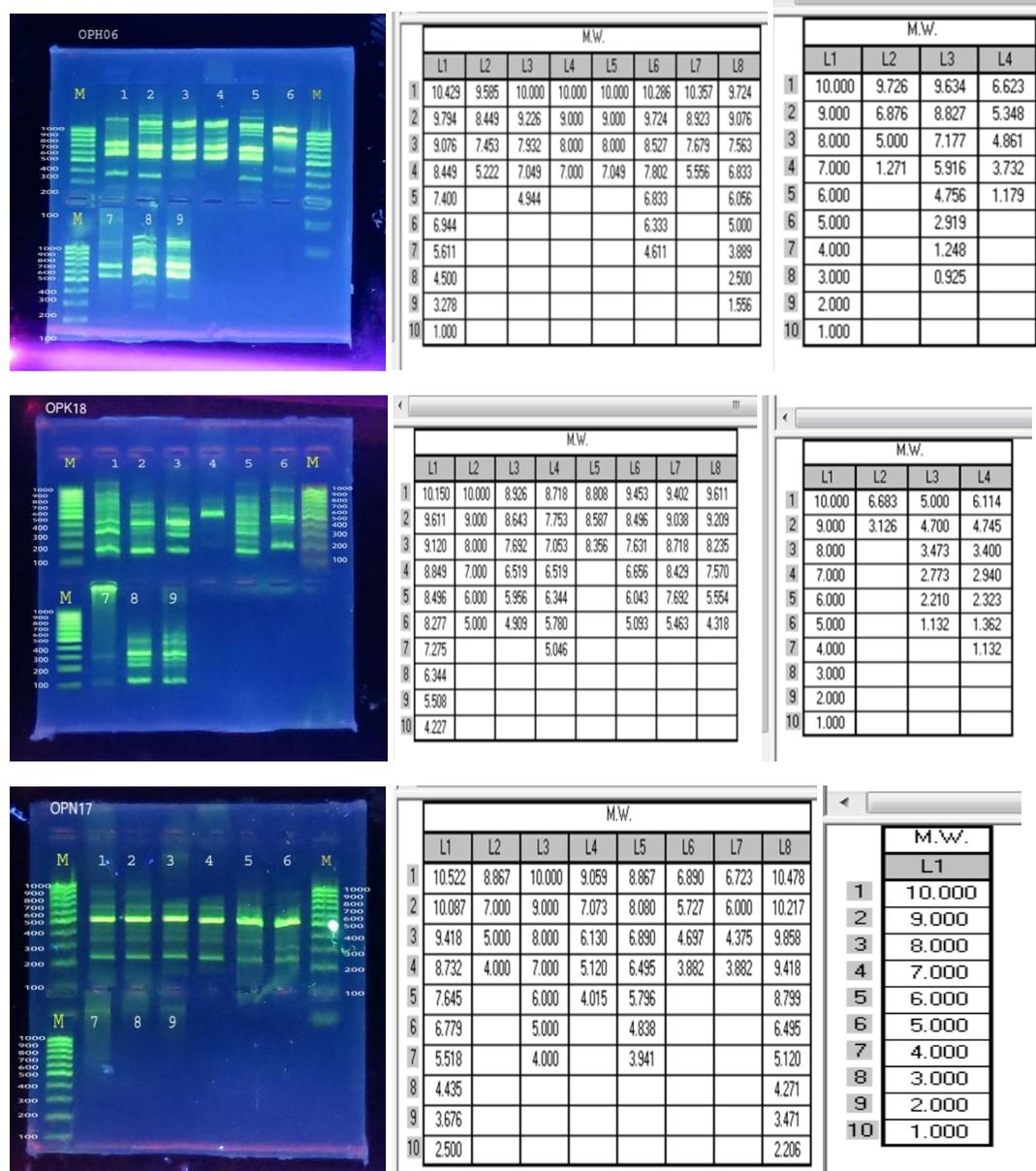


Figure (1) The primers were carried out using the electrophoresis device in the presence of acarose and the bands were identified using the Photocapt program.

- 1- local Barhi 2- Barhi produced by tissue culture 3- Balka produced by tissue culture 4- local Balka 5- local Sukkari 6- Sukkari produced by tissue culture 7- Khalas produced by tissue culture 8- Majhool produced by tissue culture 9- Saqai produced by tissue culture .



The results showed in figure (1) and table (2) that the properties of the studied prefixes carried over to the agarose gel differed between them in determining the ratio of genetic variation between the cultivars studied and in the number of bands, diagnostic strength, efficiency, number of different and similar bands and other traits, The total number of bands submitted by all primers was (530 bands) divided between (345) different bands and (185) similar bands.

Primer OPH03 gave the highest number of total bands (61 bands), followed by primer OPB07 which gave (58 bands), primer OPA12 gave (53 bands), primers (HB12 and OPC09) gave a number of bands up to (51 bands) each, while primer OPN17 gave the lowest number of bands (31 bands), while the rest of the primers had property values ranging between these values (highest value and lowest value).

The highest number of bundles for the variety was given by the primer OPH03, which amounted to (10 bundles), followed by the primer OPA12, with an average of (9 bundles). The primers (HB12, OPB07, OPC09, OPC13, and OPH06) gave the highest number of bundles for the variety, which amounted to (8 bundles) for each of them. As for the primers (OPD10, OPK18, and OPN17), the highest number of bundles for the variety was (7 bundles) for each of them. As for the primer OPD20, it gave the lowest number of bundles for the variety, which amounted to (6 bundles).

In the characteristic of the number of similar bundles, the primer gave the HB12 the highest rate. (32 bands ) followed by primer OPB07 giving a similar number of bands (27 bands), and the primers were given OPD20 (26 bundles) similar, and the primer OPC13 gave a similar number of bands amounting to (17 bands ), and the primer OPD10 gave (16 bands) the same, and gave the primer (OPC09 and OPN17) The number of similar bands was (14 bands ) each, and the primer OPA12 gave the number of bands amounted to (12 bands ) similar, followed by primer OPH03 which gave the number of bands similar to (10 bundles), The primer OPH06 gave a number of similar bands (9 bundles), while the primer OPK18 recorded a number of similar bands (8 bundles).

In the characteristic of the number of different bands, the primer scored OPH03 the highest number of different bands. (51 bands), the primers (OPA12 and OPK18) gave each (41 bands) varied, and the primer gave OPC09 (37 bands) varied, followed by the primer OPH06 which gave (36 bands) disparate, and give primer OPB07 the number of different bands reached (31 bands), as well as primer record OPD10 the number of different bundles reached (30 bands ), while the primer recorded OPC13 the number of different bands reached (27 bands), Primer recorded HB12 number of different bands (19 bands), while Primer recorded (OPN17) the number of different bands (17 bands) while the lowest rate of disparate bands recorded by Primer (OPD20) was (15 bands).

The percentage of variance recorded the highest rate with primer OPK18 at (83.67%), followed by primer OPH03 with a rate of (83.60%), and primer OPH06 with a rate of (80%). The primer OPA12 showed a variance percentage of (77.35%), while primer OPC09 recorded a variance percentage of (72.54%), followed by primer OPD10 with a rate of (65.21%). Primer OPC13 recorded a rate of (61.36%), and primer OPN17 recorded a rate of (54.83%). Primer OPB07 showed a variance percentage of (53.44%), whereas primer HB12 recorded a variance percentage of (37.25%). The lowest variance percentage was recorded by primer OPD20 at (36.58%).

In terms of primer efficiency, OPH03 primer recorded the highest efficiency among primers (11.50%), followed by OPB07 primer with an efficiency rate of (10.94%), OPA12 primer with an efficiency rate of (10%), and both primers (HB12 and OPC09) recorded an efficiency rate of (9.62%) each, while OPK18 primer recorded an efficiency rate of (9.24%), Followed by primer OPD10 at a rate of 8.67%, while primer OPH06 recorded a rate of (8.49%), primer OPC13 recorded an efficiency rate of (8.30%), primer OPD20 with a rate of (7.73%), and the lowest efficiency rate recorded by primer OPN17 at a rate of (5.84%).



In terms of diagnostic power, primer OPH03 recorded the highest rate among all primers at (14.69%). Both primers OPA12 and OPK18 recorded a rate of (11.81%) each, followed by primer OPC09 with a rate of (10.66%). Primer OPH06 recorded a diagnostic power of (10.37%), while primer OPB07 recorded a rate of (8.93%). Primer OPD10 showed a diagnostic power of (8.64%), and primer OPC13 recorded a rate of (7.78%). Primer HB12 recorded a rate of (5.47%), while primer OPN17 showed a diagnostic power of (4.89%). The lowest diagnostic power was recorded by primer OPD20 at (4.34%).

Table (2) Some properties of primers used in RAPD-PCR technology

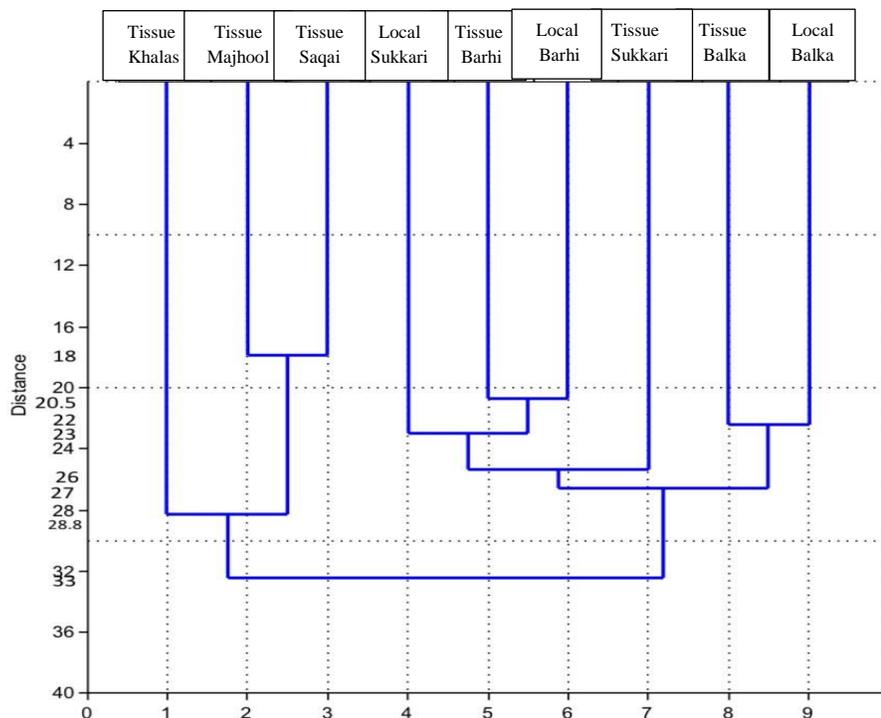
Primer name	Total number of bundles	Maximum number of bundles/variety	Number of similar bundles	Number of different bundles	Variance Percentage (%)	Primer efficiency (%)	Discrimination power (%)
HB12	51	8	32	19	37.25	9.62	5.47
OPA12	53	9	12	41	77.35	10	11.81
OPB07	58	8	27	31	53.44	10.94	8.93
OPC09	51	8	14	37	72.54	9.62	10.66
OPC13	44	8	17	27	61.36	8.30	7.78
OPD10	46	7	16	30	65.21	8.67	8.64
OPD20	41	6	26	15	36.58	7.73	4.34
OPH03	61	10	10	51	83.60	11.50	14.69
OPH06	45	8	9	36	80	8.49	10.37
OPK18	49	7	8	41	83.67	9.24	11.81
OPN17	31	7	14	17	54.83	5.84	4.89
<b>Total</b>	<b>530</b>	<b>86</b>	<b>185</b>	<b>345</b>			

Through the results of the cluster tree analysis and all the primers of the cultivars studied (Figure, 2) The cultivars were divided into two groups, the first group included cultivars (Khalas, Majhool and Saqai produced by tissue culture), a cultivars record (Majhool and Saqai produced by tissue culture) The lowest genetic distance was (18%) with a genetic similarity of (% 82), followed by the cultivars (Khalas produced by tissue culture), which recorded the largest genetic distance reached (28.8%) with a genetic resemblance of (71.2%), thus being the most genetic distance from other studied cultivar.

The second group included the cultivars (local sukkari, barhi produced by tissue culture, local barhi, sukkari produced by tissue culture, balka produced by tissue culture, local balka), and the (barhi produced by tissue culture and local barhi) cultivars recorded a genetic distance of (20.5%) with a genetic similarity of (79.5%) with the other cultivars, and a genetic similarity of (100%) due to the close genetic distance between them, followed by the cultivars (balka produced by tissue culture and local), which recorded a genetic distance of (22%) and a genetic similarity of (78%) with the other cultivars. and a genetic similarity of (100%) between them because they are at the same genetic distance.

Followed by the local Sukkari cultivar, which recorded a genetic distance of (23%) and genetic similarity of (77%). Finally, in the group came the Sukkari produced by tissue culture cultivar, which recorded a genetic distance of (26%) and genetic similarity of (74%), making it the closest genetically to the local Sukkari, with a genetic similarity rate of (97%) between them, and the second most distant genetically cultivar after the Khalas produced by tissue culture cultivar from the rest of the studied cultivars.

Figure (2) Cluster analysis of cultivars studied and for all primers



Through the results, especially in figure (2) and table (2), primers are shown to have been a good tool in finding the genetic variation between the cultivars studied and determining their genetic footprint using RAPD, which is a powerful tool in finding the genetic variation between date palm cultivars and has been used by many researchers. (Haider *et al.*, 2012 and Abd, 2015 and Abdul Al- Wahid, 2018 and Kareem *et al.*, 2018) The difference in the number of bands and in the molecular weights of these studies is consistent with the current study.

The primers studied differ in determining the genetic distance or the genetic convergence between the cultivars studied, and this confirms that each first has a certain specificity in finding the genetic variation between the categories, and a particular primer may have a greater effect than another primer, as demonstrated by the results of the primers studied.

Alansari *et al* (2014) used in their study to estimate the genetic dimension of certain Iraqi date palm cultivars using random multiplier indicators of the DNA chain, and using (10) random primers, the results show that the total of bands of (14 cultivar) (Al-Barahi, Al-Maktoum, Al-Asharsi, Jamal Al-Din, Barbn, Khustawi, Zahdi, Sultani, Hilali, Esta Imran, Khodrawi, Mandali and Khadrawi Baghdad) It is (1,243 bands), and giving Primeran (OPC13 and OPD20) a bands count of (136 and 78) bands.

The primers OPH03 and OPH06 were also used in this study, and they had previously been employed by (Abdul Al-wahid, 2018) to assess the genetic variation among six date palm cultivars (Daghla Mousa, Daghla Hussein, Auwaina Ayoub, Bobki, Aweed, and Red Barhi) The primers produced distinct banding patterns, with OPH03 generating (24 bands), while OPH06 produced (25 bands).

The primers (OPA12, OPB07, OPC09, OPC13, OPD10, OPD20, OPK18 and OPN17) used in this study have been used by (khair allah *et al.*, 2017 ) to diagnose the sex of date palm initiations using some molecular DNA indicators to distinguish between (14 cultivar) of which (7 male cultivars are red ganami, green ganami, khakri, gritly, khakri smesmi , khakri Adey, Rasasi and glami) and (7 female varieties, Barhi, Taparzel, Maktoum, Ashrasi, Khastawi, Esta Imran and Khadrawi), These primers showed a difference in the appearance of polymerization products, giving a number of bands (72, 60, 44, 87, 118, 91, 34 , 66) bands respectively, and the difference in the number of packages and in the molecular weights of the bands in the previous two studies is consistent with the current study.

The DNA chain multiplier reaction technique has led to the development of a molecular screening system in which RAPD technology is used, which is based on the use of nitrogen base sequences from the primer of DNA. When the primer finds similar areas in the DNA strip, the output doubles and when the product is analyzed, different bundles, called multiple appearance forms or multiple formats, appear. (Wang *et al.*, 1994), this is consistent with many researchers who have demonstrated the efficiency of RAPD technology in recognizing the degree of variation between plants (Geisteira *et al.*, 2002; Bennici *et al.*, 2003).

The genetic variation among the studied date palm cultivars may be attributed to differences in the primer binding sites, indicating that these are distinct cultivars. Moreover, the polymorphism observed in the banding patterns suggests the presence of genetic differences among the cultivars, which may result either from stable genetic changes caused by external factors, hybridization, or mutations. In cases where the variation is due to hybridization, this implies that the date palm cultivars exhibited polymorphism and were initially derived from seedlings progeny, which were later propagated through offshoots. These offshoots produced fruits with phenotypic traits similar to those of a known cultivar and were subsequently propagated and distributed as offshoots of that known cultivar. This conclusion is consistent with the findings of (Devanand and Chao, 2003).

In other words, variations in the number of multiplier bands and their molecular size depending on the primer used and resulting from the difference in the number of sites supplementing that primer in the plant's genome or the palm cultivars studied, The appearance or absence of different packages may be due to a difference in the order of the rules, Because the correlation sites of the used primers are randomly spread on the genome of the individuals studied, So any change in the sequence of nucleotides as a result of the addition, or deletion or rearrangement of nucleotides in the genome of individuals studied for any reason such as changes in chromosomal structure, Point mutation, binding, Somatic crossing over , DNA polymerization, the presence of so-called jumping genes, or Change in organelles DNA. The occurrence of one or more of these changes will cause a change in the correlation sites in the primer and then change the size of the multiplier piece resulting in these varying bundles or their absence in specific locations on the gel (Williams *et al.*, 1990).



From the current study, the technique of random multiplication of the DNA series (RAPD) is a powerful tool in finding genetic differences and determining the degree of kinship between date palm cultivars, and all the primers used in this study were good at determining the genetic variation between the studied cultivars. Cluster analysis is an important tool for studying similarity, difference and extraction of genetic relationships and distance distances between different palm cultivars.

#### IV. References

- **Abd, Abdulkareem Mohammad (2015).** Study of genetic relationship and distance among rare cultivars of date palm using some Molecular markers. *Basrah Journal of Date Palm Research*, 14(1): 39-50.
- **Abdul Wahid, Aqeel Hadi (2011).** Study of DNA Fingerprinting of Two Date Palm *Phoenix dactylifera* L. Male Cultivars and the Effect of Their Pollens on Some Physical and Chemical Traits of cv. Hillawi Fruit. Doctoral thesis - College of Agriculture - University of Basrah- Iraq: 263 P.
- **Abdul Wahid, Aqeel hadi (2018).** DNA fingerprint determination for six date palm *Phoenix dactylifera* L. cultivars using of RAPD-PCR molecular technique . *Basrah Journal of Date Palm Research*, 17 (1-2):1-17.
- **Al-Ansari, M. S.; Al-kazazi, A.K.A. and Hussam, S. K. (2014).** Assessment of Genetic Distance Among Some Iraqi Date Palm Cultivares *Phoenix dactylifera* L. Using Randomly Amplified Polymorphic DNA. *Iraqi Journal of Science*, 55(4B):1833-1843.
- **Al-Bakr, Abdul-Jabbar (1972).** The Date Palm: Its Past, Present, and New Developments in Its Cultivation, Industry, and Trade. Al-Ani Press, Baghdad, Iraq: 1085 pp.
- **Al-Najjar, Mohammed Abd al-Amir and Al-Abresme, wasen Fawzi Fadel and Alhamd, Abdul Rahman Dawood Salh (2020).** A review study on diversity markers in date palm. *Basra Journal of Palm Date Research*. 19(1): 73-46.
- **Bennici, A.; Anzidei, M.; and Vendramin, G.G. (2003).** Genetic stability and uniformity of *Foeniculum vulgare* Mill, regenerated plants through organogenesis and somatic embryogenesis. *Plant Science*, 166: 1-7.
- **Devanand ,P.S. and Chao ,C.T.(2003).** Identification of genetic strains Medool and Deglet Noor date *Phoenix dactylifera* L. cultivars in California using Amplified Fragment Length Polymorphism (AFLP) markers: *Acta Hort.*, 623: 333-340.
- **Doyle, J. J. and Doyle, J.I. (1990).** Isolation of plant DNA from fresh tissue. *Focus* 12(13): 39-40.
- **Dumireih, Jihad and Houmydan, Marwan and Khanshour, Anas and Abdul-kader, Ahmed (2010).** Molecular Characterization of Some Wild Genotypes of Hawthorn (*Crataegus azarolus* L.) using RAPD Technique. *Damascus University Journal of Agricultural Sciences*, (11) 26: 106-93.
- **Geisteira, A.S.; Otoni, W.C.; Barros, E.G. and Moreira, M.A. (2002).** RAPD-based detection of genomic instability in soybean plants derived from somatic embryogenesis. *Plant Breeding*, 121: 269-271.
- **Haider, N.; Nabulsi, I. and MirAli, N. (2012).** Phylogenetic relationships among date palm *Phoenix dactylifera* L. cultivars in Syria using RAPD and ISSR markers. *Journal of Plant Biology Research*, 1(2): 12-24
- **Ibrahim, Abdel baset Odeh and Mohammed, bin Saleh (2018).** Atlas the most important date palm varieties in the Gulf Arab States. International Center for Agricultural Research in Dry Areas - ICARD: 153 p.



- 
- **Kader, A.A. and Hussein, A. (2009).** Harvesting and Postharvest handling dates. *ICARDA*, Aleppo, Syria : iv+ 15 pp.
  - **Kareem, M. A. H.; Ali, H. A.S.; Hassan, F. N. (2018).** Genetic Diversity of Iraqi Date Palm *Phoenix dactylifera* L. by using RAPD Technique. *Journal of University of Babylon, Pure and Applied Sciences*, 26(1): 114-131.
  - **Khanam, S.; Sham, A.; Bennetzen, J. L. and Aly, M. A. M. (2012).** Analysis of molecular marker-based characterization and genetic variation in date palm *Phoenix dactylifera* L. *AJCS*. 6(8):1236-1244.
  - **Khierallah, Husam Saad Al-Ain Mohammed And al-Ani, Muid Rajab and al-Rawi, Thairia Khairi Othman (2017).** Sex Identification of date palm by using DNA molecular markers. *The Iraqi Journal of Agricultural Sciences*, 48 (5): 1197-1205.
  - **Mathew, L. S.; Spannagl, M.; Al-Malki, A.; George, B.; Torres, M. F.; Al-Dous, E. K. and Malek, J. A. (2014).** A first genetic map of date palm *Phoenix dactylifera* L. reveals long-range genome structure conservation in the palms. *BMC genomics*, 15, 1-10.
  - **Wang, G.; Castiglione, S.; Zhang, J.; Fu, R.; Ma, J.; Li, W.; Sun, Y. and Sala, F. (1994).** Hybrid rice *Oryza sativa* L. identification and parentage determination RAPD fingerprinting. *Plant Cell Rep.*, 14: 112-115.
  - **Williams, J. G.; Kubelik, A. R.; Livak, K. J.; Rafalski, J. A and Tingey, S. V. (1990).** DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids research.*, 18(22): 6531-6535.