A morpho-genetic approach to characterize genetic diversity in date palms (*Phoenix dactylifera* L.)

Nasser S. Al-Khalifah, Shanavaskhan, A.E. and Ejaz Askari
Natural Resources and Environment Research Institute,
King Abdulaziz City for Science and Technology
Post Box No.6086, Riyadh 11442,
Kingdom of Saudi Arabia.

Abstract

Date palm (*Phoenix dactylifera* L.), a highly out breeding, dioecious plant species has enormous amount of genetic diversity. Genotype identification of date palm is an intricate empirical exercise based on morphological characters. These cultivars are recognized by their morphologic features like tree posture, leaf arrangements, spines, pinnae and mostly by fruit characters. Lack of an authenticated manual describing the diagnostic characters of cultivars grown in a particular area and the problems associated with the local names have created a lot of confusion in cultivar identification, especially before the fruiting age. In this study an attempt was taken to enumerate and characterize some elite date cultivars of Saudi Arabia. Fourteen cultivars of date palms were selected from two orchards in Al-Qassim area and their morphological features were characterized. Fruit shape, length width ratio, base, colour variations during ripening etc were the morphologic parameters studied. Random Amplified Polymorphic DNA (RAPD) analysis was also performed using standard protocols to detect genetic variability among them. All the genotypes revealed unique profiles with selected primers and showed a range of 44.1- 73.0 % of genetic similarity. The two morphologically narrow distinguishable cultivars, Wannanah and Shagra showed only 56.9% similarity indicating variability between

them. On the other hand two morphologically very close cultivar pairs, Khalas - Makhtomi and Hilaliah – Barhy also showed close genomic similarity between them (61.6 and 64.6 %). Correlation of morphologic characters with genomic similarity showed that the fruit shape is one of the characteristics mostly influenced by genetic variation. Where ever there was insignificant length-width ratio between cultivars, more similarity in their genomic structure was observed. This study indicated that morphology of fruits along with RAPD markers can be successfully employed as tools for identifying and enumerating date palm cultivars of the world. Addition of tree characteristics, protein and sugar content of each cultivar, to this data will make a perfect tool which can be used to identify the presently known cultivars of date palms.

Key words: Morphology, fruit shape, length-width ratio, genetic diversity, RAPD.

Introduction

Date palm (*Phoenix dactylifera* L.) cultivation is the main source of agricultural income in many countries of arid regions of West Asia and North Africa. With its ability to accumulate exceptionally high level of metabolites under extreme arid conditions, it is a unique physiological entity (Al-Khalifah *et al*. 2006). Being a key species, adapted to the harsh environmental conditions of arid zones date palms are regarded as one of the important components of biodiversity in the inhospitable areas of deserts. *Phoenix dactylifera* L. is inter fertile with its allied species (Muirhead, 1961) and are successfully pollinated with *P.rectinata* and *P.atlantica* in Africa. In India and Pakistan it is pollinated with *P.sylvestris* and in Spain with *P.canariensis* (Oudejans, 1979; Benbades, 1992). This highly out breeding behavior has brought about immense genetic diversity in this species. Zaid and de Wet (1999) reported the occurrence of 3000 cultivars all around the world. There are about 450 cultivars in Saudi Arabia (Bashah, 1996), 400 in Iran (FAO, 1996), 370 in Iraq (Dowson, 1923),

250 in Tunisia (Kearney, 1906), 244 in Morocco (Saaidi, 1979) as well as many others in other date growing countries (Zaid and de Wet, 1999).

Most of these cultivar identification works are of enumerative type based on local names which varies from place to place. These cultivars are location specific, known by different names at different places or one name is assigned to different cultivars at different places. This has created lot of ambiguity in enlisting the cultivars based on local names. A scientific approach of characterizing cultivars and assigning a more acceptable legitimate name to the cultivars was seldom attempted in this species, especially in Saudi Arabia.

Genotype identification of date palm is an intricate empirical exercise based on morphological characters (Sedra *et al*., 1998). In date palms most of the female cultivars are recognised by their fruit characteristics such as size, shape, colour and taste. Morphologic characters of the tree are also taken into consideration for the cultivar identification. During the ripening process, the date fruits pass through 4 distinct stages of maturity *viz.* 'Kemri', 'Beser', 'Rutab' and 'Tamar' (Al-Ghamdi,1993). When the fruits are young they are green in colour (varies in different cultivars) and are termed 'Kemri'. Beginning of ripening marks the 'beser' stage, half ripened stage is called 'rutab' and fully ripened, soft textured stage is called 'Tamar'. These colour variations during the ripening of fruits are important morphological markers for the cultivar identification.

However some date palms have similar or narrow distinguishing morphological characters that complicate cultivar identification and demand genetic evidence to prove phylogenetic relationships at the inter specific level. Random Amplified Polymorphic DNA (RAPD) analysis is a comparatively simple, quick and less expensive procedure for generating genomic markers (Welsh and Mc Clelland,

1990; Williams et al., 1990). This technique has been successfully applied for cultivar identification of date palms (Saker *et al*., 2000; Al-Khalifah and Askari, 2003; Askari *et al*., 2003; Al-Khalifah, 2006).

The objectives of this study were to characterize some elite cultivars of date palms using morphological characters of fruits and to correlate these results with RAPD markers.

Materials and methods

Fourteen well known cultivars of date palm ('Barhy', 'Deglet Noor', 'Hilaliah', 'Hilwa', 'Khalas', 'Makhtomi', 'Moneifi', 'Nabtet Ali', 'Omal Khashab', 'Rothana', 'Sabbaka', 'Shagra', 'Sukkary', 'Wannanah') trees were tagged in two orchards of Al-Qassim area, Saudi Arabia. Colour variations during the three fruit ripening stages ('beser', 'rutab' and 'tamar') were recorded directly from the tagged trees. Hundred fruits from each cultivar were collected during 'rutab' stage and their length and width were measured using Vernier Calipers. Shape and colour of the fruits was documented using digital camera. Base and apex of the fruits were also noted carefully and the diameter of the fruit cap (persistent calyx) was measured using a millimeter scale. Based on this data the total area of the fruit base covered by the fruit cap was calculated.

For Random Amplified Polymorphic DNA (RAPD) analysis young sprouting leaves from each cultivar were collected. Total genomic DNA was extracted using the protocol of Dellaporta *et a*l. (1983). After determining the quality and quantity of extracted DNAs with a UV Spectrophotometer, the stock DNA samples were diluted in distilled water to make a working solution of 10 ηg/µl.

Polymerase Chain Reaction (PCR) was performed as described by Al-Khalifah and Askari, (2003) using 130 random 10-mer RAPD primers (OPERON Tech.) of A to G series. PCR products of each primer were separated by electrophoresis according to

their molecular weight on 1.4% (w/w) agarose gels. The profiles of each primer were then documented by Gel Documentation System of Bio Rad. (Hercules, Calif.). The length of the amplified RAPD fragments was estimated by running Kilo Base DNA marker (Amersham Pharmacia Biotech.) in the gel as standard size marker. Amplification profiles of all the cultivars were compared with each other using the Diversity Data Base software package (Bio-Rad).

Results and Discussion

Analysis of the morphological data of fruits showed high level of diversity in length-width ratio, colour, shape of the fruit, fruit-base and in the percentage of area covered by the fruit cap (Table1). Their shape varied from globular, elliptic, ovate, oblong, and to linear oblong as in 'Deglet Noor' (Figs 1&2). Many intermediary forms or combination of one or two forms were also observed. The length-width ratio of these fourteen cultivars ranged from 1.1 to 2.62, indicating a great variation in their shape. Even within the cultivars having same or insignificantly different length-width ratio there was variation in shape mainly due to the position of the widest portion, i.e. widest near the base in 'Shagra' and 'Wannanah' and widest near the middle as in 'Moneifi'. Fruit base varied from truncate to cordate or sometimes oblique. During 'Kemri' stage all these cultivars had green coloured fruits which turned to yellow or red or various degrees of combination of red and yellow in 'beser' stage. During 'rutab' stage ripening process usually starts from the tip of the fruit which brought different colouration to the fruits (Table1). 'Tamar' is the harvesting stage in which they showed colour variation from amber, golden brown, reddish brown to chocolate brown. The size of the fruit cap and percentage of the fruit-base covered by the fruit cap are important morphological markers to distinguish between cultivars. This marker showed variations from 25 % coverage to 90% in different cultivars (Table1).

Random Amplified Fragment DNA (RAPD) markers were also produced for the identification of these cultivars. Out of 130 primers screened for reproducible and polymorphic DNA amplification patterns 42 were selected for DNA fingerprinting. The DNA profiles produced by 14 cultivars with OPERON A06 primer are presented in Figs 1&2 along with their fruit morphology. The analysis of pair-wise genetic distance and similarity matrix based on Nei and Li's (1979) similarity coefficient showed an average of more than 50 % similarity among the cultivars (Table-2, from Al-Khalifah,2006). Cluster analysis using unweighted pair group method of arithmetic means (UPGMA) and the dendrogram (Fig.3 from Al-Khalifah,2006) showed maximum similarity between Makhtomi and Nabtet Ali (0.70) followed by Barhy and Hilaliah (0.65). Out of the 19 cultivars screened by Al-Khalifah, (2006) 12 formed couples and the rest showed various percentages of similarity with either to one of the couples or to more than one couples.

Correlation of morphologic characters with genomic similarity showed that the fruit shape is one of the characteristics mostly influenced by genetic variation. Where ever there was insignificant length-width ratio between cultivars, more genomic similarity was observed. In the case of Makhtomi and Nabtet Ali, where the maximum genomic similarity was observed, their length-width ratios were only different by 1.46 and 1.44 respectively. The second genomically similar couplet (Hilaliah-Barhy) also showed a very narrow variation in their length-width ratio (1.1-1.2). The other pairs that followed the same rule were Khalas-Makhtomi (1.46-1.46), Sabakka-Rothana (1.5-1.4), and Shagra-Wannanah (1.32-1.44). But there was an exception exhibited by a pair Nabtet Ali-Wannanah, where their length-width ratio was similar (1.44) to each other but their genomic similarity was the least (44.1%). But in this case, irrespective

to their similar length-width ratio they were very distinct in their fruit morphology, i.e. their shape (Elliptic-oblong and ovate), colour and fruit base.

The present data generated by using different primers suggests genetic diversity among date palm cultivars. Molecular phylogeny of 13 date palm cultivars studied by Al-Khalifah and Askari (2003) and 7 cultivars by Askari *et al*. (2003) also showed the same tendency of genetic diversity. These genetic variations at the molecular level have resulted in the production of many elite cultivars which are highly variable in fruit size, shape, colour, texture, sugar and protein content. The methods followed in this study can be extended to other cultivars also, which may ultimately result in the making of an authenticated manual describing the diagnostic characters of date palm cultivars with their available synonyms. Addition of tree characteristics, protein and sugar content of each cultivar, to this data in future will make a perfect manual that can be used as reference book to identify the presently known cultivars of date palms. The RAPD analysis will help to solve the ambiguity regarding the identity of narrowly distinguishable cultivars and to assess genetic diversity for the conservation of date palm germplasm in Saudi Arabia.

Acknowledgement

Authors are thankful to the King Abdulaziz City for Science and Technology, Riyadh for providing technical and financial support to this study.

References

Al-Ghamdi, A.S. (1993).True-to-type date palm (*Phoenix dactylifera* L.) produced through tissue culture techniques: inflorescence and pollen grain evaluation. *In:* Proc.3rd symposium on the date palm in Saudi Arabia. King Faizal Univ., Al-Hassa. Vol.1:93-103.

Al-Khalifah, N.S. and Askari, E. (2003). Molecular phylogeny of date palm (*Phoenix dactylifera* L.) cultivars from Saudi Arabia by DNA fingerprinting. *Theor. Appl. Genet.* 107: 1266-1270.

Al-Khalifah, N.S. (2006). Micro propagation and DNA fingerprinting of date palm trees of Saudi Arabia. Association of Agricultural Research Institutions in the Near East and North Africa, Amman, Jordan.

Al-Khalifah, N.S., F.A.Khan, E.Askari and S.Hadi (2006). In Vitro Culture and Genetic Analysis of Male and Female Date Palm (*Phoenix dactylifera* L.). In: Fari, M.G., I. Holb and Gy.D.Bisztray (Eds). Proc.Vth International Symposium on In Vitro Culture and Hort.Breeding. Acta Hort.725, ISHS 2006.

Askari, E., Al-Khalifah, N.S., Ohmura, T., Al-Hafidh, Y.S., Khan, F.A., Al-Hindi, A., and Okawara, R. (2003). Molecular Phylogeny of seven date palm (*Phoenix dactylifera* L.) cultivars by DNA fingerprinting. *Pak. J. Bot*. 35: 323-330.

Bashah, M.A. (1996). Date Variety in the Kingdom of Saudi Arabia. Guidance booklet: Palms and Dates. King Abdulaziz University Press, Riyadh, Saudi Arabia. pp 1225-1319.

Benbades, A.K. (1992). Coconut and Date palm. In: Benbades, A.K. and F.E.Hammerschlag (Eds.), Biotechnology of Perennial fruit crops. pp 383-400.

Dellaporta, S.L., Wood, J. and Hicks, J.B. (1983). A plant DNA minipreparation: version II. *Plant Mol. Biol. Rep*. 1: 19-21.

Dowson, V.H.W. (1923). Dates and date cultivation of Iraq. Part III. The varieties of date palms in the Shalt-Al Arab. Agr. Directorate of Mesopotamia, Memoir III, Heffer, Cambridge.

FAO (1996). Agro-statistics Database.

Kearney, T.H (1906). Date varieties and date culture in Tunis. US. Dept.Agr.Bur.Plant Industry Bull. 92:112.

Muirhead, D. (1961). Palms. Dale Stuart King Publishers, Arizona

Nei, M. and Li, W. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci., USA. 76:5269-5273.

Oudejans, J.H.M. (1979). Date Palm (Phoenix dactylifera, Palmae) In: Simmonds, N.W. (Ed.). Evolution of Crop Plants. Longman, London.

Saaidi, M (1979). Contribution a la lutte contre le bayoud, fusariose vasculaire du palmier dattier. These d'Universite, Universte de Dejon.

Saker, M.M., Bekheet, S.A., Taha, H.S., Fahmy, A.S. and Moursy, H.A. (2000). Detection of somaclonal variations in tissue culture-derived date palm plants using isoenzyme analysis and RAPD fingerprints. *Biologia Plantarum* 43 (3): 347-351.

Sedra, MyH. Lashermes, P., Trouslot, P., Combes, M.and Hamon, S. (1998). Identification and genetic diversity analysis of date palm (*Phoenix dactylifera* L.) cultivars from Morocco using RAPDmarkers. *Euphytica* 103: 75-82.

Welsh, J. and McClelland. M. (1990). Finger printing genomes using PCR with arbitrary primers. *Nucleic Acid Research* 18:7213-7218.

Williams, J.G.K., Kubelik, A.R., Kenneth, J.L., and Tingy, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Research* 18: 6522-6531.

Zaid, A. and de Wet, P.F. (1999). Botanical and Systematic description of the date palm. In: Zaid, A. (Ed.). Date Palm Cultivation. FAO, Rome.

Table 1. Comparative fruit morphology of fourteen date palm cultivars.

Cultivar	Shape	Colour variation during ripening			Length-width ratio	Fruit Cap (%)	Base
		'Beser'	'Rutab'	'Tamar'			
Barhy	Globular-Broadly elliptic	Lemon yellow	Amber	Golden brown	1.21	40	Truncate
Deglet Noor	Linear-oblong	Lemon yellow	Reddish brown	Amber	2.62	90	Truncate
Hilaliah	Globular	Yellow with rose tinge	Amber	Reddish brown	1.1	90	Truncate
Hilwa	Oblong	Scarlet red	Dark red	Chocolate brown	1.5	50	Shallowly cordate
Khalas	Ovate	Light yellow	Amber	Amber	1.46	30	Oblique
Makhtomi	Ovate-oblong	Greenish-yellow	Amber	Amber	1.46	60	Shallowly cordate
Moneifi	Elliptic	Yellow	Amber	Reddish brown	1.51	50	Truncate
Nabtet Ali	Elliptic oblong	Light yellow	Reddish brown	Reddish brown	1.44	25	Shallowly cordate
Omal Khashab	Oblong	Reddish-yellow	Amber	Amber	1.84	60	Truncate
Rothana	Elliptic-oblong	Lemon yellow	Amber	Reddish brown	1.4	30	Deeply cordate
Sabbaka	Oblong	Light yellow	Reddish brown	Light brown	1.5	50	Shallowly cordate
Shagra	Ovate-oblong	Yellow with red dots	Brown	Reddish-brown	1.32	33	Cordate
Sukkary	Ovate	Reddish yellow	Reddish brown	Reddish brown	1.43	60	Cordate
Wannanah	Ovate	Yellow with red dots	Chocolate brown	Chocolate brown	1.44	30	Oblique

Table 2. Similarity matrix based on Nei and Li's coefficients of 19 date palm cultivars obtained from RAPD markers (Al-Khalifah, 2006).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Nabtat Ali	1	100.0																		
Maktoomi	2	70.2	100.0																	
Moneifi	3	60.6	67.4	100.0																
Mowakil	4	60.5	63.1	57.0	100.0															
Omal Khashab	5	60.0	59.3	57.6	70.2	100.0														
Bayadh	6	57.5	60.6	60.0	70.3	59.9	100.0													
Khalas	7	56.6	61.6	58.0	73.0	67.3	71.3	100.0												
Rothana	8	54.4	51.6	48.0	55.1	55.4	52.1	51.9	100.0											
Hilaliah	9	53.7	56.4	47.6	64.9	56.0	57.1	59.7	50.9	100.0										
Madjhool	10	52.4	54.5	49.3	63.1	55.0	57.2	61.5	50.1	56.8	100.0									
Sukarry	11	51.9	58.1	54.6	66.0	57.2	64.7	59.3	57.9	57.6	52.7	100.0								
Kwairiah	12	51.7	56.8	50.7	55.7	54.0	53.6	53.9	56.3	58.7	51.3	55.1	100.0							
Roshodyia	13	50.5	51.9	57.8	53.9	56.8	49.6	54.5	54.0	49.5	46.5	50.5	63.1	100.0						
Hilwa	14	50.1	49.4	50.6	58.7	59.0	55.0	60.8	52.5	55.3	61.6	50.6	51.6	55.8	100.0					
Barhy	15	50.0	54.3	52.4	57.5	57.8	61.2	63.8	50.9	64.8	53.8	54.4	59.8	53.0	59.0	100.0				
Sabaka	16	46.8	48.0	53.9	52.2	48.8	56.2	48.9	58.8	47.4	44.6	58.9	44.7	48.2	43.2	48.9	100.0			
Shagra	17	46.4	48.2	44.6	62.7	54.8	50.0	54.9	44.8	55.2	64.4	48.4	56.8	48.7	54.9	52.6	46.2	100.0		
Deglet Noor	18	44.6	50.4	50.6	51.9	59.3	52.3	54.0	42.2	51.3	59.8	47.3	52.4	51.6	52.1	54.4	42.8	56.9	100.0	
Wanana	19	44.1	47.5	48.2	59.2	47.3	62.6	57.3	49.1	54.5	54.8	58.6	62.4	55.6	56.2	56.4	48.9	51.0	54.1	100.0

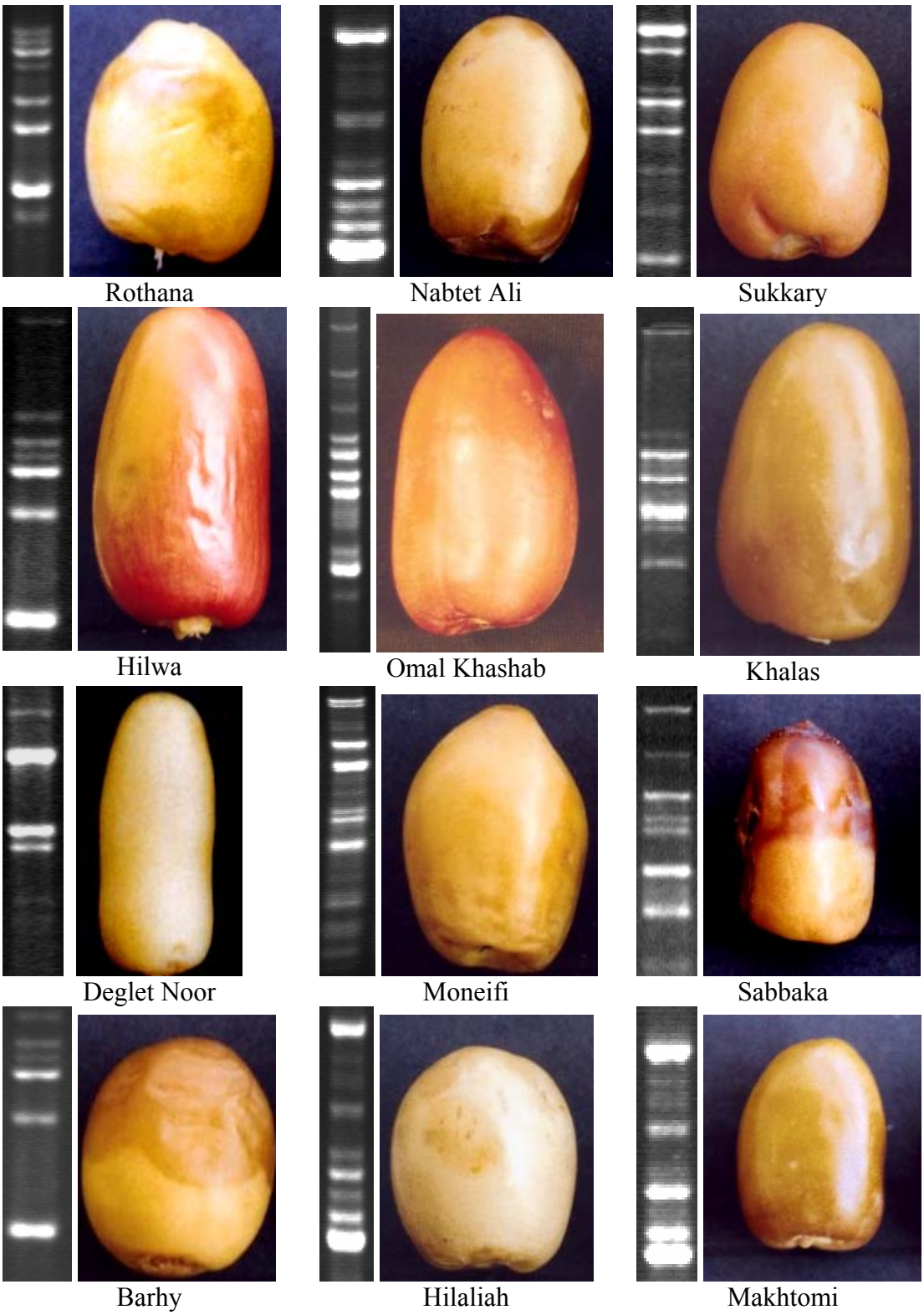
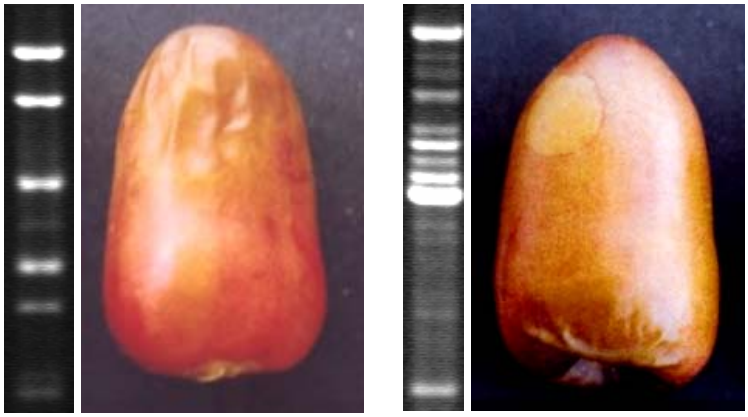


Fig.1. Fruit morphology and DNA profiles of twelve cultivars produced by A-06 OPERON primer.



Shagra Wannanah

Fig.2. Fruit morphology and DNA profiles of two morphologically similar cultivars produced by A-06 OPERON primer.

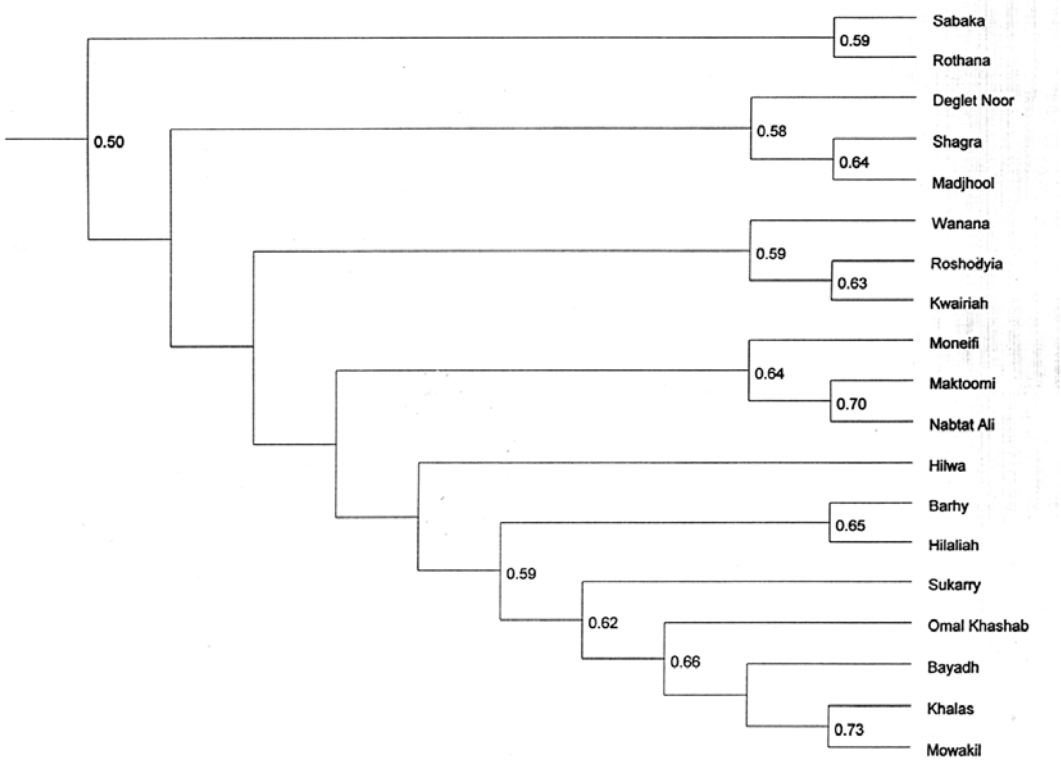


Fig. 3: A dendrogram of phylogenetic relationships among 19 cultivars of date palm based on the RAPD analysis using 42 primers (Al-Khalifah, 2006).