

Full Length Research Paper

In vitro multiplication of the semi-arid forest tree, *Balanites aegyptiaca* (L.) Del.

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Procedures were developed for micropropagation of *Balanites aegyptiaca* using axillary bud explants obtained from mature trees. Cultures were established in Murashige and Skoog (MS) medium supplemented with 2.5 mg/l 6-benzylaminopurine (BAP) and 0.1 mg/l naphthalene acetic acid (NAA). The effects of kinetin on shoot growth and proliferation *in vitro* was also investigated. Results show that shoot multiplication required 2.5 mg/l of BAP. Shoot length was significantly affected by the presence of BAP or 6-furfurylaminopurine (Kin). Rooting of shoots *in vitro* was achieved on MS medium containing 20 mg/l of the auxin, indolebutyric acid (IBA). Rooted shoots acclimated and were successfully transferred into soil, with 48% of the plantlets surviving.

Key words: *Balanites aegyptiaca*, micropropagation, rooting.

INTRODUCTION

Balanites aegyptiaca an evergreen tree is a multipurpose plant known for its many uses as fodder, charcoal, timber, fuelwood, antifeed etc (Von Maydell, 1984). It is widely distributed from Guinea through the Sahara into Egypt, and as far as Yemen, Iran to India. 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 occurs in arid zones, grows very slowly and has a slow fruit development. *B. aegyptiaca* is ecologically very flexible with excellent persistence. It is adaptable to a wide range of sites and climatic conditions (Von Maydell, 1984).

The tree is drought and fire resistant, and withstands up to 2 months flooding in areas near a river but it can not tolerate prolonged water logging (IPGRI, 1984). In Senegal, *B. aegyptiaca* was formerly protected. However, with the increased human pressures for its valuable timber and fodder, the tree is being continuously felled. 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).

Micropropagation of tree species offers a rapid means of producing clonal planting stock for afforestation, woody

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Abbreviations. IAA: Indole-3-acetic acid. IBA: indol-3-butyric acid. NAA: α-naphthalene acetic acid. BAP: 6-benzylaminopurine. Kin: 6-furfurylaminopurine. MS: Murashige and Skoog medium.

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. Woody taxa are generally difficult to regenerate by *in vitro* techniques, but some success has been achieved in a few leguminous tree species (Dhawan, 1987). This paper describes preliminary observations on clonal multiplication of axillary buds of mature *B. aegyptiaca* tree, popularly known as “desert date”.

MATERIALS AND METHODS

Plant material and explant source

Vegetative shoots were collected from ten year old tree of *Balanites aegyptiaca* located in Hann forestry Park in Senegal (14°43'N; 17°26'W). Nodes (1.5 to 3 cm) were used as source of explant. Nodes segments bearing axillary buds were surface sterilized by quick dip in 90% ethanol followed by first, sodium hypochlorite solution for 5 min, and rinsed 5 times in sterilised distilled water, then in 0.1% HgCl₂ containing tween 20, finally 5 rinsed times in sterilised distilled water.

Culture medium and condition

The explants were placed on solid basal MS (Murashige and Skoog, 1962) medium supplemented with different concentrations and combinations of BAP: 0, 1, 2.5, 5 mg/l; kin: 0, 1, 2 and IAA 0, 0.1 mg/l for shoot proliferation and multiplication. The pH was adjusted to 5.6 using 0.1 N NaOH or 0.1 N HCl before autoclaving (110°C, 20 min). 20 ml of medium were dispensed into culture tubes (25 x 150 mm). The cultures were maintained at 25±2°C either under 16 h/day photoperiod from cool white fluorescent lamps (3000 lux). The shoots were maintained by regular subcultures at 4-week intervals on fresh medium with the same compositions.

Induction of rooting and acclimatization

For root induction, excised microshoots (1-2 cm length) were transferred in liquid or solidified MS basal medium supplemented with different concentrations of NAA or IBA (5 and 20 mg/l). One excised shoot was placed in each tube (25 x 150 mm) containing 15 ml of the culture media. All the cultures were incubated at 25±2°C under 14 h photoperiod with cool, white fluorescent lamps. Rooted explants were planted in pots containing a sterile soil and kept in the greenhouse for acclimatization.

Observation of cultures and presentation of results

24 explants were used per treatment. The data pertaining to mean percentage of cultures regeneration, number and length of shoots/explant and mean percentage of rooting were statistically analysed by the Fisher's test.

RESULTS AND DISCUSSION

Among all the used media, the explants in MS medium

were healthy and grew vigorously. 100% of segment explants produced shoots within 2 weeks and explants possessed 2.25 shoots having broad leaves. B5 (Gamborg et al., 1968) and SH media (Schenk and Hildebrandt, 1972) induce vitrification with an average of 97% of new shoots produced. B5 medium produced rosette plants. Cultured axillary buds started to grow within 5 to 10 days. Excised explants cultured on MS medium formed callus at the cut and along the margin which remained unorganised. Callus formation at the proximal ends of the node explants in the present study is in line with reports on *Peganum harmala* (Saini and Jaiwal, 2000) and *Holostemma ada-kodien* (Martin, 2000). Marks and Simpson (1994) suggested that this callus formation may be due to the action of accumulated auxin at the basal cut ends, which stimulates cell proliferation especially in the presence of cytokinins. According to Preece et al. (1991) the formation of callus at basal cut ends of nod explants on cytokinin enriched medium is frequent in species with strong apical dominance.

Addition of BAP (1 – 5 mg/l) increased the number of shoots. Medium supplemented with 5 mg/l BAP induced a mean of 4.30 shoots per node explant (Table 1), while medium supplemented with Kin resulted in a reduced number of shoots although with longer internodes. Shoot multiplication required BAP, and Kin was found to be less effective than BAP. 2.5 mg/l BAP in combination with 0.1 mg/l NAA was the most effective combination for axillary bud multiplication, resulting in 3.13 shoots per node explant. This synergistic effect of BAP and auxin has been demonstrated in many plants including *Santolina canescens* (Casado et al., 2002), *Bupleurum fruticosum* (Fraternali et al., 2002), *Acacia nilotica* (Sane et al., 2001), and *A. albida* (Gassama et al., 1986).

Shoot length was significantly affected by the presence of BAP or Kin (Table 1). The highest BAP concentration (5 mg/l) reduced shoot length (2.16 cm) in cultures. 2.5 mg/l BAP was optimal for growth in this study. The overall number of shoots produced was significantly higher in the presence of 5 mg/l BAP. Other forestry species where BAP induced shoot multiplication are *A. tortilis* subsp. *raddiana* (Sane et al., 2000), *A. Koa* (Skolmen and Mapes, 1976) and *Leucaena leucocephala* (Dhawan and Bhojwani, 1985). Kin promoted shoot elongation in *B. aegyptiaca* but led to decline of shoot multiplication. In *Prosopis*, however, Kin with IAA induced higher rate of shoot multiplication than BAP (Goyal and Arya, 1979, 1984).

Individual shoots were excised from 4 – 6 week old cultures on multiplication medium and placed on the rooting medium. Root on induced shoots has been observed 10 days after rooting induction. Rooting of shoots was best achieved using high concentrations (10 to 20 mg/l) of auxin (Table 2). Shoots produced *in vitro* rooted well when treated with 20 mg/l of IBA or NAA. The number of roots per shoot is rather variable with NAA

Table 1. Effect of cytokinins on multiple shoot induction from nodal shoot segments of *B. aegyptiaca* cultured on MS medium supplemented with NAA (0,1 mg/l) over a period of 4 weeks.

Treatment mg/l	Explants with shoot formation (%)	Number of shoots per explant	Shoot length (cm)
Control	100	2.25a	2.20a
BAP 1	100	3.13b	1.89b
2.5	91.60	3.13b	2.29a
5	95.80	4.30b	2.16a
KIN 1	95.80	1.12c	2.25a
2	95.80	1.40c	2.24a

Means within a column followed by a same letter was not significantly different (P= 0,05) according to Fisher's test.

Table 2. Eft of media and auxin on root induction from cultured shoots of *B. aegyptiaca*.

Supplement (mg/l)	Treatment period	Shoots rooted %	Root numbers	Root length (cm)
IBA 20	48 h	18.8	11c	3.2a
NAA 20	48 h	21.40	3.1b	3.9a
IBA 5	10 days	48.5	12.5a	4.5b
NAA 5	10 days	11.60	2.6b	4.3a

Means within a column followed by a same letter was not significantly different (P= 0.05) according to Fisher's test.

treatment. Higher concentrations of NAA caused formation of callus. For the control (without auxin), the rooting was 0% after 30 days. According to El Nour et al. (1991), the IBA hormone did not improve rooting significantly, which is contrary to our results. After 4 weeks, plantlets were transferred to pots and later established in soil. 48% of transferred plantlets survived with subsequent establishment.

The results of this study show that axillary bud explants of *B. aegyptiaca* from mature mother plants can be readily established in MS medium containing 2,5 mg/l BAP to produce shoot multiplication. Multiple shoot regeneration has also been reported in *Ziziphus mauritiana* Lam. (Goyal and Arya, 1985; Mathur et al., 1995; Sudharsan et al., 2001). However, there are no published reports on the micropropagation of *B. aegyptiaca*. MS media without any growth hormones enhanced shoot growth and elongation, while media with BAP enhanced axillary branching explants furthering multiple shoot development of nod.

REFERENCES

- Bonga JM, Durzan DJ (1982). Tissue culture in forestry. Martinus Nijhoff, The Hague, Boston London, 387 p.
- Casado JP, Navarro MC, Utrilla MP, Martínez A, Jiménez J (2002). Micropropagation of *Santolina canescens* Lagasca and in vitro volatiles production by shoot explants. Plant Cell Tissue Organ Cult. 69: 147-153.
- Dhawan V, Bhojwani SS (1985). In vitro vegetative propagation of *Leucaena leucocephala* (Lam.) de wit. Plant Cell Rep. 4: 315 – 318.
- Dhawan V (1989). Application of biotechnology in forestry and horticulture. Plenum Publishing Corp, New York, pp. 285-296.
- El Nour M, El Khalifa, Massimo K, El Hassen B. (1991). Preliminary study on seed pregermination treatment and vegetative propagation of *Balanites aegyptiaca* (L.) Del. In : Physiology des Arbres et Arbustes en zone arides et semi-arides. Groupe d'Etude de l'arbre – Paris, France, pp. 413 –415.
- Fraternal D, Giamperi L, Ricci D, Rocchi MBL (2002). Micropropagation of *Bupleurum fruticosum*: the effect of triacontanol. Plant Cell Tissue Organ Cult. 69: 135-140.
- Gassama YK, Duhoux E (1986). Micropropagation d'*Acacia albida* Del. (Légumineuses) adulte. Bulletin de l'IFAN. Cheik Anta Diop: T. 46, Ser A. N° 34: 315-320.
- Giffard PL (1974). L'arbre dans le paysage sénégalais. Sylviculture en zone tropicale sèche. Centre Tech. Forest. Tropical. Dakar 431 p.
- Gamborg OL, Miller RA, Ojima K (1968). Nutrients requirements of suspension cultures of soybean roots. Exp. Cell. Res. 50: 150 – 158.
- Goyal Y, Arya HC (1979). Clonal propagation of *Prosopis cineraria* Linn. Through tissue culture and differentiation. J. Indian Bot. Soc. 58 – 61.
- Goyal Y, Arya HC (1984). Tissue culture of desert trees. Clonal multiplication of *Prosopis cineraria* by bud culture. J. Plant Physiol. 115: 183 – 189.
- Goyal Y, Arya HC (1985). Tissue culture of desert tree II. Clonal multiplication of *Zizyphus* in vitro. J. Physiol. 119:398-404.
- IPGRI (1984). Forage and browse plants for arid and semi-arid Africa. International board for plant genetic resources/Royal Botanic Gardens Rome. Pp. 101-102.
- Marks TR, Simpson SE (1994). Factors affecting shoot development in apically dominant *Acer* cultivars in vitro. J. Hort. Sci. 69: 543-551.

- Martin KP (2000). Rapid propagation of *Holostemma ada-kodien* Schult., a rare medicinal plant, through axillary bud multiplication and indirect organogenesis. *Plant Cell Rep.* 21: 112-117.
- Mathur N, Ramawat KG, Nandwani D (1995). Rapid in vitro multiplication of jujube through mature stem explants. *Plant Cell Tissue Organ Cult.* 43: 75-77.
- Murashige T, Skoog F (1962). A revised medium of rapid growth and essays within tobacco tissue culture. *Physiol. Plant.* 15: 573-497.
- Preece JE, Huttremann CA, Ashby WC, Roth PL (1991). Micro and cutting propagation of silver maple. I. Results with adult and juvenile propagules. *J. Am. Soc. Hortic. Sci.* 116 : 142-148.
- Saini R, Jaiwal PK (2000). In vitro multiplication of *Peganum harmala* an important medicinal plant. *Indian J. Exp. Biol.* 38 : 499-503.
- Sane D, Borgel A, Chevallier MH, Gassama/Dia YK (2001). Induction *in vitro* de l'enracinement de microboutures d'*Acacia tortilis* subsp. raddiana par traitement transitoire à l'auxine. *Ann. For. Sci.* 58: 43-437.
- Sane D, Borgel A, Verdeil JL et Gassama/Dia YK (2000). Régénération de vitroplants par embryogenèse somatique à partir d'embryons zygotiques immatures chez une espèce adaptée à la sécheresse : *Acacia tortilis* subsp. raddiana (Savi.) Brenan. *Acta Bot. Gall.* 147: 257-266.
- Schenk RU, Hildebrandt AC (1972). Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. *Can. J. Bot.* 50:199-204.
- Skolmen RG, Mapes MO (1976). *Acacia Koa* Gray plantlets from somatic callus tissue. *J. Hered.* 67: 114 – 115.
- Sudharsan C, Abo el-Nil M, Hussain J (2001). In vitro propagation of *Ziziphus mauritiana* cultivar Umran by shoot tip and nodal multiplication. *Curr. Sci.* 80: 290-292.
- Von-Maydell H-J. (1984). Arbres et arbustes du Sahel: leurs caractéristiques et leurs utilisations. Eschborn : GTZ, 531p.