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THE GENETIC SIMILARITY OF THE MICROPROPAGATED *BALANITES AEGYPTIACA* PLANTS

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Random amplification polymorphism of DNA for the micropropagated *Balanites aegyptiaca* plants by using eight of ten mere operon primers showed that there were high similarity among the amplified DNA of samples, while there were quite variations between DNA samples that may be due to mismatching occurred between primers and DNA of some samples.

Keywords: *Balanites aegyptiaca*, micropropagation, genetic similarity.

Balanites aegyptiaca as an evergreen tree is a multipurpose plant known for many uses as fodder, charcoal, fuel wood etc. It is widely distributed from Guinea through the Sahara into Egypt. The tree can be propagated naturally by seeds. Nevertheless, the species is endangered because of the high rate of clearance and low rate of seeds germination. consequently, micropropagation offers a rapid method of producing clonal planting stock for afforestation, production of woody biomass and conservation of elite germ plasm. Woody taxa are generally difficult to regenerate by *in vitro* techniques, but some success has been achieved in a few leguminous tree species.

The Random Amplification Polymorphism DNA (RAPD) assay is mainly used to study the genetic diversity between species, but also to find differences between cultivars and to support the prospects for selection and breeding (Sangwan *et al.*, 1999). As RAPD is used to study the diversity inter and intra genera and species, it is also used to stimulate the genetic stability of micropropagated plants to keep the genetic resources (Moncketon and Jeffreys 1993; Bartish *et al.*, 2000; Beng *et al.*, 2000, McArthar *et al.*, 1998 and Ashworth *et al.*, 1999).

Genetic stability is very important requirements to check the genetic stability for similarity micropropagated plants.

MATERIALS AND METHODS

Plant material was collected from El-Kharga Oasis in which

Balanites aegyptiaca is found in Paris Oasis as an adult vigor evergreen tree. Explants were taken from the clonal culture at the tissue culture unit of Desert Research Center (DRC) during the Micropropagation of *Balanites aegyptiaca*.

Samples for RAPD analysis were chosen (6 plants) from the rooted plants during acclimatization process and exposed simultaneously at once with liquid nitrogen and stored at –80°C in ultra deep freez.

Polymerase Chain Reaction (PCR) Conditions

DNA was extracted according to Dellaporta *et al.* (1983). DNA concentration and quality was determined using a spectrophotometer at wave length 260 nm and 280 nm. The RAPD reaction was carried out in a final of volume 25 µl containing 5 µg of genomic DNA template, 5 pmol of each primer, 5 μM of each dNTPs (dATP, dTTP, dCTP and dGTP) and 25 μM KCl.

Different oligonucleotides (Operon Technology) were used for generating polymorphisms, their sequences were as follows:

1-OP A15 (5`TTCCGAACCC3`),
2- OP A20 (5`GTTGCGATCC3`),
3-OP B14 (5`TCCGCTCTGG3`),
4- OP CO2 (5`GTGAGGCGTC3`),
5-OP CO4 (5`CCGCATCTAC3`),
6- OP CO7 (5`GTCCCGACGA3`),
7-OP CO9 (5`CTCACCGTCC3`),
8- OP C16 (5`CACACTCCAG3`).

RESULTS AND DISCUSSION

To determine the genetic stability of the micropropagated plantlets of *Balanites aegyptiaca*, RAPD analysis was applied. In the preliminary test, eight 10-mer primers (Operon technology) were evaluated for their ability to amplify genomic DNA fragments from *B. aegyptiaca.* Fig. (1) shows that the random amplified banding patterns obtained using each of the oligo primers indicates a high level of similarity between samples, while in some primers polymorphic fragments were amplified. Primer OP A15 (5`TTCCGAACCC3`) showed a high similarity between samples, but sample 6 showed dissimilarity. Primer OP A20 (5`GTTGCGATCC3`) showed about 14 different bands ranging from total RF 0.45 to 0.92 and molecular size from 833 base pair (bp) to 380 bp. The first four samples produced high similarity but the last two samples had few absent bands at 833 to 380 bp which reflects some dissimilarity. Amplification with primers [B14 (5`TCCGCTCTGG3`), CO_2 (5`GTGAGG CG TC 3`) and CO_4 (CCGCATCTAC3`)] produced high polymorphism with the amplified samples which are shown in 9 bands with each primer, The size of these

bands was ranging from 1171 bp to 25 bp despite of the absence of some bands in the sample No.6 with the primer CO_2 at the band size 904 bp, 698 bp and 283 bp. Samples with CO7(5`GTCCCGACGA3`) exhibited 9 bands with fragments sizes ranging between 1287 bp and 340 bp. Primer CO9 (5`CTCACCGTCC3`) produced 11 amplified regions with fragment size ranging from 1275 bp to 98 bp. The similarity among samples is high in spite of the absent band No. 7 in the samples No. 2 and No. 6 at 294 bp. Some dissimilarity was found with primer C16 (5`CACACTCCAG3`) in the different samples. Some bands (3 bands) are absent in sample No. 2 at fragment size of 622, 546 and 395 bp. Also in the sample No. 5, four bands are absent at fragment size 622, 546, 94 and 73 bp. In sample No. 6, two bands are absent with fragment size of 622 and 546 bp, while in sample No. 4 only 2 bands at 285 bp and 149 bp were detected.

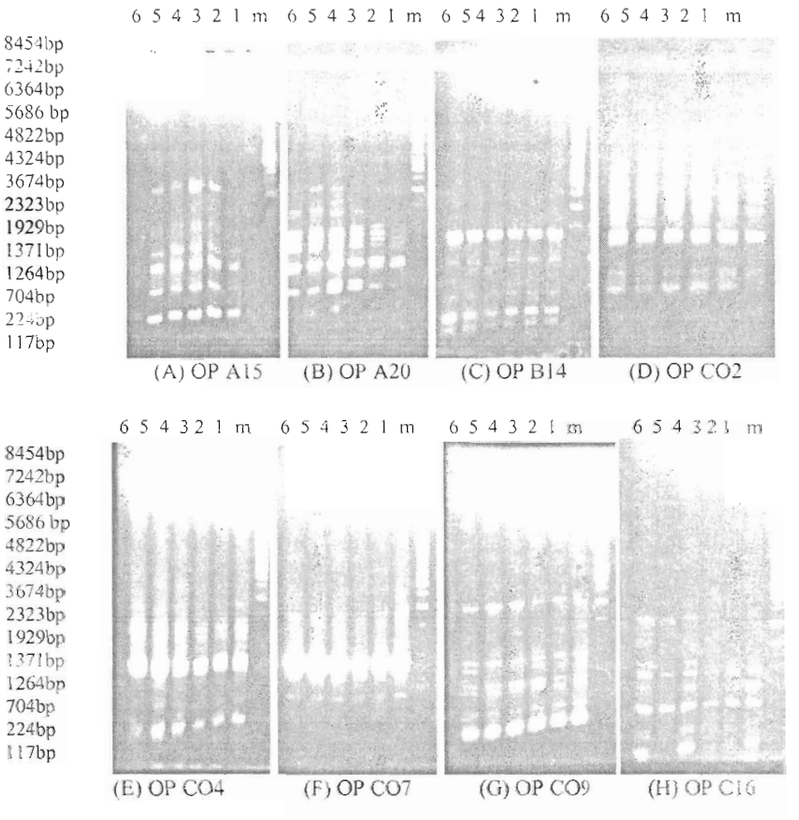

Fig. (1). The random amplified banding patterns obtained by using each of the oligo primers of operon for the micro propagated plants of *Balanites aegyptiaca* as detected.

Table (1). Genetic similarity matrix among *Balanites aegyptiaca* micropropagated samples plants as computed according to Dice coefficient from RAPD.

Dice (Czekanowski or Sorenson) Measure						
VAR00006	VAR00005	VAR00004	VAR00003	VAR00002	VAR00001	
						VAR00001
					0,928	VAR00002
				0,945	0,938	VAR00003
			0,893	0,915	0,874	VAR00004
		0,879	0,879	0,885	0,86	VAR00005
	0,866	0,784	0,811	0,815	0,807	VAR00006

Finally, data in table (1) showed the similarity matrix among samples by using 8 primers. The similarity among sample No.1 and the other 5 samples is ranging between 0.938 to 0.807 bp. The high similarity is shown between sample No. 2 and the other samples which were ranging from 94.5 to 81.5% and sample No.6 is far from the other samples. The relationship among samples is clear from the dendrogram shown in Fig. (2). It is obvious from the dendrogram that there is similarity among samples which are close together except sample No. 6 which showed some differences from the other samples, this difference may be due to some mismatching occurred between the oligonucleotides and DNA strands in this samples.

The obtained results in this study were in agreement with that mentioned by Bartish *et al.* (2000), McArther (1998) and Beng *et al* (2000), as they used RAPD to estimate and also to determine the difference between and within genera. This study used RAPD between the micropropagation individuals to estimate the similarity and the quality control produced many thousands of the true to type plants which should be under control to prevent any mutation. Mutations can occur during the subculturing process.

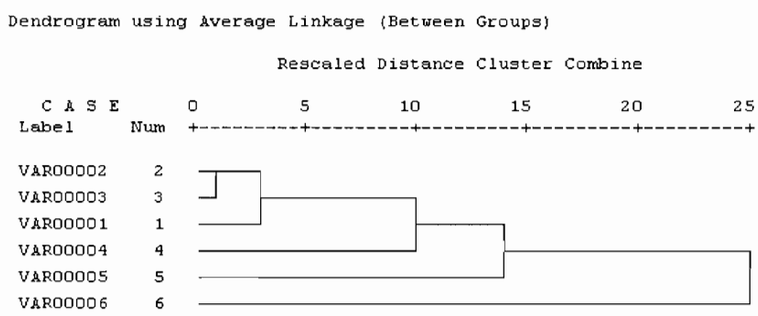

Fig. (2). Dendrogram of the micropropagated *Balanites aegyptiaca* using average linkage between plants.

REFERENCES

Ashworth, V.E.T.M.; BC.O'Brien and EA.Friar (1999). Finger printing *Juniperus communis* L.cultivars using RAPD markers, *Madrono*, 46:134-141.

Bartish, I.V; L.P. Garkava; K. Rumpunen and H. Nybom (2000). Phylogenetic relationships and differentiation among and within populations of *Chaenomeles* Lindl. (Rosaceae) estimated with RAPDs and isozymes. *Theor. Appl. Genet.* 101: 554-563.

Beng,K.; M.C.Mahani; W.Ratnam; N.N. Mohd and K.S. Beng (2000). Genetic relatedness among *Lansium domesticum* accessions using RAPD markers. *Annals of Botany*. 86: 299-307.

Dellaporta,S.L.; J.Wood and J.B. Hicks (1983). A plant DNA mini preparation. Version III. *Plant Mol. Biol.*, Rep. 1: 19-21.

McArthur, E.D; Buren, R. Van; Sanderson, SC; Harper, KT; Van, Buren, R. (1998). Taxonomy of Sphaeromoria, Artimisia, and Tanacetu (compositae, Anthemicha) based on randomly amplified polymorphic DNA (RAPD). *Great Basin Naturalist*, 58: 10-11; 2pp of ref.

Moncketon, D.G. and A.J.Jeffreys (1993). DNA profiling. *Curr. Opin. Biotechnol.*, 4: 660-664.

Sangwan;R.S.; N.S. Sangwan; D.C. Jain; S. Kumar and S.A. Ranade (1999). RAPD profile based genetic charecterization of chemotypic variants of *Artemisia anna* L. *Biochemistry and molecular Biology international*, 47: 435-944.

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المطابقة الوراثية لنباتات الهجليج الناتجة معملياً

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أظهر التضاعف العشوائى للـــ د ن ا المستخلص من نباتات الهجليج المنتجـــه معمليـــاً باستخدام ثمانية من البادئات العشوائية ذات العشرة قواعد نيتروجينية أن-هناك درجة عالية مـــن التماثل بين النباتات وبعضها وأنه يوجد درجة من عدم التماثل بين بعض العينات وذلك قد يرجـــع الى حالة عدم الإلتحام الجيد بين البادئ العشوائى مع دن ا نباتات الهجليج المنتجه معملياً.