# Rooting of microshoots of *Mangifera indica* L. cv. Amrapali

**Hussain Ara, Uma Jaiswal and V. S. Jaiswal**

Laboratory of Morphogenesis, CAS in Botany, Banaras Hindu University, Varanasi 221 005, India

**A two-step protocol has been developed for *in vitro* rooting of microshoots excised from plantlets obtained from nucellar somatic embryos of Amrapali cultivar of mango. Role of auxins, their concentrations, treatment periods and culture illumination conditions for conferring competence for rooting have been ascertained. Indole-3-butyric acid (IBA) was found to be the most potent among three auxins (IBA, indoleacetic acid and $\alpha$-naphthaleneacetic acid) tested for gaining competence for efficient rooting and root growth. Maximum rooting (89.71%) was found on auxin-free agar-gelled rooting medium after 24 h pulse treatment with 5.0 mg/l IBA in liquid root induction medium in dark. Dark condition favoured root induction and root growth, whereas light incubation (16 h photoperiod with 60 $\mu$E m$^{-2}$ s$^{-1}$ light intensity) was inhibitory.**

AMRAPALI, a compact regular bearing hybrid scion cultivar of *Mangifera indica* L. (family Anacardiaceae) resulting from controlled pollinations between 'Neelum' and 'Dashehari'[1] is vegetatively propagated by grafting. This requires rootstocks either from seedling of its own or from other mono/polyembryonic rootstock cultivar. However, *in vitro* rooting of shoots can facilitate direct planting. Despite this, it can also help in achieving the goals of clonal multiplication and generation of homogenous clones. Efficient rooting system from cuttings is an urgent need for exploitation of culture methods at commercial level.

From the field-grown shoots it is very hard to regenerate roots *in vitro* due to phenolic exudation and contamination during culture initiation. These problems are not encountered with shoots derived from *in vitro*-grown cultures. Hence *in vitro*-grown shoots can serve as an easy experimental unit for research in such plants. We describe here the results of experiments for *in vitro* rooting of microshoots (excised from plantlets obtained from somatic embryos originated from nucellus) of Amrapali cultivar. To the best of our knowledge this has been not reported in any cultivar of mango so far.

Immature fruits of Amrapali cultivar ranging in length between 1.0 and 3.5 cm, were collected from Horticultural Garden, Institute of Agricultural Sciences, BHU, Varanasi, India. Following surface sterilization with 1% (v/v) sodium hypochlorite solution, 3% cetrimide solution and 2–3 drops of Tween-20 for 15 min, the nucellus explants were isolated under aseptic conditions. Somatic embryogenesis was induced from these explants by inoculating them on modified Murashige and Skoog[2] (MS) medium consisting of half-strength major salts, full-strength minor and organics, 400 mg/l L-glutamine, 100 mg/l ascorbic acid, 6.0% (w/v) sucrose, 1.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) gelled with 0.8% (w/v) agar for 8–10 weeks in dark[3]. The somatic embryos were matured on 0.8% agar-gelled medium with 6.0% sucrose and converted into plantlets (Figure 1 *a*) in liquid medium with 3.0% sucrose + 1.0 mg/l gibberellic acid ($GA_3$) at 25 ± 2°C in 16 h photoperiod provided by cool white daylight tubes (Philips, 60 $\mu$E m$^{-2}$ s$^{-1}$). The maturation and germination medium contained Gamborg *et al.*[4] (B5) major salts (full-strength for maturation and half-strength for germination) with full-strength MS minor and organics.

In the first step, microshoots (*in vitro*-raised shoots from somatic embryos, Figure 1 *a*) 1.5 to 2.5 cm long, both from apical and nodal regions were removed (Figure 1 *b*) under aseptic conditions, surface sterilized with 0.005% $HgCl_2$ solution for 1–2 min, and dipped individually in about 4 ml liquid root induction medium (RIM) consisting of full-strength B5 major salts with MS minor and organics, 6.0% sucrose and four different concentrations (0.1 to 5.0 mg/l) of indole-3-butyric acid (IBA), indoleacetic acid (IAA) or $\alpha$-naphthaleneacetic acid (NAA), (Sigma), in each test tube (25 × 150 mm). Later on, nine concentrations (0.001 to 20.0 mg/l) of IBA were tested. The control (auxinless RIM) was kept for comparison. The cultures were incubated in dark at 25 ± 2°C for 24 h unless otherwise mentioned.

In the second step, the microshoots from liquid RIM were transferred to auxin-free 0.6% agar-gelled rooting medium (ROM) (Figure 1 *b*) consisting of half-strength B5 major salts with MS minor and organics and sucrose after 24 h. The cultures were maintained either in complete darkness or in 16/8 h light (60 $\mu$E m$^{-2}$ s$^{-1}$) dark cycle at 25 ± 2°C for two weeks to induce rooting. The effect of treatment periods (0, 8, 24, 48 and 72 h) and illumination conditions (darkness and 16/8 light-dark) on rooting and root growth was also studied with 5.0 mg/l IBA in RIM. pH of all the media was adjusted to 5.80 ± 0.05 prior to autoclaving at 108 kPa and 121°C for 15 min. After two weeks of culture, the number of microshoots rooted (for % rooting), root length (mm) and number of roots per microshoots were recorded for each treatment. The values presented are mean ± SD of three independent experiments with 12 microshoots for each treatment. The rooted microshoots were then transferred to 16 h photoperiod (60 $\mu$E m$^{-2}$ s$^{-1}$) after transferring to fresh medium (ROM) for shoot elongation and growth.

One way analysis of variance (ANOVA) was used to analyse the effect of treatments and comparison among specific pairs of treatment was made by the least significant difference (LSD) test at 5% or 1% probability level.

Among the three auxins tested, significant variation was observed in rooting percentage, root growth (measured as root length) and time required for root initiation. The microshoots treated with RIM containing IBA showed root initiation from day 10 onwards (Figure 1 *c*). IBA concentrations were highly significant for rooting ($F = 23.56$; d.f. = 9, 20; LSD = 15.44) at 1% probability level. Per cent rooting gradually increased with increase in IBA concentration in RIM. Maximum (89.71 ± 4.62%) rooting was observed with 5.0 mg/l IBA in dark, whereas in light-incubated cultures only 2.78% microshoots were rooted with the same concentration of IBA. Pulse treatment (24 h) with higher concentration of IBA (more than 5.0 mg/l) caused reduction in rooting percentage as 47.22 ± 10.39% and 41.66 ± 11.79% rooting was found with 10.0 and 20.0 mg/l respectively. Average root length

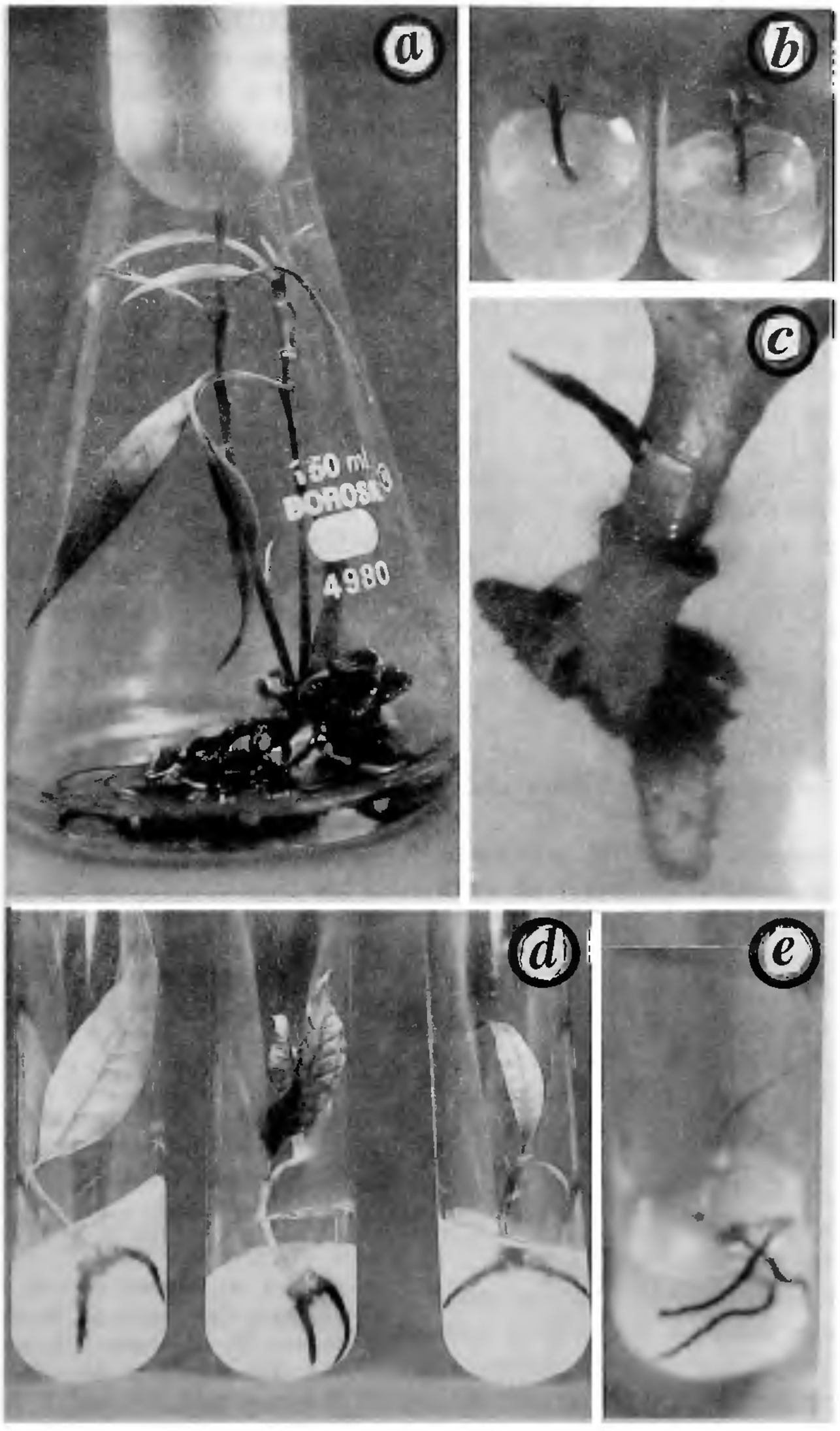


**Figure 1.** Rooting of microshoots of *Mangifera indica* L. cv. Amrupali. *a*, Plantlets regenerated from somatic embryos (×0.86); *b*, Isolated microshoots on rooting medium (×0.80); *c*, Microshoot showing root initiation after 10 days on rooting medium treated with 5.0 mg/l IBA for 24 h (×10); *d*, Rooted microshoots after 12 days showing developing roots (×0.76); *e*, Rooted microshoots after 15 days (×1).

after two weeks was 25.62 mm (ranging between 15 and 40 mm, Figure 1 *d*, *e*) when treated with 5.0 mg/l IBA and comparatively shorter roots (4–20 mm) were produced at lower (0.001 to 1.0 mg/l) and higher (10.0 and 20.0 mg/l) concentrations of IBA. The number of root per microshoot was only one when IBA concentration in RIM was below 0.5 mg/l but varied between 1 and 5 when IBA concentration was 0.5 mg/l or higher. Roots induced from microshoots with IBA were soft, reddish brown to pinkish brown with root hairs and white or yellowish white root tip.

Rooting was also induced with other auxins (IAA and NAA). However, the maximum rooting was only 19.45% with an average root length of 10 mm with 5.0 mg/l IAA after 24 h pulse treatment in dark. Gradual increase in response was observed with the increasing concentrations of IAA (0.1 to 5.0 mg/l) in contrast to NAA. In general, 24 h pulse treatment of microshoots in RIM containing NAA (0.1 to 5.0 mg/l) showed callusing at basal end of microshoots in dark. However, $11.11 \pm 4.82\%$ microshoots rooted with 0.1 mg/l NAA. Comparatively shorter root length (approximately 5 mm) was observed in cultures treated with NAA. Solitary root was observed in all the rooted microshoots induced with IAA or NAA. Roots were smooth, dark brown to black, hard brittle and the time taken for root initiation ranged between 12 and 15 days with IAA or NAA.

Microshoots (5.55%) produced one root each when treated with RIM without any auxin for 24 h. However, the root did not grow beyond 5.0 mm of length. The presence of auxins in ROM or direct culture of microshoots on auxin-containing medium completely suppressed rooting.

Duration of pulse treatment with RIM containing 5.0 mg/l IBA and 6.0% sucrose was observed as a highly significant factor for rooting ($F = 40.40$; $df = 4$, 10; $p < 0.01$) but nonsignificant for root growth ($F = 0.04$; $df = 4$, 10; $p < 0.05$). Microshoots did not root when pulse treatment period was zero hour (i.e. just dipped in RIM and cultured on ROM), whereas increase in pulse treatment period (0 to 24 h) caused enhancement in rooting percentage. Longer treatment periods (48 & 72 h) lowered the percentage of rooting. However, the time taken for root initiation, the number of roots per microshoot and the root length were not significantly affected by varying periods of treatment.

The results clearly demonstrate feasibility of the application of the tissue culture technique for inducing roots from *in vitro* shoots of mango. Variations with respect to per cent rooting, root length, number of roots per microshoot and nature of roots were controlled by auxin type and their concentrations in RIM and periods of induction treatment as well as culture illumination conditions.

Among the three auxins (IBA, IAA and NAA) tested, IBA was found to be highly potent for rooting and root development in mango microshoots as has been reported in cashewnut[5], another member of Anacardiaceae. The maximum percentage of rooting and root growth was observed in cultures treated with IBA followed by IAA and NAA. Presence of NAA induced callusing at explant base. In the present study treatment with auxin and auxin levels that avoided development of callus from shoots were preferred as suggested by Gaspar and Coumans[6]. With the increasing concentration of IBA (0.5 to 20.0 mg/l), the number of roots increased but root length and rooting percentage were significantly decreased at 10.0 and 20.0 mg/l IBA. Such inhibition of root development by very high concentration of auxin may be due to enhancement of ethylene biosynthesis in the root tissues[7].

The two-step procedure proved to be effective for rooting of mango microshoots which was not possible in direct culture of microshoots on rooting medium containing auxin. The two-step rooting procedure has also been reported in teak[8]. The present study suggests that microshoots acquire competence with auxins in specific time during first step and induce rooting on auxin-free rooting medium, whereas continuous presence of auxin suppresses rooting. Transferring to an auxin-free medium was necessary for rooting and root growth in guava[9] and hybrid larch[10], whereas auxin-free medium reduced the rooting frequency in mexican redbud[11]. Dark incubation favoured root initiation and growth. This may be due to alterations of endogenous levels of growth inhibitors and promoters, auxin transport or balance of auxin and inhibitors in cultures incubated in dark and light as reported by Economou and Read[12].

1. Sharma, D. K., *Acta Hortic.*, 1987, **196**, 61–67.
2. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473–497.
3. Litz, R. E., *HortScience*, 1984, **19**, 715–717.
4. Gamborg, O. L., Miller, R. A. and Ojima, K., *Exp. Cell Res.*, 1968, **50**, 151–158.
5. Das, S., Jha, T. B. and Jha, S., *Plant Cell Rep.*, 1996, **15**, 615–619.
6. Gaspar, T. and Coumans, M., in *Cell and Tissue Culture in Forestry* (eds Bonga, J. M. and Durzan, D. J.), Martinus Nijhoff/Dr W. Junk, The Hague, 1987, vol. 2, pp. 202–217.
7. Wareing, P. F. and Phillips, I. D. J., in *Growth and Differentiation in Plants*, Pergamon Press, UK, 1981, 3rd edn.
8. Rathore, T. S., Singh, R. P. and Shekhawat, N. S., *Plant Sci.*, 1991, **79**, 217–222.
9. Amin, M. N. and Jaiswal, V. S., *Plant Cell Tiss. Org. Cult.*, 1987, **9**, 235–243.
10. Brassard, N., Brissette, L., Lord, D. and Laliberte, S., *Plant Cell Tiss. Org. Cult.*, 1996, **44**, 37–44.
11. Mackay, W. A., Tipton, J. L. and Thompson, G. A., *Plant Cell Tiss. Org. Cult.*, 1995, **43**, 295–299.
12. Economou, A. S. and Read, P. E., *J. Am. Soc. Hort. Sci.*, 1986, **111**, 146–149.

ACKNOWLEDGEMENTS. Financial assistance provided by Department of Biotechnology, New Delhi is gratefully acknowledged.

Received 3 July 1997; revised accepted 26 December 1997