Agricultural and Marine Sciences, 9(1):1-3 ((2004)
©2004 Sultan Qaboos University

Identification of Some Date Palm (*Phoenix dactylifera* L.) Cultivars in Saudi Arabia Using RAPD Fingerprints

A.M. Al-Moshileh*, M.I. Motawei, A. Al-Wasel and T. Abdel-Latif

Plant Production and Protection Department, College of Agriculture and Veterinary Medicine, King Saud University, Al-Qassim, Saudi Arabia

استخدام البصمة الوراثية (RAPD) للتعرف على بعض أصناف نخيل التمر في المملكة العربية السعودية

عبدالرحمن المشيلح و محمد مطاوع و عبد الرحمن الواصل و طه عبد اللطيف

خلاصة: تم اختبار مدى ملاءمة طريقة التكبير العشوائي لقطع الحامض النووي الديوكسي ريبوز RAPD كأدلة وراثية في نخيل التمر، فتم تحليل البصمة الوراثية لخمسة أصناف من نخيل التمر المعروفة في المملكة العربية السعودية؛ وهي البرحي و نبتة علي و روثانا و عجوة وسكري. من بين ٢٠ بادئا جزيئيا تم اختيار ١٢ بادئا فعالا معطيا ٦٤ حزمة. كانت قطاعات نواتج الـ PCR بطريقة الـ RAPD المتحصل عليها ناجحة تماما في التمييز بين السلالات. باستخدام المقارنة بين كل زوجين من الحزم الناتجة تم حساب التشابه الوراثي و لقد أظهرت الأصناف الخمسة اختلافات على مستوى الحامض النووي. كما تم باستخدام التحليل التجميعي للأصناف الخمسة بيان الاختلافات على مستوى جزيء الحامض النووي. و تراوحت نسب التشابه الوراثي بين أصناف نخيل التمر من ٧٠% إلى ٨٥%. وكان الصنف سكري أبعد الأصناف من حيث درجة التشابه الوراثي عن بقية الأصناف الأخرى. و على أساس التحليل الوراثي قسمت الأصناف إلى مجموعتين: المجموعة الأولي تشمل علي صنفي البرحي وعجوة و المجموعة الثانية تشتمل على أصناف نبتة علي و روثانا و سكري. لذلك يقترح استخدام هذه الطريقة في التعرف على أصناف النخيل المختلفة وكذلك في دراسة التباعد الوراثي بين الأصناف.

ABSTRACT: The suitability of randomly amplified polymorphic DNA (RAPD) fingerprints as genetic markers in date palms was tested. Five date palm cultivars (Barhi, Nabtet Ali, Rothanah, Ajwa, and Sokkari) from Saudi well-known dates were subject to DNA fingerprint analysis. From 20 primers tested, only 12 were selected as reproducible, giving 64 bands. The RAPD profiles obtained were successfully used to differentiate the genotypes. Based on the pair-wise comparison of amplification products, the genetic similarity was estimated. The five date palm cultivars showed variation at the DNA level. The genetic similarity among all date palm cultivars ranged from 70 to 85%. Sokkary was quite distant from Barhi and Ajwa cultivars. A dendrogram was constructed using UPGMA analysis. On the basis of this analysis, the populations were clustered into two clusters: cluster I contained Barhi and Ajwa cultivars, and cluster II contained Nabtet Ali, Rothanah and Sokkari cultivars. Therefore, the polymorphism detected and its reproducibility suggest that RAPD markers are reliable for identification of Saudi date palm cultivars.

Keywords: Date palm, genetic markers, Saudi Arabia.

Date palms are essential integral components of farming systems in dry and semi-arid regions. In Saudi Arabia, date palms are grown on about 90% of the cultivated land (Shaheen, 1990). The date palm is considered one of the most important commercial crops in the Arab world (Anonymous, 1984). Increasing date productivity can be achieved through increasing the productivity of the existing palms or expanding the palm cultivation area. Slow growth, dioecy, the slow offshoot-based propagation system and the impossibilities of predicting adult characteristics of the seedling have severely restricted improvement of this ancient tree crop. Characterization and analysis of the available genetic diversity therefore constitute indispensable steps with regard to the development of breeding strategies.

Correct identification of palms is usually not possible until fruits are produced. In addition, the characterization of cultivars and evaluation of genetic diversity require a large set of phenotypic data that are often difficult to assess and sometimes variable due to environmental influences (Sedra *et al.*,1993, 1996). Recently, molecular techniques based on DNA have been very successful in typing cultivars of a variety of crop plants. Randomly amplified polymorphic DNA (RAPD) markers (Williams *et al.*, 1990) have been evaluated for

*Corresponding author.

identification of date palm varieties (Sedra *et al.,* 1998; Ben-Abdallah *et al.,* 2000).

Therefore, the objective of this study was to test the reliability of the RAPD-PCR system as a tool for the identification of date palm cultivars grown in Saudi Arabia.

Materials and Methods

DNA EXTRACTION: Genomic DNA of five cultivars (Barhi, Nabtet Ali, Rothanah, Ajwa, and Sokkari) was extracted from 0.5 gm leaflets. The leaves were first ground into fine powder with liquid nitrogen. DNA was extracted in 15 ml of extraction buffer (350 mM sorbitol, 5 mM EDTA, 100 mM Tris pH8, 0.5% sodium bisulphate), and the solution was filtered through a muslin cloth. The extract was centrifuged at 3000 g for 20 minutes, and the supernatant discarded. The precipitate was incubated in 30 ml of lysis buffer (1.5 M NaCl, 100mM Tris-HCl pH 8, 20 mM EDTA, 4% CTAB) for 4 hours at 65˚C with occasional mixing. Following incubation, 5 ml of chloroform/isoamyl-alcohol (24:1) was added to the tubes, mixed, and centrifuged at 2600 g for 10 minutes. The aqueous phase was removed to a fresh tube and an equal volume of ice-cold isopropanol was added, followed by centrifugation as above to precipitate the DNA. The pellet was washed in 70% ethanol and dissolved in TE buffer (10mM Tris-HCl, pH8.0, 0.1mM EDTA).

RAPD-PCR AMPLIFICATION: Twenty random primers were obtained from Amersham Biosciences International (Amersham Biosciences UK Limited, England HP7–9NA). RAPD analysis was conducted in a thermal cycler (Thermolyne Amplitron). The reaction mixture (25 μL) contained 1x PCR buffer with Mg Cl2 (50 mM K Cl, 10 mM Tris- HCl (pH 9.0), 2 mM Mg Cl2 and Triton X-100), 200 μM each of dATP, dCTP, dGTP, and dTTP, 30 ng template DNA, 50 pM of oligonucleotide primer and 1.5 unit of Taq polymerase. The mixtures were subjected to the following conditions: hold at 94˚C for 2 min, followed by 40 cycles of 94˚C for 1 min, 36˚C for 1 min and 72˚C for 2 min, and a final hold at 72˚C for 5 min. PCR products were visualized along with a DNA marker on 2% agarose gel with 1X TAE buffer and detected by staining with ethidium bromide. Gels were photographed on Polaroid films under UV light.

DATA HANDLING AND CLUSTER ANALYSIS: Data were scored for computer analysis on the basis of the presence or absence of the amplified products for each random primer. If a product was present in a genotype, it was designated "1", if absent it was designated "0" after excluding irreproducible bands. Pair-wise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to generate similarity coefficients based on simple matching. The similarity coefficients were then used to construct a dendogram by UPGMA (Unweighted Pair-Group Method with Arithmetical Averages) using NTSYS-PC (Rohlf, 1993).

Results and Discussion

SCREENING FOR POLYMORPHIC PRIMERS: Twenty primers of arbitrary nucleotide sequence were used to amplify DNA segments from the five Saudi date palm cultivars. The number of amplification bands per primer varied between 0 and 8. Twelve primers out of 20 were selected for further analysis based on the intensity, size and number of amplified products (Table 1). To ensure reproducibility and genetic pertinence of RAPD marker data, the primers generating no, weak or complex patterns were discarded.

Analysis of the 12 selected primers among the five cultivars included in this study generated 64 bands, 29 of which were polymorphic. There were 2.4 polymorphic bands per primer on average. Examples of polymorphism are shown in Figure 1. All five date palm

TABLE 1

Selected primers with the numbers of amplified products and polymorphic fragments.

Primer Number	Nucleotide Sequence (5' to 3')	No. of Amplification Products	No. of Polymorphic Products
2	GTTTCGCTCC	8	5
5	AACGCGCAAC	7	3
6	CCCGTCAGCA	3	2
7	CAGGCCCTTC	8	6
9	AGTCAGCCAC	7	0
11	AGGGGTCTTG	4	2
12	GGTCCCTGAC	4	0
13	GAAACGGGTG	5	4
14	GTGACGTAGG	6	2
15	GGGTAACGCC	3	1
16	GTGATCGCAG	4	1
19	CAGCACCCAC	5	3

M	Barhi	Nabtet Ali	Rothanah	Ajwa	Sokkari

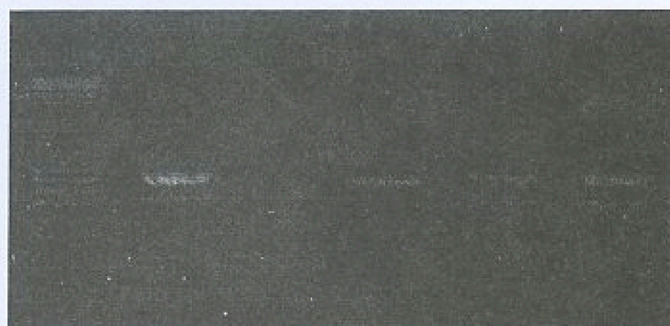

Figure 1. Polymorphism revealed using primer 2 to amplify genomic DNA purified from five cultivars of date palm (left to right) Barhi, Nabtet Ali, Rothanah, Ajwa and Sokkari. M lane is 1 kbp ladder DNA marker.

TABLE 2

Simple matching coefficients of similarity determined from analysis using 64 RAPD loci.

Cultivars	Barhi	Nabtet Ali	Rothanah	Ajwa	Sokkari
Barhi	1.000				
Nabtet Ali	0.750	1.000			
Rothanah	0.773	0.843	1.000		
Ajwa	0.821	0.750	0.774	1.000	
Sokkari	0.702	0.764	0.755	0.732	1.000

cultivars were distinguishable by their band patterns. Polymorphism between cultivars can arise through: nucleotide changes that prevent amplification by introducing a mismatch at one priming site; deletion of a priming site; insertions that render priming sites too distant to support amplification; and insertions or deletions that change the size of the amplified product (Williams *et al*., 1990). The overall polymorphism exhibited in this study is rather high in comparison with results reported by Sedra *et al.* (1998) and Ben Abdallah *et al*. (2000). The last study indicated that 11 of the 122 primers tested were selected as reproducible among four cultivars of date palm.

CLUSTER ANALYSIS: The RAPD markers produced by 12 primers were tested to construct a similarity matrix (Table 2). The genetic similarity among all date palm cultivars ranged from 0.70 to 0.84. Rothanah cultivar was the closest relative (0.84) to Nabtat Ali cultivar. On the other hand, Sokkari cultivar exhibited the greatest genetic difference from Barhi and Ajwa cultivars.

Cluster analysis was conducted to generate a dendogram showing relationships between date palm cultivars. The dendrogram constructed from simple matching with UPGMA analysis revealed two main clusters (Fig. 2). Cluster I contained Barhi and Ajwa cultivars. Cluster II contained Nabtet Ali, Rothanah and Sokkari cultivars. The fact that Nabtet Ali and Rothanah cultivars did not markedly diverge from Sokkari cultivar suggests a narrow genetic diversity of populations from which these cultivars have been derived. On the other hand, the latter cultivars were less similar to Barhi and Ajwa cultivars. This result may be due to the fact that Nabtet Ali, Rothanah and Sokkari cultivars were from Al-Qassim region, but Barhi cultivar is introduced from Iraq and Ajwa cultivar is cultivated in the Almadina area. RAPD- markers should therefore be of high value for date palm germplasm characterization and genetic maintenance (Sedra *et al*.,1998). In either case, a prediction of genetic similarity based on genetic markers provides more information than can be gained from pedigree information alone (Tinker *et al*., 1993). Moreover, Ben-Abdallah *et al*., (2000) suggested that RAPD markers can be used for varietal identification, evaluation of date palms and for studying the genetic diversity of cultivars.

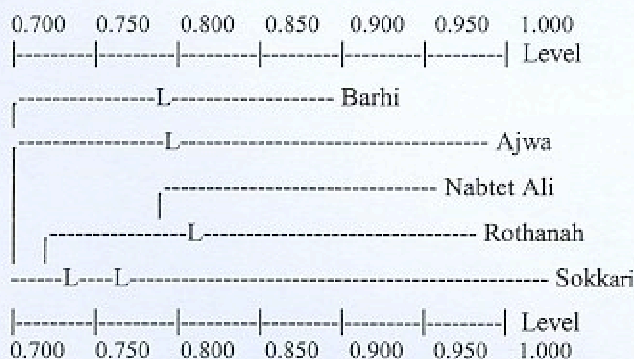

Figure 2. Dendrogram constructed from similarity coefficients showing the clustering of five date palm cultivars.

Conclusions

The results from this study suggest that the use of RAPD markers to detect polymorphism is reliable for identification of data palm cultivars in Saudi Arabia. Rothanah cultivar was the closest to Nabtet Ali cultivar, while Sokkari cultivar exhibited the greatest genetic difference from Barhi and Ajwa cultivars.

References

Anonymous. 1984. FAO Production Year Book. Food and Agriculture Organization of the United Nation, Rome.

Ben-Abdalla, A., K. Stiti, P. Lepoivre and P.D. Jardin. 2000. Date palm (*Phoenix dactylifera* L.) cultivar identification using random amplified polymorphic DNA (RAPD). *Cahiers/ Agricultures* 9:103-107.

Rohlf, F.J. 1993. NTSYS-PC Numerical Taxonomy and Multivariate System, Version 1.8. *Applied Biostatistics Incorporated*, New York.

Sedra, My. H., H. El-Filali and D. Frira. 1993. Observation sur quelques caracteristiques phenotypiques et agronomiques du fruit des varietes et clones du palmier dattier selectionnes. *Al Awamia* 82:105-120.

Sedra, My. H., H. El-Filali, A. Benzine, M. Allaoui, S. Nour and Z. Boussak. 1996. La palmeraie dattere marocaine: Evaluation du patrimone phenicicole. *Fruits* 1:247-259.

Sedra, H., P. Lashermes, P. Trouslot, C. Combes and S. Hamon. 1998. Identification and genetic analysis of date palm (*Phoenix dactylifera* L.) varieties from Morocco using RAPD markers. *Ephytica* 103:75-82.

Shaheen, M.A. 1990. Propagation of date palm through tissue culture: A review and an interpretation. *Annals of Agricultural Sciences*, Ain Shams Univ., Cairo, Egypt. 35:895-909.

Tinker, N.A., M.G. Fortin and D.E. Mather. 1993. Random amplified polymorphic DNA and pedgree relationships in spring barley. *Theory of Applied Genetics* 79:976-984.

Williams, G.K., A.R. Kubelik, K.L. Livak, J.A. Rafalski and S.V. Tingey. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18:6531-6535.

Received December 2003.
Accepted June 2004.