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Genetic comparisons of Egyptian date palm cultivars (*Phoenix dactylifera* L.) by RAPD-PCR

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Random amplified polymorphic DNA technique was used to compare genetic material from four females date palm and four unknown male trees of Egyptian date palm. The genetic similarity between the four females date palm (Zaghloul, Amhat, Samany and Siwi) ranged from 87.5 to 98.9%. The banding profiles obtained suggested that both males 3 and 4 are genetically related to the four female cultivars.

Key words: Date palm, cultivars, RAPD-PCR, genetic similarity.

INTRODUCTION:

Date palm *(Phoenix dactylifera* L.), a long-living monocotyledon plant, is of economic importance in Egypt and all of North Africa. It presents a source of income to oases inhabitants and creates favorable conditions for improving secondary crop culture like barley, alfalfa and clover as forage.

The recently developed techniques, based on the polymerase chain reaction (PCR), offer a new tool for genetic analysis and construction of linkage maps. The random amplified polymorphic DNA (RAPD) technique utilizes arbitrary primers for the amplification of template DNA (Welsh and McClelland, 1990). The use of arbitrary primers for evolution studies and linkage analysis has been found effective in several plant species (Halward et al., 1992; Carlson et al., 1991). The objectives of the present study were to determine genetic similarity between four females date palm based on RAPD markers and identify unknown males of Egyptian date palm through known female cultivars.

MATERIALS AND METHODS

Plant materials

Leaves of date palm were collected from the Egyptian Ministry of Agriculture Experiment Station at Al-Kanater Al-khairia, Kalubia Governorate during 2002 season. The date palm studied were four males and four females (Zaghloul, Amhat, Samany and Siwi)].

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Extraction of DNA

DNA was extracted from fresh materials according to modified mini-prep CTAB method (Harris, 1995). The polymerase chain reaction (PCR) mixture (25 ul) consisted of 0.8 U of Taq DNA polymerase, 25 pmol dNTPs, and 25 pmol of random primer, and 50 ng of genomic DNA. The reaction mixture was placed on a DNA thermal cycler (Perkin Elmer 9700). The PCR programme included an initial denaturation step at 94°C for 2 mins followed by 45 cycles with 94°C for 1 min for DNA denaturation, annealing as mentioned with each primer, extension at 72°C for 30 seconds and final extension at 72 °C for 10 minutes were carried out. The amplified DNA fragments were separated on 2% agarose gel and stained with ethidium bromide. The amplified pattern was visualized on a UV transilluminator and photographed.

The RAPD bands were scored for their presence (1) or absence (0). The index of similarity between each two varieties was calculated using the formula: $B_{ab}=2\ N_{ab}/(N_a + N_b)$, where Nab is the number of common fragments observed in individuals a and b, and N_a and N_b are the total number of fragments scored in a and b respectively (Lynch, 1990). The genetic similarity was calculated for each primer separately and average for all primers was carried out with each comparison. The genetic similarity was calculated for each primer separately and average for all primers was carried out with each comparison. Dendrogram was constructed using the average linkage between groups (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

All the five primers (Table 1) examined produced different RAPD fragment patterns (Figure 1). The number of fragments generated per primer varied between 4 to 12. Genetic similarity estimated between different male and each female date palm plants is presented as a dendrogram (Figure 2). The highest values of genetic

Table 1. Primers used and their annealing temperatures.

Primer	Sequence 5'- 3'	Annealing Tm °C / Sec
1	AGG CCC CTG T	30/30
2	ATG CCC CTG T	
3	AAA GCT GCG G	
4	CAG GCC CTT CCA GCA CCC AC	50 / 30
5	GAA ACG GGT GGT GAT CGC AG	

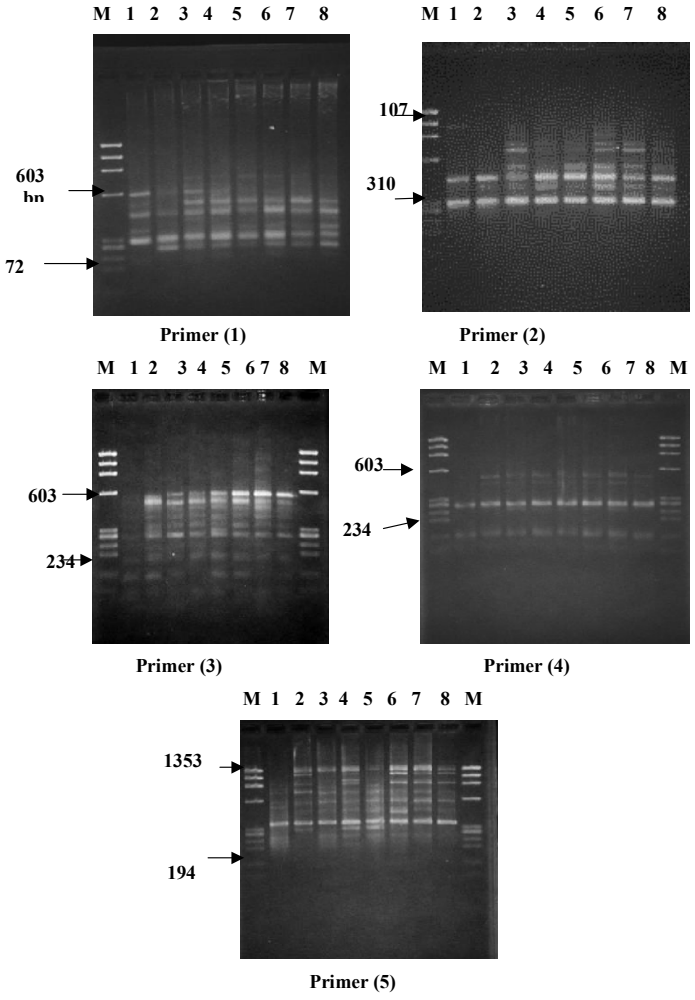

Figure 1. RAPD patterns in 8 samples of date palm obtained with the five random primers. M is DNA marker, lanes 1- 4 are the four samples of males and lanes 5–8 are the four samples of females (Zaghloul, Amhat,, Samany and Siwi).

similarity were observed between both male 3 and 4 with female cultivars, and it ranged from 88.9 to 95.3%. This result reflects the similarity between unknown male trees and female cultivars, but this data is not sufficient to identify unknown male. Identification of male variety exactly needs more advanced molecular studies. We also observed that the small alterations in PCR parameters or quality of target DNA can alter RAPD patterns (see also, Williams et al., 1993; Bardakci and Skibinski, 1994). Thus there may be reason to view with caution systematic conclusions based on RAPD analysis alone. On the other hand, the possibility of carrying out compatibility analysis with unlimited numbers of primers, each detecting variation at several regions in the genome, provides an advantage over other techniques. Even if some primers amplify identical regions of the genome or if the technique itself is noisy, it should be possible to build up quickly a consensus from patterns of inter-population variation.

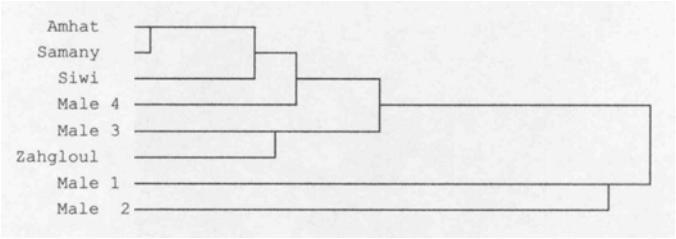

Figure 2. Dendrogram using Average Linkage (Between Groups) Rescaled Distance Cluster Combine for 4 females and 4 males using data of all primers.

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