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# INVESTIGATION THE PROTEIN PATTERN OF LEAVES AND ROOTS OF BARHI AND KHALAS DATE PALM (*Phoenix dactylifera* L.) CULTIVARS PROPAGATED BY OFFSHOOTS AND TISSUE CULTURE TECHNIQUES

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**ABSTRACT**

**The present study was conducted to investigate the protein pattern of leaves and roots of Barhi and Khalas date palm cultivars propagated by offshoots and tissue culture plants and grown in one of the private orchards in Ktaban region in Basrah governorate, Iraq. Results of the protein pattern showed that protein bundles on polyacrylamide gel were differed by their molecular weights, the number of protein bundles ranged from 6 to 8 according to the female cultivar, propagation method and plant part used.Leaves and roots of Barhipropagated by offshoots and tissue culture techniques showed the same site and molecular weight for the first protein bundle 220.000 KDa and for the second protein bundle 222.976 KDa. The cluster analysis of the two cultivars propagated by offshoots and tissue culture techniques depending on the molecular weights of the protein bundles showed two main groups with the degree of difference between them amounting to (7.5%), the first main group included Barhipropagated by offshoots and tissue culture techniques, for both leaves and roots .Whereas Khalas propagated by offshoots and tissue culture techniques, for both leaves and roots grouped in the second main group.**

**Keywords:** Protein pattern; Barhi and Khalas date palm; offshoots; tissue culture echniques.

## INTRODUCTION

Date palm tree (*Phoenix dactylifera* L.) belongs to the family Arecacea that includes about 220 genus and 2600 species. It is a dioeciousplant, where male and females flowers carried on separate trees and the date palm fruits have a high nutritional value, which made it an important reliable food source. Taain, [1], Shabana et al., [2].

Proteins are about 71% of the weight of a dry cell. They are of high molecular weights and have the advantage of not penetrating through the permeable membranes. Mattar [3] mentioned the possibility of distinguishing between palm cultivars by using the gel electrophoresis technique for proteins.

Al-Sahi and Al-Amber [4] studied the electrophoresis of proteins of three cultivars of date palm (Halawi, Sayer and Barhi), and observed three protein bundles for each of the three cultivars that differed in their molecular weights.

Al-Essa [5] studied the protein pattern of three date palm cultivars viz., Khalas, Shishi, and Rises and were found differences in the protein pattern during the different growth stages.

Study conducted by Abd et al. [6] on twelve date palm male cultivars (Khadairy, Shalhoum, Ardawi, Bandar, Ereojan, Habiti, FahlZuhdi, Abu Kafisha, Atishi, Sweihi, Abu Tair, Nabhar) grown in the orchards of the Samawah city,Iraq to identify their molecular characteristics. Genetic differences were studied using RAPD indicators through the use of four primers. The results showed a difference in the number of DNA bundles and their molecular weights according to the primerused. Some of the cultivars corresponded to the number of bundles. The two cultivars Bandar and Sweihi were corresponded, as well as Abu Kafisha and Nabhar cultivars. In addition, Ereojan, Atishi and Abu Tair were in the same line of correspondence, that proved that these cultivars are different names to one cultivar that given by gardeners and according to their regions.

The present study was conducted to investigatethe protein pattern of leaves and roots of Barhi and Khalas date palm cultivars propagated by offshoots and tissue culture plants.

## MATERIALS AND METHODS

The present study was conducted in one of the private orchards in Ktaban region in Basrah governorate to investigate the protein pattern of leaves and roots of Barhi and Khalas date palm cultivars propagated by offshoots and tissue culture plantlets. Selected trees were as similar as possible in age (5 years), growthand had the same agricultural practices.

Samples of leaves and roots were collected from the trees and were dried using the lyophilization technique by using Freeze Dryer at a temperature of -26°C. Protein extracted from the samples according to the method described in Al-Najar [7] by taking 1 g of leaves and roots and placing them in a ceramic mortar with 3 ml of Tris-HCl-buffer (0.1M, pH7.5) containing Phenyl methane sulfonyl fluoride on temperature of 4°C, then centrifuged at the same temperature and at the speed of 18000 r / s for half an hour, then took 40 microliters from the leachate to the device using the Polyacrylamide gel.

Proteins electrophoresis was carried out on a polyacrylamide gel using the Slab-Electrophoresis method with the presence of SDS according to the method described Bavei et al. [8] and the Broad Range Protein Molecular Weight Markers were used from Promega company. Molecular weights of proteins were determined and drew through a special software Photo Capt Mw version 17. Molecular weights were used to plot the relationship between species by cluster analysis Ander berg, [9]. The following are the treatment details

1.Marker 2. Roots of Barhi collected from tissue culture raised plants3 .Leaves of Barhi collected from tissue culture raised plants4 .Leaves of Barhi collected fromoffshoots5 .Roots of Barhicv. collected from off shoots 6. Roots of Khalas collected from tissue culture raised plants7 .Leaves of Khalascollected from tissue culture raised plants9 .Roots of Khalas collected from off shoots.

## RESULTS AND DISCUSSION

The results of the protein pattern of the studied cultivars (Fig. 1a, b, c,d,e,f,g,h,i) show that there are differences between these cultivars in size, area and height by cultivar, propagation method and plant part used. Protein bundles on polyacrylamide gel were differed by their molecular weights, the number of protein bundles

ranged from 6 to 8 according to the female cultivar, propagation method and plant part,so eight protein bundles were recorded in the roots and seven protein bundles in the leaves of Barhi cv. propagated by tissue culture techniques, whereas seven protein bundles recorded in roots of Khalascv propagated by tissue culture techniques.

Six protein bundles were recorded in the leaves and roots of Barhi and Khalascvs. propagated by offshoots, in addition to six protein bundles for leaves of Khalas cv. propagated by tissue culture techniques.

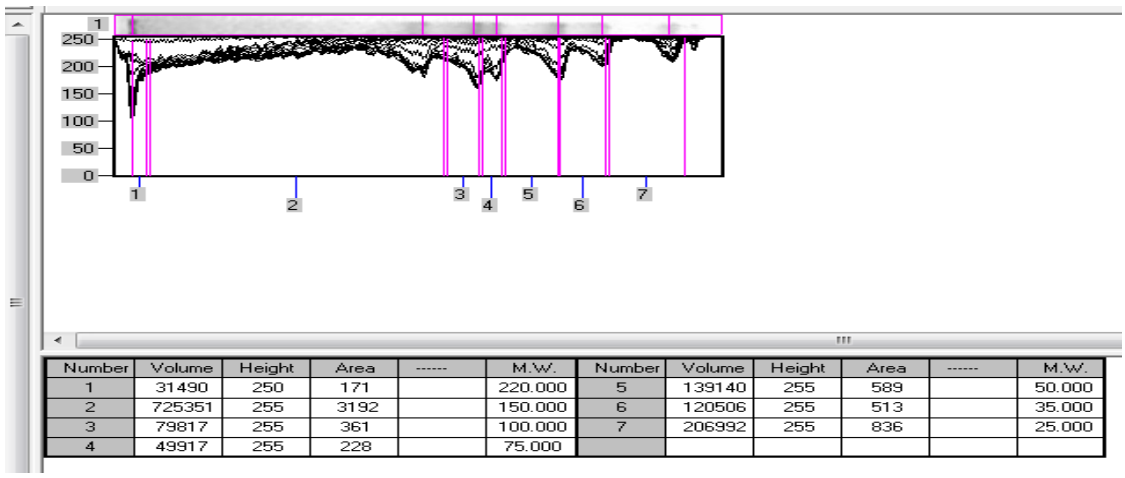
As for the sites of the protein bundles on polyacrylamide gel and molecular weights, differed according to the cultivar, propagation method and the plant part, since all the treatments had the same site as the first bundle and the same molecular weight 220.000 KDa. The molecular weight of the third protein bundle was 157.254 KDa in the leaves and roots of Barhi cv. propagated by offshoots and the roots of Khalas cv propagated by tissue culture techniques in addition to the fourth protein bundle for the leaves of Barhi cv. propagated by tissue culture techniques, the leaves of Khalas cv propagated by tissue culture techniques and the leaves and roots of the Khalas cv propagated by offshoots. This indicated to the genetic convergence of the two cultivars, regardless of the method of propagation and the plant part used they belong to the same species " dactylifera", genus " phoenix" and family "Arecacea".

Results presented in Fig. 2 indicated that leaves and roots of Barhi cv. propagated by offshoots and tissue culture plants showed the same site and molecular weight for the first protein bundle 220.000 KDa and for the second protein bundle 222.976 KDa. The roots and leaves of Barhi cv. propagated by offshoots were similar to the same protein site and the same molecular weight 157.254 KDa, while the same bundle was different for the leaves and roots of Barhi cv. propagated by tissue culture techniques. All treatments of Barhi cv. were similar in the same site and molecular weight of the sixth and seventh protein bundles, 47.284 KDa with the exception of the treatment of propagated by tissue culture techniques which amounted to100.000 KDa.

As for Khalas the figure itself indicated that the roots and leaves had the same site and molecular weight for the first protein bundle 220.339 KDa. The leaves and roots of Khalas cv. propagated by tissue culture techniques were similar in site and the molecular weight of the second protein bundle which was 223.189 KDa. The third and fourth proteins of the leaves and roots of Khalas cv. propagated by offshoots and the leaves of Khalas cv. propagated by tissue culture techniques were similar 202.994 KDa and 157.254 KDa respectively. The same bundles for the roots of Khalas cv. propagated by tissue culture techniques were differed 157.254 KDa and 122.116 KDa respectively. The fifth protein bundle for leaves of Khalas cv. propagated by tissue culture techniques and off shoots were similar which amounted to109.588 KDa. All treatments were similar in the molecular weight of the sixth protein bundle that recorded 24.211KDa.

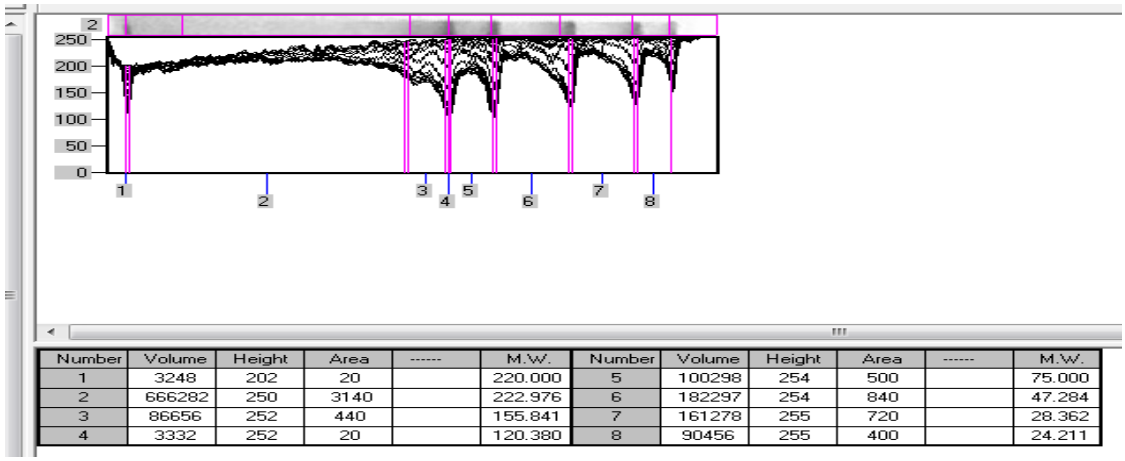
The variation of the cultivars propagated by offshoots to those propagated by tissue culture techniques in the number of bundles and their sites may be due to some simple genetic variations resulting from the use of different hormones in the technique of micropropagation, which appeared in the form of protein bundles varied in number, site and molecular weight.

1.Marker 2 . Roots of Barhi cv. propagated by tissue culture plants 3. Leaves of Barhi cv. propagated by tissue culture techniques4 . Leaves of Barhi cv. propagated by off shoots 5. Roots of Barhi cv. propagated by off shoots6. Roots of Khalas cv. propagated by tissue culture techniques7. Leaves of Khalas cv. propagated by tissue culture techniques9 .Roots of Khalas cv. propagated by offshoots.



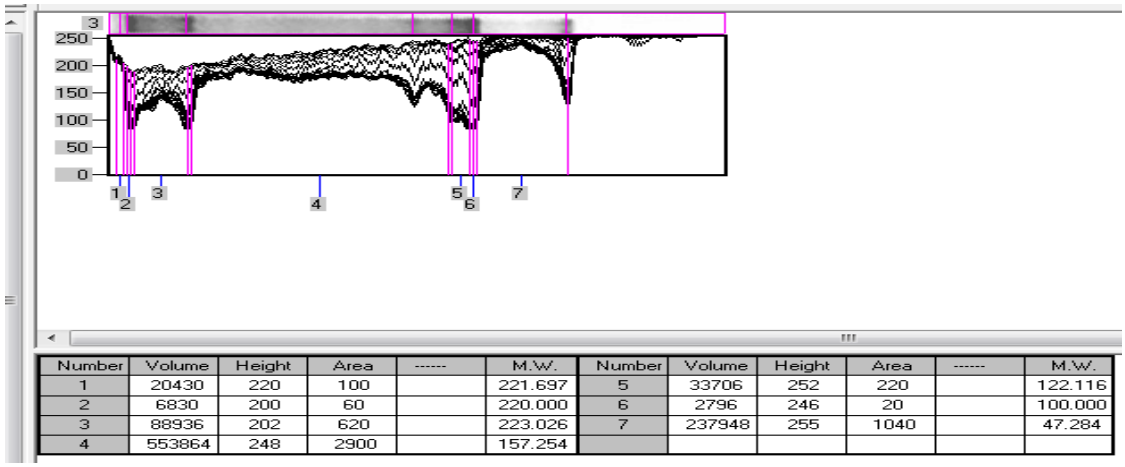

| Number | Volume | Height | Area | ------ | M.W. | Number | Volume | Height | Area | ------ | M.W. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 31490 | 250 | 171 | | 220.000 | 5 | 139140 | 255 | 589 | | 50.000 |
| 2 | 725351 | 255 | 3192 | | 150.000 | 6 | 120506 | 255 | 513 | | 35.000 |
| 3 | 79817 | 255 | 361 | | 100.000 | 7 | 206992 | 255 | 836 | | 25.000 |
| 4 | 49917 | 255 | 228 | | 75.000 | | | | | | |

**a**



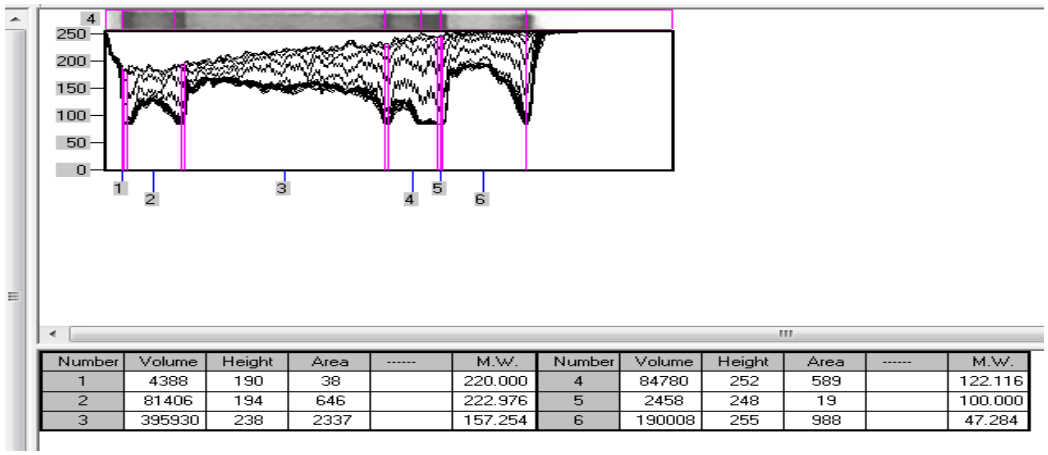

| Number | Volume | Height | Area | ------ | M.W. | Number | Volume | Height | Area | ------ | M.W. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3248 | 202 | 20 | | 220.000 | 5 | 100298 | 254 | 500 | | 75.000 |
| 2 | 666282 | 250 | 3140 | | 222.976 | 6 | 182297 | 254 | 840 | | 47.284 |
| 3 | 86656 | 252 | 440 | | 155.841 | 7 | 161278 | 255 | 720 | | 28.362 |
| 4 | 3332 | 252 | 20 | | 120.380 | 8 | 90456 | 255 | 400 | | 24.211 |

**b**



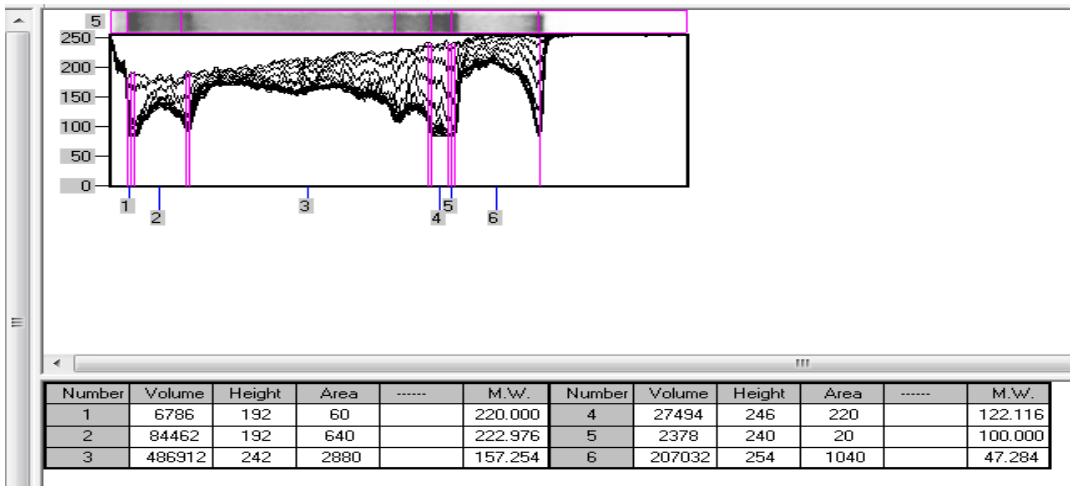

| Number | Volume | Height | Area | ------ | M.W. | Number | Volume | Height | Area | ------ | M.W. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 20430 | 220 | 100 | | 221.697 | 5 | 33706 | 252 | 220 | | 122.116 |
| 2 | 6830 | 200 | 60 | | 220.000 | 6 | 2796 | 246 | 20 | | 100.000 |
| 3 | 88936 | 202 | 620 | | 223.026 | 7 | 237948 | 255 | 1040 | | 47.284 |
| 4 | 553864 | 248 | 2900 | | 157.254 | | | | | | |

**c**



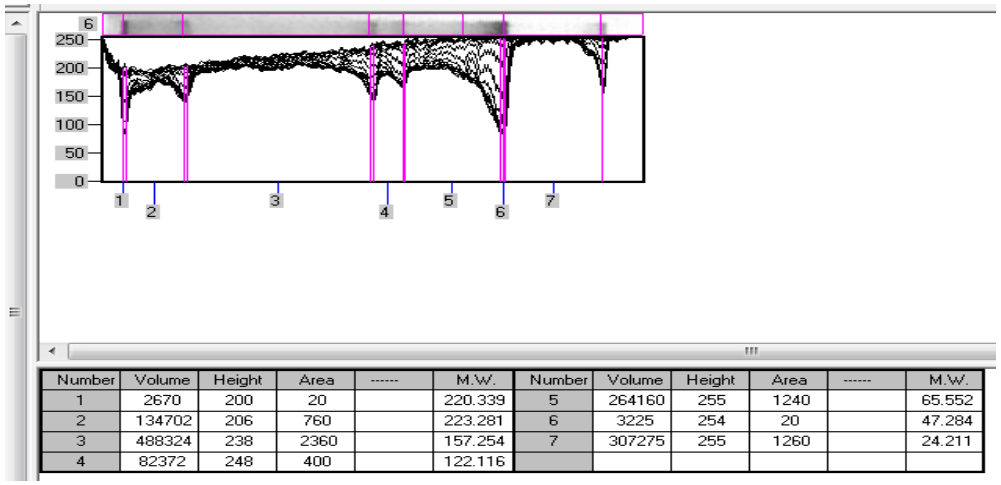

| Number | Volume | Height | Area | ------ | M.W. | Number | Volume | Height | Area | ------ | M.W. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 4388 | 190 | 38 | | 220.000 | 4 | 84780 | 252 | 589 | | 122.116 |
| 2 | 81406 | 194 | 646 | | 222.976 | 5 | 2458 | 248 | 19 | | 100.000 |
| 3 | 395930 | 238 | 2337 | | 157.254 | 6 | 190008 | 255 | 988 | | 47.284 |

d



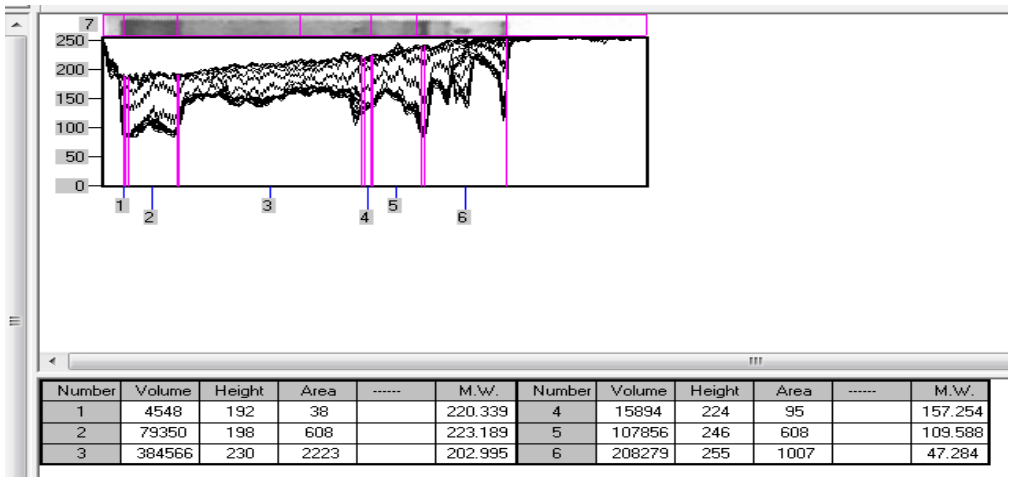

| Number | Volume | Height | Area | ------ | M.W. | Number | Volume | Height | Area | ------ | M.W. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 6786 | 192 | 60 | | 220.000 | 4 | 27494 | 246 | 220 | | 122.116 |
| 2 | 84462 | 192 | 640 | | 222.976 | 5 | 2378 | 240 | 20 | | 100.000 |
| 3 | 486912 | 242 | 2880 | | 157.254 | 6 | 207032 | 254 | 1040 | | 47.284 |

**e**



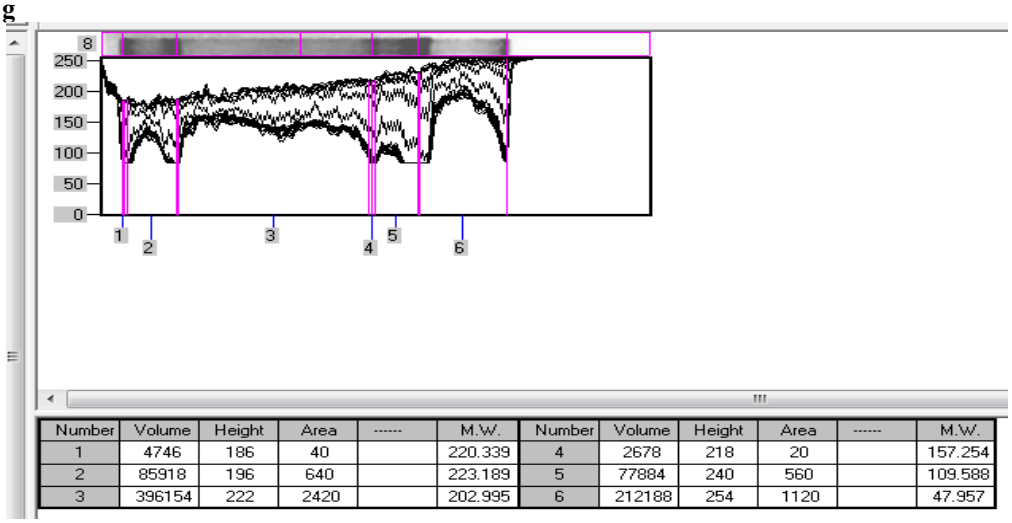

| Number | Volume | Height | Area | ------ | M.W. | Number | Volume | Height | Area | ------ | M.W. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2670 | 200 | 20 | | 220.339 | 5 | 264160 | 255 | 1240 | | 65.552 |
| 2 | 134702 | 206 | 760 | | 223.281 | 6 | 3225 | 254 | 20 | | 47.284 |
| 3 | 488324 | 238 | 2360 | | 157.254 | 7 | 307275 | 255 | 1260 | | 24.211 |
| 4 | 82372 | 248 | 400 | | 122.116 | | | | | | |

f



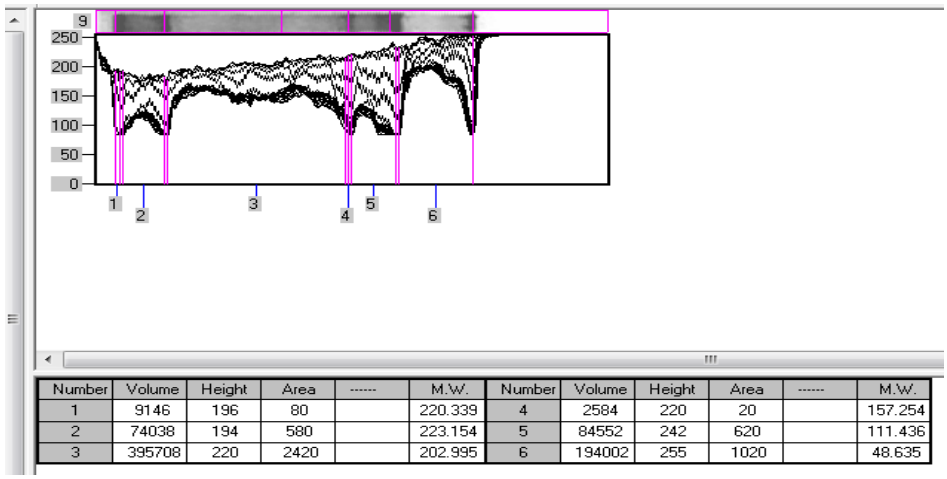

| Number | Volume | Height | Area | ------ | M.W. | Number | Volume | Height | Area | ------ | M.W. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 4548 | 192 | 38 | | 220.339 | 4 | 15894 | 224 | 95 | | 157.254 |
| 2 | 79350 | 198 | 608 | | 223.189 | 5 | 107856 | 246 | 608 | | 109.588 |
| 3 | 384566 | 230 | 2223 | | 202.995 | 6 | 208279 | 255 | 1007 | | 47.284 |

**g**



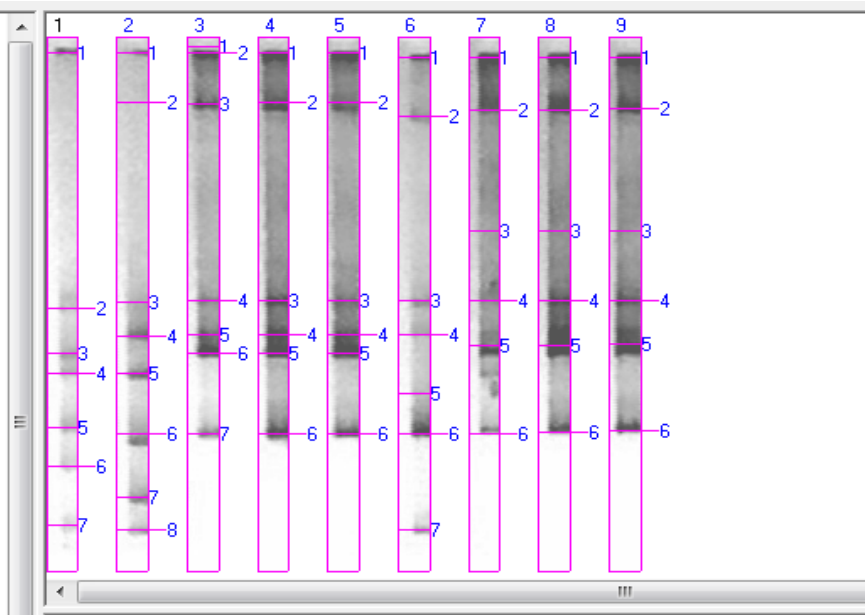

| Number | Volume | Height | Area | ------ | M.W. | Number | Volume | Height | Area | ------ | M.W. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 4746 | 186 | 40 | | 220.339 | 4 | 2678 | 218 | 20 | | 157.254 |
| 2 | 85918 | 196 | 640 | | 223.189 | 5 | 77884 | 240 | 560 | | 109.588 |
| 3 | 396154 | 222 | 2420 | | 202.995 | 6 | 212188 | 254 | 1120 | | 47.957 |

**h**




| Number | Volume | Height | Area | ------ | M.W. | Number | Volume | Height | Area | ------ | M.W. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 9146 | 196 | 80 | | 220.339 | 4 | 2584 | 220 | 20 | | 157.254 |
| 2 | 74038 | 194 | 580 | | 223.154 | 5 | 84552 | 242 | 620 | | 111.436 |
| 3 | 395708 | 220 | 2420 | | 202.995 | 6 | 194002 | 255 | 1020 | | 48.635 |

**Fig. 1. (a,b,c,d,e,f,g,h,i) :Protein pattern of date palm cultivars**



| | M.W. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | L1 | L2 | L3 | L4 | L5 | L6 | L7 | L8 | L9 |
| 1 | 220.000 | 220.000 | 221.697 | 220.000 | 220.000 | 220.339 | 220.339 | 220.339 | 220.339 |
| 2 | 150.000 | 222.976 | 220.000 | 222.976 | 222.976 | 223.281 | 223.189 | 223.189 | 223.154 |
| 3 | 100.000 | 155.841 | 223.026 | 157.254 | 157.254 | 157.254 | 202.995 | 202.995 | 202.995 |
| 4 | 75.000 | 120.380 | 157.254 | 122.116 | 122.116 | 122.116 | 157.254 | 157.254 | 157.254 |
| 5 | 50.000 | 75.000 | 122.116 | 100.000 | 100.000 | 65.552 | 109.588 | 109.588 | 111.436 |
| 6 | 35.000 | 47.284 | 100.000 | 47.284 | 47.284 | 47.284 | 47.284 | 47.957 | 48.635 |
| 7 | 25.000 | 28.362 | 47.284 | | | 24.211 | | | |
| 8 | | 24.211 | | | | | | | |

**Fig. 2. Protein bundles sites and their molecular weights of date palm cultivars**

Fig. 3 indicated to the cluster analysis of the two cultivars under study depending on the molecular weights of the protein bundles resulting from the leaves and roots of the two cultivars propagated by offshoots and tissue culture techniques. These treatments grouped in two main groups with the degree of difference between them amounting to (7.5%), the first main group included Barhi cv. propagated by offshoots and tissue culture techniques, for both leaves and roots.Whereas Khalas cv. propagated by offshoots and tissue culture techniques, for both leaves and roots grouped in the second main group.

The same figure also indicated that the first main group was distributed under two main groups with a degree of difference between them amounting to (6.0%), the first included the roots and leaves of Barhicv. propagated by tissue culture techniques, while the group under the second main included the roots and leaves of the Barhi cv. propagated by offshoots.

The second main group was divided into two main groups with a difference degree between them amounting to (6.2%), the first included the roots and leaves of Khalascv. propagated by offshoots, while the group under the second main group included the roots and leaves of Khalas cv. propagated by tissue culture techniques. The lowest degree of difference between the leaves and roots of the Barhi cv. propagated by offshoots and the leaves and roots of Khalas cv. propagated by offshoots was (3.0%), while the greatest degree

of difference was between the leaves and roots of Khalas cv. propagated by tissue culture techniques which was (4.7%).

These results indicated that these two female cultivars propagated by offshoots and tissue culture techniques have increased in affinity with each other and it is possible that the genetic factors that carry them may be due to the fact that they originated from one origin and became independent from each other over time, and that the resulting differences between the two methods of propagation for both cultivars maybe due to the difference in the process of gene expression in both methods because of the use of plant hormones in the method of micro-propagation of date palms or the result of gene expression during the hardening stage. This study was similar to the study of Abdul Wahid and Aati [10] for some date palm cultivars using the gel electrophoresis technique, where the results of the study showed the differences in the number and sites of protein bundles and their molecular weights ranged between (32.58 and 67.939 kDa). The current study also agreed with the results of Al-Najjar [7] for some male cultivars of date palm, that showed the difference among the studied cultivars in the number, sites and characteristics of protein bundles.

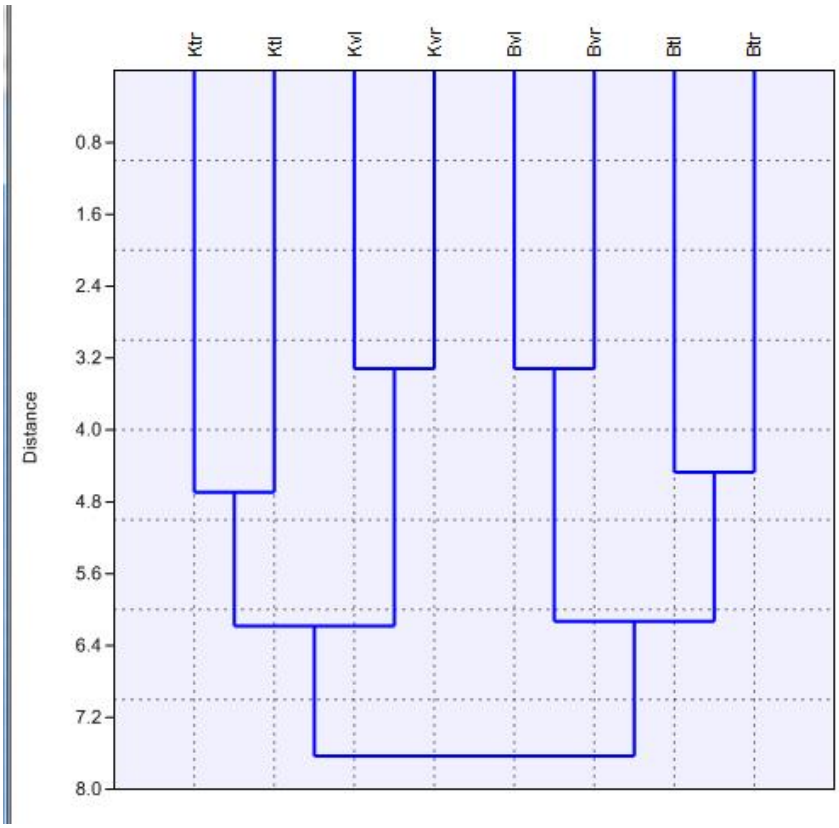



**Fig. 3. Custer analysis of Barhi and Khalas cultivars of date palm propagated by offshoots and tissue culture techniques**

Btr = Roots of Barhi cv. propagated by tissue culture techniques ,Btl = Leaves of Barhi cv. propagated by tissue culture techniques, Bvl = = Leaves of Barhi cv. propagated by offshoots, Bvr = Roots of Barhi cv. propagated by offshoots , Ktr = Roots of Khalas cv. propagated by tissue culture techniques , Ktl = Leaves of Khalas cv. propagated by tissue culture techniques, Kvl = Leaves of Khalas cv. propagated by offshoots, Kvr = Roots of Khalas cv. propagated byoffshoots

## CONCLUSION

The present study clearly indicated that protein bundles on polyacrylamide gel were differed by their molecular weights, the number of protein bundles ranged from 6 to 8 according to the female cultivar. The cluster analysis of the two cultivars propagated by offshoots and tissue culture techniques depending on the molecular weights of the protein bundles showed two main groups with the degree of difference between them amounting to (7.5%).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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