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Molecular Comparison Between Some Tissue-Planted Date Palm Taxa And Natural-Planted Date Palm Taxa In Iraq Using Rapd-Pcr

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Abstract
This study uses the RAPD-PCR technique to carry out a molecular analysis using 12 different varieties of domestic and foreign date palm cultivars to determine the genetic similarity and relationship between some tissue propagating and non-tissue propagated date palm cultivars. 870 bundles, including 113 distinct bundles, were created by these primers. The double bundles had sizes ranging from 100 to 2000 base pairs. With a percentage of polymorphism of 20.87%. The OPC-02 primer had the highest values for firmness and reach (91) and (19) bundles with polymorphism. Its primer efficiency was 10.45%, and its discriminatory ability was 16.81. While the primer N17 provided the value for the primer efficiency (1.14) but the primer FO6 and M19 provided the least prefixes in the number of polymorphic bunds and the percentage of polymorphism amounting to (0), and the primer N17 provided the fewest output bunds (10). The two cultivars Umm al-Dahn (locally) and Majhoul (International tissue) are the most closely related cultivars from a genetic point of view with a similarity ratio of (0.714), while Barhi (local) and Umm al-Dahn (tissue) are more distinct and have a similarity ratio of (0.040). The percentage of genetic similarity between the studied date palm cultivars ranged from 0.040 to 0.714, Except for the two varieties Barhi (locally) and Sakkai (International tissue), the cluster analysis revealed the convergence of the free and (international tissue) varieties in one subgroup and the separation of each tissue variety into a separate group.

Keywords : Umm al-Dahn , Medjool , Barhi , Sakkai , RAPD-PCR

Introduction

One of the oldest fruit trees is the date palm, *Phoenix dactylifera* L. (Drasfield *et al.,* 2005). According to khankahdani and Bagheri , (2019), it is a monocotyledonous perennial tree that is a member of the Arecaceae family. It is very significant in terms of the economy, society, and medicine (Rami *et al.,* 2021; Abd Rabou and Radwan, 2017). Due to the date palm's high nutritional value, high yield, and longevity (El-sohaimy and Hafez, 2010), date palm cultivation is only permitted in western India (El-shafie, 2012) and Iraq between Mandali and Tikrit at a latitude of 35 degrees north and a latitude of 30 degrees south (Ibrahim, 2019). Date palm cultivation is thought to have started 7,000 years ago in Mesopotamia. The date palm is one of the plants that can withstand both drought and high temperatures, which allowed it to adapt to the climate in central and southern Iraq and produce a significant number of commercial varieties, close to 627 in number (Al-Bakr, 1972; Ibrahim and

Khalif, 2004). In many nations where date production is widespread, dates are the primary source of income and food for the local population (Aleid, 2012). Size, weight, color, texture, and other morphological traits can be used to distinguish between different date palm species (Saboori *et al.,* 2020). However, these morphological traits have a limited ability to discriminate between different species due to their strong reliance on environmental factors and geographic location. As a result, some cultivars with similar morphological attributes are given the same cultivar name, and when the environmental component is removed, these traits show strong genetic influence (Hamza *et al.,* 2011). Communities of date palms evolved that adapted to these changes, and they appeared within the members of the type continuous changes and They are inherited in physiological, morphological, and genetic traits. The genetic and morphological developments that occurred in the type of date palm *P.dactylifera* over thousands of years led to the emergence of natural changes within the members of that type as a result of changes in the environment during those ages.

The study's goal was to use the RAPD-PCR technique with 24 random primers for molecular comparison between some local and international date palm cultivars propagated by tissue culture and propagated by natural cultivation, in order to avoid cultivar confusion and to understand the genetic relationship between date palm tree species, and to discover the genetic dimension and the similarity ratio between them.

Materials and Methods

Locations for Collecting Plant Samples:

As shown in Table 1, samples from the Al-Rashidiya farm, Mandalay Agriculture directorate of the Ministry of Agriculture, the Fadak farm connected to the Husseiniya shrine, and 12 date palm cultivars were used in this investigation.

Table (1) The date palm cultivars that were used in the study, where they were collected, and the latitude and longitude coordinates.

NO.	Taxa	Location	longitude	latitude
1	Barhi (locally)	Al-Rashidiya	33.52677	44.33841
2	Barhi (tissue)	Al-Rashidiya	33.52677	44.33841
3	Umm al-Dahn (locally)	Al-Rashidiya	33.52677	44.33841
4	Umm al-Dahn (tissue)	Al-Rashidiya	33.52677	44.33841
5	Medjhool (international tissue)	Al-Rashidiya	33.52677	44.33841
6	Sakkai (international tissue)	Al-Rashidiya	33.52677	44.33841
7	Challas (international tissue)	Al-Rashidiya	33.52677	44.33841
8	Abo-maan (international tissue)	Al-Rashidiya	33.52677	44.33841
9	Merhage (locally)	Mandalay	33.71727	45.48599
10	Koronfully (locally)	Mandalay	33.71727	45.48599
11	Merhage (tissue)	Karbala	32.72204	43.87423
12	Koronfully (tissue)	Karbala	32.72204	43.87423

Genomic DNA extraction :

According to Garcia-Alegria et al. (2020), genomic DNA was extracted from leaf tissue using the ZR plant/seed DNA MiniPrepTM method, and the purity and concentration of DNA were assessed using a UV-Vis NanoDrop spectrophotometer (Thermoscientific). The agarose gel was then subjected to electrophoresis using a

concentration of (1%), ethidium bromide dye, and the gel was analyzed using ultraviolet light with two wavelengths of 260 and 280 nm.

The primers :

To compare the various date palm cultivars in the study, 24 random primers were employed. These primers were created by Operon Technologiec Inc. in Alameda, California, USA, and are listed in Table 2.

Table (2) Types of random primers used in the search with their nucleotide sequences

NO	Primer symbol	Nucleotide sequences (5"- 3")	NO	Primer symbol	Nucleotide sequences (5"- 3")
1	OPC-02	GTGAGGCGTC	13	W10	TCGCATCCCT
2	OPB-10	CTGCTGGGAC	14	X11	GGAGCCTCAG
3	N17	CATTGGGGAG	15	MO8	TCTGTTCCCC
4	R13	GGACGACAAG	16	D20	ACCCGGTCAC
5	B15	GGAGGGTGTT	17	W03	CTCCGGAGTG
6	P18	TGCTTGGCCT	18	M06	CTGGGCAACT
7	P12	AAGGGCGAGT	19	A12	TCGGCGATAG
8	E19	ACGGCGTATG	20	OPA-11	CAATCGCCGT
9	T16	GGTGAACGCT	21	GO8	TCACGTCCAC
10	PO9	GTGGTCCGCA	22	PO6	TCGGCGGTTC
11	LO5	GACTGCACAC	23	M19	CCTTCAGGCA
12	CO5	GATGACCGCC	24	FO6	GGGAATTCGG

The Go Taq Green Master Mix from Promega (USA) was used in a volume of 25 L for the DNA amplification reaction. It contains 10 mM Tris-HCl, pH = 8, 50 mM KCL, 1.5 mM MgCl2, 200 µM nitrogenous bases, and one volume of DNA polymerase at a concentration of 1X. The samples were put inside a revolving thermoplastic apparatus while the DNA strand was initially deformed at 94 ° for 5 minutes (one cycle). Furthermore, mold deformation at 94 degrees for one minute, primer bonding at 36 degrees for one minute, initial elongation at 72 degrees for two minutes (45 cycles), and ultimate elongation at 72 degrees for seven minutes (one cycle).

In order to detect the amplification products on an agarose gel with a concentration of 1.5% added with ethidium bromide dye 0.5 micrograms/ml for an hour and 15 minutes at a voltage difference of 90 volts, 5 microliters of the amplification product were taken. A volumetric DNA indicator was also used. Use an ultraviolet instrument to see and photograph the bundles , then use the following parameters to determine whether the necessary bundles are present. 100 bp (100-2000 bp) from (Promega-USA).

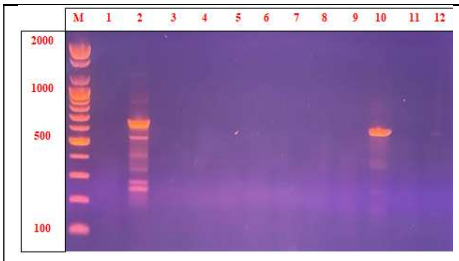
The following law was used to compute the primer efficiency%.

Efficiency of The primer (Starting From Total Yield bunds) / (Primers Per Total bunds) x 100.

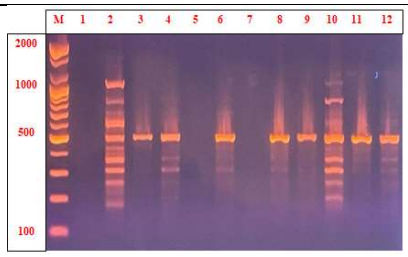
According to the following law, the polymorphism (variance)% percentage was determined: The polymorphism percentage is calculated as follows (the primer of the polymorphic number's polymorphic result) / (the primer of the entire polymorphic result's number of bunds) x 100.

The polymorphic result of the number of bundles' primer divided by each bundle's primers, multiplied by 100, gives the primers discriminatory power (Grudman *et al.*,1995). Then, using the pre-made statistical program SPSS, the similarity ratio and the genetic relationship tree (dendogram) were estimated.

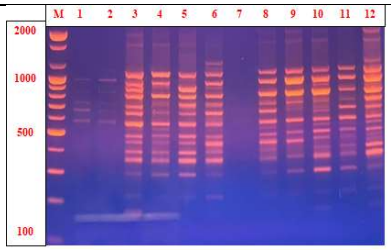
Results:



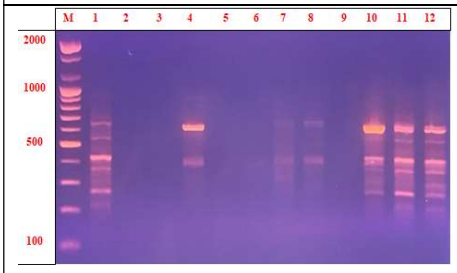
3. Primer amplification N17 output for the current search



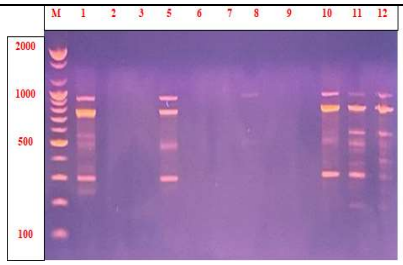
2. Primer amplification OPB-10 output for the current search



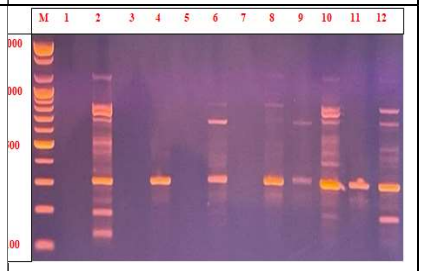
1. Primer amplification OPC-02 output for the current search



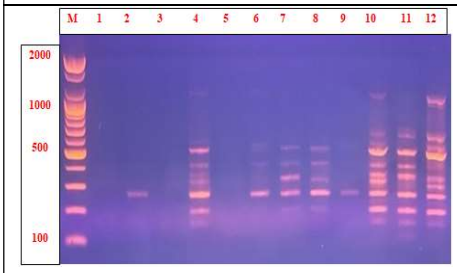
6. Primer amplification P18 output for the current search



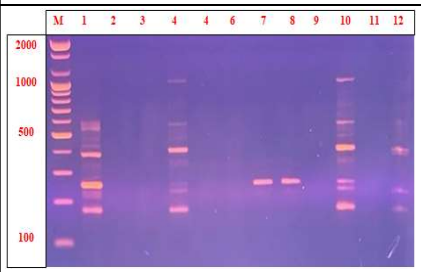
5. Primer amplification B15 output for the current search



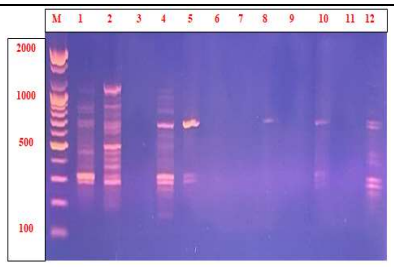
4. Primer amplification R13 output for the current search



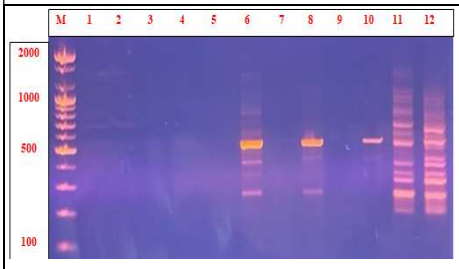
9. Primer amplification T16 output for the current search



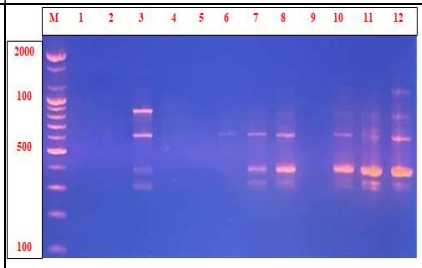
8. Primer amplification E19 output for the current search



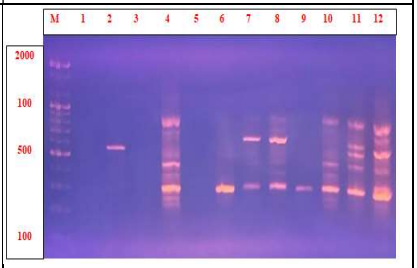
7. Primer amplification P12 output for the current search



12. Primer amplification CO5 output for the current search



11. Primer amplification LO5 output for the current search



10. Primer amplification PO9 output for the current search

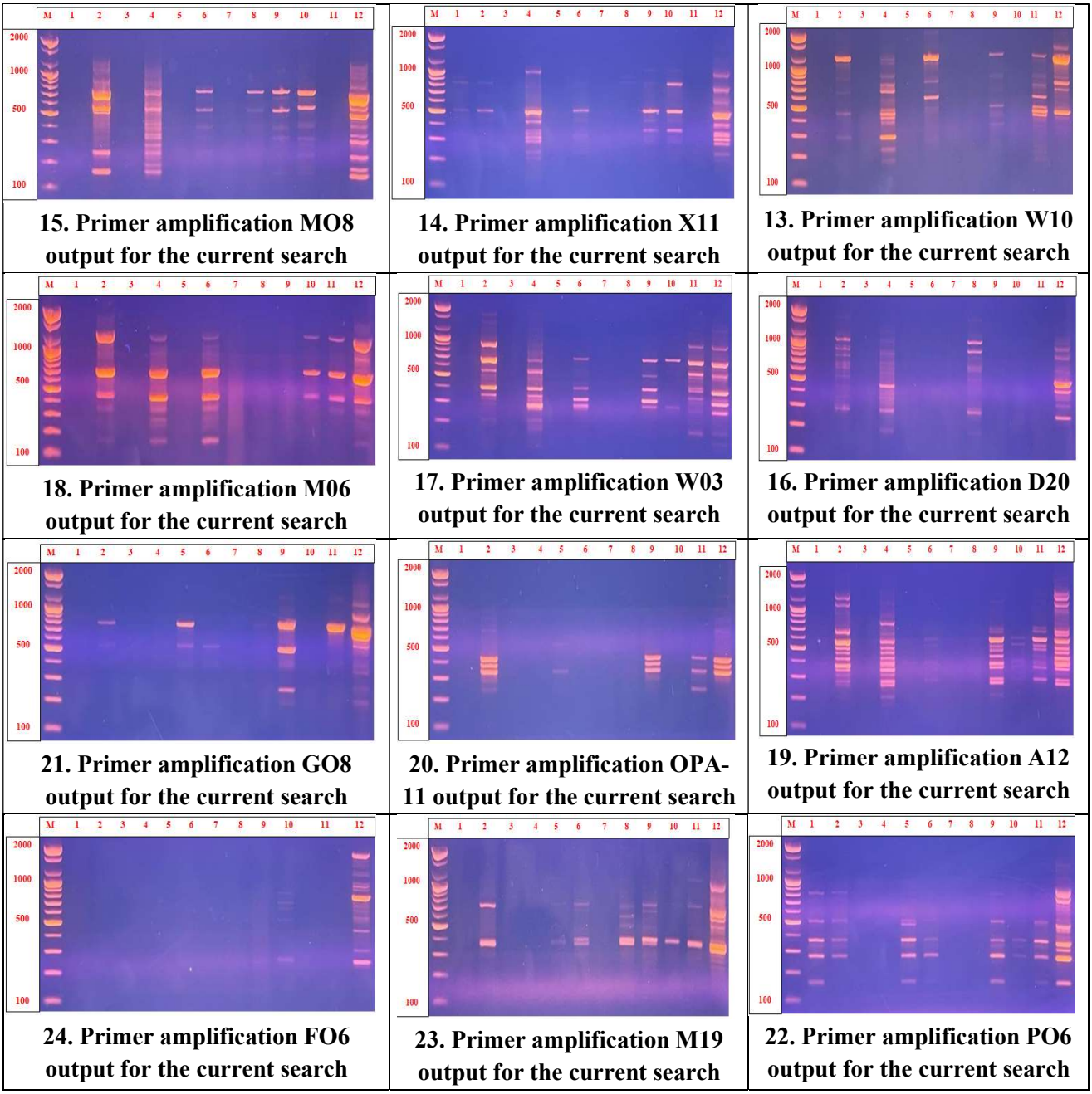


Figure (1) The result of amplification of random primers and relay on agarose gel with a concentration of 1.5% for an hour and an electric potential difference of 100 volts and after staining with ethidium bromide, M = Marker (100-2000 bp), for taxa 1- Merhage (tissue), 2- Challas (international tissue), 3- Sakkai (international tissue), 4- Koronfully (tissue), 5- Umm al-Dahn (tissue), 6 – Majhoul (international tissue), 7- Barhi (locally), 8- Barhi (tissue), 9- Merhage (locally), 10- Koronfully (locally), 11- Abu Maan (international tissue), 12- Umm Al Dahn (locally) .

Table (3) shows the results of random primers with their efficiency and discriminating ratios from whole

and differentiated bundles.

Primers symbol	Total number of amplified fragment	Number of polymorphic fragments	Primer polymorphism percentage	Primer efficiency percentage	Primer discriminative ability Percentage
OPC-02	91	19	20.87	10.45	16.8
OPB-10	43	8	10.60	4.94	7.07
N17	10	1	10	1.14	0.88
R13	40	5	12.5	4.59	4.42
B15	45	4	8.88	5.17	3.53
P18	35	3	8.57	4.02	2.65
P12	26	2	7.69	2.98	1.76
E19	16	1	6.25	1.83	0.88
T16	32	5	15.62	3.67	4.42
PO9	36	2	5.55	4.13	1.76
LO5	24	3	12.5	2.75	2.65
CO5	42	5	11.90	4.82	4.42
FO6	38	0	0	4.02	0
W10	35	7	20	4.02	6.19
X11	43	5	11.62	4.94	4.42
MO8	32	3	9.37	3.67	2.65
D20	54	6	11.11	6.20	5.30
W03	35	7	20	4.02	6.19
M06	68	11	16.17	7.81	9.73
A12	16	2	12.5	1.83	1.76
OPA-11	22	3	13.63	2.52	2.65
GO8	40	5	12.5	4.59	4.42
PO6	30	6	20	3.44	5.30
M19	17	0	0	1.95	0
Total	870	113			

Table (4) The proportion of palm cultivars that are identical in the current study,
S1= Merhage (tissue), S2= Challas (international tissue), S3= Sakkai (international tissue), S4= Koronfully (tissue), S5= Umm al-Dahn (tissue), S6= Majhoul (international tissue), S7= Barhi (locally), S8= Barhi (tissue), S9= Merhage (locally), S10= Koronfully (locally), S11= Abu Maan (international tissue), S12= Umm Al Dahn (locally) .

S12	S11	S10	S9	S8	S7	S6	S5	S4	S3	S2	S1	Taxa
											0.000	S1
										0.000	0.235	S2
									0.000	0.050	0.158	S3
								0.000	0.097	0.500	0.360	S4
							0.000	0.181	0.053	0.226	0.120	S5
						0.000	0.212	0.467	0.077	0.630	0.273	S6
					0.000	0.167	0.040	0.214	0.231	0.135	0.263	S7
				0.000	0.304	0.419	0.125	0.379	0.154	0.375	0.308	S8
			0.000	0.313	0.118	0.556	0.214	0.355	0.059	0.600	0.226	S9
		0.000	0.353	0.448	0.226	0.630	0.135	0.500	0.118	0.484	0.357	S10
	0.000	0.467	0.400	0.355	0.200	0.615	0.200	0.333	0.133	0.467	0.212	S11
0.000	0.548	0.655	0.500	0.455	0.171	0.714	0.171	0.500	0.091	0.655	0.263	S12

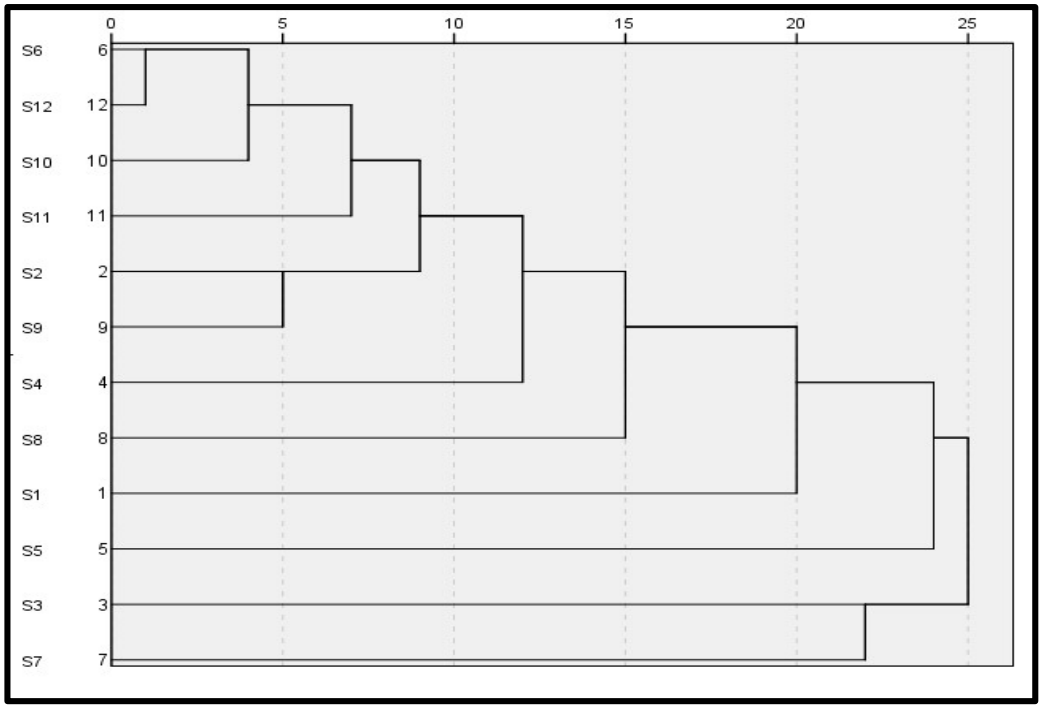


Figure (2) Phylogenetic tree diagram of some phylogenetically propagated and naturally propagated date palm cultivars . S1= Merhage (tissue), S2= Challas (international tissue), S3= Sakkai (international tissue), S4= Koronfully (tissue), S5= Umm al-Dahn (tissue), S6= Majhoul (international tissue), S7= Barhi (locally), S8= Barhi (tissue), S9= Merhage (locally), S10= Koronfully (locally), S11= Abu Maan (international tissue), S12= Umm Al Dahn (locally) .

Discussion:

DNA amplification and identification of cultivars

Using RAPD markers and 24 randomly selected primers, the genetic diversity and genetic relationships between date palm cultivars were assessed in the current study. The capacity of the primers to distinguish between different date palm cultivars varied. Table (3)'s findings demonstrate that 870 bands are created as a result of the amplification of 24 genomic DNA primers at a rate of 36.2 per primer. There were (10) bands for the N17 primer, which had a lower efficiency (1.14%), and (91) bands for the OPC-02 and OPC-02 primer, respectively. The primers (M19, FO6) had the lowest discriminating power (0%) whereas the OPC-02 primer had the highest discriminatory power (16.81%), with the maximum efficiency (10.45%). The OPC-02 primer displayed the highest polymorphism percentage values (20.87%), while the polymorphism percentage with the lowest value was (10%) and had a discriminating power of 0.88%. All primer polymorphism for the 24 different date palm kinds is (4.70). Table 3's results show that the polymorphism rate (4.70) is comparable to those of other studies that used RAPD indicators, which ranged between 3.1 and 3.5 for each primer (Askari *et al.,* 2003; Kichaoui *et al.,* 2013). This discrepancy is caused by the differences in cultivars or the primers used (Alansari *et al.,* 2014).

Genetic affinity between date palm cultivars in the current research:

The percentage of similarity between 12 taxa locally, tissue and (international tissue) date palm varieties that were discovered by RAPD indicators is shown in table (4). The similarity values ranged from (0.040-0.714), with the local cultivar Umm Al-Dahn being more similar to the Majhoul (international tissue) cultivar, with a similarity ratio of 0.714 , while the two cultivars, Umm Al-Dahn (tissue) and Barhi (Locally), had the lowest similarity ratio of 0.040, making them the most genetically researched cultivars. The similarity ratios for the cultivars Umm al-Dahn, Merhage, Koronfully, and Barhi were 0.171, 0.226, 0.500, and 0.304, respectively, when compared to the free and histological variants. Regardless of the type of cultivar, when comparing the (international tissue) cultivars with the local cultivars, we discovered that the Umm al-Dahn (free) cultivar and the (international tissue) Medjhool cultivar shared the highest similarity values (genetically closer), amounting to 0.714, and the (international tissue) Sakkai cultivar shared the lowest similarity values (farther genetically), amounting to 0.059. When comparing the locally tissue varieties with the (international tissue) varieties, we discover that the locally tissue variety Koronfully and the (international tissue) variety Khalas share the highest similarity value of 0.500, while the locally tissue variety Koronfully and the (international tissue) variety Sakkai share the lowest similarity value of 0.097. The findings revealed that the cultivar Umm al-Dahn (tissue), with a similarity ratio of 0.040-0.226, is thought to be the genetically remotest from the other cultivars.

Cluster analysis:

The phylogenetic tree based on the RAPD-PCR technique's amplification of 24 random primers from 12 different taxa (4 free, 4 tissue, and 4 international (tissue) is shown in Figure (2). Figure (2) depicts how the examined palm cultivars were divided into two major groups, which included Five sub-groups made up the first main group. Two subgroups made up the first group. The tissue Majhool International and Umm Al-Dahn (Free) were the two cultivars that shared the most genetic similarity, from which Koronfully (Free) and Abu Ma'an (international tissue) were branched out. The second group contained the two categories Khalas (International tissue), Merhaj (Free), while separated, while classified, the second main group, the two categories (International tissue Saqai, Barhi free). The findings of Table (4) and Figure (2) demonstrate that there is genetic

diversity among the examined date palm varieties. Genetic diversity is crucial for protecting plant species in their natural habitats and defending them against environmental stresses, the effects of which vary depending on phenotypic, physiological, or genetic traits. And that the genetic variance between the examined palm kinds may result from the regional differences and the genetic transmission that takes place through pollen grains (Gross-Balthazard, 2013). In addition to the impact of tissue culture and the characteristics of the histologically abundant part that led to the separation of each tissue variety as an independent group and differed in the percentage of similarity genetic diversity with that of free varieties according to the type of cultivar (Al-Khalifah and Askari, 2007), environmental and geographical factors such as rainfall, temperature, and soil type also have an impact on genetic variation (AL-Qurainy *et al.,* 2015).

Conclusion:

The OPC-02 random primer, which produced the maximum number of bundles compared to the other random primers employed in this study, was able to distinguish between tissue-grown palm cultivars and naturally growing local and foreign palm cultivars. The local cultivar Umm al-Dahn shared a higher similarity ratio (0.714) with the (international tissue) cultivar Majhoul, whereas the two local cultivars Um al-Dahn (tissue) and Barhi shared the lowest similarity ratio (0.040), making them the cultivars that have undergone the most extensive genetic research.

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