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Genetic distinction among cultivars of date palm using some molecular markers

Abdul Kareem Mohammed Abd

Palm Research Cente University of Basrah Iraq – Basrah

Abu_zahra19662yahoo.com

ABSTRACT

The experiment was carried out in the laboratories of biological techniques in the Department of Livestock, Agriculture College, Basrah University. The Randomly Amplified Polymerase Chain Reaction technique was used with four primers to find out the exactration DNA and the percentage of genetic divergence in six cultivars of date palm (Al_khasab, Allylwi,Bent Alsabaa, Dekal Ahmer, Alfersee and AlKhdrawee).Also, the study involved isolating DNA from the newly grown leaves of the study's date palm cultivars, and showing differences among the amplified pieces for each cultivar by deporting samples to the Electrophorses in the RAPD interactions .The results stated that there are considerable genetic differences among the cultivars of the current study. This was in terms of both the number of amplified bands and their molecular weight according to the primer used. The total number of the bands used was 64 for all primers. Moreover, the cultivars showed huge variations except ,Bent Alsabaa and Dekal Ahmer which stood at 75% and Dekal Ahmer and AlKhdrawee stood at 66.7%.The data was then collected and analyses, and the cultivars were classified into two separate groups. The cultivars Dekal Ahmer and Allylwi were in the same group where the former recorded less distance. Finally, the study concluded that the types are separated from each other reflected by phenotypic differences among them.

Indexing terms/Keywords

Date palm; DNA; Genetic; Cultivars.

Academic Discipline And Sub-Disciplines

Biotechnology

SUBJECT CLASSIFICATION

Date palm

TYPE (METHOD/APPROACH)

The study of the genetic diversity of the cultivars of date palm.

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INTRODUCTION

The date palm, *Phoenix dactylifera* L., belongs to a palm family called Arecaceae and a class called Palmalea. This family includes 200 species, and the most significant species, from the economic point of view and according to their relationship with human beings, are four, one of which is Phoenix. Date palms belong to the said species, Phoenix. And these aforementioned species are followed by nearly 400 types of date palms (1).Date palms started to appear in the Arab Gulf Region thousands of years ago. It's believed that most date palm types in Iraq had originally emerged from the seed date palms. The best of the types were selected and propagated by planting shoots that had already formed around the mother palm tree. Through this, the seed species of desired specifications could be protected in terms of vegetative and fruit qualities (2).In a study conducted by(3),13 species of Saudi date palms were researched where 37 primers had been used. The results of this study showed that multi-configuration was between 50-89%, which was in line with. (4). The latter study was carried out to check the genetic stability of certain kinds of date palm, such as Birhi and Maktum, yielded as a result of tissue culture.Through applying 20 primers, obvious results were obtained in which occurrence of the genetic variation in plants, resulting from formation of cross-buds, was revealed.Having made use of genetic fingerprinting through a random technique called RAPD on some Saudi date palms (5) suggested the emergence of difference on the level of DNA.(6) experimented with the suitability of random magnification technique of DNA pieces as genetic evidence in date palms. And the genetic fingerprint of five common types of date palm in Saudi Arabia was analysed: Birhi, Nebtet Ali, Ruthana, A'jua, and Sukeri. Then 12 primers and 64 bands were the content of an experiment. The percentage of genetic similarity among types of date palm ranged between 70-85%, whereas Al-Sukeri, as a date palm type and in terms of genetic similarity, was the furthest from the rest. In order to be much more acquainted with the types of date palm and range of differences, this study has been carried out.

2-Materials and Method:

2-1-DNA Extraction:

A DNA was extracted by grinding 0.1 gm of green leaves after being dried by a device called Freeze Dry in a solution (100mMTris PH=8.5 MEDTA, 500Mm NaCL, 1.5%SDS, 0.7% B-Mercaptoethanol). This was conducted through adopting a modified technique(*7*).

The samples were placed in a water tub at a temperature 60 Celsius for 30 minutes with a slight stirring. The plant residue was separated from DNA dissolved in the solution of extraction by the process of centrifugation by a centrifuge at a speed of 4000 rpm for 10 minutes. Then deposition of the DNA was achieved by adding 0.6 volume of Isopropanol to an aqueous and DNA was collected at the bottom of a tube by a technique called centrifugation at a speed of 10000 rpm for 10 minutes.

The DNA needed a wash by ethyl alcohol. Later DNA was dried, then dissolved in 300-400 ml with sterile distilled water. In the end, samples were diluted to attain a concentration of 25 ng/ml. To amply specific pieces of the DNA where their outsets have had nucleotide structure completing the primer used, the RAPD technique was used. Then the DNA pieces resulting from Agarose gel were separated and compared with different species (*8*). Four primers, where each of which consisted of 10 nucleotide bases, were under an experiment. Those said bases were products of Operon Technologies inc Alamenda Calif. USA), (Table 1).

An interaction, which was based on a size of 25ml, occurred in a medium consisted of 10 Mm HCl, PH:8.3, 1.5 Mm MgC12, 50Mmkcl) of the four nucleotides, and 15 m of a primer and an enzyme unit of the condensing enzyme, Taq DNA polymerase.

Propagation, as a process, was carried out in a Gene Amp PCR System 9600, Perkin Elmer, Foster city, Calif. The system included a rotation per 4 minutes at 94 Celsius, then followed by 35 rotations each of which lasted for a minute at 94 Celsius, 2 minutes at 36 Celsius, 2 minutes at 72 Celsius with a rotation per 7 minutes at 72 Celsius as a final protracted rotation. It's important to say that the system was fixed at 4 Celsius. Finally, the gel electrophoresis was done in line with (9).

2-2-Electrophoresis and Colouring and Imaging:

Outputs of the propagating processes were separated in the electrophoresis device at a concentration 1.5% in a solution called TAE(0.04M Tris- Acetate, 0.01 MEDTA, PH=7.8) for 3.5 hrs / 90 volts. The Agrose Gel was coloured in a solution of Bromide Alathidiom of a concentration 0.5 mg/ml. Then by using a gel documentation device, the gel was photographed. Later, the percentage of the genetic distance among cultivars, which depend on the results of the genetic similarity, was estimated according to the following formula:

Genetic distance=1-(2nxy/nx+ny)^{x} 100

Whereas **nxy** represents the number of common bands between two samples X and Y which are represented two categories.

Nx: a total number of the bands in the sample X

Ny: a total number of the bands in the sample Y

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2-3-Statistical Analysis:

Results of the propagating processes for the four primers were collected and inserted in special tables according to the comparison of availability or not DNA pieces for different samples. Availability of the DNA piece was symbolised by no. 1, and non-availability by no.0. Coefficient of the genetic distance and input similarity was calculated by using formula (10).

Then a cluster analysis was carried out and an outline of the genetic distance among inputs was drawn through adopting " The unweighted pair group method for the arithmetic average (UPGMA)" (11). All statistical analyses were conducted by using a program called "Numerical Taxonomy and Multivariate Analysis System" already installed in a computer (NTSYS-pc,12). Also, they made use of a program called Photocapt in identifying bands' values and demonstrating their specifications.

Table (1) primers used in study

No	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%

3-Results and discussion :

The process of electrophors indicated that the total number of DNA's pieces resulted from amplifying using the four primers was 64 pieces. And there were no cases of resemblance among the packets of the present study recorded. Through comparing the results of the propagation process among different primers, an apparent difference in the number of the resulting bands was observed, depending on the primer used. Also, a clear variation in their molecular weights was found among different cultivars. It is also noted that some primers, such as OPA-09 and OPC-09, were capable of unveiling a big percentage of differences among cultivars, whereas the capacity of the primer OPB-09 and OPC-08 revealed fewer differences. As the study revealed, the average of the DNA pieces propagated was 11.75.Analysis of the results of the genetic relationship relied on the availability or not of the bands resulting from multiplyin,g of specific pieces of genes of the plants used in this study, and relied on molecular weights as well as the number and locations complementing sequences of primers on the bar of the DNA. Light bands were neglected which is consistent with (13).As to the variation, relying on differences in the prominence of bands which normally result from the appearance of some multiplying bands together in the same molecular weight, this appeared as a thick band, which is in reality come grating bands. This band might result from the phenomenon called " homozygotsity," where multipyling prominence might be in the same molecular weight. Therefore, the said pieces clustered together in these locations. The increase in the concentration of DNA sometimes leads to the recurrence of the targeted DNA copies that caused the same location to be multiplied more than once. And since the adequate concentration of DNA is hard to identify due to being affected by a number of factors, it would be quite difficult to use differences in the thickness of the resulting band as a measure of the genetic variation. This is consistent with what was mentioned by (14).The results of the primers used in estimating genetic distance between every two cultivars studied and described by (10) which were counted on the availability of come grating bands between a pair of these plants. After inserting data into the program, the genetic distance among the selected cultivars was found as illustrated in (Tables 2,3,4,5).Results in table 4 suggested there were clear differences among cultivars of date plam involved in this study. This means that there is an obvious genetic distance among them where the distance reached at 100%, except for categories such as BentAlsabaa and Dekal Ahmer, where distance stood at 75%. This brought to light that these cultivars are separatedfrom each other and are of special features. This was evident through phenotypic differences for the plant and fruit.As for (Tables 3,4,5), cultivars were characterised by their high genetic distance through using the primer OPB-09, OPC-09 and OPC-08. Also, categories Dekl Ahmer and Khadhrawi recorded genetic difference standing at 66% through using primer OPB-09.As can be seen from figure 1, cultivars were distibuted into two independent groups. Cultivars such BentAlsabaa, Alfersee and Al_khasab were put in one group, and the rest - Allylwi , Dekal Ahmer and AlKhdrawee - were put in another group.Less distance was recorded between Allylwi and Dekal Ahmer, then, according to the genetic distance, followed by Alfersee and Bent Alsabaa. Also, figure 2 showed photos of the DNA's products using 4 primers with the assistance of Photocapt program in identifying the numbers and values of bands.

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Table (2) the genetic distance between cultivars of date palm using the primer OPA-09 RAPD indicators

Simple	Total	Monomorphic		Polymorphic%
Al_khasab × Allylwi	3	00	3	%100
Al_khasab × Bent Alsabaa	6	00	6	%100
Al_khasab × Dekal Ahmer	4	00	4	%100
Al_khasab × Alfersee	5	00	5	%100
Al_khasab × AlKhdrawee	4	00	4	%100
Bent Alsabaa× Allylwi	5	00	5	%100
Allylwi × Dekal Ahmer	3	00	3	%100
Allylwi ×Alfersee	4	00	4	%100
Allylwi × AlKhdrawee	3	00	3	%100
BentAlsabaa× Dekal Ahmer	6	1	5	% 83.4
Alfersee× Bent Alsabaa	7	00	7	%100
AlKhdrawee ×Bent Alsabaa	6	00	6	%100
Alfersee× Dekal Ahmer	5	00	5	%100
Dekal Ahmer× AlKhdrawee	4	00	4	%100
Alfersee× AlKhdrawee	5	00	5	%100

Table (3) the genetic distance between cultivars of date palm using the primer OPB-09 RAPD indicators

Simple	Total	Monomorphic		Polymorphic
Al_khasab × Allylwi	6	00	6	%100
Al_khasab × Bent Alsabaa	3	00	3	%100
Al_khasab × Dekal Ahmer	6	00	6	%100
Al_khasab × Alfersee	4	00	4	%100
Al_khasab × AlKhdrawee	5	00	5	%100
Bent Alsabaa× Allylwi	3	00	3	%100
Allylwi × Dekal Ahmer	6	00	6	%100
Allylwi ×Alfersee	4	00	4	%100
Allylwi × AlKhdrawee	5	00	5	%100
Bent Alsabaa× Dekal Ahmer	3	00	3	%100
Alfersee× Bent Alsabaa	1	00	1	%100
AlKhdrawee ×Bent Alsabaa	2	00	2	%100
Alfersee× Dekal Ahmer	4	00	4	%100
Dekal Ahmer × AlKhdrawee	5	1	4	%80
Alfersee× AlKhdrawee	3	00	3	%100

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Table (4) the genetic distance between cultivars of date palm using the primer OPC-09 RAPD indicators

Simple	Total bands	Monomorphic		Polymorphic
Al_khasab × Allylwi	2	00	2	%100
Al_khasab × Bent Alsabaa	1	00	1	%100
Al_khasab × Dekal Ahmer	4	00	4	%100
Al_khasab × Alfersee	0	00	0	%00
Al_khasab × AlKhdrawee	5	00	5	%100
Bent Alsabaa× Allylwi	3	00	3	%100
Allylwi × Dekal Ahmer	7	00	7	%100
Allylwi ×Alfersee	2	00	2	%100
Allylwi × AlKhdrawee	7	00	7	%100
Bent Alsabaa× Dekal Ahmer	4	00	4	%100
Alfersee× Bent Alsabaa	0	00	0	%00
AlKhdrawee ×Bent Alsabaa	5	00	5	%100
Alfersee× Dekal Ahmer	4	00	4	%100
Dekal Ahmer × AlKhdrawee	8	00	7	%87.5
Alfersee× AlKhdrawee	5	00	5	%100

Table (5) the genetic distance between cultivars of date palm using the primer OPC-08 RAPD indicators

Simple	Total bands	Monomorphic		Polymorph
Al_khasab × Allylwi	5	00	5	%100
Al_khasab × Bent Alsabaa	0	00	0	00
Al_khasab × Dekal Ahmer	5	00	5	%100
Al_khasab × Alfersee	0	00	00	00
Al_khasab × AlKhdrawee	0	00	0	%100
Bent Alsabaa× Allylwi	5	00	5	%100
Allylwi × Dekal Ahmer	10	00	10	%100
Allylwi ×Alfersee	5	00	5	%100
Allylwi × AlKhdrawee	0	00	0	%100
Bent Alsabaa× Dekal Ahmer	5	00	5	%100
Alfersee× Bent Alsabaa	0	00	0	00
AlKhdrawee ×Bent Alsabaa	0	00	0	00
Alfersee× Dekal Ahmer	5	00	5	%100
Dekal Ahmer × AlKhdrawee	5	00	5	100%
Alfersee× AlKhdrawee	5	00	5	%100

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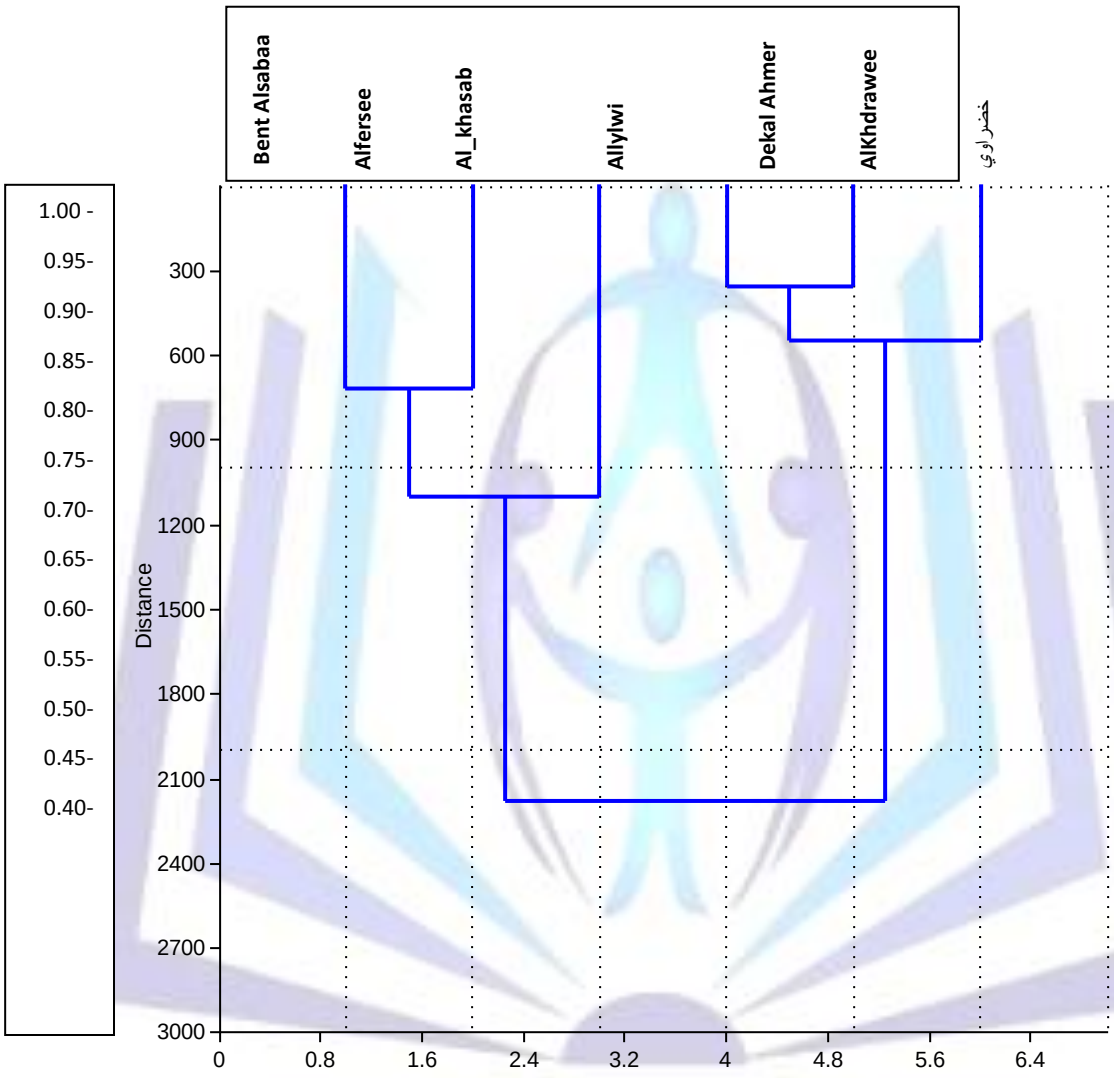

Figure (1) cluster analysis of a number of cultivars date palm (Al_khasab and Allylwi , Bent Alsabaa, Dekal Ahmer, and Alfersee and AlKhdrawee)using four indicators of prefixes in the RAPD

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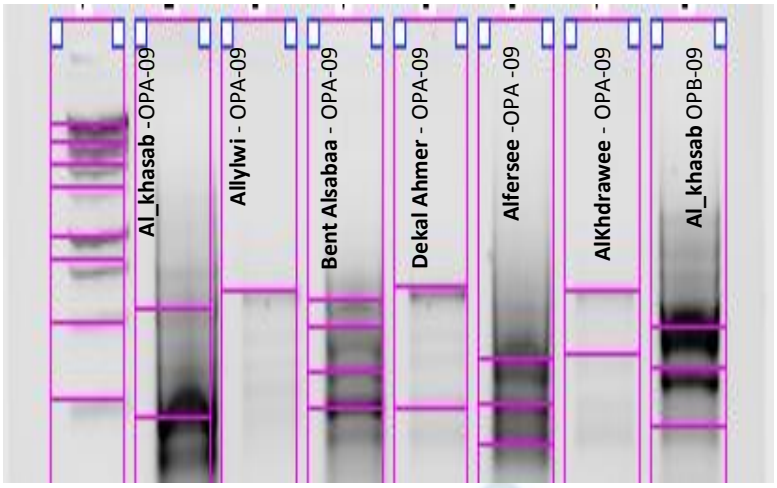

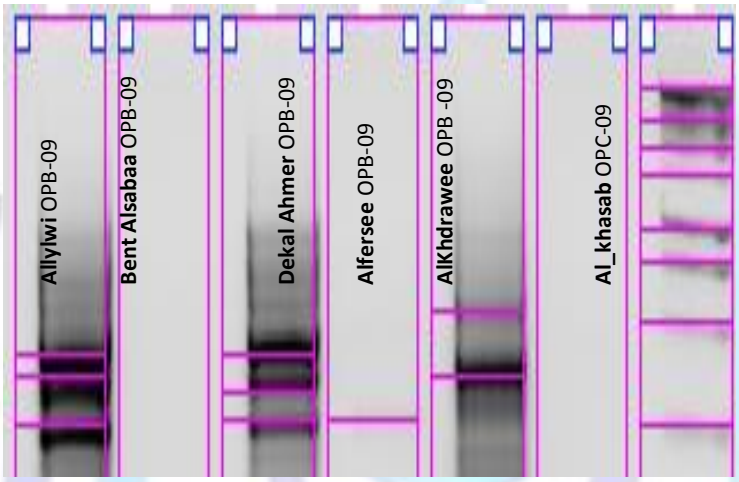

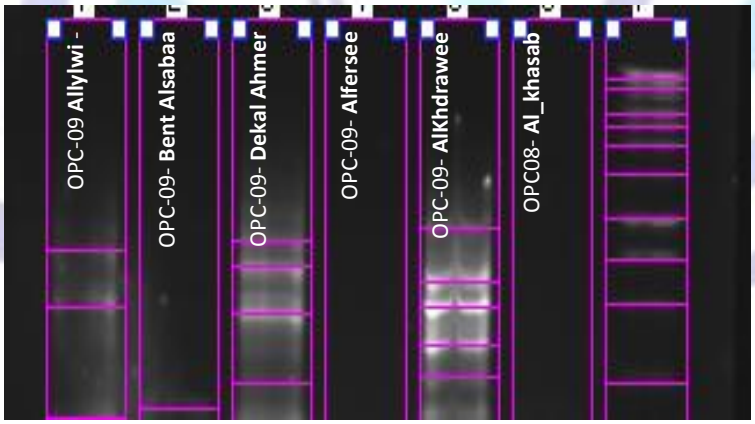

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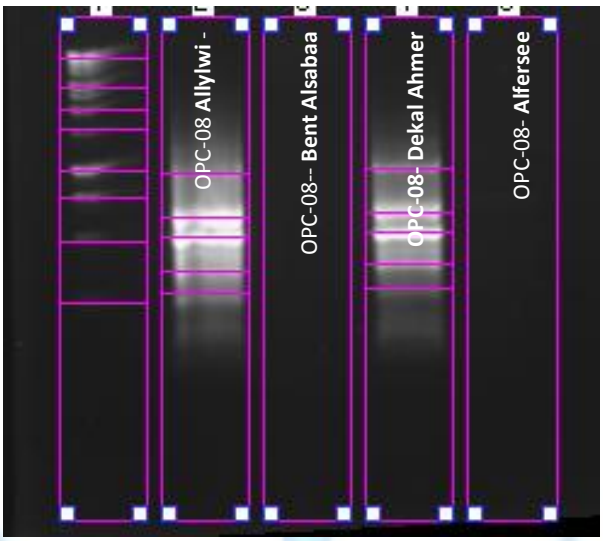

Figer(2) polymorphism revealed using RAPD primerOPA-09 ,OPB-09,OPC-09,OPC-08 to amplify genomic DNA purified from cultivars of date palm

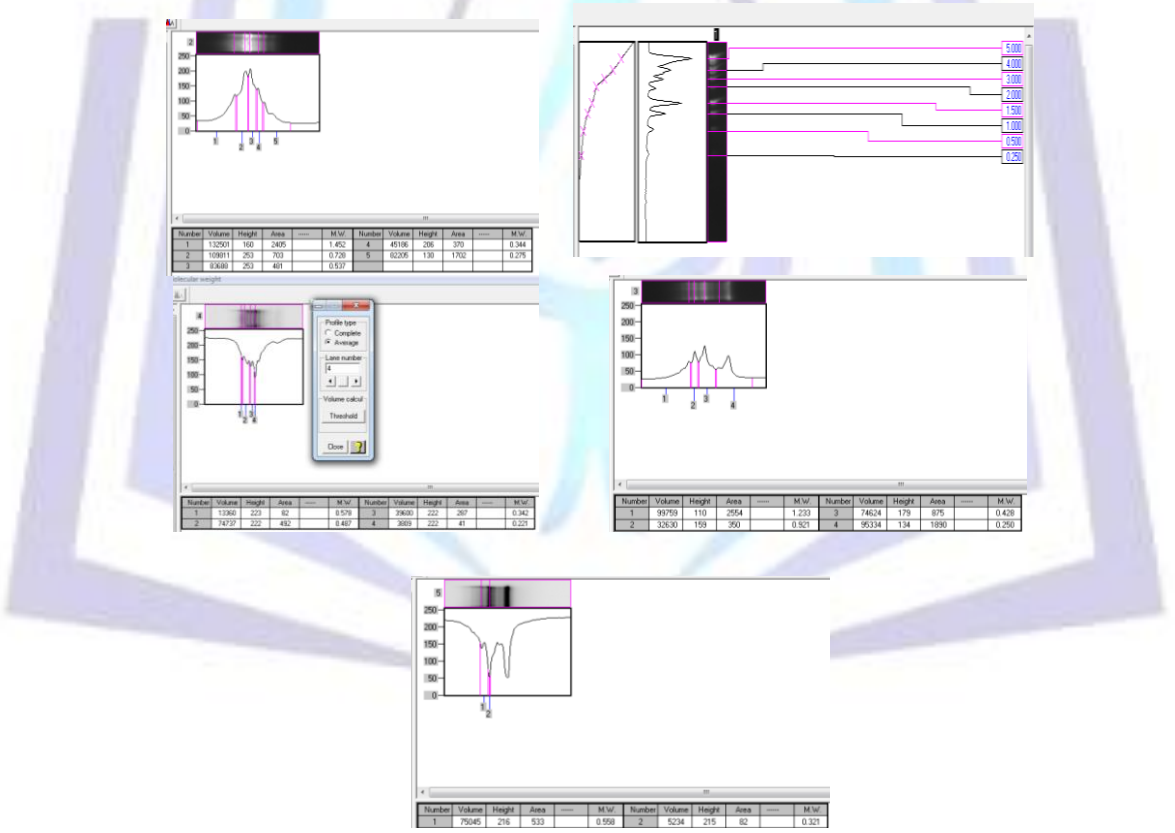

Figure (3) analysis of the results by using the program photocapt

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