Plant Tissue Cult. **14**(2) : 135-142, 2004 (December)

PTC

In vitro Propagation of Banyan Tree (*Ficus benghalensis* L.) - A Multipurpose and Keystone Species of Bangladesh

M. M. Rahman, M. N. Amin* and M. F. Hossain

Department of Botany, University of Rajshahi. Rajshahi-6205, Bangladesh

Key wards: In vitro, Propagation, Banyan tree, *Ficus benghalensis*

Abstract

Nodal segments of about 100-year-old banyan tree (*Ficus benghalensis* L.) was found to be the best for multiple axillary shoot production on MMS1 medium supplemented with 0.5 mg/l BA. Subculturing of the nodal segments from regenerated shoots to the same medium profoundly stimulated shoot proliferation and elongation. These shoots were devoid of any root and induced to develop roots by culturing the isolated individual shoots on MMS2 containing 0.1 mg/l IBA. The success of rooting was 100%, while only 65% of the plantlets thus obtained were finally established in soil.

Introduction

Ficus benghalensis L. (syn. *F. indica* L.) commonly known as banyan tree belongs to the family Moraceae. The plant seems to be indigenous in the sub-Himalayan forests and is widely distributed through the slopes, hill ranges in peninsular India and planted in Bangladesh, Myanmar, Sri-Lanka and Malaysia (Santapau 1981, Khan and Alam 1996). Banyan tree is multipurpose in use and very popular for their medicinal, socio-cultural and ethnobotanical properties (Agarwal 1990, Ambasta *et al*. 1992, Nath and Debnath 1947). The tree provides habitat for a number of animals and plants and hence it is considered as one of the most important keystone species in the Gangetic flood plain and other ecosystem of Bangladesh. It is also considered as a most suitable plant for community plantation as a shade tree (Khan and Alam 1996). Thus, there are good demands for transplants of banyan tree for mass scale plantation programme in different countries of arid and semi-arid region. The banyan tree is conventionally propagated by seeds. But the seeds of *F. benghalensis* germinate to seedlings, only when they pass through the

*Author for correspondence.

alimentary system of birds that restricts easy propagation of the tree by seedlings. It can also be propagated by hard wood cuttings, but it is a very slow and unreliable process. Tissue culture techniques have advantages that may provide methods for both large-scale propagation and improvement of tree species like the banyan tree (Bajaj 1986, Dunstan and Thorpe 1986, Boulay 1987). Reports are available on tissue culture of different species of *Ficus* like *F. elestica* (Battle and Mele 1984), *F. religiosa* (Narayan and Jaiswal 1986), *F. auriculata* (Amatya and Rajbhandary 1989), *F. carica* (Pontikis and Melas 1986) and *F. benjammina* (Kristainsen 1992). So far there is no report on *in vitro* plant regeneration in *Ficus benghalensis* - the great banyan tree. Therefore, the present investigation was undertaken to establish a protocol for regenerating a large number of plantlets from the nodal segment cultures of the banyan tree.

Materials and Methods

Shoot apices (8 - 10 cm) were collected from an approximately 100-year-old tree of *Ficus benghalensis* growing near the bus terminal in Rajshahi University campus. The shoot segments were defoliated after bringing to the laboratory and treated with 1% Savlon (an antiseptic plus surfactant) for about 10 min. The material was then washed thoroughly under tap water and finally surface-sterilized with a 0.1% $HgCl_2$ for 10 min followed by washing thrice in autoclaved distilled water. The explants (ca. 1.5 cm) consisting of shoot tips and nodal segments were prepared from the surface sterilized materials. They were then implanted on agar-gelled modified MS medium such as MS with half strength of major salts (MMS1) for shoot proliferation and MS with half strength of both major and minor salts (MMS2) for rooting. The MMS1 was supplemented with various concentrations of either BA and Kn alone or in different combinations of BA, Kn, NAA for shoot bud initiation and multiplication. Microcuttings prepared from the *in vitro* proliferated shoots were cultured on MMS2 medium fortified with 0.1 - 1.0 mg/l of either IBA, NAA or IAA for rooting. All media were enriched with 3% sucrose, adjusted to pH 5.7 ± 0.1, gelled with 0.6% agar and steam sterilized for 20 min at 121_C under 1.1 kg/cm^2 pressure. The cultures were grown at 26 ± 1_C under 16 h photoperiod with a photon flux density of about 70 μ $mol.m^{-2}.s^{-1}$.

Results and Discussion

The *in vitro* experiments of the present study initially involved the establishment of nodal- and shoot tip explants in aseptic cultures, which resulted in the induction of multiple shoot formation, elongation of shoots,

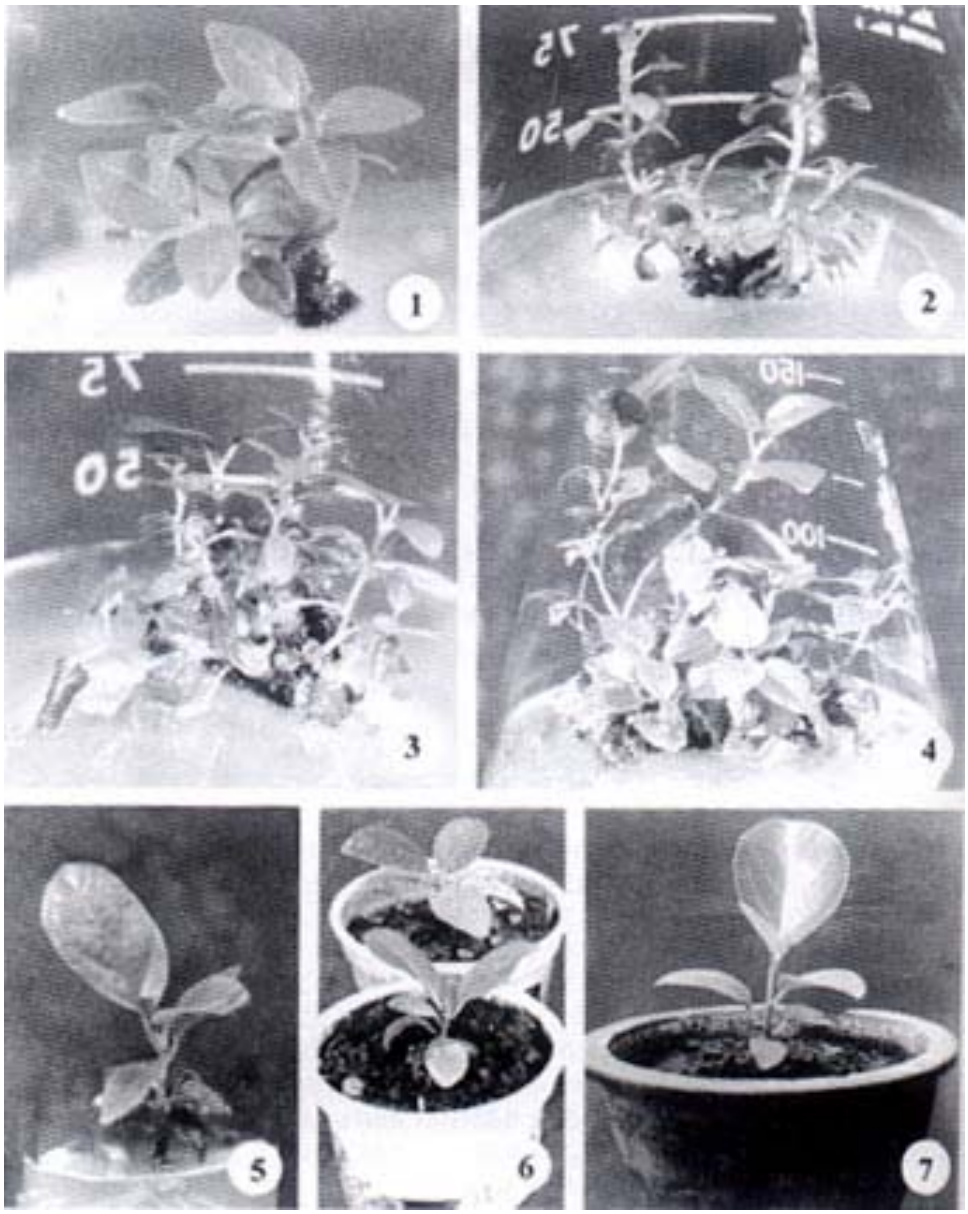
development of roots for plantlet formation and finally the establishment of plantlets under *ex vitro* condition. Two types of explants showed different responses when they were cultured on MMS1 with multiple concentrations and combinations of cytokinins and auxins. Of the two cytokinins (BA and Kn) used, BA was found to be comparatively better for shoot proliferation (Table 1). Similarly, the nodal segments responded better than the shoot tip explants and 90% cultures of the nodal explants produced shoots on MMS1 with 0.5 mg/l BA.

Table 1. Effect of growth regulators in MMS1 medium on shoot proliferation from the nodal explants of the banyan tree.*

Growth	% of shoot	No. of total	No. of usable	Average length
BA				
0.1	65	4.85 ± 0.24	4.00 ± 0.24	3.24 ± 0.15
0.2	80	8.52 ± 0.25	7.55 ± 0.21	4.00 ± 0.18
0.5	90	12.65 ± 2.16	9.60 ± 1.11	4.52 ± 0.14
1.0	70	6.33 ± 0.18	5.62 ± 0.17	3.54 ± 0.12
1.5	50	4.50 ± 0.25	3.90 ± 0.23	3.10 ± 0.13
Kn				
0.2	45	3.80 ± 0.24	3.40 ± 0.23	2.85 ± 0.13
0.5	70	5.20 ± 0.12	4.86 ± 0.20	3.25 ± 0.18
1.0	80	6.23 ± 2.17	5.45 ± 0.18	3.50 ± 0.14
1.5	65	5.00 ± 0.10	4.56 ± 0.17	3.11 ± 0.12
2.0	35	3.25 ± 0.23	3.10 ± 0.21	2.80 ± 0.18
BA + NAA				
0.2+ 0.1	55	4.50 ± 0.20	3.65 ± 0.23	3.22 ± 0.18
0.2+ 0.2	60	5.32 ± 0.24	4.35 ± 0.24	3.55 ± 0.12
0.5+ 0.1	50	3.62 ± 0.18	3.00 ± 0.18	2.62 ± 0.17
0.5+ 0.2	40	3.50 ± 0.42	2.15 ± 0.20	2.25 ± 0.19
1.0+ 0.1	75	8.65 ± 0.32	6.12 ± 0.20	4.10 ± 0.14
1.0+ 0.2	70	6.00 ± 0.22	5.24 ± 0.21	3.85 ± 0.16
Kn + NAA				
0.5+ 0.1	50	3.54 ± 0.23	2.86 ± 0.20	2.82 ± 0.21
0.5+ 0.2	55	4.25 ± 0.21	3.20 ± 0.20	2.85 ± 0.18
1.0+ 0.1	60	4.85 ± 0.23	3.55 ± 0.28	3.05 ± 0.13
1.0+ 0.2	70	6.10 ± 0.25	4.25 ± 0.22	3.15 ± 0.18
1.5+ 0.1	40	3.20 ± 0.20	2.50 ± 0.10	2.65 ± 0.13
1.5+ 0.2	35	2.85 ± 0.13	2.00 ± 0.26	2.55 ± 0.17

*Data were collected from 15 - 20 explants after eight weeks of their culture.

In this medium, the highest number of shoots per culture was 12.65 ± 2.16 and the average length of shoots per culture was 4.52 ± 0.14 cm. The nodal explants initially produced two to three shoots within three - four weeks after inoculation on BA supplemented medium (Fig. 1). On the other hand, the



Figs. 1-7: Regeneration of plantlets *in vitro* from nodal explants obtained of field grown *Ficus benghalensis* plants. 1. Development of axillary shoots from the nodal explant after four weeks of culture. 2. Development and multiplication of axillary shoots on 1/2 MS medium containing 0.5 mg/l BA after eight weeks of culture. 3. Development and multiplication of axillary shoots on the same medium after twelve weeks of culture. 4. Elongation of multiple axillary shoots from the same explant and on the same medium after fifteenth weeks of culture. 5. Adventitious root formation on regenerated shoots. 6. *In vitro* raised plantlets grown under *ex vitro* environment in plastic pots containing garden soil, compost and sand (2 : 2 : 1). 7. Establishment of *in vitro* grown plantlets outside in earthen pots containing soil and organic manure.

highest of 80% cultures of nodal explants produced shoots on the medium with 1.0 mg/l Kn, where the number of multiple shoots was 6.23 ± 2.17 and average length of shoots was 3.50 ± 0.14 cm. With respect to both shoot induction and increased shoot number, in BA proved better than Kn and it was found to be as the most effective cytokinin for shoot induction as well as shoot proliferation in *Ficus benghalensis*. The findings of the present study are in conformity with those of many others where BA performed better than Kn. As a synthetic cytokinin BA has the advantage over other cytokinins in inducing *in vitro* shoot production in woody plant like *Artocarpus heterophyllus* (Amin and Jaiswal 1993), *Ficus religiosa* (Amin et al. 2001), *Azadirachta indica* (Islam et al. 1997) and chestnut (Vieitez and Vieitez 1980). Among cytokinin and auxin combinations, 1.0 mg/l BA + 0.1 mg/l NAA induced maximum proliferation of shoots. On this medium, 75% explants responded by producing 8.65 ± 0.32 shoots per culture within six weeks of culture. Gill et al. (1997), Islam et al. (1994), Sen et al. (1992), Kumer et al. (1991) and Sita (1986) reported that a combination of BA and NAA (5 : 1/10 : 1) was suitable for shoot multiplication in woody plants.

Shoot initiation was observed in each subculture and the number of shoots increased gradually with the age of the culture (Figs. 2 - 4). At the initial stage of culture two - three shoot buds sprouted from a nodal explant on a medium containing 0.5 mg/l BA within three weeks. When it was subcultured in the same medium, a cluster of 12 - 14 shoots was produced (Fig. 2). This cluster of shoots was divided into pieces and subcultured individually on the same medium. Each culture then produced a bunch of 15 - 20 shoots within four weeks of subculture (Figs. 3, 4). Results of this study indicate that a large scale propagation of *Ficus benghalensis* is feasible by tissue culture methods and hundreds of plants can be regenerated from one nodal explant within fourth subculture. However, after fourth subculture the cultured explants reach a stage where the shoot multiplication rate of subcultured material remained constant. The same result was also reported by Amin and Jaiswal (1978) and Norton and Norton (1986).

Microcuttings (3 - 4 cm) were prepared from the *in vitro* proliferated shoots and cultured on MMS2 with 0.1-1.0 mg/l of either IBA, NAA or IAA for adventitious rooting. The effects of these three auxins on percentage of root formation, number of roots per shoot and length of the longest roots were recorded after six weeks of culture. The rooting responses to different concentrations and types of auxins are shown in Table 2.

Both the concentration and the nature of auxins used markedly influenced frequency of root formation and the number of roots per shoot. Among the three

types of auxin used, IBA was found to be the best for root induction. Cent per cent of shoot cuttings produced roots when they were cultured on the medium with 0.1 mg/l IBA. In this experiment, the highest number of roots per microcutting was 6.84 ± 0.12 and the maximum root length was 3.15 ± 0.21 cm (Fig. 5). A higher concentration (>1.0 mg/l) of IBA, NAA or IAA could not form any root but produced only callus at the cut ends of the shoot. Use of IBA as auxin for successful root induction was reported in *Albizia lebbeck* (Reza et al. 1996), *Azadirachta indica* (Sarker et al. 1997) and *Ficus religiosa* (Amin et al. 2001). Plantlets were at first established in small plastic pots with a view to easy handling and maintenance during acclimatization and transplantation. About

Table 2. Effect of different concentrations of auxins on adventitious root formation from the *in vitro* grown microcuttings cultured on MMS2.

Different conc. of auxins (mg/l)	% of microcutting rooted	No. of root per microcutting	Average length of the root (cm)	Callus formation at the cutting base
IBA				
0.1	100	6.84 ± 0.12	3.15 ± 0.21	–
0.2	95	5.36 ± 0.21	2.86 ± 0.46	–
0.5	80	4.45 ± 0.15	2.54 ± 0.18	+
1.0	65	3.72 ± 0.15	2.35 ± 0.15	++
NAA				
0.1	65	3.75 ± 0.18	2.30 ± 0.23	–
0.2	80	4.50 ± 0.21	2.82 ± 0.24	–
0.5	70	4.00 ± 0.23	2.65 ± 0.14	–
1.0	50	3.20 ± 0.15	2.10 ± 0.15	+
IAA				
0.1	50	2.00 ± 0.21	2.20 ± 0.13	–
0.2	60	2.25 ± 0.23	2.60 ± 0.18	–
0.5	55	2.10 ± 0.26	2.25 ± 0.16	-
1.0	40	1.95 ± 0.15	2.00 ± 0.15	+

'–' indicates no response, '+' indicates slight callusing, '++' indicates considerable callusing. *There were 15 - 20 microcuttings in each treatment. Data were recorded after four - six weeks of culture.

80% of the plantlets established under *ex vitro* condition when they were initially transferred on coco-peat as potting mix. On the other hand, 70% survival rate of plantlets was observed when they were transferred on ice cream pots (Fig. 6) containing garden soil, compost and sand (2 : 2 : 1). It was also observed that 65% of the plantlets could be established under *ex vitro* conditions when they were transferred on earthen pots containing soil and organic manure (Fig. 7). The reason behind variation in the survival rate of the plantlets on different potting mix is that coco-peat is a soil-free material with very porous but good water holding capacity and do not allow microbial growth easily, while a mix of garden soil, compost and sand has a moderate water

holding capacity and allows microbial growth in the interface of plantlets and potting mix. The third category potting mix, fresh soil plus organic manure have least water retaining capacity and allow rapid microbial growth over the surface. The use of sufficiently porous potting mix that allows adequate drainage and aeration has been recommended for fast acclimatization of *in vitro* regenerated plants (Dunstan and Turner 1984 and Kozai et al. 1987). Nevertheless, excellent success has been obtained with soil-free potting mix like vermiculite, perlite, peat plugs or small foam blocks (Miller 1983 and McCown 1986). At the age of two and a half years no morphological abnormality was noticed in the transplants and hence all the transplanted plants were assumed to be morphologically identical to the mother plants from which explants were taken for *in vitro* culture.

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