INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2004/06–2–321–323
http://www.ijab.org

In Vitro Clonal Propagation of Banyan (*Ficus benghalensis* L.) Through Axillary Bud Culture

M.K. MUNSHI, L. HAKIM[1], M.R. ISLAM AND GOLAM AHMED
Institute of Food and Radiation Biology, Atomic Energy Research Establishment, GPO Box 3787, Dhaka 1000, Bangladesh
[1]Corresponding author's e-mail: lhakim@dhaka.net

ABSTRACT

Nodal segments containing axillary buds of *Ficus benghalensis* L. were induced to produce a large number of multiple shoots by culturing on MS medium supplemented with 1.0 BA+ 0.1 NAA (mg/L) and 20% (v/v) coconut milk. Excised shoots from this culture were rooted best on ½ MS medium fortified with 0.5 mg/L IBA. The complete plantlets thus obtained were successfully transferred to soil.

Key Words: Regeneration; Clonal propagation; Axillary bud; Banyan; *Ficus benghalensis*

INTRODUCTION

In most of the tropical regions including Bangladesh, there is an urgent need to protect the existing forest cover from further deterioration and enhance reforestation. Induction of forest trees could slow the daily rate of denudation in these areas. The application of tissue culture methods offers new prospects for the rapid multiplication of many plants. In recent years, studies on *in vitro* propagation of woody plants have shown that these techniques may be a solution for rapid propagation of selected forest trees (Bonga & Durzan, 1987; Ahuja, 1991). However, woody plants have been found to be in general recalcitrant to *in vitro* regeneration. Banyan (*Ficus benghalensis* L.), belongs to the family Moraceae, is a woody plant with long life span and attains large dimensions. Its leafy crown sometimes attains a circumference of 1000-2000 feet. It is evergreen except in dry localities where it is leafless for a short time. The tree occurs throughout the forest tracks of India and sub-Himalayan region. The wood of banyan is durable under water and used for well curbs. Stronger and more elastic aerial roots are used for tent poles, cart yokes, carrying shafts etc. Various parts of the plants are considered medicinal (Sharma, 1997). Seeds of this plant do not germinate directly on soil until they pass through the digestive system of the bird. Generally the fruits are eaten by the birds and the seeds are excreted along with faeces and these seeds usually grow on the old walls, cracks of the building and pockets of trees. So propagation of this tree through seed germination is complicated and is beyond our control. Keeping in view these facts, an attempt was made to develop in *vitro* regeneration system of this giant tree.

MATERIALS AND METHODS

Actively growing shoots apices were collected from the young plants growing wildly. The leaves were removed and stem apices were washed thoroughly under running tap water. Shoot apices having nodal segments with axillary buds were surface sterilized with 0.1% $HgCl_2$ solution and were cut into pieces a few mm below and above each node. These nodal segments were used as primary explants and cultured on MS (Murashige & Skoog, 1962) medium supplemented with different concentrations of BA in single or in combination with NAA for shoot induction. Different concentrations of coconut milk were also added in the medium to increase the number of shoots. Excised shoots from these cultures were implanted onto ½ MS added with IBA or NAA at different concentrations for root induction. The pH of the medium was adjusted to 5.8 and gelled with 3.6% phytagel and autoclaved for 20 minutes at 121°C under 1.05 kg/cm^2. All cultures were maintained in a culture room at 26±2°C and were exposed to continuous fluorescent light for 16 h per day.

RESULTS AND DISCUSSION

Nodal explants showed their first response by enlarging and bursting of axillary buds within 1-2 weeks of culture (Fig. 1A). New shoot development from axillary bud was observed within three weeks of culture and more shoots were found to develop during subcultures. The number of shoots per explant was found in the range of 2.15 to 4.17 under different concentrations of BA when used singly and 2.0 mg L^{-1} BA yielded maximum shoots (Fig. 2A). In different concentrations and combinations of BA+NAA, multiple shoot formation was recoded in the range of 3.09 to 5.70 and best response was found under 1.0 BA+0.1 NAA mg L^{-1} combination (Fig. 2B). The combined effects of BA+NAA on multiple shoot induction were reported earlier in different plants (Lauzer & Vieth 1990; Abubacker & Alagumanian, 1999; Lal & Ahuja, 2000). Further attempts were made to develop profuse multiple shoots by adding different concentrations of coconut milk (5 to 40% v/v) to

Fig. 1. Proliferation of axillary bud in MS medium added with 2.0 BA (mg/L) (A); development of multiple shoot in MS medium fortified with 1.0 BA + 0.1 NAA (mg/L) + 20% v/v coconut milk (B); rooting of excised shoot in ½ MS medium supplemented with 0.5 IBA (mg/L); establishment of plants in plastic pot (D) and small ploythene bags (E)

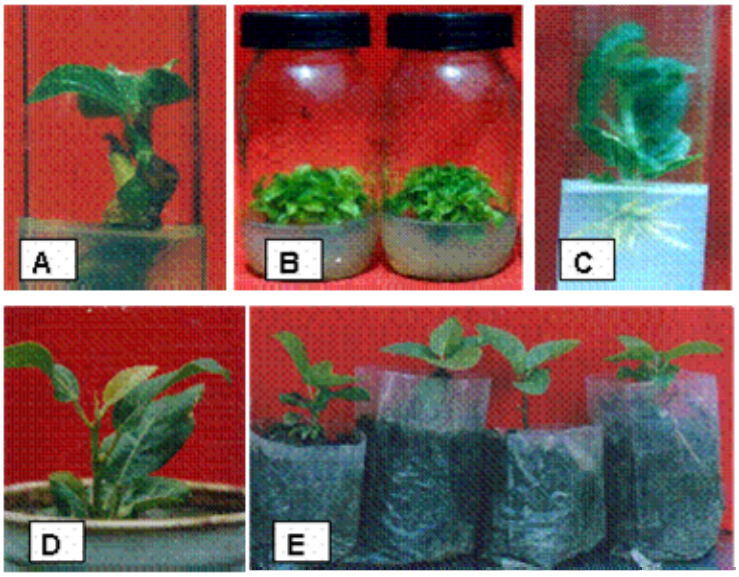


Fig. 2. Response of axillary bud towards multiple shoot induction on MS medium added with different concentrations of BA (A); BA+NAA (B); coconut milk with 1.0BA+0.1NAA combination (C)

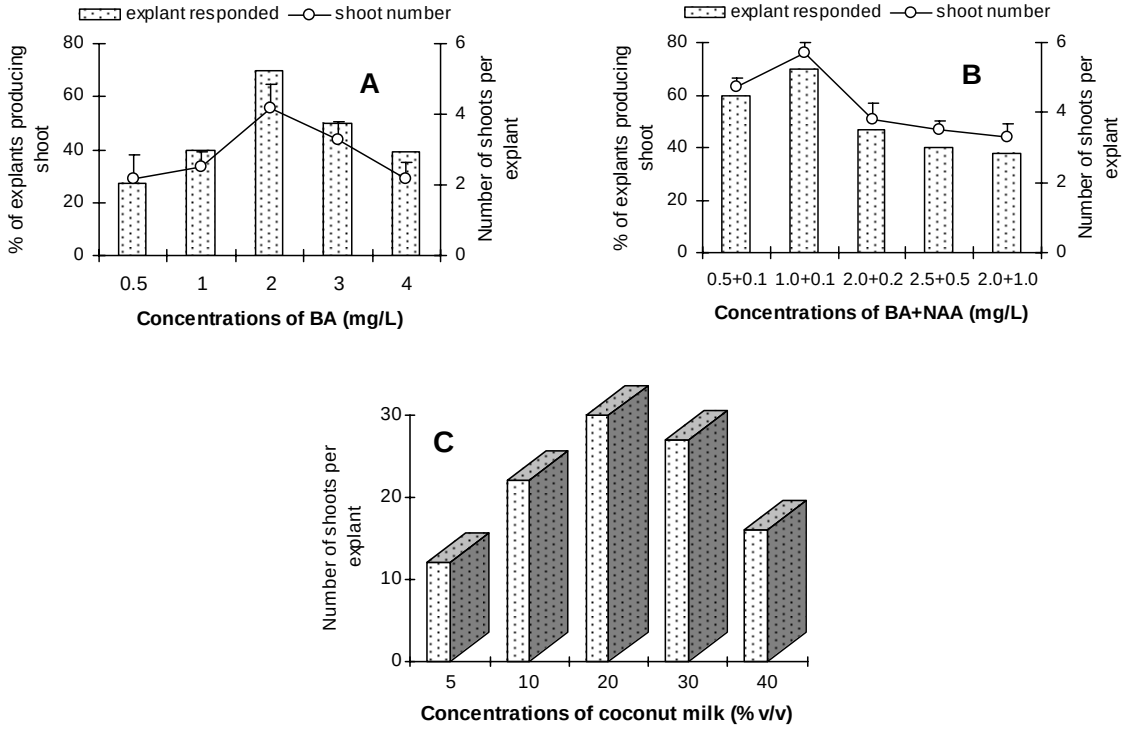
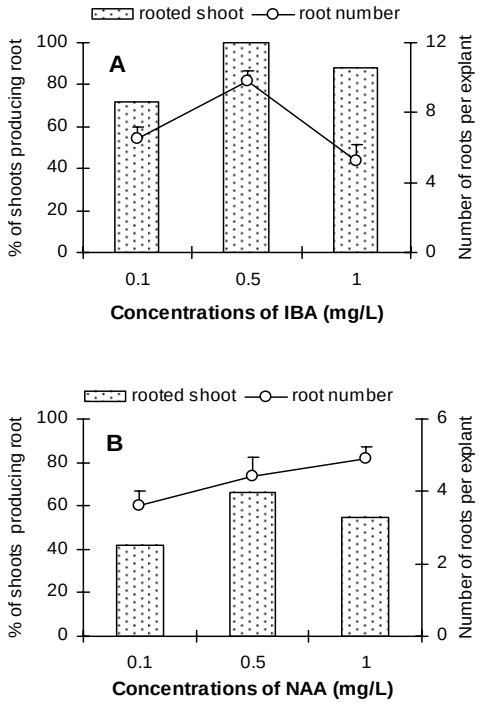


Fig. 3. Effects of different concentrations of IBA (A) and NAA (B) on rooting of excised shoot in banyan



MS medium fortified with 1.0 BA+0.1 NAA mg L^{-1}. Coconut milk resulted in dramatic increase of multiple shoot development and best performance was recoded under 20% v/v (Figs. 1B, 2C). Stimulatory effects of coconut milk towards multiple shoot induction in *Elaeocarpus robustus* was reported by Roy *et al*. (1998). Nodal segments have been demonstrated to be useful materials for mass propagation of woody plants because of high regeneration ability of the explants (Kaur *et al*., 1998; Kumar *et al*., 1998; Quraishi & Mishra, 1998). It is considered that shoots originated from axillary buds are preferable for vegetative propagation because of reduced risk of genetic instability (Hussey, 1986).

Individual shoots from these cultures were excised and cultured onto ½ MS medium supplemented with IBA or NAA at different concentrations for root induction to raise full-fledged plantlets. All the treatments resulted in root formation but NAA exhibited poor response (Fig. 3A) compared to IBA. IBA at a concentrations of 0.5 mg L^{-1} showed best response with respect to rooting percentage, rooting quality and number of roots per cutting (Figs. 1C, 3B). IBA is considered as the most effective auxin in root induction (Litz & Jaiswal, 1990). Microcuttings of *Ficus carica* cv. Gular were easily rooted on ½MS medium supplemented with 2.0 mg L^{-1} IBA+0.2% activated charcoal (Kumar *et al*., 1998). The complete plantlets were transferred to small plastic pots (Fig. 1D) and also small polythene bags (Fig. 1E) containing a mixture of soil and compost. They were gradually acclimatized and eventually transferred to the field. About 80% plants survived under natural environment. Kumar *et al*. (1998) found 68% survival rate of *Ficus carica* cv. Gular in the field condition. This experimental finding established a protocol for *in vitro* regeneration of this giant tree.

REFERENCES

Abubacker, M.N. and S. Alagumanian, 1999. *In vitro* organogenesis of various explants of *Azadirachta indica* A. Juss. *Plant Tissue Cult*., 9: 177–80

Ahuja, M.R., 1991. *Woody Plant Biotechnology*, p. 373. Plenum Press, New York

Bonga, J.M. and D.J. Durzan, 1987. *Cell and Tissue Culture in Forestry*, Vol. 1, 2 and 3. Martinus Nijhoff Publ., Dordrecht

Hussey, G., 1986. Vegetative propagation of plants by tissue culture. *In:* Yeoman, M. (ed.), *Plant Cell Culture Technology*, pp: 29–58. Blackwell Scientific Publ., London

Kaur, K., B. Verma and U. Kant, 1998. Plant obtained from Khair tree (*Acacia catechu* Willd.) using mature nodal segments. *Plant Cell Rep*., 17: 427–9

Kumar, V., A. Radha and C.S. Kumar, 1998. *In vitro* plant regeneration of fig (*Ficus carica* L. cv. Gular) using apical buds from mature trees. *Plant Cell Rep*., 17: 717–20

Lal, N. and P.S. Ahuja, 2000. Adventitious shoot bud formation from cultured leaf explants of *Rheum emodi* Wall. *Plant Tissue Cult*., 10: 17–24

Lauzer, D. and J. Vieth, 1990. Micropropagation of seed derived plants of *Cynara scolymus* L. cv. Green clove. *Plant Cell, Tissue and Organ Cult.*, 21: 237–44

Litz, R.E. and V.S. Jaiswal, 1990. Micropropagation of tropical and sub-tropical fruits. *In:* Debergh and R.H. Zimmerman (eds.), *Micropropagation Technology and Application*, pp: 247–66. Kluwer Academic Publishers, Dordrecht

Quraishi, A. and S.K. Mishra, 1998. Micropropagation of nodal explants from adult tree of *Cleisanthus collinus*. *Plant Cell Rep*., 17: 430–3

Murashige, T. and S. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*., 15: 473–9

Roy, S.K., M.S. Islam and S. Hadiuzzaman, 1998. Micropropagation of *Elaeocarpus robustus* Roxb. *Plant Cell Rep*., 17: 810–3

Roy, S.K., P.K. Roy and M.S. Haque, 2000. Mass clonal propagation of *Artocarpus heterophyllus* through *in vitro* culture. *In:* Cubota, C. and C. Chun (eds.), *Transplant Production in the 21st Century*. pp: 219–25. Kluwer Academic Publishers, Dordrecht

Sharma, D., 1997. *Shyamolee Nisharga* (in Bengali), pp: 148–51. Bangla Academy, Dhaka, Bangladesh

(Received 03 January 2004; Accepted 10 January 2004)