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# Regeneration of plantlets from the callus of stem segments of adult plants of *Ficus religiosa* L.

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ABSTRACT

Stem segments of adult plants of Ficus religiosa L. cultured on MS medium containing 1.0 mg/l 2,4-D produced callus. Shoots were regenerated when the induced calli were transferred to medium supplemented with 0.05 to 2.0 mg/l BAP. Callus derived shoots produced roots and developed into plantlets when transferred to medium supplemented with 1.0 mg/l NAA.

Abbreviations: MS, Murashige and Skoog (1962); BAP, 6-benzylaminopurine; NAA, naphthaleneacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid.

## INTRODUCTION

Tissue culture technique is becoming an important tool for the rapid clonal multiplication of selected plants (Jones, 1983). Although tissue culture of herbaceous angiosperms have been extensively studied (Murashige, 1974), trees have not received sufficient attention. Since the majority of trees are not amenable to vegetative propagation by rooting of excised branches and grafting, tissue culture is attracting considerable attention for obtaining pure elite populations under in vitro conditions (Sita et al. 1982). The special growth characteristics of trees pose difficult problems for tissue culturists; they are slow growing coupled with long dormancy and their calli in particular when excised from mature trees are rarly responsive to conditions designed to stimulate plant regeneration (Anand and Bir, 1984). Sporadic differentiation of leafy shoots and/or roots from the tissue culture of several trees species has been reported (Mehra and Mehra, 1974; Mukhopadhyay and Mohan Ram, 1981; Upadhyay and Chandra, 1983; Bapat and Rao, 1984). However, in the majority of the reports the explants used have been seeds, seedlings or parts of juvenile plants. Since the desirable character in trees are identifiable only after their maturity, these studies are not effective for immediate use in tree improvement programs.

The present investigation on Ficus religiosa L. a hardwood tree of great commercial importance in India was undertaken to develop tissue culture protocol for large scale propagation.

## MATERIALS AND METHODS

About 3.0 cm long stem segments were taken from young branches of an approximately 20-year-old tree of F. religiosa L. situated in the campus of Banaras Hindu University. They were washed in running tap water for about 30 min and then treated with 1% solution (v/v) of Cetavlon (a detergent and antiseptic) for 5 min. After rinsing with distilled water several times, the segments were surface sterilized with 0.1% (w/v) mercuric chloride solution for 5 min followed by thorough washing with double distilled water. Segments (5-8 mm) were aseptically excised and were cultured on solidified MS medium (Murashige and Skoog, 1962) supplemented with various concentrations and combinations of BAP, NAA and 2,4-D. Cultures were maintained at 25 ± 2°C with 12 h illumination at about 4000 lx. For each treatment 24 cultures were raised and all experiments were repeated at least thrice.

## RESULTS AND DISCUSSION

The stem segments cultured on MS medium supplemented with 1.0 mg/l 2,4-D showed growth of callus all over the surface of the segment after 2 weeks of the culture and within 5 weeks the entire segment turned into a mass of friable greenish yellow callus (Fig. 1).

Five to six-week-old calli obtained from these cultures and subcultured on medium supplemented with NAA in concentrations of 0.05 to 2.0 mg/l began to form roots after 4 weeks of subculture. NAA at 1.0 mg/l induced root formation in 45.8 ± 6.7 percent cultures. However, the percentage of root formation in cultures decreased if the level of NAA was above 1.0 mg/l (Table 1). On medium supplemented with 0.05 to 2.0 mg/l BAP rapid growth of the callus took place

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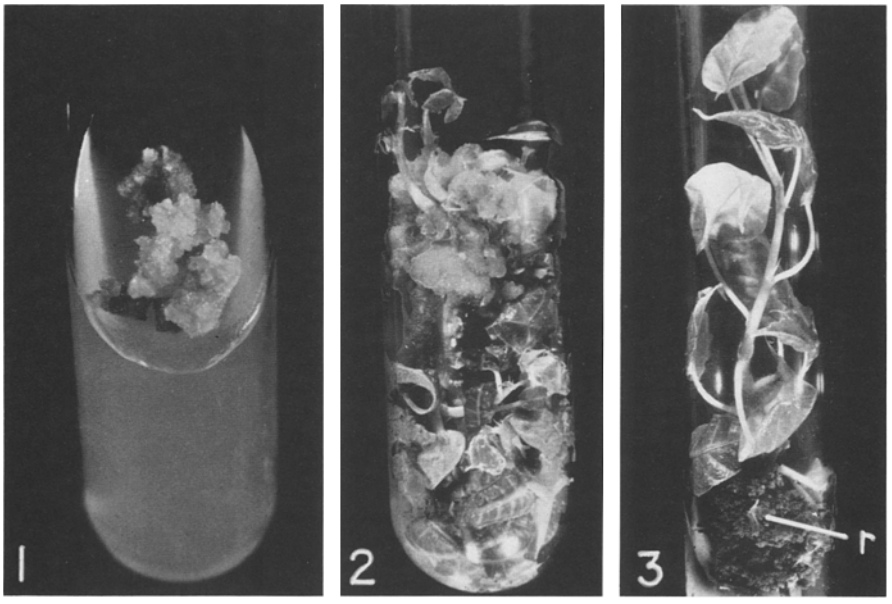


Fig. 1. Proliferation of callus from stem segment on MS + 2,4-D (1.0 mg/l). Five-week-old culture x 1.0

Fig. 2. Multiple shoots developing from callus subcultured on MS + BAP (1.0 mg/l). Seven-week-old culture x 0.9

Fig. 3. Induction of root (r) on shoot transferred on MS + NAA (1.0 mg/l). Six-week-old culture x 0.8

and light green nodular structures subsequently differentiated into shoot buds after 4-5 weeks of subculture and developed further into shoots. The percentage of cultures showing shoot formation was maximum (50.0 ± 6.2) at 1.0 mg/l BAP. Each responsive culture showed the production of 6-10 shoots along the periphery of the callus after 7 weeks of subculture and a few of them penetrated into the medium (Fig. 2). However, BAP above 1.0 mg/l inhibited shoot formation (Table 1). Medium containing BAP (0.05 to 2.0 mg/l) in combination with NAA (0.05 to 2.0 mg/l) decreased the percentage of shoot forming cultures (Table 1). BAP 2.0 mg/l in combination with 2.0 mg/l NAA inhibited organogenesis and produced callus only.

When well developed shoots were isolated and transferred to MS medium supplemented with 1.0 mg/l NAA, callus developed at the shoot base and a few roots were also produced within 3 weeks of transfer. These plantlets grew further and bore several leaves and attained a length of 8-10 cm after 6 weeks of transfer (Fig. 3).

The present study clearly demonstrates the formation of shoot buds from the callus of stem segments of Ficus religiosa L. in presence of BAP. Such an effect of BAP of inducing shoot buds has also been reported in hypocotyl callus of Santalum album (Bapat and Rao, 1984). The presence of BAP alongwith NAA decreased the percentage of shoot forming cultures. These results indicate that NAA counteracts the effect of BAP. Similar antagonistic effect

Table 1. Effect of BAP and NAA on the regeneration frequency of shoots and roots from stem callus of F. religiosa L. Each treatment consisted of 24 cultures and results were scored after 8 weeks of subculture.

| MS + Growth regulators (mg/l) | | Percentage of cultures showing organogenesis ± S.D. | |
|---|---|---|---|
| BAP | NAA | shoots | roots |
| 0 | 0 | 0 | 0 |
| 0.05 | 0 | 29.1 ± 3.6 | 0 |
| 0.5 | 0 | 37.4 ± 4.5 | 0 |
| 1.0 | 0 | 50.0 ± 6.2 | 0 |
| 2.0 | 0 | 20.8 ± 3.5 | 0 |
| 0 | 0.05 | 0 | 25.0 ± 3.2 |
| 0 | 1.0 | 0 | 45.8 ± 6.7 |
| 0 | 2.0 | 0 | 18.8 ± 2.3 |
| 0.5 | 0.05 | 25.0 ± 3.2 | 0 |
| 1.0 | 0.05 | 41.6 ± 6.7 | 0 |
| 1.0 | 0.5 | 27.0 ± 3.5 | 0 |
| 0.05 | 0.5 | 13.5 ± 1.6 | 26.4 ± 3.5 |
| 0.05 | 1.0 | 0 | 38.1 ± 4.7 |
| 2.0 | 2.0 | 0 | 0 |

of cytokinin to auxin was obtained in callus cultures of Aegle marmelos as well (Arya et al. 1981). The present findings show the potential for the production of

large numbers of plants through rapid shoot proliferation in culture followed by the rooting of individual cultured shoots *in vitro*.

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