

Micropropagation of curry leaf (*Murraya koenigii* L.)

K MARY MATHEW, Y S RAO¹, K PRADIP KUMAR, SALLYKUTTY JOSEPH,
R LAKSHMANAN & K J MADHUSOODANAN

Indian Cardamom Research Institute

Kochi - 682 025, India.

Abstract

Micropropagation of curry leaf (*Murraya koenigii*) was standardized using nodal segments from mature trees on Murashige and Skoog media supplemented with 6-benzylaminopurine, kinetin, ∞ -naphthaleneacetic acid and indole-3-butyric acid as growth regulators. A multiplication ratio of 1:3 was observed over a 5 week culture period on media with benzylaminopurine (1.0 mg l⁻¹), kinetin (0.5 mg l⁻¹) and naphthaleneacetic acid (0.1 mg l⁻¹). The best response of 1-2 roots per shoot was obtained on medium containing naphthaleneacetic acid and indolebutyric acid (0.1 mg l⁻¹ each).

Key words: curry leaf, micropropagation, *Murraya koenigii*.

Abbreviations

- BAP : 6-Benzylaminopurine
IBA : Indole-3-butyric acid
Kin : Kinetin
MS : Murashige and Skoog medium
NAA : α -Naphthaleneacetic acid

Murraya koenigii L., popularly known as curry leaf, is widely cultivated in India, for its aromatic leaves and extensively used for flavouring curries and chutneys. The leaves as well as root and bark are also highly valued in ayurvedic medicine mainly as tonic, sotmachic and carminative. Conventional propagation of curry leaf is through seeds and suckers. No published reports are available on commercially viable methods for vegetative propagation of curry leaf. The present study outlines a

simple and efficient protocol for micropropagation of elite curry leaf genotypes.

Tender shoot segments (2-3 cms) were collected from healthy, elite curry leaf trees; washed under tap water and surface sterilized with 0.06% mercuric chloride for 10-12 min. After repeated rinsing with sterile distilled water, single node sections were dissected and planted on various media combinations. Murashige & Skoog (1962) basal me-

¹Corresponding author

dium supplemented with BAP, Kin and NAA was utilized for culture stabilisation and multiplication. After adjusting pH to 5.7, the medium was solidified with 0.7% agar (bacteriological grade) and autoclaved. For rooting of shoots, MS liquid medium was used at half strength concentration with auxins NAA and IBA. All the cultures were maintained at $25 \pm 1^\circ\text{C}$ under a 16 h photoperiod. Hardening of plantlets was done in sand filled cups under a mist chamber with 80% RH at a temperature not exceeding 30°C .

Nodal segments displayed morphogenic response within 14 days of culture initiation. Latent fungal contamina-

tions occurred in approximately 5% of cultured explants which is low when compared to the high infection rates of several tropical woody species grown *in vitro* (Enjarlic *et al* 1988). Axillary buds developed at an average rate of 1:3 over a 5 week culture period on media with BAP (1.0 mg l^{-1}), Kin (0.5 mg l^{-1}) and NAA (0.1 mg l^{-1}). However, an asynchronous proliferation of adventitious buds from and around the nodal regions was also observed (Fig. 1). These buds were relatively shorter and thicker, ranging from 0.3-0.5 cm in length and could be elongated on the same media but with lower levels of BAP (0.1 mg l^{-1}). In the present observation, a callus phase was

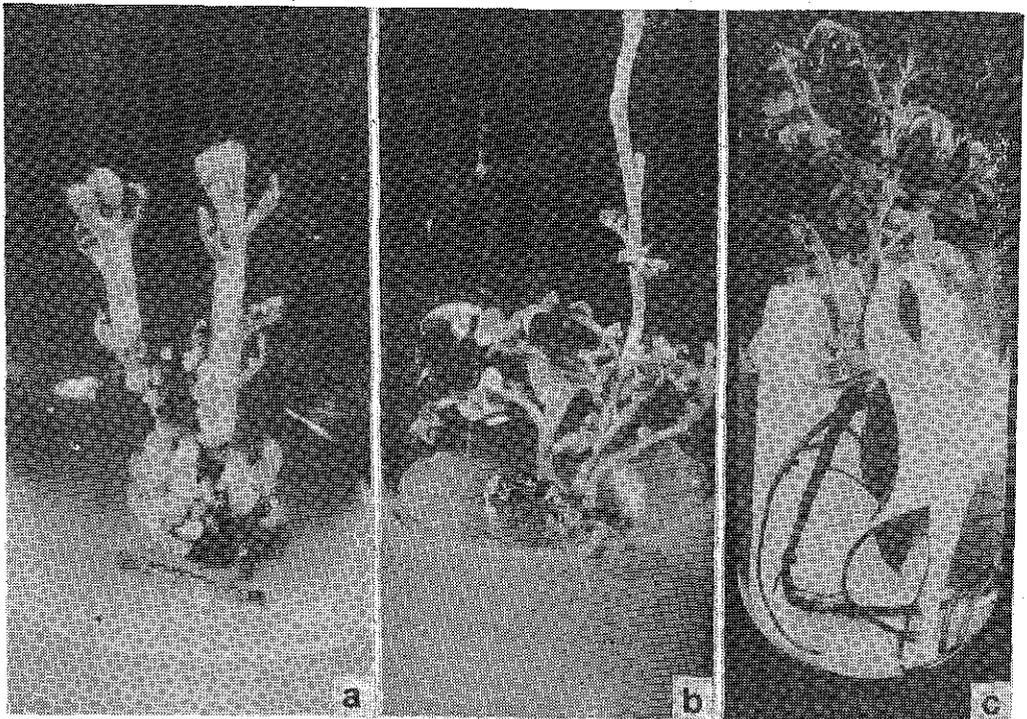


Fig. 1. Micropropagation of curry leaf

- a. Development of adventitious buds on nodal explants b. Formation of multiple shoots
c. *In vitro* rooted plantlet

not detected and such proliferations were completely avoided for obvious reasons. When the BAP content in the medium was reduced to 0.5 mg l^{-1} , proliferations of that kind were not observed. The resultant shoots had an average length of 1.5 cm with 2-3 healthy leaflets (Fig. 2). As suggested by Swartz (1991), it is reasonable and preferable to attempt to grow plants on simple media with lower and balanced hormone combinations and from organized meristems.

The cultures were multiplied and maintained through regular passages at 6-7 weeks intervals which resulted in a three fold increase in the number of shoots produced. Amply developed multiple shoots with 3-4 leaflets were exposed to rooting trials. Rhizogenesis was relatively easy and was observed within 20 days of culture initiation in 60% of shoots. The best response of 1-2 roots per shoot was on medium containing NAA and IBA at 0.1 mg l^{-1} (Fig. 3). The roots attained a length of 2-3 cm with well-developed laterals that enabled the plantlets to successfully adapt to greenhouse environment and a survival percentage of 80% was attained.

Micropropagation techniques for woody perennials have not been as successful as that of herbaceous plants due to reasons such as, low rate of shoot proli-

feration, persistent apical dominance, endogenous contaminants, phenolic exudations and difficulty in rooting, to mention a few. However, curry leaf which is a slow growing woody perennial has not exhibited any of these problems in culture thus indicating the possibility of using tissue culture techniques for its large scale propagation. Since an intermediary callus phase could be avoided in the present study the plants developed can be anticipated to be genetically uniform as that of the mother plant.

References

- Enjarlic F, Carron M P & Lardet L 1988 Contamination of primary cultures in tropical areas : The case of *Hevea brasiliensis*. Acta Hort. 225 : 57-65.
- Murashige T & Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15 : 473-497.
- Swartz H J 1991 Post culture behaviour : Genetic and epigenetic effects and related problems. In : Debergh P C & Zimmerman R H (Eds.) Micropropagation : Technology and Application (pp. 95-121). Kluwer Academic Publishers, The Netherlands.