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REGULAR ARTICLE

Molecular and morphological identification of some elite varieties of date palms grown in Saudi Arabia

Nasser S. Al-Khalifah*, Ejaz Askari and A. E. Shanavas Khan

King Abdulaziz City for Science and Technology (KACST), P.O. Box 6086, Riyadh, Saudi Arabia

Abstract

Date palm (*Phoenix dactylifera* L.), is a highly out breeding, dioecious plant species of enormous genetic diversity. Genotype identification of date palm is an intricate empirical exercise based on morphological characters. In date palms most of the female cultivars are recognised by their fruit characteristics such as size, shape, colour and taste. Morphological characters of the tree are also taken into consideration for cultivar identification. However some date palms have similar or narrow distinguishing morphological characters that complicate cultivar identification and require genetic evidence to prove phylogenetic relationships. RAPD analysis has been successfully applied for cultivar identification of date palms. The objectives of the present study were to characterize some elite cultivars of date palms using morphological characters of fruits and to correlate the results with RAPD markers. A total of 14 well-known cultivars of date palm (Barhy, Deglet Noor, Hilaliah, Hilwa, Khalas, Makhtomi, Moneifi, Nabtet Ali, Omal Khashab, Rothana, Sabbaka, Shagra, Sukkary, Wannanah) were selected from Saudi Arabia. Analysis of the morphological data of fruits revealed a 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. The length-width ratio of these 14 cultivars ranged from 1.1 to 2.62, indicating a great variation in their shape. Correlation of morphologic characters with genomic similarity using RAPD markers showed that the fruit shape is one of the characteristics most influenced by genetic variation. Wherever there was insignificant length-width ratio among cultivars, more genomic similarity was observed. Genetic variations at the molecular level have resulted in the production of many elite date palm cultivars which are highly variable in fruit size, shape, colour, texture, sugar and protein content. The methodology followed in this study can be extended to other cultivars, which may ultimately result in the compilation of an authentic manual describing the diagnostic characters of date palm cultivars with their existing synonyms. The RAPD analysis will help to resolve the ambiguity regarding the identity of narrowly-distinguishable cultivars and to assess genetic diversity for the conservation of date palm germplasm in Saudi Arabia.

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 levels 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 desert areas. *Phoenix dactylifera* L. is interfertile with its allied species (Muirhead, 1961) and 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 3,000 cultivars around the world. There are about 450 cultivars in Saudi Arabia (Bashah, 1996), 400 in Iran (FAO, 1996), 600 in Iraq (FAO, 2008), 250 in Tunisia (Kearney, 1906), 244 in Morocco (Saaidi, 1979) as well as many cultivars in other date-growing countries (Zaid and de Wet, 1999).

Most of the cultivar identification studies are of an enumerative type based on local names which vary 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 much ambiguity in listing the cultivars based on local names. A scientific approach of characterizing cultivars and assigning a more

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*Corresponding Author

Nasser S. Al-Khalifah
King Abdulaziz City for Science and Technology (KACST), P.O. Box 6086, Riyadh, Saudi Arabia

Email: abujawad@kacst.eud.sa

acceptable legitimate name to the cultivars has seldom been attempted for this species, especially in Saudi Arabia.

Genotype identification of date palm is commonly based on morphological characters (Sedra et al., 1998). In date palm most of the female cultivars are recognised by their fruit characteristics such as size, shape, colour and taste along with the morphological characters of the tree for cultivar identification. During the ripening process, the date fruits pass through four distinct stages of maturity, i.e. *kemri, beser (khalal), rutab* and *tamar* (Al-Ghamdi, 1993). When the fruits are young they are green in colour (varying in different cultivars) and are termed *kemri*. The beginning of ripening marks the *beser* stage, the half ripened stage is called *rutab* and fully ripened, soft textured stage is called *tamar*. Colour variations during the ripening of fruits are important morphological markers for the cultivar identification.

Some date palm cultivars have similar or narrow distinguishing morphological characters that complicate cultivar identification and require evidence to prove phylogenetic relationships at the interspecific level. RAPD analysis is a comparatively simple, quick and inexpensive 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 palm using morphological characters of fruits and to correlate these results with RAPD markers.

Materials and methods

A total of 14 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 the Al-Qassim area, Saudi Arabia. Colour variations during the three fruit ripening stages (*beser, rutab* and *tamar*) were recorded directly from the tagged trees. One hundred fruits from each cultivar were collected during *rutab* stage and their length and width measured using Vernier Calipers. Shape and colour of the fruits was documented using a digital camera. The 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 these data the total area of the fruit base covered by the fruit cap was calculated.

For RAPD analysis young sprouting leaves from each cultivar were collected. Total genomic DNA was extracted using the protocol of Dellaporta et al. (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.

A Polymerase Chain Reaction (PCR) was performed as described by Al-Khalifah and Askari (2003) using 130 random 10-mer RAPD primers (OPERON Tech., USA) of A to G series. The 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 the Kilo Base DNA marker (Amersham Pharmacia Biotech.) in the gel as a 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 a 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 (Table 1). Fruit shape varied from globular, elliptic, ovate, oblong, to linear oblong as in Deglet Noor (Figure 1,2). Many intermediary forms or combination of one or two forms were also observed. The length-width ratio of these 14 cultivars ranged from 1.1 to 2.62, indicating a great variation in their shape. Even within cultivars having the 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 the *kemri* stage all cultivars had green coloured fruits which turned to yellow or red or various degrees of a combination of red and yellow in the *beser* stage. During the *rutab* stage the ripening process usually starts from the tip of the fruit which brought different colorations to the fruits (Table 1). *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 cultivars. This marker showed variations of 25-90% coverage in different cultivars (Table 1).

Table 1. Comparative fruit morphology of 14 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

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 the Nei and Li (1979) similarity coefficient showed an average of more than 50% similarity among the cultivars (Table 2, from Al-Khalifah, 2006). Cluster analysis using the 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 to either one couple or to more than one couple.

A correlation of morphologic characters with genomic similarity showed that fruit shape is one of the characteristics most influenced by genetic variation. Wherever there was an 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 only differed 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 the Nabtet Ali-Wannanah pair, where the length-width ratio was similar (1.44) but the genomic similarity was the least (44.1%). But in this case, irrespective of 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.

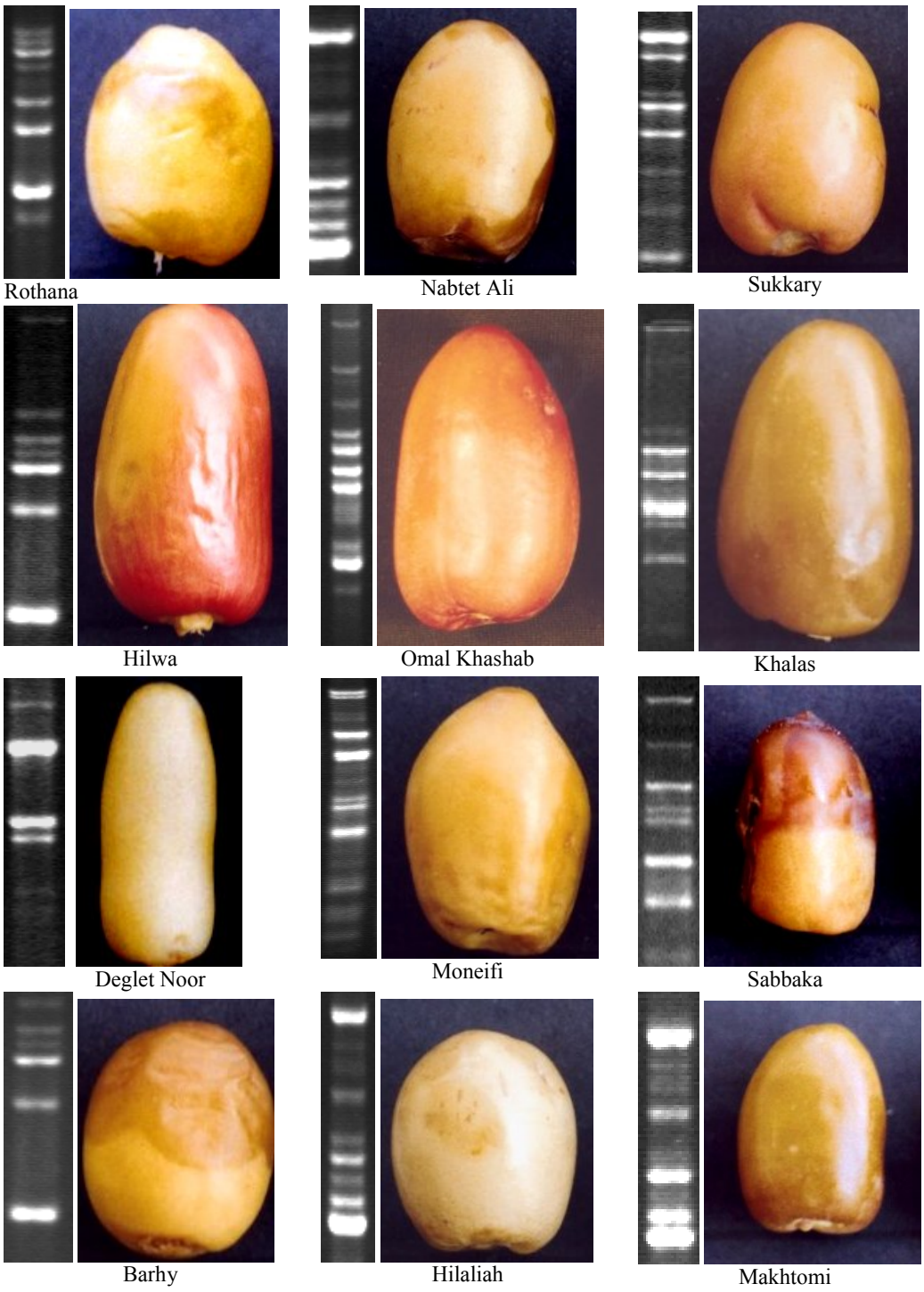


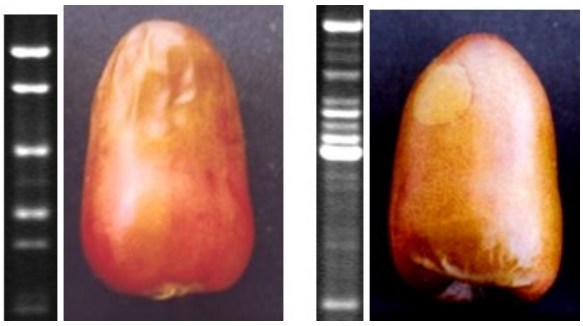
Figure 1. Fruit morphology and DNA profiles of 12 cultivars produced by A-06 OPERON primer.

Results generated by the present data using different RAPD primers suggest 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.

Table 2. Similarity matrix based on the Nei and Li 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
1	Nabtat Ali	100.0																		
2	Maktoomi	70.2	100.0																	
3	Moneifi	60.6	67.4	100.0																
4	Mowakil	60.5	63.1	57.0	100.0															
5	Omal Khashab	60.0	59.3	57.6	70.2	100.0														
6	Bayadh	57.5	60.6	60.0	70.3	59.9	100.0													
7	Khalas	56.6	61.6	58.0	73.0	67.3	71.3	100.0												
8	Rothana	54.4	51.6	48.0	55.1	55.4	52.1	51.9	100.0											
9	Hilaliah	53.7	56.4	47.6	64.9	56.0	57.1	59.7	50.9	100.0										
10	Madjhool	52.4	54.5	49.3	63.1	55.0	57.2	61.5	50.1	56.8	100.0									
11	Sukarry	51.9	58.1	54.6	66.0	57.2	64.7	59.3	57.9	57.6	52.7	100.0								
12	Kwairiah	51.7	56.8	50.7	55.7	54.0	53.6	53.9	56.3	58.7	51.3	55.1	100.0							
13	Roshodiyia	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						
14	Hilwa	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					
15	Barhy	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				
16	Sabaka	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			
17	Shagra	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		
18	Deglet Noor	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	
19	Wanana	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

The methodology followed in this study also can be extended to other cultivars, which may ultimately result in the compilation of an authentic manual describing the diagnostic characters of date palm cultivars, with the existing synonyms. The addition of tree characteristics, protein and sugar content of each cultivar to these data in future will make an ideal manual that can be used as a reference to identify the presently-known date palm cultivars. The RAPD analysis will help to resolve the ambiguity regarding the identity of narrowly-distinguishable cultivars and to assess genetic diversity for the conservation of date palm germplasm in Saudi Arabia and other countries.



Shagra **Wannanah**

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

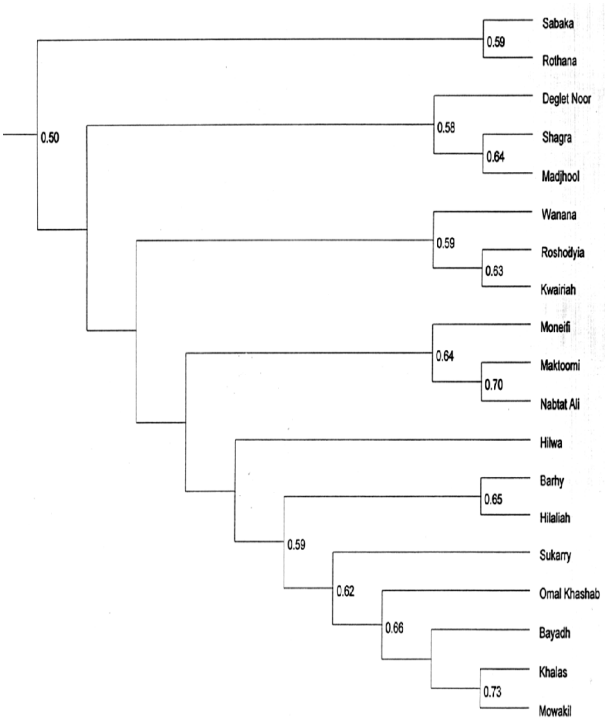


Figure 3. A dendrogram of phylogenetic relationships among 19 cultivars of date palm based on the RAPD analysis using 42 primers (Al-Khalifah, 2006).

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