The possibility of early detection of date palm seedling Al-Barhi cultivars by using RAPD technique and comparing it with the mother

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Abstract

DNA of seven date palm seedling resulting from the planting of the seeds of Al-Barhi cultivars has been analyzed and compared with the original mother (Al-Barhi) . Four primers (OPA-09,OPB-09, OPC-9,OPC08)have been used for DNA estimation by using RAPD technique.

The results showed the presence of multiple configurable (polymorphic) between studied seedling ,i.e. there is a difference at genotype level .The study suggested that the differences as aresult of seedling arising from seed

Introduction

Date palm belonging to the family Arecaceae has more than 200 genus and about 4.00 spicees .The order Arecae is one of the most important plant orders to which date palm belong and many other species of palm .Date palm belong to the genus *Phoenix dactilifera* L. (Ibrahim,2008).

Key words: **date palm**, **Al-Barhi cultivars**, **RAPD technique**

Genotypic and phenotypic development and change that have occurred on date palm over thousands of years have led to the natural variation within the same member (the same type) as a result of changes in the environment during this period .These changes appeared in a form of physiological ,morphological and genetic cultivars .The index genetic marker is a genetic characteristic used to deduce the presence of particular site locus on chromosome or genome and understanding this site helps on studding certain character or specific gene and genes closed with each other with marker and inherited with it (khairallah,2008).Random Amplified Polymorphic DNA (RAPD)is one of the DNA indicators used in PCR (Polymers chain reaction) leading to multiple outputs from certain sites spread over the genome by using random primer (Williams *et al* ,1990).Khalifah and Askari (2006)from their study of three cultivars (Barhi,Skari,and Khlass) noted that RAPD technique is very important tool in the early detection of genetic variations that occurs in plants resulting from tissue culture .RAPD technique is also used to distinguish between many date palm cultivars (Coeniquuel and Mercier,1994).In a study of Issa *et al* .(2008) of finger print of three date palm cultivars in Al-Hasaa an Qatif in Saudi Arabia has received a multiple configurable between varieties .Said *et al* (2003)by using RAPD technique in their study and 5 primers have received difference between studied cultivars (Zaglol,Imahat ,Sananyand Sawee).

Due to the great importance of date palm in Iraq and to facilitate the early distinguish between seedling produced by seed in early stages ,RAPD technique is used for this purpose.

Material and Methods

Eight leaf samples was collected from date palm seedling resulting from planting seed of cultivars (Al-Barhi) ,Samples were preserved under the temperature -20c until use .

Genetic material (DNA) were isolated from seedling leaf (8 sample) according CTAB described by Weigand *et al* .(1993).

Prepared RAPD reactions

Four Random Decamer Primers were used during this from Operon company (Table 1).The reaction solution used the PCR (Amersham Biotech Sweeden) size 25 micro liter consisting of .

-10xPCR buffer	2.5µl
-25-50 mM $MgCl_2$(50m M)	1.0µl
-primer (100pmole)	1.0µl
-DNA Template (25-50)mg/ml	1.2µl
-Tag DNA polymerase (5unit/µl)	0.25µl
-Distilled water	17.25 µl

Buffer solution consisting of (KCl 50mM,,10M.Tris- HCl pH8.3)

Amplitron Thermal Cycler ,Thermolyne was used for DNA extraction and according to this protocol:

One cycle for 4 minutes at a temperature 94 C followed by 35 cycles include one minute run at 94 C ,2 minutes run at 36C and 2 minutes at 72 C with one run for 7 minutes at 72 as a final for DNA extraction .The device has been programmed on 4 degrees Celsius .Separation has been done on gel a grouse according to Bornet and Branchard (2001).

Table (1)The set of Operon primers used in RAPD reactions

N.	Operon cod	Primer sequence	GC%
1	OPA-09	GGGTAACGCC	70%
2	OPB-09	TGGGGGACTC	70%
3	OPC-09	CTCACCGTCC	70%
4	OPC-08	TGGACCGGTG	70%

Analysis of Result

The number of replication bands was calculated from that with lower molecular weight to those bands with higher molecular weight (Cao *et al* .2002).Replicated band was represented by (1) and its absence by (0) .Date were analyzed by using the program similarity for quantitative data (SIMQUAL),according to equation (Nei ,1973), and used similarity to sketch Dendrogram using Unweighed pair –Group Method with Arithmetic Averages (UPGMA)and Numerical Taxonomy (NTSYS-PC,1.80)(Rohlf,1993) on computer by using photocapt program me for band identification and their characters or specifications (shape 1,2,3,4 and 6).

Results

It is clear from the result obtained during this study that there are differences between the fingerprinting of seedling resulting from planting the seeds of date palm (Al-Barhi) cultivar ,in terms of length their location and number of bands. Notes from form (1a) that mother plant and seedling number seven showed the least number of bands by using (OPB-09) primer while the seedling fourth got the largest number of bands .The seedling 6

and 7 have got band with about 370 nucleotides .We find from table (1b) less degree of constitutes between seedling 6 and 7 in 85.5% of identity .The reaming seedling showed 100% of identity.

Table (2a)showed different number of bands that have been separated by using (OPC-09) primer for the seedling planted ,seedling sixth showed one band while reaming seedling showed two bands .Seedling number 2 was similar to number 3,4,5 and 6 by containing band with 2155 nucleotides ,the other seedling was different in their nucleotides length .It is clear from table (2b)that showed fingerprint result by using (OPC-09) primer with an existence of polymorphic between seedling planted from seeds of Al-Barhi cultivars .Less polymorphic found between seedling and seedling 6 in 50% of identity ,while the presence of polymorphic of other seedling in this study are different.

We can also see from table (3a)that there are different seedling bands by using primer (OPC-08).Two bands is recorded in seedling number 2 and 5 while the fourth seedling recorded five bands .Mother plant as well as fourth and seventh recorded band with 356 nucleotides .As seen from table (3b) that a polymorphic between seedling was observed and less polymorphic between seedling number 5 and 6 in 75% ,as well as for the seedling 3 and seedling 7 in the same proportion with seedling1 and 4 .88.8% was recorded between seedling 4 and 7 .The rest seedling showed 100% polymorphic .

From table (4a) we observed that there are differences in the number of bands between seedling in this study .2 bands in fifth seedling and mother plant ,4 bands in third, fourth, sixth and seventh seedling .Mother plant and the second seedling recorded band with 762 nucleotide ,fourth and fifth

seedling with 700 nucleotide band ,third and fifith seedling with 362 nucleotide band ,the rest of seedling differs in bands length .From table (4b) we find the existence of polymorphic that reached 100% .Except third,fourth (85.5%)and third,sixth (80%)and third ,seventh(85.5%) and fifith ,sixth (80%)third ,fifth and sixth seedling with 87.5% and seedling number five and seedling number seven with 83.3%.

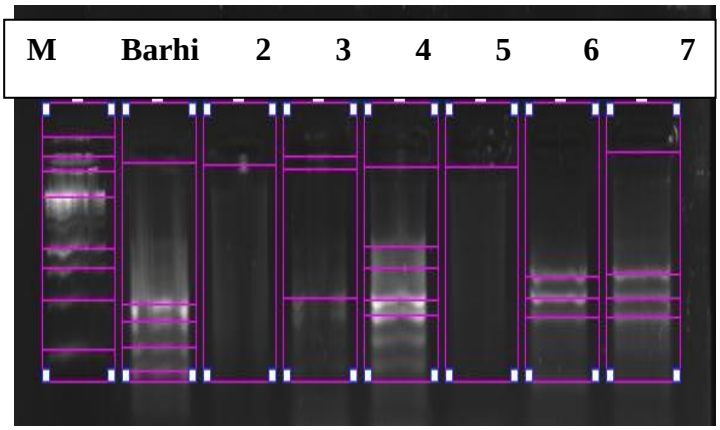
We also find from dendrogram table (5) clear segregation of seedling into two groups .Third ,fourth, fifth and six seedling are in the same group ,while the rest of seedling is in the other group .Seedling 5 and seedling 6 are closer to each other and recorded less variation between them .The rate of similarity between the studied seedling has recorded 53%.

Discussion

The result showed the presence of genetic differences in the genetic structure of the studied seedling depending on the used primer the result also showed the presence of polymorphic and that as a result of genetic segregation in the seed (Ibrahim,2008).As evidence from the polymorphic of these bands these differences are caused by fixed genetic changes ,whether due to external factor like hybridization or internal factor like mutation (Al-Barghouthi,1997),In spite that the cytological study indicates that the chromosomes of advanced organisms are fixed in shap and number in the tissue of the same species (Mutaina .1997),and the change in the chromosomes leads to the change in the location of primer sites ,leading to the formation of bands on the gel that differs in length and number while those species without genetic change appear on the gel on the from band with similar monomorphic, i.e. similar in the number and length separated

bands on the gel(Jurani,1989).Ahmed (1999)Explained that these difference in the number of bands between varieties due to difference in site and in the number of bands between cultivars are due to difference in site recognized by the primer with in genome (either by deletion or addition or replacement of single nucleotide in the DNA sequence of date palm),and the difference in the length of bands ,which reflects the difference in length between sites link with the primer of nucleotide sequencing that complete DNA template of studied cultivars.

Table (1a) number and types of bands for seedlings and mother plant (Al-Barhi) by using primer OPB-09



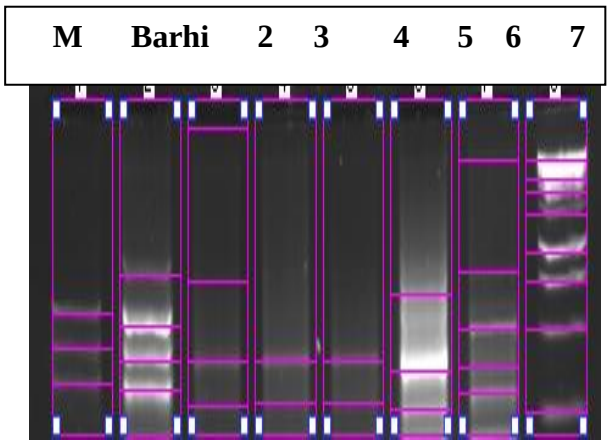
Form 1.polymorphism revealed using primer opb-09 to amplify genomic DNA purified from six seedling and mother plant of Al-Barhi of date palm

opb-09	1	2	3	4	5	6	7
4000	0	0	1	0	0	0	1
3548	1	0	0	0	0	0	0
3403	0	1	0	0	0	0	0
3262	0	0	0	1	1	0	0
3128	0	0	1	0	0	0	0
1548	0	0	0	1	0	0	0
1000	0	0	0	1	0	0	0
824	0	0	1	0	0	1	1
522	0	0	0	0	0	1	1
500	0	0	0	1	0	0	0
461	1	0	0	0	0	0	0
383	0	0	0	1	0	0	0
370	0	0	0	0	0	1	1
355	0	0	0	0	0	0	0
347	1	0	0	0	0	0	0
256	1	0	0	0	0	0	0

Table (1b)The genetic distance between seedling and plant Mother (Al-Barhi)by using primer OPB-09 to the RAPD marker

Simple	Total bands	Monomorphic	polymorphic	Polymorphic%
2*1	5	00	5	%100
3*1	7	00	7	%100
4*1	9	00	9	%100
5*1	5	00	5	%100
6*1	7	00	7	%100
7*1	8	00	8	%100
3*2	4	00	4	%100
4*2	6	00	6	%100
5*2	2	00	2	%100
6*2	4	00	4	%100
7*2	4	00	4	%100
4*3	8	00	8	%100
5*3	4	00	4	%100
6*3	6	00	6	%100
7*3	6	00	6	%100
5*4	5	00	5	%100
6*4	7	00	7	%100
7*4	8	00	8	%100
6*5	4	00	4	%100
7*5	5	00	5	%100
7*6	3	2	1	%85.5

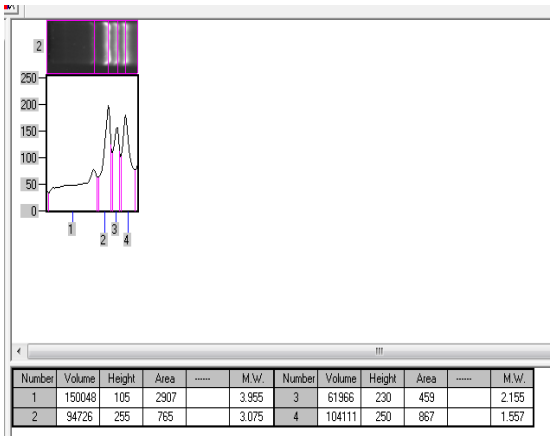
Table (2a) number and types of bands for seedlings and mother plant(Al-Barhi) by using primer OPC-09



7	6	5	4	3	2	1	Pbopc-09
0	0	0	0	1	0	0	5286
0	0	0	0	0	1	0	3955
0	0	0	0	1	0	0	3865
0	0	0	0	0	0	1	3345
0	0	1	0	0	0	0	3262
0	0	0	0	0	1	0	3075
0	0	0	0	0	0	1	2495
0	1	1	1	1	1	0	2155
0	0	0	0	0	0	1	1673
0	0	0	0	0	1	0	1557
1	0	0	1	0	0	0	1313
0	1	0	0	1	0	0	1250
1	0	0	0	0	0	0	865

Form.polymorphism revealed by using primer opc-09 to amplify genomic DNA purified from six seedling and mother plant of Al-Barhi of date palm

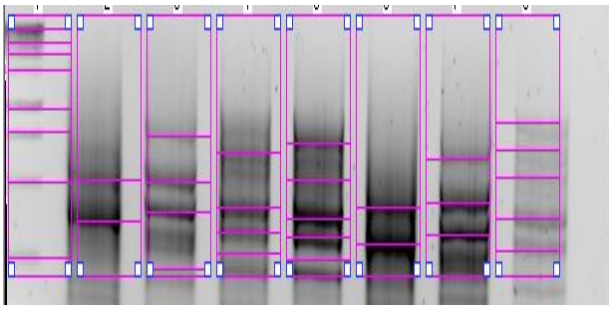
Table (2b)The genetic distance between seedling and plant Mother Al-Barhi)by using primer OPC-09 to the RAPD marker)



Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	150048	105	2907		3.955	3	61966	230	459		2.155
2	94726	255	765		3.075	4	104111	250	867		1.557

Simple	Total bands	Monomorphic	polymorphic	Polymorphic%
2*1	7	00	7	%100
3*1	7	00	7	%100
4*1	6	00	6	%100
5*1	5	00	5	%100
6*1	5	00	5	%100
7*1	5	00	5	%100
3*2	7	1	6	%88.5
4*2	5	1	4	%80
5*2	5	1	4	%80
6*2	5	1	4	%80
7*2	6	00	6	%100
4*3	5	1	4	%80
5*3	5	1	4	%80
6*3	4	2	2	%50
7*3	6	00	6	%100
5*4	3	1	2	%88.5
6*4	3	1	2	%88.5
7*4	3	1	2	%88.5
6*5	3	1	2	%88.5
7*5	4	00	4	%100
7*6	4	00	4	%100

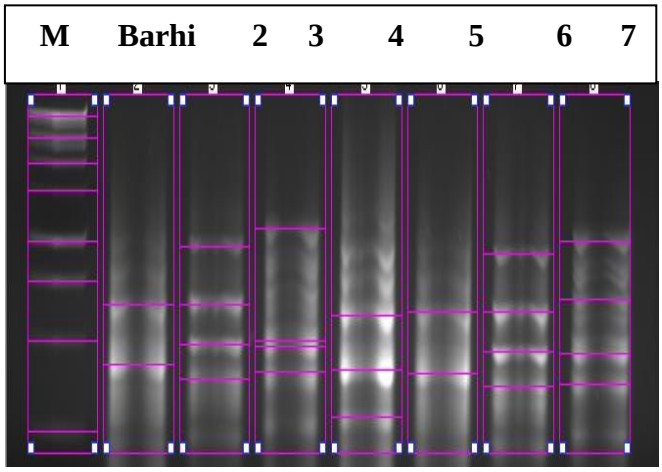
Table (3a) number and types of bands for seedlings and mother plant(Al-Barhi) by using prim



Form 3.polymorphism revealed by using primer opc-08 to amplify genomic DNA purified from six seedling and mother plant of Al-Barhi of date palm

7	6	5	4	3	2	1	Opc-08Pb
1	0	0	0	0	0	0	1188
0	0	0	0	0	1	0	921
0	0	0	1	0	0	0	819
1	0	0	0	0	0	0	735
0	0	0	0	1	0	0	710
0	1	0	0	0	0	0	646
1	0	0	0	0	0	1	523
0	0	0	1	0	0	0	511
0	0	0	0	0	1	0	500
0	1	1	0	1	0	0	394
0	0	0	0	0	1	0	378
1	0	0	1	0	0	1	356
0	0	0	0	1	0	0	315
0	1	0	0	0	0	0	309
0	0	0	1	0	0	0	303
0	0	1	0	0	0	0	285
1	0	0	0	0	0	0	267
0	0	0	0	1	0	0	261
0	0	0	1	0	0	0	242
0	0	0	0	0	1	0	212

Table (3b)The genetic distance between seedling and plant Mother (Al-Barhi) by using primer OPC-08 to the RAPD marker



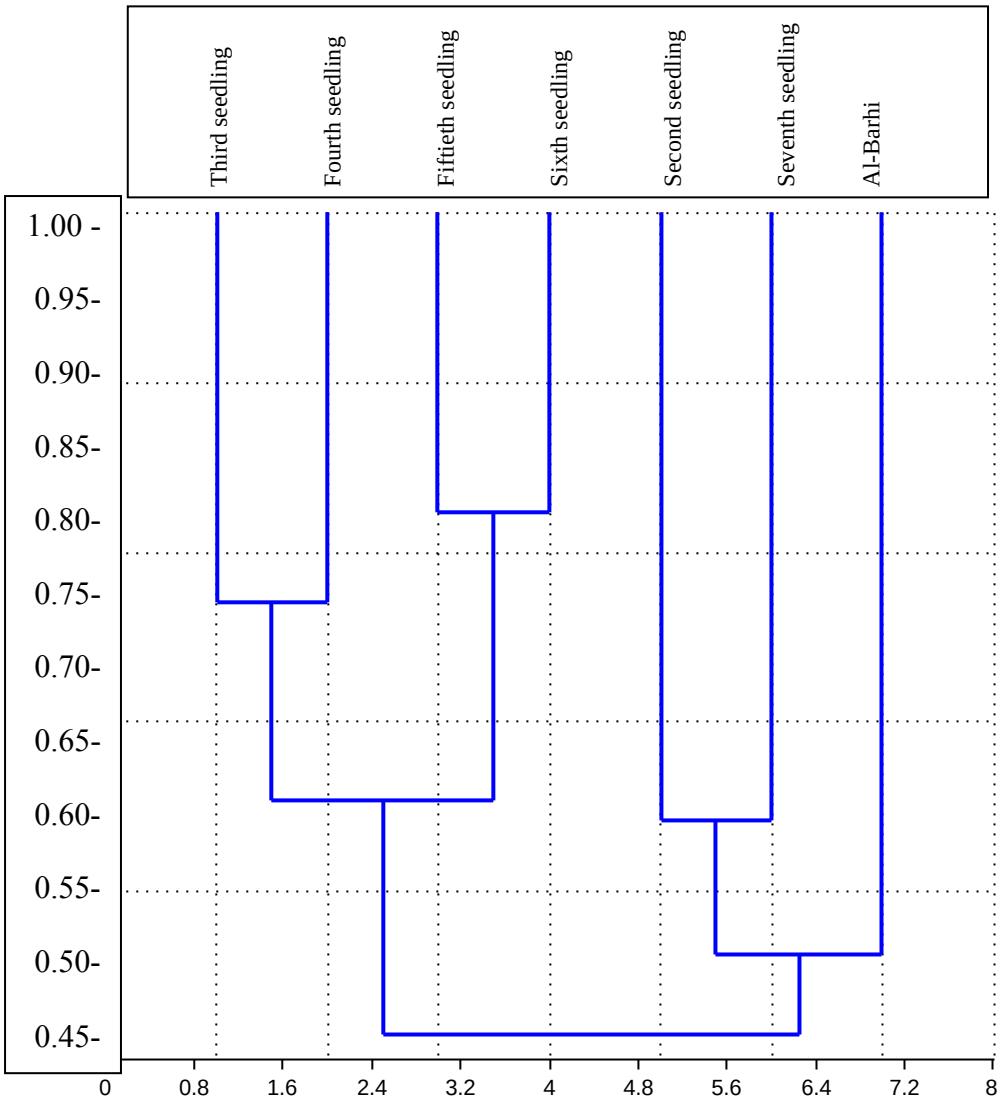
Form 4.polymorphism revealed using primer opa-09 to amplify genomic DNA purified from six seedling and mother plant of Al-

7	6	5	4	3	2	1	Pbopa-09
0	0	0	0	1	0	0	1642
1	0	0	0	0	0	0	1500
0	0	0	0	0	1	0	1428
0	1	0	0	0	0	0	1333
1	0	0	0	0	0	0	812
0	0	0	0	0	1	1	762
0	1	1	1	0	0	0	700
0	0	0	0	0	0	0	671
0	0	0	1	1	0	0	500
0	0	0	0	0	1	0	481
1	0	0	0	1	0	0	471
0	1	0	0	0	0	0	445
0	0	0	1	0	0	1	374
0	0	1	0	1	0	0	362
0	0	0	0	0	1	0	346
1	0	0	0	0	0	0	332
0	1	0	0	0	0	0	320
0	0	0	1	0	0	0	270

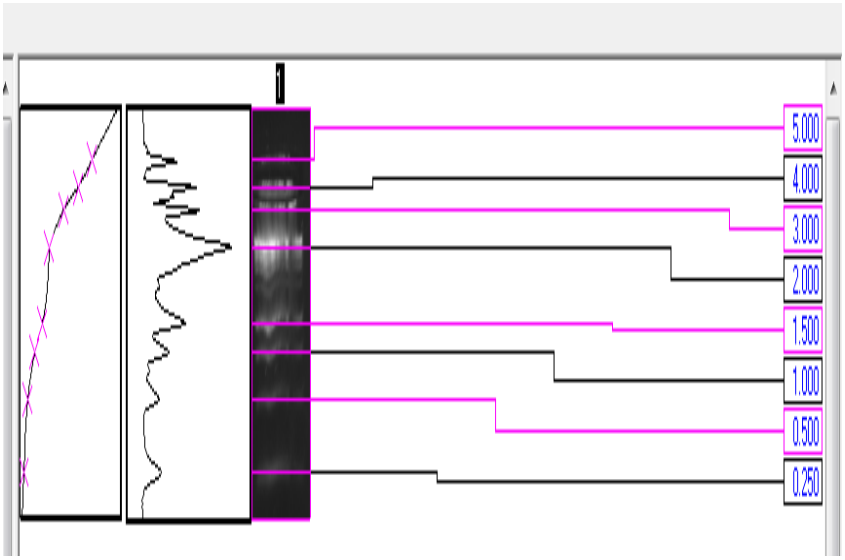
Table (4a) number and types of bands for seedlings and mother plant (Al-Barhi) by using primer OPA-0

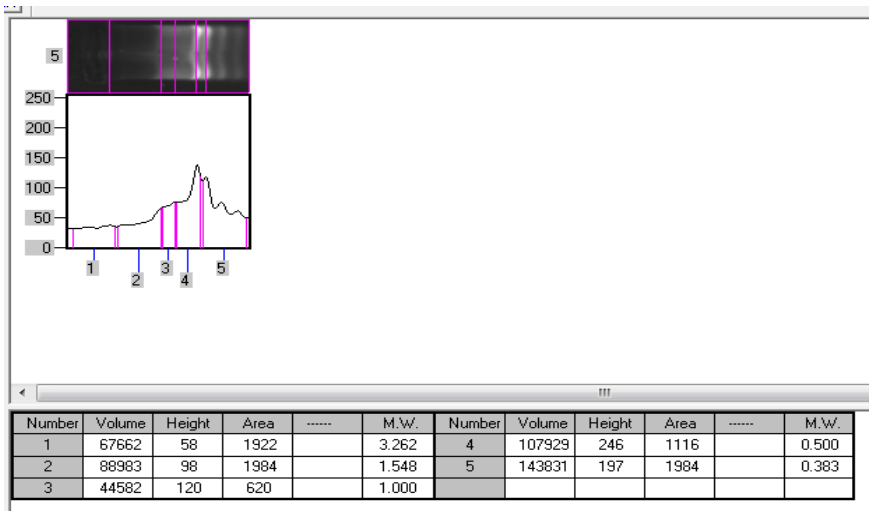
Simple	Total bands	Monomorphic	polymorphic	Polymorphic%
2*1	6	00	6	%100
3*1	6	00	6	%100
4*1	5	00	5	%100
5*1	4	00	4	%100
6*1	6	00	6	%100
7*1	6	00	6	%100
3*2	8	00	8	%100
4*2	8	00	8	%100
5*2	6	00	6	%100
6*2	8	00	8	%100
7*2	8	00	8	%100
4*3	7	1	6	%85.5
5*3	5	1	4	%80
6*3	8	00	8	%100
7*3	7	1	6	%85.5
5*4	6	00	6	%100
6*4	6	00	6	%100
7*4	8	00	8	%100
6*5	5	1	4	%80
7*5	6	00	6	%100
7*6	8	00	7	%100

Table (4b)The genetic distance between seedling and plant Mother (Al-Barhi)by using primer OPA-09 to the RAPD marker

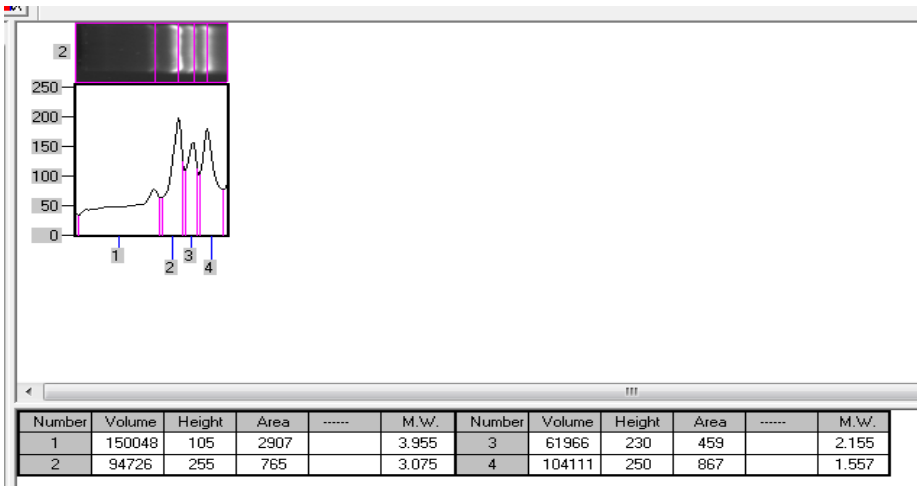


Form (5) cluster -analysis (dendrogram) for a number of date palm seedlings and mother plant(AL-Barhi).





Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	67662	58	1922		3.262	4	107929	246	1116		0.500
2	88983	98	1984		1.548	5	143831	197	1984		0.383
3	44582	120	620		1.000						



Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	150048	105	2907		3.955	3	61966	230	459		2.155
2	94726	255	765		3.075	4	104111	250	867		1.557

Form (6) analysis of the results by using the program photocapt

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إمكانية الكشف المبكر لبادرات نخيل التمر صنف البرحي باستخدام مؤشر الـRAPD ومقارنتها بنبات الأم

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الخلاصة

تم تحليل البصمة الوراثية لسبعة بادرات من نخيل التمر الناتجة من زراعة بذور صنف البرحي ومقارنتها مع نخيل التمر صنف برحي(نبات الام) وقد استخدم فيها أربعة بادئات وهي (OPA-09 ,OPB-09,OPC-09,OPC-08) وقد تم تقدير البصمة الوراثية طريقة الـ(RAPD) وقد أظهرت النتائج وجود تعدد شكلي (polymorphic) بين البادرات المدروسة . أي وجود اختلاف على مستوى التركيب الوراثي . وقد رجحت الدراسة أن الاختلافات نتيجة للبادرات الناشئة من البذرة.