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Electrophoretic Analysis of Protein Patterns in Date Palm "Khalas" Cultivar Leaflets Among Different Locations of Al-Ahsa

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ABSTRACT

Proteins extracted from different date palm (*Phoenix dactylifera* L.) trees "Khalas" Cultivar leaflets, which were collected from 10 different locations of Al-Ahsa oasis in the eastern province of Saudi Arabia, have been analyzed by SDS-PAGE electrophoresis. The present study showed different protein patterns, which revealed by NJ and UPGMA clustering methods into three major clusters 1, 2 and 3. Cluster 1 occupies distinct place in the dendrogram that composed of OY, OM, T and SH locations samples were reflected (monomorphism). Cluster 2 included A and AM locations samples that distinguished each by unique band. While the Cluster 3 included B, G, J and M locations samples which separated singly one by one, since there are no enough sharing bands to cluster them together except they were low in their protein content. The results collectively indicated that the difference in clustering patterns is mainly due to genetic variations. Additionally, cluster 1 location samples may represent the proposed original "Khalas" cultivar, while the other tree samples originated from seeds resembled the original cultivar. More genetic and biochemical studies should be conducted to support this conclusion. However, identification of the original "Khalas" cultivar is important to guaranty the superior characteristics of cultivated cultivar trees.

Keywords: *Phoenix Dactylifera* L., Clustering, Phylogenetic, UPGMA, Proteins Patterns, Genetic Variations

1. INTRODUCTION

Date palm (*Phoenix dactylifera* L., 2n = 2× = 36) is a monocotyledonous and dioeciously species belonging to Arecaceae family (ITIS, 2010; Elmeer *et al*., 2011). It widely cultivated in arid regions of the Middle East and North Africa, (Hamza *et al*., 2011; Al-Yahyai and Al-Khanjari, 2008; Khierallah *et al*., 2011; Elmeer *et al*., 2011; Jain, 2011; Abul-Soad and Madi, 2010). It is widely distributed in the Eastern Province of Kingdom of Saudi Arabia. There are more than 70 cultivars that have been grown there for ages (Al-Abduolhadi *et al*., 2011; Al-Issa *et al*., 2009). Date palm can be propagated by seeds, which usually produce trees bearing inferior fruits. Offshoots are more preferred for conventional propagation because they produce true-to-type trees with fruit quality identical to the mother tree (Al-Khalifah *et al*., 2013; Jain, 2011). The high stability of protein profile makes protein electrophoresis a powerful tool in elucidating the origin and the evolution of cultivated plants (Ladizinsky and Hymowitz, 1979). Therefore, protein pattern analysis by gel electrophoresis has been used in higher plants to study various problems in breeding, genetics, taxonomy, physiology, (Leonardo *et al*., 2010; Chang *et al*., 2010; Castillejo *et al*., 2012; Elshibli and Korpelainen, 2008; Davar *et al*., 2012) as it has been appreciated as a biochemical tool for studying the phylogentic relationships (Al-Yahyai and Al-Khanjari, 2008).

The phylogenetic analysis based on protein patterns were used to study the genetic relatedness between and among cultivar (El-Hady *et al*., 2010; Abdulla and Gamal, 2010; Haider *et al*., 2012). So, it would be helpful in recognizing the originals cultivars. On the other hand, "Khalas" cultivar is one of the most economical date palm cultivars grown in the eastern province of the Saudi Arabia, in Al-Ahsa oasis where known locally that "Khalas" Cultivar

trees are differed in their fruit quality depending on the location even within the oasis (Al-Mulla, 1997; Asif *et al*., 1982). Al-Issa (2006) showed in their comparative study to genetic and biochemical differences within "Khalas" cultivar trees growing in two localities. The main aim of the present study is to analyze whether their genetic diversity among "Khalas" cultivar trees according to ten locations of Al-Ahsa oasis using protein profiling. Consequently, "Khalas" cultivar have been selected for electrophoretic proteins analysis by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), leaflet tissues collected from 10 different locations within Al-Ahsa oasis which considered as the origin of this cultivar.

2. MATERIALS AND METHODS

The samples were collected from juvenile green leaflets from 30 years old and above "Khalas" cultivar trees in each location, the area were samples collected covered the most of Al-Ahsa oasis areas for date palm cultivation, which divided in to three areas: North, Middle and East villages (**Table 1 and Fig. 1**). The trees were offshoot of previous mother trees which were grown in the same location. The samples transferred immediately to liquid nitrogen, then to deep freezer-20°C until the time of usage. The samples were cut into 2×2 mm before ground in mortar with liquid nitrogen prior to be subjected to protein electrophoretic analysis using the SDS-PAGE.

2.1. Protein Extraction

The samples were ground in a mortar by liquid nitrogen, then the proteins extracted by homogenizing 100 mg of ground leaflet samples by half volume stainless steel beads with 1 mL extracting buffer (0. 5 M Tris/HCl buffer, pH 6.8+10% Glycerol v/v+4% PVP w/v) at 9/4 min., followed by 10/2 min. by using of (Bullet Blender Homogenizer), then incubated overnight at 4°C. The crude extract was vortexed by using of (VELP vortex mixer), then centrifuged at 12500 rpm for 10 min by using of (Eppendorf centrifuge 5424), the supernatant was moved to new tubes, while discarded the debris.

2.2. Protein Concentration

To the supernatant 1 ml of chilled acetone was added, vortexed, centrifuged at 12500 rpm for 10 min., then acetone discarded, this step repeated twice. After the acetone have been discarded the precipitated protein was incubated at room temperature for 15 min.

2.3. Resuspending Proteins

The proteins are redissolving in 150 µL. of extracting buffer, vortexed, boiled at 95°C for 4 min and then vortexed, centrifuged at 12500 rpm for 10 min, after that loaded in 10 µL.

2.4. Electrophoresis

Discontinuous vertical SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was carried out according to Laemmli (1970) with some modifications, in 12.5% resolving gels [6.67 mL Acrylamide-bis acrylamide (30%\2.6%)+4 mL (1.5 M tris/HCl buffer pH 8.8)+5.05 mL DW+0.16 mL (10% SDS)+freshly prepared 0.12 mL (10% amm. persulphate)+0.008 mL TEMED] and stacking gels [0.95 mL Acrylamide-bis acrylamide (30%\2.6%)+1.25 mL (0.5 M tris/HCl buffer pH 6.8)+2.72 mL DW+0.05 mL (10% SDS)+freshly prepared 0.025 mL (10% amm. persulphate)+0.005 ml TEMED]. The run buffer was prepared by dissolving 3.0 gm Tris+14.1 gm Glycine+1 gm SDS, in DW to 1 L, while the pH adjusted to 8.3 and the electrophoresis was carried out by using of (BioRad, Broka 0.75 mm) mini electrophoresis system with (BioRad PowerPac Basic) at 100-150 Volts.

2.5. Staining and Destaining

The gel was washed with 100 mL of Gel fixing solution (Ethanol: Acetic Acid: Distilled Water, 5:1:4% respectively-GFS) for 1 h. with agitation at room temperature, then removed the (GFS). The gel covered with 100 mL of Gel washing solution (Methanol: Acetic Acid: Distilled Water, 5:1:4% respectively-GWS) for overnight with agitation at room temperature, then removed the (GWS). The gel covered with 100 mL of Coomassie brilliant blue R-250 (0.4 g+200 mL (40% Methanol), after filtered added 200 mL (20% acetic acid) for 3-4 h with agitation at room temperature, removed the stain off the gel, then distained with 100 mL of (GWS) several times.

2.6. Band Scoring and Analysis

Protein bands have been scored, only unambiguous bands coded for presence/absence (I/O). Quantitative evaluation of the protein bands have been done by eye.

2.7. Protein Profiling

Molecular weight of electrophoretic protein bands have been calculated according to Weber and Osborn (1969) method using standard protein marker with the following bands starting at the top with 225 K.D. band followed by 150, 100, 75, 50, 35, 25, 15 and 10 K.D. band, (**Table 2**).

Table 1. Areas of the samples, locations with corresponding abbreviations

Area	North				Middle			East		
No.	1	2	3	4	5	6	7	8	9	10
Location	SH[a]	G[b]	J[c]	M[d]	OY[e]	AM[f]	B[g]	A[h]	OM[i]	T[j]

a. Al-Shoa`bah, b. Al-Gurain, c. Julaijalah, d. Al-Mutairfi, e. Al-Oyuni, f. Ain Merjan, g. Al-Battalia, h. Al-Aqar, i. Al-Omran, j. Al-Taraf

Table 2. The marker and the samples electrophoretic protein band

Marker	OY[e]	OM[i]	T[j]	M[d]	SH[a]	B[g]	J[c]	AM[f]	G[b]	A[h]
225										
										168
150										
100										
								998		
75										
	52	52	52		52			52	52	52
50										
	43	43	43		43	43	43	43	43	43
35										
25										
	20	20	20	20	20	20	20	20	20	20
	17	17	17	17	17	17	17	17	17	17
15										
	14	14	14		14	14		14		14
	12	12	12	12	12	12	12	12	12	12
10										
	9	9	9	9	9	9	9	9	9	9

a. Al-Shoa`bah, b. Al-Gurain, c. Julaijalah, d. Al-Mutairfi, e. Al-Oyuni, f. Ain Merjan, g. Al-Battalia, h. Al-Aqar, i. Al-Omran, j. Al-Taraf

Fig. 1. Map of Al-Ahsa oasis were cultivar samples locations mentioned by abbreviations, SH. Al-Shoa`bah, G. Al-Gurain, J. Julaijalah, M. Al-Mutairfi, OY. Al-Oyuni, AM. Ain Merjan, B. Al-Battalia, A. Al-Aqar, OM. Al-Omran,T. Al-Taraf

2.8. Data Analysis

The results obtained from protein patterns were analyzed statistically, while molecular weight of each protein band was determined (**Table 2**), Protein bands were scored depending on their presence (I) or absence (O). Jaccard's distances was determined and hierarchical clustering was constructed by Neighbor-Joining Method (NJ) and Unweighted Pair Group Method With Arithmetic Average (UPGMA).

3. RESULTS

In the present study, the total protein extracts of different date palm trees "Khalas" cultivar leaflets samples collected from ten locations were subjected to SDS-PAGE analysis. The proteins were found to be composed of a total of nine bands (**Fig. 2, Table 2**), The Genetic Distances based on Jaccard's distances method on the basis of presence and absence of bands (**Table 3**), ranged between 0.0-0.50 (**Table 4**). Most bands number (8) occurred in A and Am locations, while the least number (4) occurred in M location.

The minimum variation value of 0.0 was observed in between OY, OM, T and SH locations samples, while the maximum variation values of 0.5 was observed between A, AM and M, locations samples. Protein banding pattern of A and AM locations samples at 98 kda and 168 kda showing allelic variations, although resolved with low protein content. There are some bands in common between all species (molecular weights: 20, 17, 12, 9 KDa). The phylogenetic analysis based on protein pattern according to UPGMA and NJ based dendrograms (**Fig. 3 and 4**) showed that the different protein patterns were placed into three main clusters. The first cluster is OY, OM, T and SH samples which represent (monomorphism) and distinguished by 52, 43, 20, 17, 14, 12 and 9 kda bands. The second cluster including A and AM locations samples which distinguished each by unique band, A sample with 168 kda band, AM sample with 98 kda band (**Fig. 3**), which is more close to cluster 1 (**Fig. 3 and 4**). The Third cluster B, G, J and M samples were distinguished by the absence of the two unique band 98 and 168 as the absence of the following bands 52, 43 and 14 kda bands from sample M, 52 and 14 kda bands from sample J, 52 kda band from sample B, 14 kda band from sample G.

Table 3. Protein pattern bands represented by O = absent, I = present

Mol. wt.	OY[e]	OM[i]	T[j]	M[d]	SH[a]	B[g]	J[c]	AM[f]	G[b]	A[h]
168	O	O	O	O	O	O	O	O	O	I
98	O	O	O	O	O	O	O	I	O	O
52	I	I	I	O	I	O	O	I	I	I
43	I	I	I	O	I	I	I	I	I	I
20	I	I	I	I	I	I	I	I	I	I
17	I	I	I	I	I	I	I	I	I	I
14	I	I	I	O	I	I	O	I	O	I
12	I	I	I	I	I	I	I	I	I	I
9	I	I	I	I	I	I	I	I	I	I
Total	7	7	7	4	7	6	5	8	6	8

a. Al-Shoa`bah, b. Al-Gurain, c. Julaijalah, d. Al-Mutairfi, e. Al-Oyuni, f. Ain Merjan, g. Al-Battalia, h. Al-Aqar, i. Al-Omran, j. Al-Taraf.

Table 4. Data matrix of Jaccard`s distance analysis within different samples

Locations	OY[e]	OM[i]	T[j]	M[d]	SH[a]	B[g]	J[c]	AM[f]	G[b]	A[h]
OY	0.000									
OM	0.000	0.000								
T	0.000	0.000	0.000							
M	0.429	0.429	0.429	0.000						
SH	0.000	0.000	0.000	0.429	0.000					
B	0.143	0.143	0.143	0.333	0.143	0.000				
J	0.286	0.286	0.286	0.200	0.286	0.167	0.000			
AM	0.125	0.125	0.125	0.500	0.125	0.250	0.375	0.000		
G	0.143	0.143	0.143	0.333	0.143	0.286	0.167	0.250	0.000	
A	0.125	0.125	0.125	0.500	0.125	0.250	0.375	0. 222	0.250	0.00

a. Al-Shoa`bah, b. Al-Gurain, c. Julaijalah, d. Al-Mutairfi, e. Al-Oyuni, f. Ain Merjan, g. Al-Battalia, h. Al-Aqar, i. Al-Omran, j. Al-Taraf.

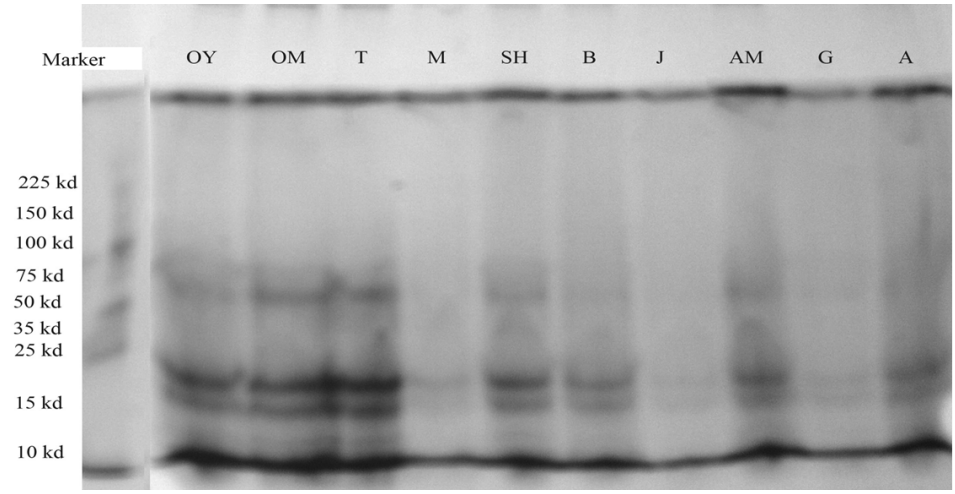

Fig. 2. Electrophoretic proteins patterns of cultivar samples with protein standard marker

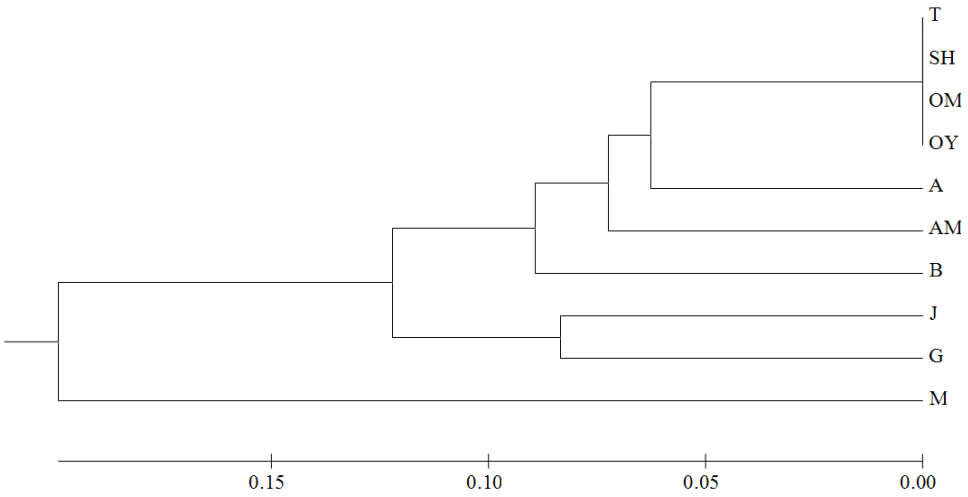

Fig. 3. UPGMA based dendrogram showing genetic relationship among the cultivar samples based on Jaccard's distance

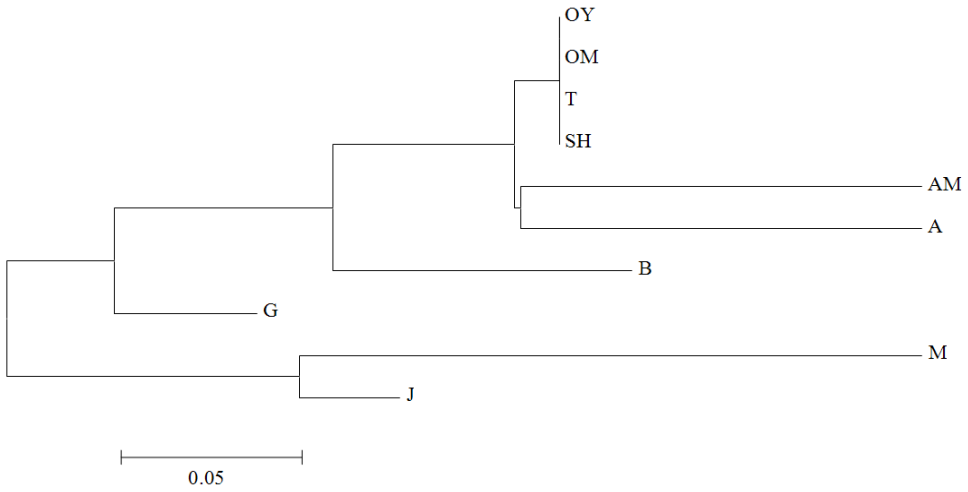

Fig. 4. Neighbor Joining (NJ) based dendrogram showing genetic relationship among the cultivar samples of based on Jaccard's distance

This cluster samples were separated singly one by one, except J and G locations samples were clustered both together under UPGMA dendrogram (**Fig. 3**), also J and M both clustered together under NJ dendrogram (**Fig. 4**). Almost each sample in cluster 3 has specific banding pattern but there are no enough sharing bands to cluster them together and to show their relatedness except they were low in their protein content.

4. DISCUSSION

Since this study have been done to recognize and identify the original "Khalas" cultivar among many "Khalas" cultivar trees growing in Al-Ahsa oasis, the results revealed that the complete similarity in the electrophoretic pattern were in between samples of cluster 1, since the minimum variation values of 0.0 based on Jaccard's distance (**Table 4**) was observed in between this cluster, which include OY, OM, T and SH locations samples (**Fig. 1 and 2**), however all the three areas of Al-Ahsa oasis of this study have been represented in this cluster, SH belong to North, OY belong to the Middle, OM and T belong to East areas (**Fig. 1**), where it could be used as an indicator to the original "Khalas" cultivar trees. Additionally cluster 2 samples for A and AM locations are the closest to cluster 1, since variation values of 0.125 based on Jaccard's distance was observed between both cluster 1 and 2 samples (**Table 4**), were each sample of cluster 2 differed with one band from samples of cluster 1, as another indicator that the cluster 1 sample trees may represent the original Khalas cultivar. It have been conducted by this study results that there is differences in protein patterns within "Khalas" cultivar trees. The genetic differences observed in this study agree with the previous study of Al-Issa (2006) and support the probability of some cultivars trees grown from seeds resembled the original one, then distributed later as an original cultivar (Devanand and Chao, 2003).

5. CONCLUSION

It could be concluded from this study that there is a genetic differences among "Khalas" cultivar date palms and the Identification of the original "Khalas" cultivar is important to guaranty the superior characteristics of cultivated trees. Undoubtedly it needs to be subject to more studies. Furthermore, DNA markers should be used to show the genetic structures and variations within "Khalas" cultivar trees, to confirm the fingerprint of the original "Khalas" cultivar. In the long term, the goals should be included the recognizing of the other original cultivars of date palm trees which represent nutritional values and economical importance.

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