

IN VITRO PROPAGATION OF *Balanites aegyptiaca* (L) DEL., AN ENDANGERED MEDICINAL PLANT.

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ABSTRACT

Young vigorous shoot tips of wild *Balanites aegyptiaca* (L) Del. were collected from Paris Oasis, Alwadi Algadeed, Egypt and used as the source of explants for micro-propagation. Shoots were established on MS basal solid medium supplemented with 6-benzyladenine (BA) at different levels combined with α -naphthalene acetic acid (NAA). MS medium without any growth regulators enhanced shoot elongation, while shoots response and axillary branching were affected by BA levels. Shoots productivity were significantly affected by the presence of BA. Shoots number and length were significantly achieved on MS medium contained 2.0 or 2.5 mg/l of BA combined with 0.2 mg/l of NAA. In vitro rooting, shoots were rooted on MS basal solid medium contained either IBA singly or mixture of IBA and NAA. Shoots were rooted well on MS basal solid medium supplemented with 2.0 mg/l of IBA. Rooted shoots were planted in pots filled with peat moss and sand (1 : 1) v/v mixture. Survival percentage for adapted plantlet were 90 % after 4 weeks. The plantlets were transferred to polyethylene bags and replanted successfully under field conditions.

Keywords: Balanitaceae, *Balanites aegyptiaca*, in vitro propagation, heglig, desert date, growth regulators.

INTRODUCTION

Balanites aegyptiaca (L) Del, (Family: Balanitaceae) an evergreen tree is a multipurpose plant known for its many uses as fodder, charcoal, timber, fuel wood, antifeed (Von Myddell, 1984). It is widely distributed from Guinea through the Sahara into Egypt. It is encountered on clay soils under rainfall of 500 mm. (Giffard, 1974). It is sporadically distributed on sandy soils under rainfall of 250 mm. The species, which occur in arid zone, grows very slowly and has a slow fruit development. *Balanites aegyptiaca* (L) Del, is ecologically very flexible with excellent persistence. It is adaptable to a wide range of sites and climatic conditions (Von Myddell, 1984). Although the tree regenerates naturally by seed or after moderate coppice, the species is endangered because of the high rate of clearance, (El-Nour, *et al.*, 1991). Micro-propagation on tree species offers a rapid means of producing Clonal planting stock for afforestation, woody biomass and conservation of elite germplasm (Bonga and Durzan, 1982). The tissue culture of forest trees has shown promise in obtaining regenerants and Clonal multiplication for domestication of wild populations, afforestation and improvement of economically important trees that have been cultivated for generations. Plant tissue culture is an alternative method of propagation (George and Sherrington, 1984) and is being used widely for the commercial propagation of a large number of plant species, including many medicinal plants (Rout *et al.*, 2000). Since, many medicinal plant species are disappearing at an

alarming rate due to rapid agricultural and urban development, uncontrolled deforestation and indiscriminate collection. (Fay, 1992), advanced biotechnological methods of culturing plant cells and tissues should provide new means of conserving and rapidly propagating valuable, rare, and endangered medicinal plants. Micro-propagation is also, a powerful tool for the quick production of genetically homogenous plants, it allows to obtaining a large number of plants from the very limited source available. In Egypt, *Balanites aegyptiaca* (L) Del. is known as Balah haraara; Igleeg; Liglieg; Hel'eig and Hegeleeg, (Täckholm, 1974). It's considered an endangered plant, So the aim of this study was to assess the effect of growth regulators on in vitro shoot proliferation and in vitro rooting of *Balanites aegyptiaca* (L) Del. This paper describes the work carried out on in vitro propagation of *Balanites aegyptiaca* (L) Del. as an important medicinal plant for the supply of raw materials for extraction of medicinally important compounds for the pharmaceutical industry

MATERIALS AND METHODS

Plant Materials

Young vigorous shoot tips of wild *Balanites aegyptiaca* (L) Del. were collected from Paris Oasis, Alwadi Algadeed, Egypt, 2004 and immediately transferred to the laboratory and used as the source of explants.

Preparing Of Explants

Shoot tips were excised with four leaves primordia. The explants were thoroughly washed under the running tap water for 30 minutes, then disinfected in 1 % sodium hypochlorite (20 %, v/v), clorox solution plus three drops of tween 20 for 15 minutes with periodical agitation, followed by rinsing three times with sterile distilled water under aseptic conditions.

Culture Medium And Conditions

MS basal solid medium, (Murashige and Skoog, 1962) was used for culture initiation and different micro-propagation stages. Medium was supplemented with 3 % (w/v) sucrose and gelled with 0.6 % agar. The pH value of the medium was adjusted to 5.8 and the medium was sterilized by autoclaving at 15 lb /IN 2 pressure and 121°C for 20 minutes. The cultures were incubated at 26 ± 2 °C under 16 h. photoperiod with cool white fluorescent lamp.

Culture Initiation

Shoot tips were prepared and cultured onto MS basal solid medium supplemented with 0.1, 0.2, 0.5 and 1.0 mg/l of 6- benzyl adenine (BA) combined with 0.1 mg/l of α- naphthalene acetic acid (NAA) as well as control. All treatments were incubated for 4 weeks and data were taken as survival %, shoot growth as response percentage and length (cm.).

MULTIPLICATION OF SHOOTS

The responding cultures were used for the multiplication trails (multiple shoots induction). Explants at least 1 cm. long were transferred onto MS basal solid medium supplemented with 0.5, 1.0, 1.5, 2.0, and 3.0 mg/l of 6- benzyl adenine (BA) in combination with 0.2 mg/l of α- naphthalene acetic acid (NAA), the control treatment as well. All cultures incubated for 4 weeks,

and data were recorded as shoots number per explants and shoot length (cm.).

In Vitro Rooting

For root induction, healthy micro-shoots (1-2 cm. long) were transferred into MS basal solid medium contained 1.0 and 2.0 mg/l of Indol 3-butyric acid (IBA) alone or in combination with 1.0 and 2.0 mg/l of α -naphthalene acetic acid (NAA), control as well. All cultured were incubated as mentioned prior. Data were taken after 6 weeks as root formation percentage (%), roots number / micro-shoot and root length (cm.).

Hardening And Field Planting

Healthy rooted plantlets generated in vitro were transferred to small pots containing sterile mixture of peat moss and sand (1:1 v/v) and kept in incubation room for 4 weeks for first stage of hardening. They were later transferred to polyethylene bags containing the same mixture and kept in nursery for second stage of hardening. After 8 weeks of growth in the nursery, the plants were transferred to the field in the experimental station, Siwa Oasis, Matroh Governorate, Egypt.

Data Analysis

The experiment was repeated twice according to a completely randomized experimental design; 15 jars were used for each treatment. Analysis of variance was performed with model procedure (SPSS 8.0 statistical package) and mean comparison with Duncan's multiple range tests. Significance was recorded at $p \leq 0.05$.

RESULTS AND DISCUSSION

Establishment Of Culture

Among all the used treatments in Table (1), the explants in MS medium were healthy and grow vigorously. Shoot tip explants started to grow within 4 weeks. MS medium without any growth regulators failed to form any new micro-shoots as comparison to other treatments. Shoot survival and growth were affected by all treatments tested in Table (1) without significant differences. Addition of BA at level 0.5 mg/l combined with NAA in rate of 0.1 mg/l increased the survival of explants till 100 % and shoot elongated to 1.93 cm. Medium supplemented with 0.5 mg/l BA in combined with 0.1 mg/l NAA was superior in terms of shoot length as well as shoot growth as response (100 %) in Figure (1).

Table (1): Effect of growth regulators on shoot survival % and shoot length (cm.) in vitro *Balanites aegyptiaca* after four weeks

BA mg/l	NAA mg/l	No. of Replicates	Shoot Survival (%)	Shoot Length(cm.)
0.0	0.0	15	86.66 a	0.86 d
0.1	0.1	15	86.66 a	1.11 cd
0.2	0.1	15	93.33 a	1.33 bc
0.5	0.1	15	100.00 a	1.93 a
1.0	0.1	15	93.33 a	1.46 b

Different letters within columns indicate significant difference ($p \leq 0.05$)

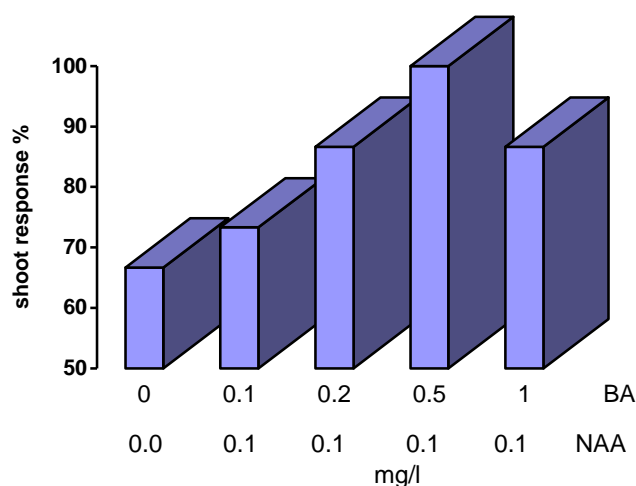


Figure (1): Effect of growth regulators on shoot response % in vitro *Balanites aegyptiaca* after four weeks

Multiplication Of Shoots

In order to select the most suitable medium for multiplication, different combinations were tried and the results are given in Table (2).

Table (2): Effect of growth regulators on shoot growth and Proliferation in vitro of *Balanites aegyptiaca* after four weeks

BA mg/l	NAA mg/l	No. of Replicates	Shoots growth	
			No. of new micro shoots /explant	Shoot length(cm.)
0.0	0.0	15	0.000 d	0.000 c
0.5	0.2	15	0.466 d	0.816 b
1.0	0.2	15	1.660 c	2.560 a
1.5	0.2	15	3.800 b	2.840 a
2.0	0.2	15	4.660 a	2.873 a
2.5	0.2	15	4.533 a	2.626 a
3.0	0.2	15	3.500 b	2.630 a

Different letters within columns indicate significant difference ($p \leq 0.05$)

The results of this study show that, axillary bud explants from mature mother plants of *B. egyptiaca* can be readily established in MS medium containing 2.0 or 2.5 mg/l BA plus 0.2 mg/l NAA to produce multiple shoots. Figure (2) shows the effect of growth regulators on shoots productivity. Increasing BA concentrations affected positively shoot number, and shoot length. Consequently, productivity was affected. BA in rate of 2.0 mg/l was the most effective among all treatments tested. The highest productivity value was achieved with shoots subcultured for one month on MS medium supplemented

with 2.0 mg/l BA plus 0.2 mg/l NAA, while From the results, it could be concluded that, shoot productivity are useful variables, since they combined the effect of the treatments on shoot number, length and nodes to give a general idea of the behavior in vitro shoots. Similar results have been reported by other workers, (Bajaj,1986 ; Chalupa, 2002 ; Sudhersen and Hussain, 2003 and Gomes *et al.*, 2003) using explants derived from woody plants .MS medium without any growth regulators enhanced shoot growth and elongation, while medium with BA enhanced axillary branching explants furthering multiple shoot development of nodes. This result agrees with the finding of Mansour *et al.*, (2003) on *B. egyptiaca*. Productivity (mm) = shoot number per explant x shoot length (mm), (Perez Tornero *et al.*,2000)

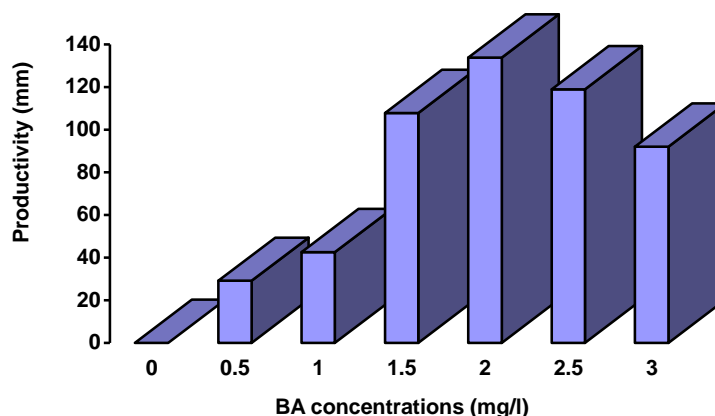


Figure (2): Effect of growth regulators on shoot productivity (mm) in vitro of *Balanites aegyptiaca* after four weeks

In Vitro Rooting

Roots began to emerge on day 8 and by day 12 were clearly visible . Rooting percentages determined on day 28 and the result presented in Table 3 and Figure (3).

Table (3): Effect of auxins as IBA and NAA on rooting stage in vitro of *Balanites aegyptiaca* after 4 weeks.

IBA mg/l	NAA mg/l	No. of Replicates	Root formation %	Root No. / explant	Root length (cm.)
0.0	0.0	15	13.33 c	0.200 c	0.500 c
1.0	0.0	15	53.33 b	0.933 b	2.820 b
2.0	0.0	15	86.66 a	2.133 a	5.066 a
1.0	1.0	15	80.00 a	1.866 a	4.433 a
2.0	2.0	15	86.66 a	2.266 a	3.866 ab

Different letters within columns indicate significant difference ($p \leq 0.05$)

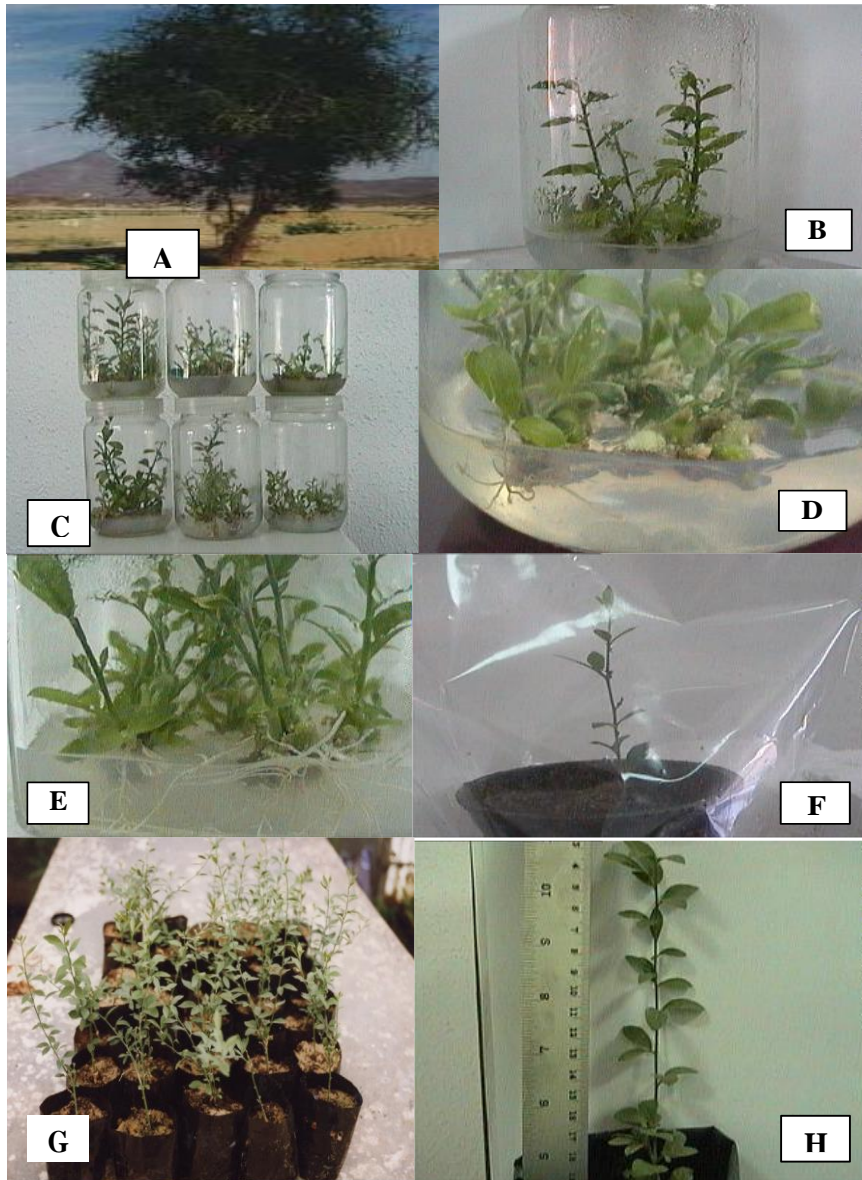


Figure (3): *In vitro* micro propagation of. A) wild *Balanites egyptiaca* (L) Del tree, B) Induction of axillary bud growth *in vitro*, C) multiple shoots in different treatments, D&E) *in vitro* rooting, F) *ex vitro* hardening, in soil G) hardening of plantlets, in nursery, H) plantlet ready to transfer into the field.

A satisfactory rooting percentage was achieved only by using IBA or in combination with NAA after 4 weeks. At 2.0 mg/L of IBA 86 % of the 15 micro shoots involved in this treatment developed roots with an average of 2.133 roots / shoot and 5.066 cm root length. The same rooting percentage was obtained by the combination of 2.0 mg/L of IBA and 2.0 mg/L of NAA, but root length was shorter (3.866 cm). Rooting formation percentage and growth was appeared the lowest value in medium without growth regulators as comparison to the other treatments .Both the concentration and nature of the auxins used markedly influenced root formation, number and length per shoot. high root numbers (fig.2) were induced by medium IBA combined with NAA concentration. MS medium without auxins, the roots failed to emerge high positive result , the rooting was 13.33 % after 4 wks, this results as contrary to the results of Mansour *et al.*,(2003) on *B. aegyptiaca*, may be due to the effect of growth regulators accumulation in the pervious stage. According to El Nour *et al.*, (1991), the IBA auxin did not improve rooting significantly which is contrary to our results. From the results, it could be concluded that, rooting percentage, root number and root length / explant obtained in medium containing 1.0 and 2.0 mg/L of IBA either individual or combined with 1.0 plus 2.0 mg/l of NAA, showed no statistical significant differences compared to that with 1.0 mg/l, although root length had decreased .In this concern, this result agrees with Llerente and Apostolo, (1998) also, confirmed with Mansour *et al.*,(2003), They state that, rooting of shoots in vitro was achieved on MS medium containing indolebutyric acid (IBA). Tyagi and Prakash, (2004) found that, no rooting was observed on MS medium devoid of auxin in Jojoba shoots. All succeeded plantlets have not any callus on base of stem. This result is considered high positive as comparison to obovious result, whereas the highest value was 48 % (Mansour *et al.*,2003), the difference may be due to callus formation. Ajithumar and Seeni, (1998) confirmed this result , they stated that, most of the roots differentiated from the calli did not have a vascular connection with the stem and establishment of the plants after hardening was poor. Rooted shoots were transferred to peat moss: sand mixture and acclimatized in the greenhouse under high humidity conditions and transferred to soil. The percentage survival of plantlets was 90 %. The plants did not show any morphological variation.

Conclusion

Induction of multiple shoots was achieved in the axillary buds of the shoot tip explants on MS basal medium supplemented with 0.5 mg/l BA plus 0.1 mg/l NAA. An increase in the number of shoots per explant (4.6) was observed when the shoots were sub-cultured on MS medium supplemented with 2.0 or 2.5 mg/l BA plus 0.2 mg/l NAA over the other BA and NAA combination tested. The optimum rooting response was obtained on auxins enriched medium. In vitro generated plantlet could be established with 90% success on initial hardening in the incubation room. These plantlets could be established with 90% success under the nursery condition (Figure 3). Hardening plants have been planted in the field, established with 100 % success. Therefore, in vitro propagation of *B. aegyptiaca* will be of immense

importance as it allows obtaining a large number of plants from the very limited source available. Also, these results could be utilized for mass production to get active materials and also would help in conserving the germplasm and commercial cultivation of this economically important medicinal plant.

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الإكثار الخضري الدقيق لنبات الهجليح كنبات طبي معرّض للانقراض.

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أجريت هذه الدراسة بوحدة زراعة الأنسجة ، مركز بحوث الصحراء، وزارة الزراعة ، بهدف الإكثار الخضري الدقيق لنبات الهجليح كنبات طبي معرّض للانقراض. تم جمع الأجزاء النباتية (البراعم الطرفية والقمم النامية) من الأفرع الحديثة لنباتات بالانيتيس ايجيبتيكا (الهجليح)، من واحة باريس، الوادي الجديد، مصر، وإستعمل كمصدر نباتي لأكثار النبات خضريا. تم الحصول على أكبر نسبة من الأفرع الحية عند زراعة القمة النامية على بيئة موراشج وسكوج الصلبة والمحتوية على ٥,٠ بنزائل ادينين بالإضافة إلى ١,٠ ملجم / لتر من النفثالين حمض ألكليك ، كما تم الحصول على الأفرع الحديثة عند زراعة القمم النامية على بيئة موراشج وسكوج الصلبة والمحتوية على البنزائل ادينين بتركيزات مختلفة مع ٢,٠ ملجم / لتر من النفثالين حمض ألكليك . اضافة البنزائل ادينين في البيئة أثر معنويا على نسبة تكوين الأفرع الحديثة وطول الأفرع ، بينما تأثر طول الأفرع فقط في البيئات الخالية من البنزائل ادينين ، حيث تكونت أكبر نسبة من الأفرع الحديثة عند زراعة البراعم الطرفية على بيئة موراشج وسكوج الصلبة والمحتوية على ٢,٠ ، ٢,٥ ، ٢,٠ ملجم / لتر من البنزائل ادينين بالإضافة إلى ٢,٠ ملجم / لتر من النفثالين حمض ألكليك. كما تأثر معدل التضاعف والإنتاجية للأفرع معنويا لوجود البنزائل ادينين في بيئة موراشج وسكوج الصلبة. تأثر تكوين الجذور على الأفرع بإضافة اندول حمض البيوتريك سواء كان منفردا أو مع النفثالين حمض ألكليك. تكونت الجذور على الأفرع عند زراعتها على بيئة موراشج وسكوج الصلبة والمحتوية على ٢ ملجم / لتر من اندول حمض البيوتريك بعد أربعة أسابيع ، وتم أقلمة البادرات الصغيرة الناتجة من مرحلة التحذير بعد نقلها إلى اكياس بلاستيك تحتوي على مخلوط من البيت موس والرمل بنسبة (١:١) وكانت نسبة النجاح ٩٠ % بعد أربعة أسابيع ، وتم نقلها إلى المشتل ثم زراعتها بنجاح في الحقل بعد شهرين.