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Biosystematic studies for *Balanites aegyptiaca* (*Balanitaceae*) populations in Egypt

Abstract

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The study of *Balanities aegyptiaca* populations in Egypt is carried out to verify the taxonomic rank of these populations. This study was based on the morphological, soil physico-chemical characters and eighteen straight chain hydrocarbons in addition to RAPD markers. The study revealed that all the *Balanities aegyptiaca* (L.) Del. populations are belonging to *Balanities aegyptiaca* (L.) Del. var. *aegyptiaca*. This variety is represented in Egypt by three ecotypes namely: Nile valley type, Sahelian type and Xerotype.

Introduction

Balanites aegyptiaca (L.) Del. belongs to the monogeneric family *Balanitaceae*. The genus includes nine species, seven of which occur in Africa.

Balanites aegyptiaca is distributed from Senegal, Somalia, Ethiopia and Eritrea and extends south to Zimbabwe. And to the north from Egypt to the Jordan valley in Palestine and to western Arabian peninsular (Sands 1983 & 1989). In Egypt, the Nubian desert is the northern limit for the species (Sands, 1989, Springuel, 1997 and Boulos, 2000).

The taxonomic group of the genus *Balanites* has long been a source of disagreement. Bentham & Hooker (1862) include *Balanites* in the family Simaroubaceae. Engler (1896 Appendix; 1931) grouped it under Zygophyllaceae, as a subfamily. After that, Cronquist (1968) returned it to Simaroubaceae. Hegnauer (1973), cleared that *Balanites* is not chemically similar to the Simaroubaceae because it lacks the quassia-like alkaloids typical of that family. Scholz (1964), Hegnauer (1973) and Cronquist (1981) included *Balanites* in the Zygophyllaceae. The similarities between flavonoids of *Balanites* and Zygophyllaceae; Maksoud & El-Hadidi (1988) did not support treatment of *Balanites* as a distinct family; and this the same in opinion of Narayana, *et al* (1990). Sands (1989) treated *Balanites* in a separate monogeneric family Balanitaceae. This treatment is supported by Sheadan & Culter (1993), through the study of the anatomy of 37 species in 19 genera belongs to Zygophyllaceae *sensu lato* and the result of C_4 activity in 27 species; the results revealed

the exclusion of *Balanites* into a separate family. Recently, Boulos (2000) treated *Balanites* within Zygophyllaceae.

According to Sands (1983), a study of the genus is currently being completed and a full revision of the genus will be published. Sands (pers. Comm.. in Maksoud & El-Hadidi, 1988), he recognizes five varieties of *Balanites aegyptiaca* with vary in respect to the color of the leaflet, pubescence, length of spines, the number of flowers per corymb as well as the size and shape of the fruit. However, Sands (1989) treated *Balanites aegyptiaca* (L.) Del. as two varieties: var. *pallida* Sands with dense tomentellous leaflets and inflorescence always at spinless nodes with 5(-7) flowers; the type specimen was from Somalia. And the var. *aegyptiaca* with variable leaf and spine development, leaflets usually with sparse indumentum (or early glabrescent), inflorescences often at spinous nodes; type specimens was from Egypt.

For comparative studies in plant collections, a complementary approach using RAPDs may be more appropriate for generating accurate estimates of genetic diversity and relationships than either method used alone, Chan & Sun (1997). RAPD analysis enables the detection of information genetic marker at a large number of loci in both coding and non-coding regions of the genome (Willalms *et al* 1990).

This study aimed to verify the taxonomic rank of the *Balanites aegyptiaca* populations representing all the Egyptian localities. Based on morphological, ecological, chemical and Random Amplified Polymorphic DNA (RAPD) data.

Material and methods

Plant material

Eight *Balanites aegyptiaca* samples were collected from different Egyptian populations of the species (Figure 1), The habitat features for the eight sites are summarized as follows and arranged as the populations number.

1- Nile bank, Aswan city, Upper Egypt.
2- Ecotone sector of Wadi Alaqui at the entrance of the Wadi, eastern desert, Upper Egypt.
3- Down-stream of Wadi Alaqui, eastern desert, Upper Egypt.
4- Upper-stream of Wadi Alaqui, eastern desert, at the Sudano-Egyptian border.
5- Paris Oasis, western desert.
6- Halaib, Red Sea mountain escarpment, southern corner of Nubian desert at Sudano-Egyptian border.
7- Hamata mountain, Red Sea mountain escarpment, eastern desert
8- Wadi Abu Shubeira, ecotone part, 13 km north of Aswan city, eastern desert.

Soil analysis

Soil mechanical analysis was carried out according to Folk (1968). Soil texture analysis was carried out by Bouyucos Hydrometer method according to Allen & Stainer (1974). Soil reaction (pH) measured for soil extracts Potentiometrically. Soil anions: bicarbonate ion concentration measured by titration, chloride ion concentration measured by Argentometry (Lukács & Rédly, 1988). Soil cations: calcium and magnesium were measured according to Boham, *et al.* (1979). Electric conductivity (EC) measured by a Direct Conductivity Bridge (μm hos/cm).

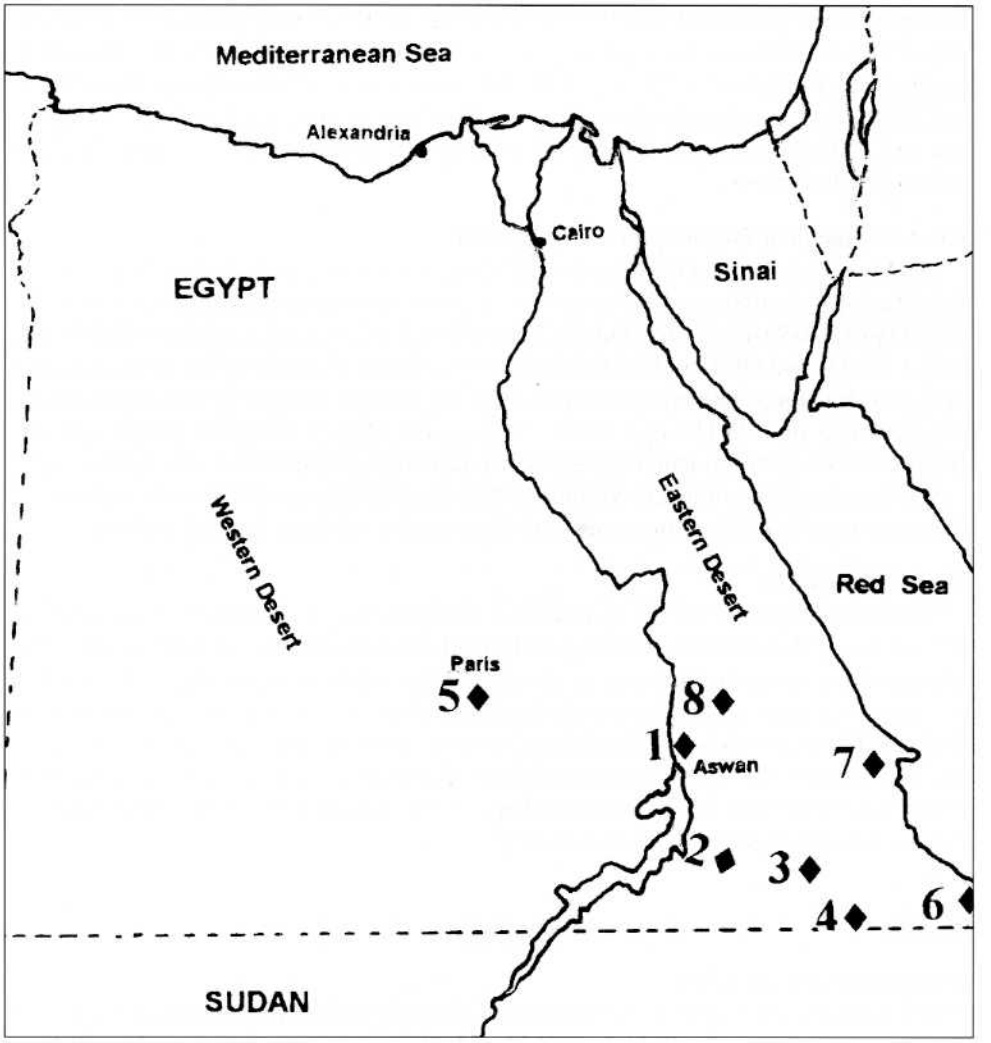

Fig. 1. Distribution of the studied Balanities aegyptiaca populations.

Vegetation

The associate plant species in the eight studied localities were collected in a circle of 50 m radius around the target *Balanites* population. The collected species were identified in Cairo University Herbarium (CAI) and South Valley University Herbarium according to Täckholm (1974), Hassan (1987); Boulos (1995, 1999 & 2000).

Extraction of hydrocarbons

Lipid material was extracted according to Amer (1999); from two grams of the powdered seeds for each sample. The non-saponified fraction of the lipid material was injected to Gas Chromatography (GC) according to (Rady, *et al* 1987 & Amer, 1999). Gas chro-

matography was performed using Perkin Elmer Sigma 2B GC equipped with SP 4270 integrator. The column was coiled glass (1.5 m x 0.4 mm) packed with OV-101. Oven temperature was isothermal at 250 °C, while detector and injector temperatures were 300 °C and 220 °C; respectively. Gas flow rates were 80 ml/min for nitrogen, 60 ml/min for hydrogen and 220 ml/min for air. The developed peaks were standardized against slandered series of hydrocarbons.

Random Amplified Polymorphic DNA (RAPD)

DNA was isolated from fresh leaves according to Sambrook *et al.* (1989). PCR was conducted using five arbitrary 10-mer primers with the following sequences: OPA01 (CAG GCC DTT C), OPA-02 (TGC CGA GCT G), OPB-04 (GGA CTG GAG T), OPB14 (TCC GCT CTG G) and OPB16 (TTT GCC CGG A). Marker of 100bp DNA ladder was also used. The reaction conditions were optimized, the reaction mixture (50 µL total volume) was consisted of : dNTPs (0.2 mM) , $MgCl_2$ (1.5 Mm), 1 X buffer primer (0.2 M), Template DNA (100 ng) and Taq polymerase (2 units). Amplification was carried out in Coy Thermocycler according to Vierling (1992). Amplification products were analysed by electrophoresis in 1.4 % agarose gels and visualized by ethidium bromide staining.

Statistical analysis

Multivariate analysis for the hydrocarbon (as percentages) and RAPD data were carried out using Hierarchical clustering analysis(Manhattan distance). RAPD amplification products were scored as: presence or absence of the amplification products. If a product was present in other genotype it was designated 2, if not shared in another genotype it was designated 1, and 0 if absent. This type of scoring was done for each amplification product. The genetic diversity was based on both shared and unique polymorphic products, which have been used to construct the dendrogram. Statistica Programme for Windows Release 4.5, copyright by StatSoft, Inc. (1993) was used.

Results

Morphological description

The collected eight specimens representing the eight studied populations are characterized by: tree form up to 8 m high. Stems with axillary naked spines, up to 8 cm long. Foliage leaves alternate, 2-foliate, minutely puberulous or glabrescent. Leaflets elliptic to broadly ovate or obovate, apex bluntly acute to obtuse or rounded and cuneate base. Flowers 5-merous, 5-12 arranged in loose corymb-like cymose often at spinous nodes. Sepals tomentellous outside and around the midrib vein of the inner side. Petals narrowly obovate-elliptic, yellow-or blue green 4.5-6 x 1-2 mm. Stamens 10; ovary mostly tomentose, to 1 mm high; style glabrous 1-2 mm long. Fruit drupe, elongated in early stages, ovoid to ellipsoidal sometimes sub-rounded on ripening, (2.3-)2.9-4(-4.5) x (1.3-)1.7-2.2 (-2.5) cm.

According to Sands (1989), the above descriptions for the eight collected specimens are belonging to *Balanites aegyptiaca* (L.) Del. var. *aegyptiaca.* However, the studied populations hold minor morphological diversity linked to the habitat of its origin and supported by the herbarium samples deposited in Cairo University Herbarium (CAI), the differences are summarized as follows (Table 1).

Soil

Table 2 outlined the physico-chemical characters of the soil samples collected from the eight habitats associated with the studied *Balanites aegyptaica* populations. The results cleared that *Balanites aegyptaica* can grow in a pH range from nearly neutral to slightly alkaline soil (6.97-8.96). Soil salinity ranges from non-saline (EC=106 µm hos/cm) to slightly saline (EC=1330 µm hos/cm). Most of the samples appeared to be grown in sandy soil with 42.3 %-90.4 % sand.

Vegetation

Table 3 outlined that a total of 127 species were traced as associated species to the studied nine *Balanites aegyptaica* populations. The associated species are mostly xerophytic especially in case of populations 2-8. Among of these species are: *Acacia tortilis* subsp. *raddiana, Aerva javanica, Citrullus colocynthis* and *Panicum turgidum*. Populations 4,6 & 7 are grown in a dense vegetation localities while populations 1 & 8 are grown in a poorer localities. Population 1 is characterized by the presence of mesophytic species among of them: *Cynodon dactylon, Medicago sativa, Oxalis corniculata and Echinochloa colona* in addition to the xerophytic species.

The Life form analysis (Hassib, 1951), of the associated species to *Balanites aegyptiaca* populations (Table 3), revealed the dominance of Chamaephytes in populations 1, 2, 3, 4 & 8 by percentages 16.36%, 26.66%, 55.55%, 29.61% and 45.45%, respectively. While Phanerophytes were the dominant life form in populations 5 and 7 by percentages 41.17% and 36.36%, respectively. On the other hand Therophytes was the dominant Life form in case of population 6, and represented by 43.47%, from the total species.

Table 1. Morphological diversity within the studied populations.

Character	Population number & ecotype		
	Nile valley type	Xerotype	Sahelian type
	1 & 2	3, 4 & 5	6, 7 & 8
Leaflet texture	Thin, papery & glabrescent	Leathery & minutely puberulous	Leathery & minutely puberulous
Leaflet length	3.5 (-4.5) cm	1.5 (-2.3) cm	3.0 (-4.7) cm
Leaflet width	1.8 (-2.4) cm	0.7 (1.5) cm	1.7 (-2.5) cm
Leaflet apex	Broad acute	Acute	Broad acute
Leaflet shape	Elliptical	Elliptical	Elliptical
Petiole length	0.5 (-1.5) cm	0.6 (-1.2) cm	0.8 (-1.2) cm
Petiolule length (mm)	2.5	1.7-2.0	(0.5-) 2.2
Spine length	1-5 (-8) cm	2.2 (-3.5) cm	0.6-6.0 cm
Floweral node	Spineless	Spiny	Spiny
Number of flowers/corymb	(7-10) -12	7-10	7 (-8)
Pedicel length	8 (-12) mm	4.2 (-8) mm	6-8 mm
Fruit size	3.8 (-4.3) x 2.0 (-2.5) cm	2.5 (-3.0) x 1.8 (-2.2) cm	3.2 (-4.5) x 2.2 (-2.3) cm
Fruit ridges	Five moderate ridges	Unclear ridges, less hairy	Clear five ridges, less hairy
Fruit shape	Elliptical-ovoid	Sub-spherical-elliptical	Elliptical

Table 2. Physico-chemical character of the soil samples collected from the eight habitats associated to the studied Balanites aegyptaica population.

Population number	Sand %	Silt %	Clay %	pH	EC μ m hos /cm	Cl mg/ 100 gm	HCO_3 mg/ 100 gm	Ca mg/ 100 gm	Mg mg/ 100 gm
1	28.7	46.6	23.93	6.0	451	1.83	0.85	22.3	22.0
2	42.3	33.7	24.53	97.0	438	23.00	67.00	14.0	0.0
3	34.1	28.6	27.78	8.15	422	16.00	11.60	8.0	1.6
4	88.3	1.0	10.50	7.78	106	4.26	12.06	5.8.	1.4
5	80.5	2.8	14.36	7.56	3690	101.60	17.94	488.2	214.5
6	90.4	0.3	9.00	8.13	140	4.02	11.70	5.6	1.1
7	82.3	5.1	12.13	7.74	1330	11.00	29.00	7.0	5.0
8	80.1	2.5	15.60	8.16	1474	12.17	25.07	8.1	4.8

Hydrocarbons

Eighteen aliphatic straight chain hydrocarbons were identified using GC. Table (4) outlines the percentages of hydrocarbons measured as a percent to the total identified hydrocarbons. The data revealed that n-decane is the dominant hydrocarbon in the studied samples; its percentages ranges from 34.80 -76.9. N-pentadecane is the co-dominant hydrocarbon with percentages range from 10.69 –21.24. Notable similarity are noticed between populations 1 & 2 and between populations 3,4,& 5 as well as between populations 6,7 & 8.

RAPD

The amplification products of the genomic DNA isolated from the eight *B. aegyptiaca* populations using five primers (OPA01, OPA02; OPB04, OPB14 and OPB16) revealed that: a total of 41 clear polymorphic bands (Fig. 2). Three major bands are shared in all the studied populations: OPA01 at 250 kbp, OPA02 at 385 kbp and OPB16 at 242 kbp.

Statistics

The constructed dendrogram (Fig. 3) based on the amplification products of the five RAPD primers and the eighteen hydrocarbons as percentages revealed that: the 8-populations are grouped into two major clusters. One cluster includes populations 6,7 & 8 and populations from 1-5 in the other cluster. The later cluster includes smaller forks one constructed from populations 1 & 2, and the other constructed from populations 3 & 4.

Discussion

Plant growth is influenced by two major groups of factors: genetic and environmental. The latter is mainly related to soil and air characteristics. The most effective environmental factors is the soil water. The water deficit (?drought) is the most limiting factor in the

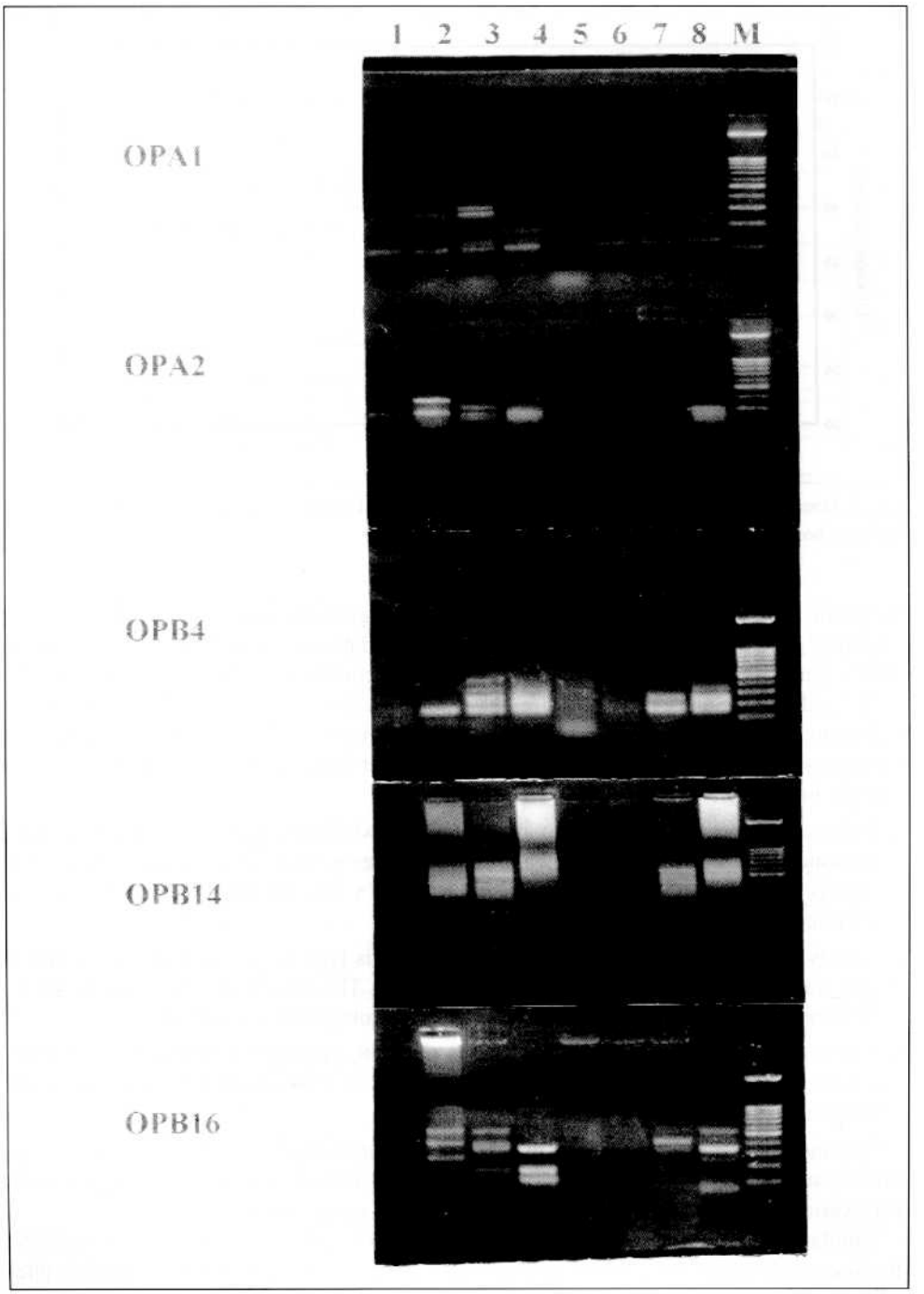

Fig. 2. DNA_RAPD pattern of the studied *Balanities aegyptiaca* populations using different primers: OPA1, OPA2, OPB4, OPB14 and OPB16. (Marker Molecular weight = 100bp ladder).