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# *In vitro* Micropropagation of Banyan Tree (*Ficus benghalensis* L.) through Shoot Tip Culture

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**ABSTRACT**

In the present investigation, shoot tips of banyan tree (*Ficus benghalensis* L.) were cultured on modified MS medium blended with BAP for shoot initiation. Then the initiated shoots were transferred to ficus multiplication medium for multiple shoot development and further transferred to ficus rooting medium supplemented with IBA for rooting. The success of rooting was 100%, while only 90% of the plantlets thus obtained were established in soil.

**Key words:** Banyan, *Ficus benghalensis*, Regeneration, Micropropagation, Shoot tip

*Ficus benghalensis* 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 (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. It is also considered as a most suitable plant for community plantation as a shade tree (Khan and Alam 1996). Thus, there is good demand 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 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. elastica* (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). Reports are also available on *in vitro* propagation of *Ficus benghalensis* L. through axillary bud culture (Munshi *et al.* 2004) and nodal segments (Rahman *et al.* 2004). Therefore, the present investigation was undertaken to establish a protocol for regenerating a large number of plantlets from the shoot tip culture of banyan tree.

Table 1 Composition of different media used in the experiment

| | Basal media | BAP (mg/l) | IAA (mg/l) | Adenine (mg/l) | IBA (mg/l) | Sucrose (%) | Agar (%) | *p*H |
|---|---|---|---|---|---|---|---|---|
| Ficus Initiation Medium (FIM) | | | | | | | | |
| FIM 1 | MS | 0.5 | - | - | - | 3 | 0.7 | 5.8 ± 0.1 |
| FIM 2 | MS | 1.0 | - | - | - | 3 | 0.7 | 5.8 ± 0.1 |
| Ficus Multiplication Medium (FMM) | | | | | | | | |
| FMM 1 | MS | 0.5 | 0.4 | 10 | - | 3 | 0.7 | 5.8 ± 0.1 |
| FMM 2 | MS | 1.0 | 0.4 | 10 | - | 3 | 0.7 | 5.8 ± 0.1 |
| FMM 3 | MS | 2.0 | 0.4 | 10 | - | 3 | 0.7 | 5.8 ± 0.1 |
| FMM 4 | MS | 3.0 | 0.4 | 10 | - | 3 | 0.7 | 5.8 ± 0.1 |
| Ficus Rooting Medium (FRM) | | | | | | | | |
| FRM 1 | ½ MS | - | - | - | 0.2 | 2 | 0.6 | 5.8 ± 0.1 |
| FRM 2 | ½ MS | - | - | - | 0.4 | 2 | 0.6 | 5.8 ± 0.1 |
| FRM 3 | ½ MS | - | - | - | 0.5 | 2 | 0.6 | 5.8 ± 0.1 |
| FRM 4 | ½ MS | - | - | - | 1.0 | 2 | 0.6 | 5.8 ± 0.1 |

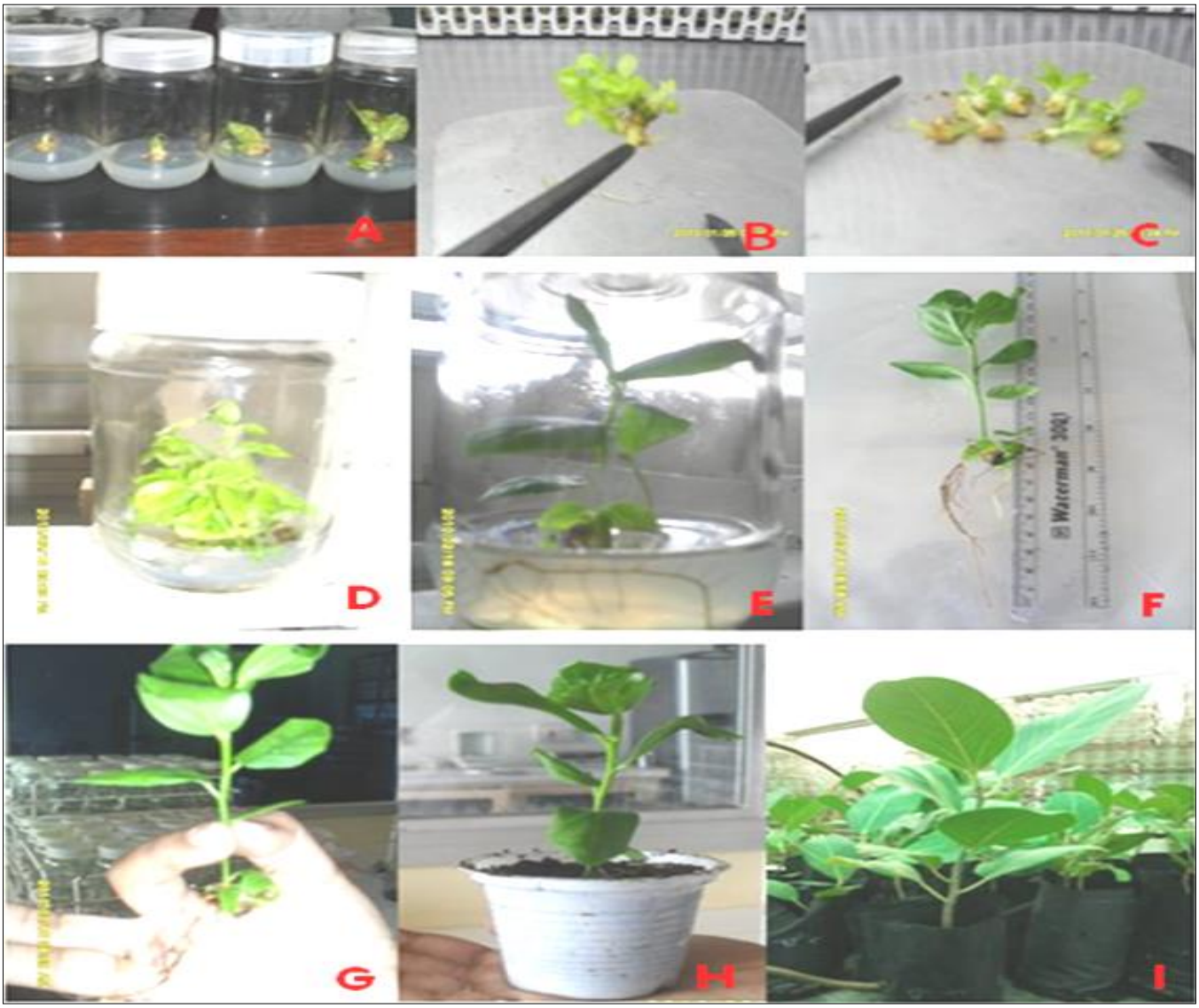


Fig 1 (A) Shoot initiation on FIM2, (B) and (C) Explants preparation for sub-culturing, (D) Shoot multiplication on FMM4, (E) Root regeneration on FRM 3, (F) and (G) Completely regenerated plant, (H) Establishment of plant in plastic pot with coco peat and (I) Plant ready for transplantation into the soil

## MATERIALS AND METHODS

Shoot apices (8-10 cm) were collected from an approximately 12 year old tree of *Ficus benghalensis*. The shoot tips were brought to the laboratory and treated with 5% Savlon (an antiseptic and surfactant) for about 20 minutes. The explants were then washed thoroughly under tap water and surface sterilized with 0.1% $HgCl_2$ for 7 minute followed by rinsing thrice with sterile distilled water. The explants (1.5 cm) consisting of shoot tips were prepared from the surface sterilized materials. Then, they were cultured on ficus initiation medium (FIM) i.e. modified Murashige and Skoog medium (MS) blended with BAP for shoot initiation. Then the initiated shoots were transferred to ficus multiplication medium (FMM) for multiple shoot development and further transferred to ficus rooting medium (FRM) supplemented with IBA for rooting. The *p*H of media was adjusted to 5.8 ± 0.1, gelled with agar and autoclaved for 20 minutes at 121°C under 1.1 kg/cm$^2$ pressure. All the cultures were maintained in culture room at 25 ± 1°C and were exposed to continuous fluorescent light for 12 hours per day.

## RESULTS AND DISCUSSION

New shoot development from shoot tip was observed within three weeks of culture on FIM2 containing 1.0 mg/l BAP (Fig 1A) while shoot development on FIM1 containing 0.5 mg/l BAP was delayed and hence not considered for further work. More shoots were found to develop during subcultures. The number of shoots per explant were in the range of 2 to 7 under different concentrations of BAP (Fig 2) and FMM4 containing 3.0 mg/l BAP yielded maximum shoots (Fig 1D). Individual shoots from these cultures were excised and cultured onto ½ MS medium supplemented with IBA at different concentrations for root induction to raise full-fledged plantlets. All the treatments resulted in root

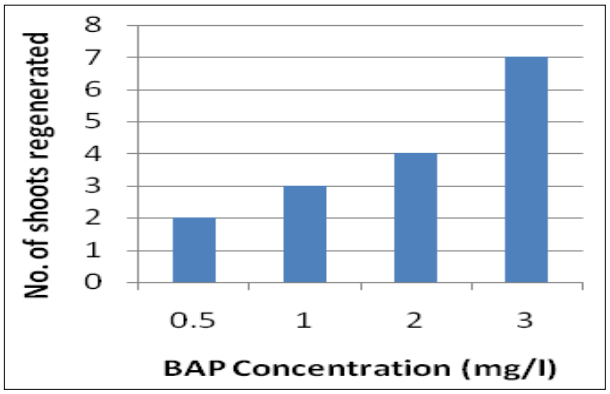


Fig 2 Effect of BAP concentration in FMM on shoot multiplication

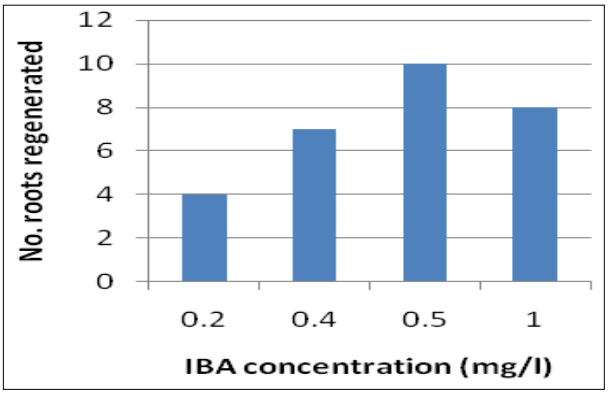


Fig 3 Effect of IBA concentration in FRM on root regeneration

formation (Fig 3) while FRM3 containing IBA at a concentration of 0.5 mg/l showed best response with respect to rooting quality and number of roots per cutting (Fig 1F, 1G). IBA is considered as the most effective auxin in root induction (Litz and Jaiswal 1990). Microcuttings of *Ficus carica* cv. Gular were easily rooted on ½MS medium supplemented with 2.0 mg/l IBA + 0.2% activated charcoal (Kumar *et al*. 1998). The complete plantlets were transferred to small plastic pots (Fig 1H) containing coco peat and soil. They were gradually acclimatized and eventually transferred to the field. About 90% 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 through shoot tip.

Therefore, it can be concluded that the initiated shoots were transferred to ficus multiplication medium for multiple shoot development and further transferred to ficus rooting medium supplemented with IBA for rooting. The success of rooting was 100%, while only 90% of the plantlets thus obtained were established in soil.

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