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Full Length Research Paper

Electrophoretic analysis of proteins from different date palm *(Phoenix dactylifera* L.) cultivars in Saudi Arabia

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Fifteen (15) samples of different date palm cultivars were collected from different locations in Al-Ahsa oasis in the eastern province of Saudi Arabia. Extracted proteins from these samples were analyzed by electrophoresis, and clustered according to the average linkage between groups hierarchical clustering method. The results reveal high degree of similarity based on Jaccard`s similarity method on the basis of presence and absence of bands, that ranged between 0.421 to 0.917, which was represented by phylogenetic dendrogram in six clusters. The closely related cultivars "Hel" and "Hat" in addition to "Khl" represent the sixth cluster, which is separated out of other cultivars with high degree of similarity that ranged between 0.636 to 0.714; it was confirmed by the first principal component with high loading (52.3%), and characterized by four bands (92, 100, 205 and 108 kda). These bands were mostly positioned close to each other in the scatter diagram. The second principal component with loading of 15.7%, which were represented by three bands 19, 25, and 37 kda, have been confirmed the first cluster of closely related cultivars "Shi" and "Shl", as well as the closely related cultivars "Mj" and "OmR" among the second cluster. It can be concluded that most of Al-Ahsa oasis date palm cultivars were from one genetic origin, however, each cultivar was grown from seed of locally known cultivar, and later was selected due to preferred fruit characteristics. More biochemical and molecular studies would be necessary to uncover the genetic relationships between area cultivars.

Key words: SDS-PAGE, bands, phylogenetic, cluster, dendrogram, similarity, principal components.

INTRODUCTION

Date palm (*Phoenix dactylifera* L., 2n=2x=36) is a monocotyledonous and dioeciously species belonging to Arecaceae family. It includes 225 genus and 2600 species (Corner, 1966), and widely cultivated in arid regions of the middle east and north Africa, (Hamza et al., 2011; Khierallah et al., 2011; El-Tarras et al., 2007; Elmeer et al., 2011). 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 (Asif et al., 1982; Al-Ghamdi and Al-Kahtani, 1993a; Al-Issa, 2009, 2013). 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

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Table 1. Cultivars names with their corresponding abbreviations.

Cultivar	Abbreviation
Mejnaz	Mj
Khalas	Khl
Shaishi	Shi
Shehil	Shl
Garrah	Grr
Berhi	Brh
Helali	Hel
Hatemi	Hat
Khonaizi	Khn
Asailah	Asi
Khosab	Khs
Tayyar	Tya
OmRohaim	OmR
Ruzaiz	Ruz
Owsaili	**Ows**

fruit quality identical to the mother tree (Asif et al., 1982; Al-Ghamdi and Al-Kahtani, 1993b, c; Khierallah et al., 2015). 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), as well as fast and less expensive alternate tool (Smykal et al., 2008). Therefore, protein pattern analysis by gel electrophoresis has been used in higher plants to study various problems in genetics (Mohammed et al., 2006; Dakhil et al., 2013; Koshroo et al., 2011), taxonomy (Barta et al., 2003), physiology (Stegemann et al*.,* 1987; Al-Helal, 1994), as it has been appreciated as a biochemical tool for studying the phylogentic relationships (Al-Yahyai and Al-Khanjari, 2008; Attaha, et al., 2013). The phylogenetic analysis based on protein patterns were used to study the genetic relatedness between and among cultivars (Abd El-Hady et al., 2010; Munshi and Osman, 2010; El Akkad, 2004; Haider et al., 2012; Khoshroo et al., 2011; Attaha et al., 2013). Protein patterns were used in identification of different date palm cultivars (Stegemann et al*.,* 1987; Munshi and Osman, 2010; Koshroo et al., 2011; Attaha et al., 2013; Koshroo et al., 2013; Al-Issa, 2013), as well as in different plants (Barta et al., 2003; El Akkad 2004; Abd El-Hady et al., 2010; Gad and Mohamed, 2012; Aejaz et al., 2014). So, it would be helpful in recognizing the relationships of different cultivars. Fifteen cultivars namely: Mejnaz, Khalas, Shaishi, Shehel, Garrah, Berhi, Helali, Hatemi, Khonaizy, Osailah, Khossab, Tayyar, Omm rhaim, Ruzaiz, and Awosaili were selected for this study, where Khonaizi and Khossab cultivars were known as Qatif origin cultivars, also Berhi cultivar was known as Iraqi origin cultivar. The main objective of this study was to enrich our knowledge about the biodiversity of Al Ahsa oasis date palm cultivars, however, it aimed to recognize inter-genetic relationships, phylogenetic revolution using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) technique.

MATERIALS AND METHODS

The samples of the 15 cultivars were collected from juvenile green leaflets of 30 years old and above of date palm cultivars trees. 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 (Table 1).

Protein extraction

The samples were ground in a mortar by liquid nitrogen, then the proteins extracted with 0.7 ml extracting buffer (0. 5 M Tris/HCl buffer, pH 6.8 + 10% Glycerol v/v + 4% PVP w/v) according to Al-Helal (1994) with some modifications, by homogenizing 50 mg of ground leaflet samples by half volume stainless steel beads at 9/4 min., followed by 10/2 min by using bullet blender homogenizer, then incubated overnight at 4°C. The crude extract was vortexed by using VELP vortex mixer, then centrifuged at 12500 rpm for 10 min by using Eppendorf centrifuge 5424; the supernatant was moved to new tubes, while the debris was discarded.

Protein concentration and resuspending

According to Wessel and Fluegge (1984), with some modifications, to 150 µl of the supernatant, 600 µl methanol was added, vortexed, next 150 µl chloroform was added, vortexed, 450 µl deionized water was added, vortexed, centrifuged at 10000 rpm for 1 min, then top aqueous layer was removed, while protein was between layers, 600 µl methanol was added, vortexed, centrifuged at 10000 rpm for 1 min, then supernatant was removed without disturbing pellet; precipitated protein was incubated at room temperature for 15 min; the precipitated proteins was dissolved in 150 µl of extracting buffer, vortexed, boiled at 95°C for 4 min, then vortexed, centrifuged at 12500 rpm for 10 min, after that loaded in 15 µl.

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 g Tris + 14.1 g Glycine + 1 gm SDS, in DW to 1 L, while the pH was adjusted to 8.3, and the electrophoresis was carried out by using (BioRad, Broka 0.75 mm) mini electrophoresis system with (BioRad PowerPac Basic) at 100 to 150 V.

Staining with coomassie and destaining

The gel was stained by coomassie brilliant blue R-250, and destained according to Meyer and Lambert (1965) with some modifications.

Table 2. Protein pattern bands represented by O = absent and I = present.

Mwt.	Mj[a]	Khl[b]	Shi[c]	Shl[d]	Grr[e]	Brh[f]	Hel[g]	Hat[h]	Khn[i]	Asi[j]	Khs[k]	Tyr[l]	OmR[m]	Ruz[n]	Ows[o]
270	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
260	O	O	O	O	O	O	I	I	O	I	I	I	I	O	O
239	O	O	O	O	O	I	O	O	O	O	O	O	O	O	O
205	O	I	O	O	O	O	I	I	O	O	O	O	O	O	I
165	O	O	O	O	O	O	O	O	O	O	O	O	O	O	I
150	O	O	O	O	I	I	I	I	O	I	O	O	O	O	O
145	I	O	O	O	O	O	O	O	O	O	O	O	O	I	I
108	O	I	O	O	O	I	O	I	O	O	O	O	O	O	O
100	O	I	O	O	O	O	I	I	O	O	O	O	O	O	O
92	O	I	O	O	O	O	O	I	O	O	O	O	O	O	O
84	I	I	I	O	O	O	O	I	O	O	O	O	I	I	I
75	I	O	O	O	O	I	I	I	O	O	O	O	I	O	O
60	I	I	O	O	I	I	O	O	O	O	I	O	I	I	O
56	I	I	I	I	I	I	I	O	O	O	I	O	I	I	I
50	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
4**6**	O	O	O	O	I	O	O	O	O	O	O	O	O	O	O
40	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
37	O	I	O	O	O	O	I	O	O	O	O	O	I	O	O
35	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
32	O	O	O	O	O	O	I	O	I	O	O	O	O	I	O
28	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
25	I	I	I	I	O	I	I	I	O	O	O	O	I	O	O
22	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
19	O	O	O	O	O	O	O	O	O	I	O	O	O	O	O
17	I	I	I	I	I	I	I	I	I	I	I	O	I	I	I
15	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
5	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Total	15	18	12	11	13	16	18	18	10	12	12	9	16	14	14

Staining with silver nitrate plus and destaining

Completely the gel was destained from coomassie brilliant blue R-250, then stained by silver nitrate according to BIO-RAD silver staining after Coomassie Brilliant Blue R-250 staining (Silver Stain Kit-161-0443).

Band scoring and analysis

Protein bands were scored, only unambiguous bandscoded for presence / absence (I/O). Quantitative evaluation of the protein bands was done by eye.

Protein profiling

Molecular weight of electrophoretic protein bands were calculated according to Weber and Obero (1969) method using standard protein marker with the following bands starting at the top with 250 K.D band followed by 150, 100, 75, 50, 37, 25, 20, 15 and 10 K.D band (Table 2).

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 similarity was determined and hierarchical clustering was constructed, principal components analysis PCA was done by using IBM SPSS Statistics for Windows software (2010).

RESULTS

The total protein extracts of the different cultivars of the date palm trees, which were collected from leaflets samples were subjected to SDS-PAGE analysis, then stained by Coomassie brilliant blue R-250, and re-stained by silver nitrate plus. Therefore, the faint bands which developed by Coomassie brilliant blue R-250 stain, became clearer when re-stained by silver nitrate plus stain. In general, the protein pattern of studied cultivars visibly looked slightly different, however, these differences were seen in the low protein content bands, as well as there is differences related to the intensity of protein bands. The proteins were found to be composed of a total of 27 bands (Figures 1 and 2, Table 2). Some

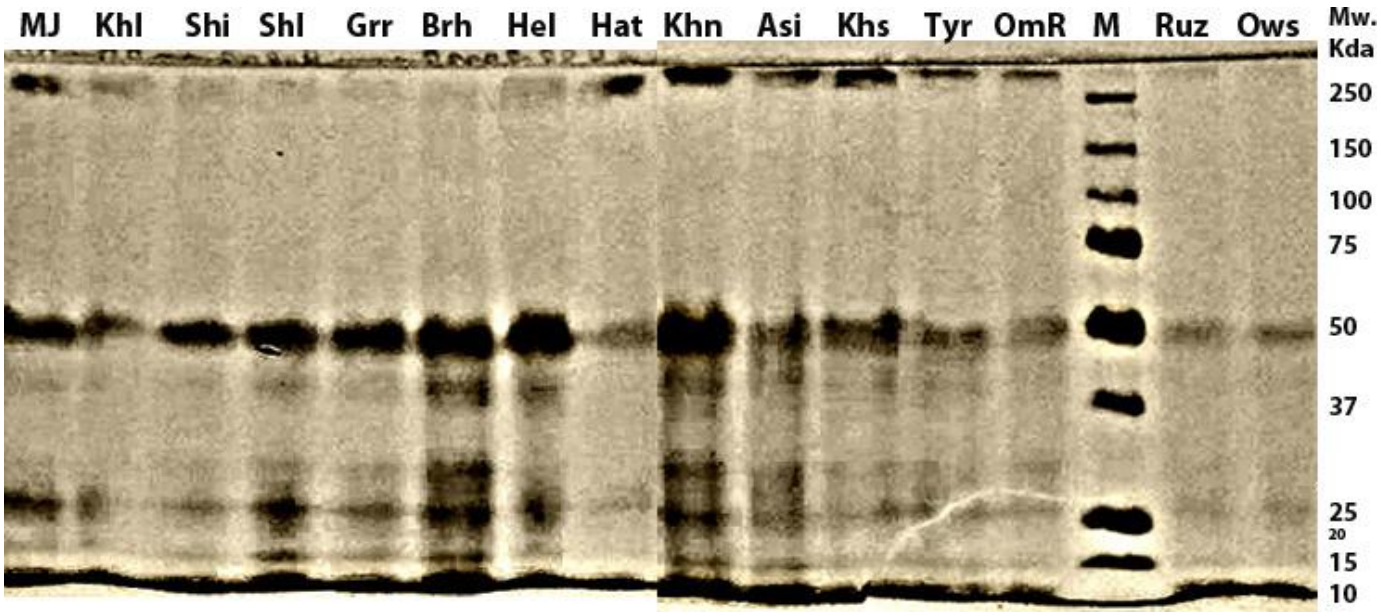

Figure 1. Electrophoretic proteins patterns of cultivars samples with protein standard marker, stained by comassie blue R-250.

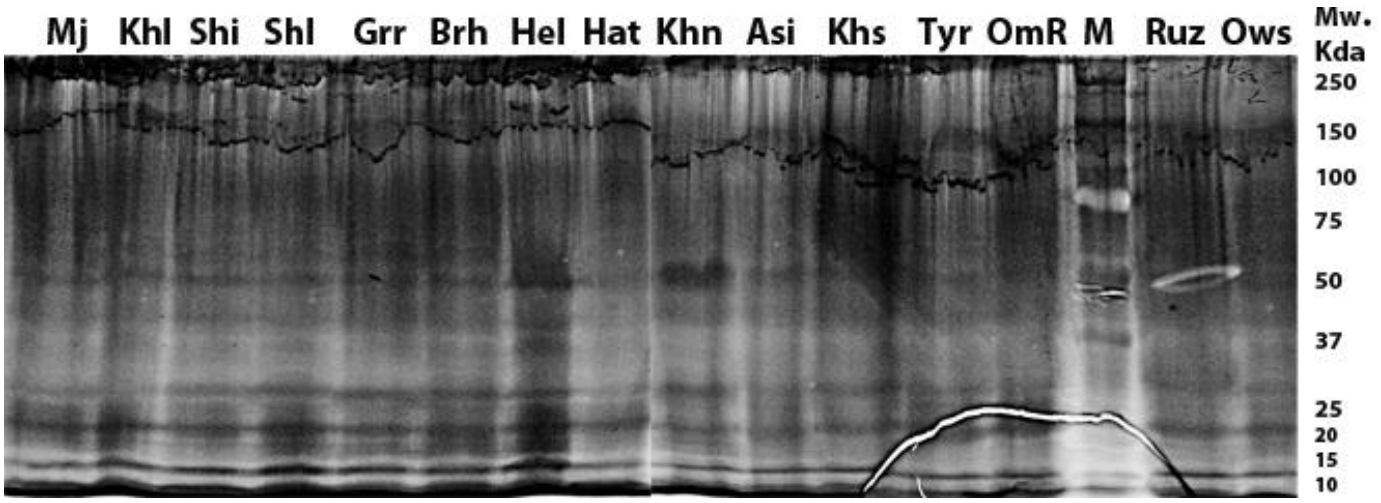

Figure 2. Electrophoretic proteins patterns of cultivar samples with protein standard marker, restained by silver nitrate.

bands were common between all cultivars at: 270, 50, 40, 35, 28, 22, 15 and 5 kda, while most bands number (18) occurred in "Khl", "Hel" and "Hat" cultivars, while the least number (9) occurred in "Tyr" cultivar. Protein bands varied in the intensity between studied cultivars; appeared higher in "Hel" cultivar, and lower in "Hat" cul-tivar. "Hel" and "Khn" cultivars seemed higher in intensity of band 50 kda than other cultivars, as well as "Khl" and "Hel" cultivars which seemed higher in intensity of band 22 kda than other cultivars bands (Figure 1 and 2, Table 2).

The genetic similarity based on Jaccard`s similarity method on the basis of presence and absence of bands (Table 2), ranged between 0.421 to 0.917. The highest similarity value of 0.917 based on Jaccard`s similarity method was observed between "Shi" and "Shl" cultivars, while the least similarity value of 0.421 was observed between "Khl" and "Tyr" cultivars. Protein banding pattern of "Brh" cultivar sample was at 239 kda, "Ows" cultivar sample was at 165 kda, "Grr" cultivar sample was at 46 kda, and "Asi" cultivar sample wat 19 kda showing allelic variations, but were resolved with low protein content (Table 3).

Phylogenetic analysis (Figure 3) showed six clusters; the first cluster composed of "Shi" and "Shl" cultivars,

Table 3. Data matrix of Jaccard`s Similarity analysis within different cultivars.

Molecular weight	Mj[a]	Khl[b]	Shi[c]	Shl[d]	Grr[e]	Brh[f]	Hel[g]	Hat[h]	Khn[i]	Asi[j]	Khs[k]	Tyr[l]	OmR[m]	Ruz[n]	Ows[o]
Mj[a]	1														
Khl[b]	0.650	1													
Shi[c]	0.800	0.667	1												
Shl[d]	0.733	0.611	0.917	1											
Grr[e]	0.647	0.550	0.667	.714	1										
Brh[f]	0.722	0.619	0.647	.688	0.706	1									
Hel[g]	0.571	0.636	0.579	.611	0.550	0.619	1								
Hat[h]	0.571	0.714	0.579	.526	0.476	0.619	0.714	1							
Khn[i]	0.563	0.474	0.692	.750	0.643	0.529	0.556	0.474	1						
Asi[j]	0.500	0.429	0.600	.643	0.667	0.556	0.579	0.579	0.692	1					
Khs[k]	0.750	0.550	0.667	.714	0.733	0.706	0.632	0.550	0.643	0.667	1				
Tyr[l]	0.500	0.421	0.615	.667	0.571	0.471	0.500	0.500	0.727	0.750	0.692	1			
OmR[m]	0.824	0.700	0.750	.688	0.611	0.684	0.700	0.619	0.529	0.556	0.813	0.563	1		
Ruz[n]	0.813	0.600	0.733	.667	0.688	0.579	0.524	0.455	0.714	0.529	0.688	0.533	0.667	1	
Ows[o]	0.706	0.600	0.733	.667	0.588	0.500	0.524	0.524	0.600	0.529	0.588	0.533	0.579	0.750	1

a, Mejnaz; b, Khalas; c, Shaishi; d, Shehel; e, Garrah; f, Berhi; g, Helali; h, Hatemi; i, Khonaizi; j, Asailah; k, Khosab; l, Tayyar; m, Omruhaim; n, Ruzaiz; o, Owsaili.

which were closely related, and distinguished by 11 common bands and degree of similarity (0.917); the second cluster composed of "Mj", "OmR" and "Khs" cultivars, were "Mj" and "OmR" were closely related, and distinguished by 14 common bands and degree of similarity of 0.824; the third cluster composed of "Ruz" and "Ows" cultivars, which were closely related, and distinguished by 12 common bands and degree of similarity of 0.750; the fourth cluster composed of "Grr" and "Brh" cultivars, which were closely related, and distinguished by 12 common bands and degree of similarity 0.706; the fifth cluster composed of "Asi", "Tyr" and "Khn" cultivars, were "Asi" and "Tyr" closely related, and distinguished by nine common bands and degree of similarity 0.750, the sixth cluster composed of "Hel", "Hat" and "Khl" cultivars, were "Hel" and "Hat" closely related, and distinguished by 15 common bands and degree of similarity 0.714 (Table 2).

Principal components analysis PCA was used to evaluate the results of electrophoretic patterns depending on presence (I) or absence (O) of electrophoretic bands (Table 2); the KMO and Bartlett`s tests for adequacy and sphericity result where 0.722 and 0.001, respectively (Table 4), the variance results were summarized in (Table 5), since the first component represent 52.3% of the total variations, the second component represent 15.7% of the total variations, hence the cumulative value represent 68% of the total variations, however the first component was represented by four variables, 92, 100, 205 and 108 kda, the second component was represented by three variables,19, 25, and 37 kda (Table 6); the variables were scattered on the 2-dimensional scatter gram, where both first and second principal components have been represented by their variables in two distinct groups (Figure 4).

DISCUSSION

Electrophoretic protein patterns technique reproduce considerable results in discrimination of differences among species and cultivars, however the date palm leaflets were wide used by researchers as a source of whole proteins for studies related to phylogenetic revolution or cultivars identification (Ahmed and Al Qaradhawi, 2009; Dakhil et al., 2013; Al-Issa, 2013; Khierallah et al., 2014). Validity of the results data was based on presence and absence of electrophoretic bands (Table 2), for principal components analysis (Andy, 2005), since the data have passed the tests of KMO and Bartlett for adequacy and sphericity, with 0.722 and 0.001, respectively. The study revealed eight bands in common between all cultivars at 270, 50, 40, 35, 28, 22, 15 and 5 kda. So, the high degree of similarity based on Jaccard's similarity method on the basis of presence and absence of bands, which ranged between 0.421 to 0.917 (Table 3), have been represented by phylogenetic dendrogram in the six clusters since each cluster includes a condition of closely related cultivars, "Shi" and "Shl", "Mj" and "OmR", "Ruz" and "Ows", "Grr" and "Brh", "Asi" and "Tyr", "Hel" and "Hat" with degrees of similarity 0.917, 0.824, 0.750, 0.706, 0.750 and 0.714, respectively. It could be supposed that each cluster were evolved from one common ancestor, as well as it could

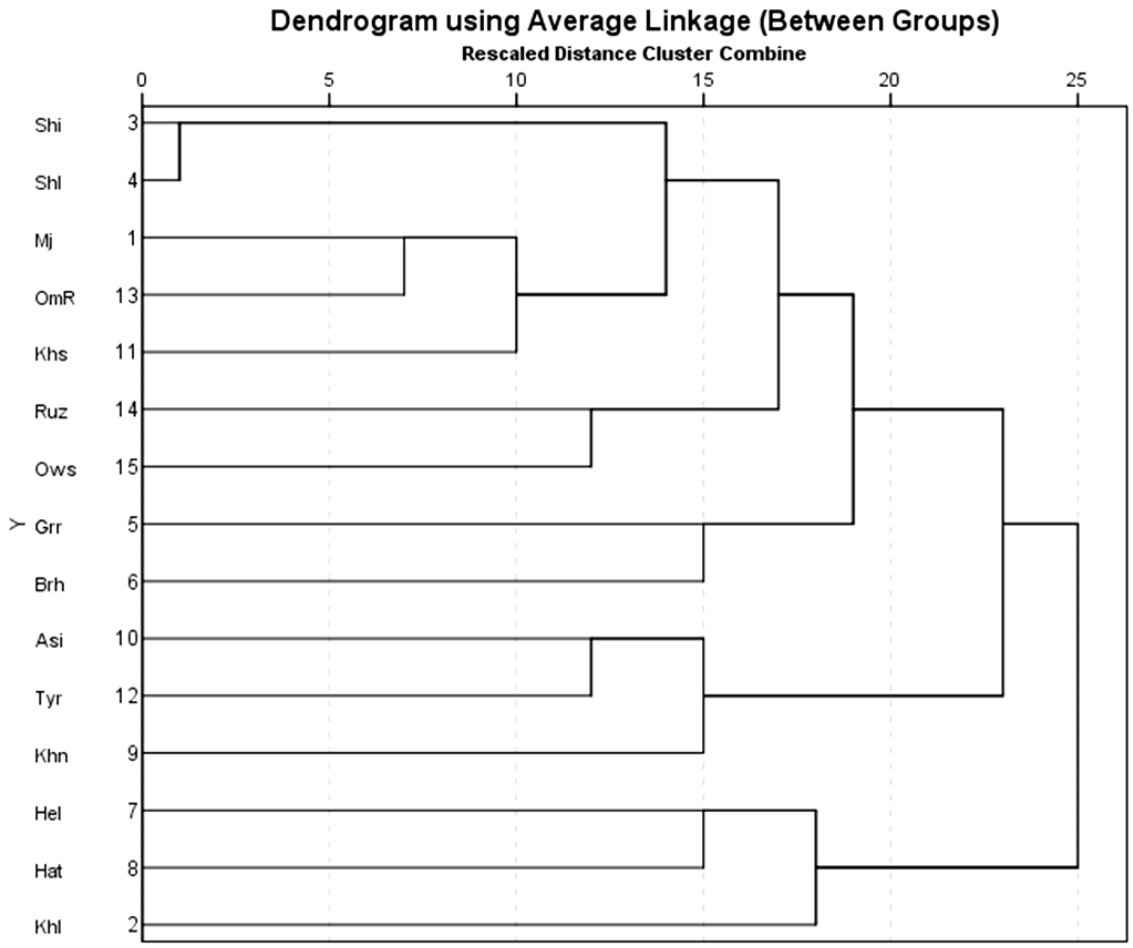

Figure. 3 Average linkage between groups hierarchical clustering dendrogram based on Jaccard`s Similarity showing genetic relationships among the different cultivars.

Table 4. The KMO and Bartlett`s tests for adequacy and sphericity.

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		0.722
Bartlett's Test of Sphericity	Approx. Chi-Square	46.550
	df	21
	Sig.	0.001

Table 5. Eigenvalues of 7 components, % of variance, % of cumulative variation for each component, first two components targeted.

Total variance explained									
Component	**Initial Eigenvalues**			**Extraction sums of squared loadings**			**Rotation sums of squared loadings**		
	Total	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**
1	3.661	52.296	52.296	3.661	52.296	52.296	3.162	45.167	45.167
2	1.100	15.712	68.008	1.100					
3	0.932	13.312	81.321						
4	0.738	10.542	91.863						
5	0.302	4.316	96.179						
6	0.174	2.485	98.664						
7	0.094	1.336	100.000						

Table 6. Displayed first two component represented by the variables and its loadings.

Rotated component matrix[a]		
	Component	
	1	2
B92	0.918	
B100	0.883	
B205	0.798	
B108	0.787	
B19		-0.773
B25		0.697
B37		0.597

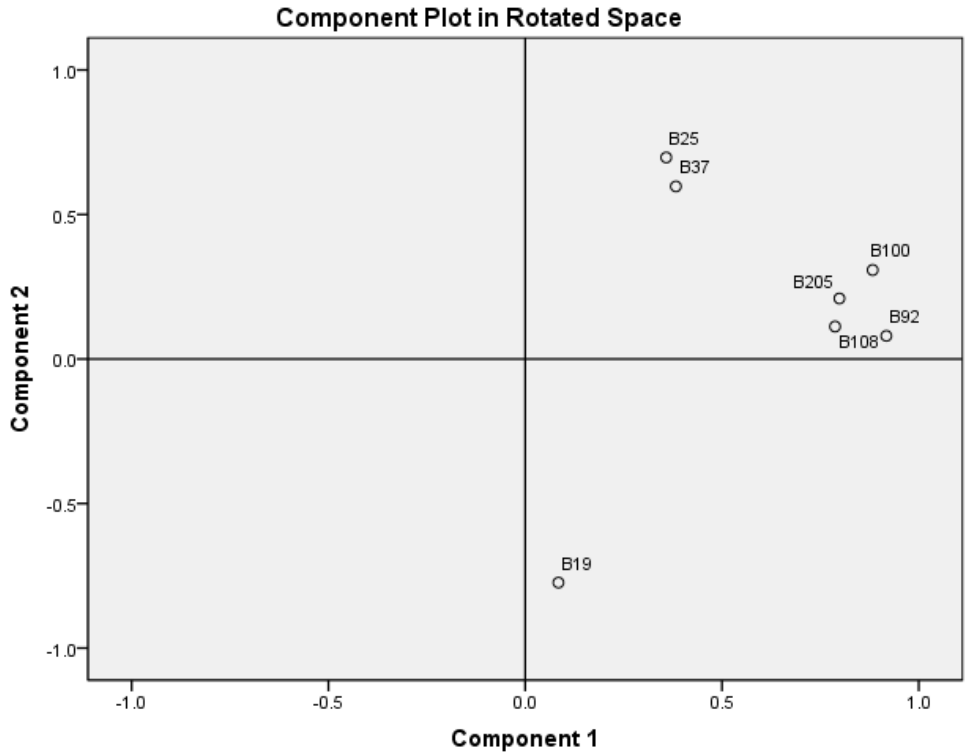

Figure. 4 Scatter diagram of electrophoretic bands according to the first two components.

be concluded that most of Al-Ahsa oasis cultivars, which were included in present study were refer to one genetic origin; such conclusions have been reported for other localities cultivars (Ahmed and Al Qaradhawi, 2009; Khierallah et al., 2014; Attaha et al., 2013; Khalifah et al., 2012), and agree with the conclusion, that the diversity of protein bands between varieties within species are generally low (Vaz et al., 2004; Hastuti and Prabang, 2009; Khalifah et al., 2012). The closely related cultivars "Hel" and "Hat" in addition to "Khl" cultivar which represent the sixth cluster, which separated out of other cultivars (Figure 3), with high degree of similarity which ranged between 0.636 to 0.714, (Table. 3), have been confirmed by the first principal component with high loading (52.3%) (Table 5), which was characterized by four bands 92, 100, 205 and 108 kda, (Table 6); these bands were mostly positioned close to each other in the scatter diagram (Figure 4). The second principal component, was characterized by low loading (15.7%) (Table 5) with three bands 25, 37 and 19 kda, (Table 6), where bands 25 and 37 were mostly positioned close to each other in the scatter diagram (Figure 4); it confirmed the first cluster of closely related cultivars "Shi" and "Shl" with high degree of similarity 0.917, as well as it confirmed

the closely related cultivars "Mj" and "OmR" among the second cluster, with high degree of similarity of 0.824.

The third cluster which is represented by closely related cultivars "Ruz" and "Ows" with high degree of similarity 0.750, (Table 3), have not been confirmed by either first or second components. The probable reason is that the cluster was not supported by enough distinguished bands being developed. Clustering of date palm cultivars by phylogenetic dendrograms and confirming this clustering by principal component analysis have been reported (Ahmed and Al Qaradhawi, 2009; Khoshroo et al., 2011; 2013; Attaha et al., 2013). The differences of protein bands intensity between cultivars protein patterns should be taken in the consideration too, since there are differences among studied cultivars in the intensity of protein bands, it appeared higher in "Hel" cultivar, lower in "Hat" cultivar, and also "Hel" and "Khn" cultivars seems higher in intensity of band 50 kda than other cultivars, as well as "Khl" and "Hel" cultivars seems higher in intensity of band 22 kda than other cultivars.

Protein banding pattern of "Brh" cultivar showed allelic variation at 239 kda, while "Grr" cultivar showed allelic variation at 46 kda; both were close related, sharing 12 common bands, with degree of similarity of 0.706, however, "Brh" cultivar was known as south Iraq originated cultivar (Al-Bakr, 1972), while "Grr" cultivar known as Al-Ahsa oasis originated (Asif, 1982); this close relation may be interpreted due to the gulf Arabic cultivars referred to one genetic origin.

Allelic variation have been shown in "Ows" cultivar at 165 kda, which was closely related to "Ruz" cultivar with 12 common bands, with degree of similarity 0.750, "Asi" cultivar showed allelic variation at 19 kda, which was closely related to "Tyr" cultivar with nine common bands, with degree of similarity 0.750, but although all allelic variations were resolved with low protein contents, they were regarded to the gene expression. However, the allelic variations could be used as an alternative, or complementary biochemical markers (Ould Mohammad et al., 2008; Abd El-Hady et al., 2010; Attaha et al., 2013). "Khs" and "Khn" cultivars which were known as Al-Qatif originated cultivars have been included in second and fifth clusters, respectively, however, this result in addition to "Brh" cultivar support previous conclusion that Arabic gulf cultivars were referred to one genetic origin, and they were distributed as a result of offshoots transportation within the Arabic gulf localities.

Conclusion

It could be concluded from the present study, even with the reality of the genetic diversity among date palm cultivars in Al Ahsa oasis that they belong to one genetic origin, since each cultivar was grown from a seed, however, the cultivar have been selected later due to preferred fruit characteristics. More and detail biochemical and molecular studies would be necessary to uncover the genetic relationships between date palm cultivars within Al-Ahsa oasis and other Arabic gulf localities.

Conflict of interests

The author(s) did not declare any conflict of interest.

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