Proc. Indian natn. Sci. Acad. **B52** No. 4 pp 505–507 (1986)

In vitro Regeneration of Plantlets from Leaf Tissue of *Ficus religiosa* L.

PRATAP NARAYAN and V S JAISWAL
Department of Botany, Banaras Hindu University, Varanasi 221005

(Received 25 April 1986; after revision 14 May 1986)

Plantlets were produced directly from leaf explants of *Ficus religiosa* L., on Murashige and Skoog's medium supplemented with 2.0 mg l^{-1} BAP in combination with 1.0 mg l^{-1} NAA. NAA alone at 0.5 to 1.0 mg l^{-1} induced only root formation. 2,4-D inhibited organogenesis.

Key Words: *Ficus religiosa* L., Leaf explant, Plantlet regeneration

Introduction

There is an urgent need for a large number of improved fast-growing trees of short rotation to meet the immediate demand for wood and wood products. The techniques of cell, tissue and organ culture are an alternative by which several plant species can be produced in multiples in a short duration (Vasil 1980). By using various parts of the plant, a few hard wood tree species have been propagated successfully through tissue culture. Gupta et al. 1980, (Sommer & Caldas 1981, Brown & Sommer 1982, Jones 1983, Bajaj 1986).

Materials and Methods

Immature leaves (5 × 2 cm) were taken from young branches of an approximately 20 years old tree of *Ficus religiosa* 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 leaves were surface sterilized with 0.1% (w/v) mercuric chloride solution for 5 min followed by thorough washing with double distilled water. The sterile leaves were punched with a sterile cork borer (5 mm diameter), and lamina explants, were inoculated on agarized MS-medium (Murashige & Skoog 1982) supplemented with various concentrations and combinations of benzylaminopurine (BAP), naphthaleneacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D). The cultures were maintained at 25 ± 2°C with 12 hr illumination at about 4000 lx. For each treatment 24 cultures were raised, and all the experiments were repeated at least thrice.

Results

Lamina explants cultured on MS-medium supplemented with 0.5 mg l^{-1} NAA produced numerous root initials with delicate root hairs (6–10 per explant) in about 50% cultures directly from their wounded margins after 3 weeks of culture (figure 1). These root initials attained a length of 1.0 to 2.0 cm after about 5 weeks. However, the medium containing 0.5 mg l^{-1} 2,4-D suppressed differentiation of organs, and initiated callus production from all over the surface of explants.

On medium supplemented with BAP (0.05 to 2.0 mg l^{-1}), the explants showed enlargement and scanty callusing from wounded margin but, later,

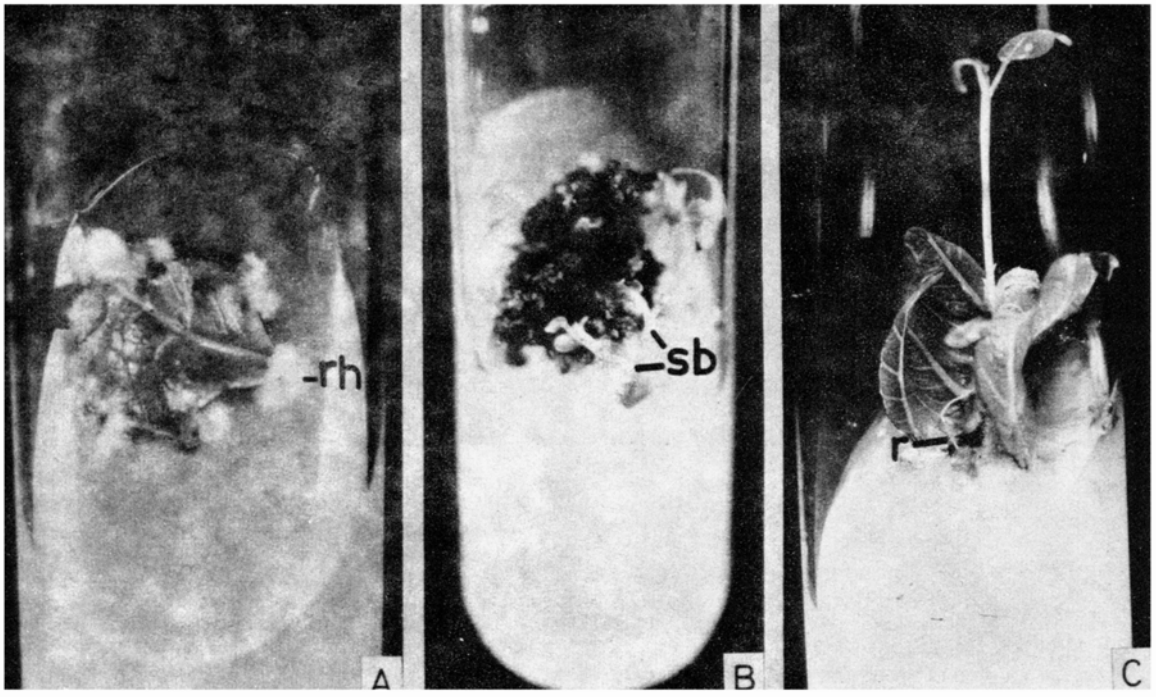

Figure 1 A–C Regeneration of plantlets from leaf explants of *Ficus religiosa;* **A**, Induction of numerous root initials with delicate root hairs from the wounded margin of lamina explant MS + NAA (0.5 mg l^{-1}). Three week-old culture. × 2.0; **B**, Production of shoot buds as well as callus proliferation from lamina explant on MS + BAP (1.0 mg l^{-1}) + NAA (0.5 mg l^{-1}). Six-week-old culture. (× 1.6); **C**, Direct regeneration of plantlet from the wounded margin of lamina explant on MS + BAP (2.0 mg l^{-1}) + NAA (1.0 mg l^{-1}). Ten-week-old culture. (× 2.0)
rh–root hair, *r*–root, *sb*–shoot bud

turned brown. The explants cultured on medium supplemented with 1.0 mg l^{-1} BAP in combination with 0.5 mg l^{-1} NAA showed differentiation of 5-8 shoot buds per explant, alongwith callusing, in about 50% cultures. The first morphological sign of differentiation was noticed after 2 weeks of inoculation with appearance of minute protuberances from the wounded margin of explants. These turned dark-green and developed into small shoot buds after 6 weeks of culture (figure 1B). If the concentration of BAP was raised to 2.0 ppm, and that of NAA to 1.0 ppm, the explant expanded slightly and developed shoot buds from the wounded margins after 7 weeks of culture. Roots were also produced in such cultures from the base of shoots resulting in complete plantlets (1–4) which attained a length of 3–4 cm within 10 weeks of culture (figure 1C). At the higher concentration of BAP (3.0 mg l^{-1}), in combination with higher concentration of NAA (2.0 mg l^{-1}), organogenesis was inhibited.

Twelve week old *in vitro* raised plantlets were transferred to pots containing a mixture of sterile soil, sand and compost (3:3:1). Potted plantlets were incubated at 25 ± 2°C under fluorescent light (16 hr photoperiod), covered with glass beakers to maintain high humidity around the plants. Pots were suitably watered from time to time. About 70% plantlets survived and started to produce new leaves after about 1 week. These plantlets were transferred to green house after about 6–7 weeks where they developed into healthy plants.

Conclusion

The production of numerous roots from wounded margin of explants, in presence of NAA in the medium, is comparable to a similar response of NAA in the leaf cultures of *Morus alba* (Oka & Ohyama 1981). BAP alone is ineffective in inducing shoot buds and requires the presence of NAA in *F. religiosa*. These results suggest the existence of some specific potential for producing buds as well as complete plantlet from leaf explants exposed to different levels of BAP + NAA. On the basis of differentiation of plantlets from the leaf of adult plant, it is suggested that the root and shoot differentiation in *F. religiosa* is the function of quantitative interaction between NAA and BAP.

Acknowledgements

The authors are thankful to the Head, Department of Botany, Banaras Hindu University, for laboratory facilities and to the Council of Scientific and Industrial Research, New Delhi, for a Research Associateship to one of them (PN).

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