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Micropropagation of curry leaf tree

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Abstract

Murraya koenigii (curry leaf tree) is cultivated for its aromatic leaves which are used as condiment. Nodal cuttings from mature curry leaf plants cultured in Woody plant basal medium (WPM) supplemented with 4.4 μM benzyladenine (BA) and 4.65 μM kinetin produced 12–30 multiple shoots per node by the eighth week of inoculation. The shoots easily rooted *in vitro* in woody plant medium contained naphthalene acetic acid 1.35 μM NAA. Ninety percent of the plants survived transfer to a hardening chamber and were transferred to the field after three months. *In vitro*-developed shoots were also rooted *ex vitro* by dipping in 2.46 μM indole-3-butyric acid for one minute. They were transplanted to sand in a hardening chamber with 70–80% relative humidity and a temperature of 28±2 °C. Eighty to ninety percent of the *ex vitro*-rooted plants survived and were transferred to the field after 3 months.

Abbreviations: BA – 6-benzyladenine; IBA – indole-3-butyric acid; NAA – ∝-naphthaleneacetic acid; WPM – Woody Plant Medium (McCown et al., 1979)

Introduction

Murraya koenigii (curry leaf tree), belonging to the family Rutaceae, is an aromatic deciduous plant found throughout India. It is cultivated for its aromatic leaves. The leaves of the plant are extensively employed as flavouring in curries and are also a source of vitamins A and C. Infusion of the leaves, roots and bark are stomachic and carminative. The leaves are used for the treatment of gastrointestinal problems. The juice of the roots is taken to relieve pain associated with the kidneys (Anonymous, 1988). The fruit contains the alkaloid koenigin. The volatile oil of *M. koenigii* is toxic to fungi (Pandey and Dubey, 1997). Conventional propagation is by seeds, which germinate under shade. Unfortunately, the seeds retain their viability only for a short period. Clonal propagation by root suckers is another method in the multiplication of elite genotypes.

Micropropagation of curry leaf tree using intact seedling was reported by Bhuyan et al. (1997). It is not entirely known what properties plants propagated from seedlings will have when they reach maturity; we decided to attempt *in vitro* propagation of mature, elite plants of *M. koenigii*. In this paper we report propagation of mature plants by shoot multiplication from nodal explants.

Materials and methods

Preparation of plant materials and multiplication of shoots

Nodal explants were taken from root suckers of a mature, 7-year old curry leaf plant. The explants were first washed using liquid soap and then treated with 0.3% copper oxychloride for 20 min to reduce surface contaminants. Surface sterilization was carried out with 0.1% mercuric chloride for 2–5 min under aseptic conditions and was followed by three washes with sterile distilled water to remove all the traces of mercuric chloride. Finally, they were trimmed to 1 cm nodal segments and inoculated in culture media.

Culture medium and culture conditions

The basal medium used for inoculation was woody plant medium (WPM-McCown et al., 1979) with 3% sucrose (Qualigens, India) and 0.7% agar (bacteriological grade, Hi media, India) and autoclaved at 120 °C for 20 min. The cultures were incubated at 25±2 °C under a 12-h photoperiod of 30 μmol m^{-2} s^{-1} irradiance provided by cool white fluorescent tubes. The basal medium was supplemented with various combinations of growth regulators, 4.44–17.76 μM benzyladenine (BA), 4.65–18.6 μM kinetin, and 2.46–14.7 μM indole-3-butyric acid (IBA). The pH of the medium was adjusted to 5.8 before autoclaving. The multiple shoots that developed were separated and transferred to culture medium of the same composition for further multiplication. The shoots were subcultured at four-week intervals.

Rooting of shoots and transfer of plantlets of soil

The shoots (2–3 cm long) were transferred to rooting medium (WPM+2% sucrose and 0.7% agar) containing 1.35–9.89 μM naphthalene acetic acid (NAA), and 4.9–19.6 μM IBA for rooting. Shoots with well developed roots were transferred to pots after washing in running water to remove agar.

Table 1. Effect of growth regulators on induction of multiple shoots from nodal explants of *M. koenigii*

BA (μM)	Kinetin (μM)	IBA (μM)	% of explants* responding	No. of shoots* after 8 weeks
4.44	0.00	0.00	25±1.56	4±0.12
8.87	0.00	0.00	25±1.36	5±0.13
13.32	0.00	0.00	30±1.45	6±0.13
17.76	0.00	0.00	30±1.82	8±0.13
0.00	4.65	0.00	20±1.62	4±0.18
0.00	9.29	0.00	20±1.63	5±0.14
0.00	13.95	0.00	30±1.53	6±0.13
0.00	18.60	0.00	30±1.26	7±0.11
4.44	4.65	0.00	60±1.32	25±0.11
8.87	4.65	0.00	60±1.53	26±0.13
8.87	9.29	0.00	60±1.44	28±0.12
13.32	4.65	0.00	60±1.62	28±0.14
4.44	4.65	2.46	55±1.23	23±0.11
4.44	4.65	4.90	55±1.21	24±0.13
4.44	4.65	9.80	60±1.27	23±0.10
4.44	4.65	14.70	60±1.11	24±0.13

*Two independent experiments with 10 replicates per treatment. Each replicate contains 5 explants (total 100 explants); data (mean±SE).

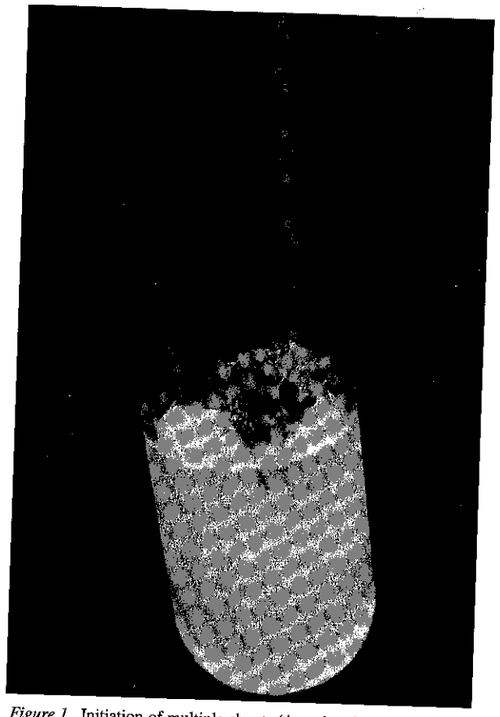

Figure 1. Initiation of multiple shoots (4 weeks after inoculation).

The pots contained sand and potting mixture in 3:1 ratio. Hardening was achieved in a chamber with 70–80% relative humidity and a temperature of 28±2 °C. After 30 days, acclimatized plants were transferred to polythene bags containing garden soil and sand in equal quantity and kept in the shade for another 4–5 weeks before being planted out.

For *ex vitro* rooting, shoots without roots (3–4 cm long) were dipped in 2.46 μM IBA for 1 min and planted in cups containing sand and hardened at 70–80% RH and 28±2 °C.

Results and discussion

BA and kinetin were found to induce multiple shoots in 30% of the cultures at concentrations of 13.32 μM and 13.95 μM respectively (Table 1). But when BA and kinetin were used in combination (each 4.44 μM and 4.65 μM) percent response (60%) doubled. This combination induced 25 harvestable shoots by the eighth week of inoculation (Figures 1 and 2).

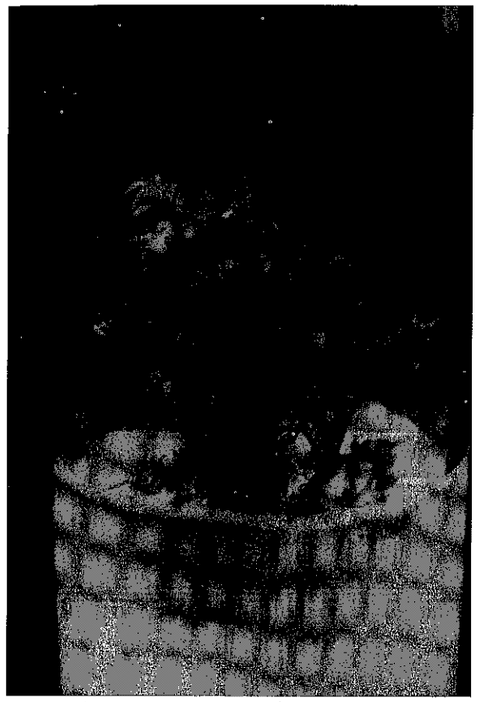

Figure 2. Well developed multiple shoots (8 weeks after inoculation).

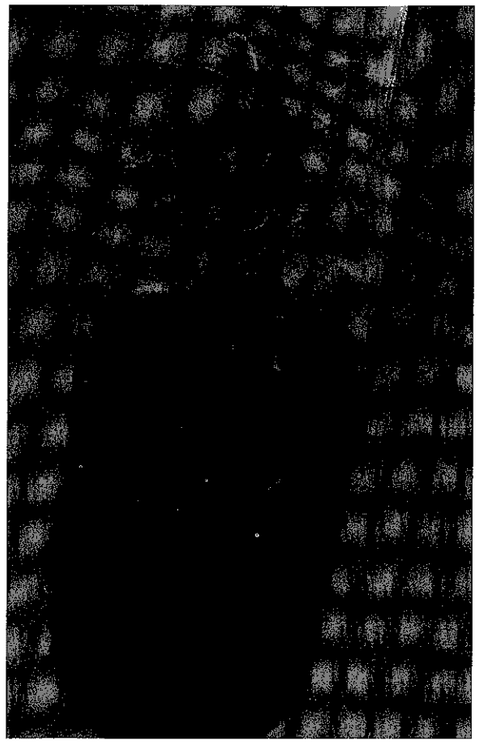

Figure 3. In vitro rooted curry leaf plant (1.35 μM NAA, 6 weeks old).

Table 2. Effect of different auxins on *in vitro* rooting of *M. koenigii*

NAA (μM)	IBA (μM)	% of cultures showing roots*	No. of roots/shoot after 8 weeks*
1.35	0.00	70±1.11	3±0.11
2.69	0.00	70±1.25	3±0.12
5.37	0.00	75±1.23	3±0.13
9.89	0.00	75±1.20	4±0.15
0.00	4.90	25±1.34	1±0.10
0.00	9.80	30±1.45	1±0.12
0.00	14.70	35±1.33	2±0.13
0.00	19.60	40±1.30	2±0.11
5.37	4.90	70±1.32	3±0.11
5.37	9.80	70±1.20	3±0.12
9.89	4.90	75±1.11	3±0.14
5.37	14.70	75±1.31	3±0.15

*Two independent experiments with 10 replicates per treatment. Each replicate contains 5 explants (total 100 explants); data (mean±SE).

For rooting of shoots, the auxins NAA and IBA were tried singly and in combinations (Table 2). NAA produced a small callus at the base of the shoot and tufted roots at higher concentrations. At 1.35 μM, NAA produced 2–3 roots of 6–7 cm length (Figure 3). IBA induced a single taproot, but the percentage of response was low.

In vitro-cultured curry leaf shoots given a pulse treatment with IBA and then planted in sand developed healthy tap roots (Figure 4). The two rooting treatments, *in vitro* and *ex vitro* did not produce significantly different rooting results. Thus this method can be used as an alternative to *in vitro* rooting and reduce the cost of production considerably. A stimulating effect of IBA on rooting of curry leaf cuttings was also reported by Lalitha et al. (1997). *Ex vitro* rooting is preferred in different crops with a view to save time and resources (Meane and Debergh, 1983; Preece and Sutter, 1991).

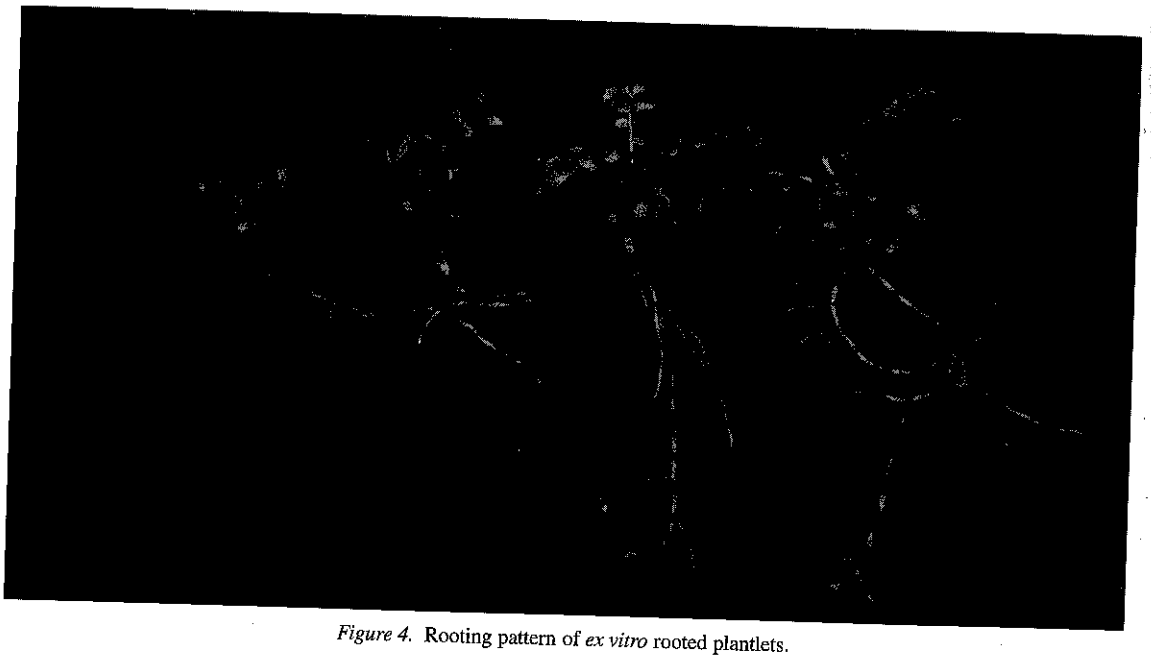

Figure 4. Rooting pattern of *ex vitro* rooted plantlets.

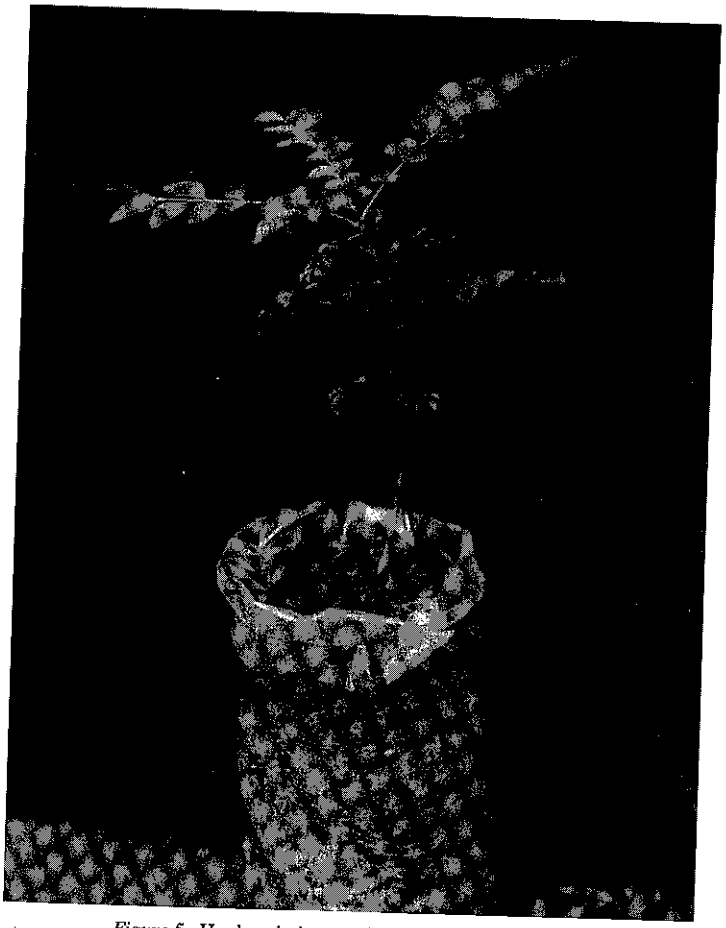

Figure 5. Hardened plant ready for transfer to the field.

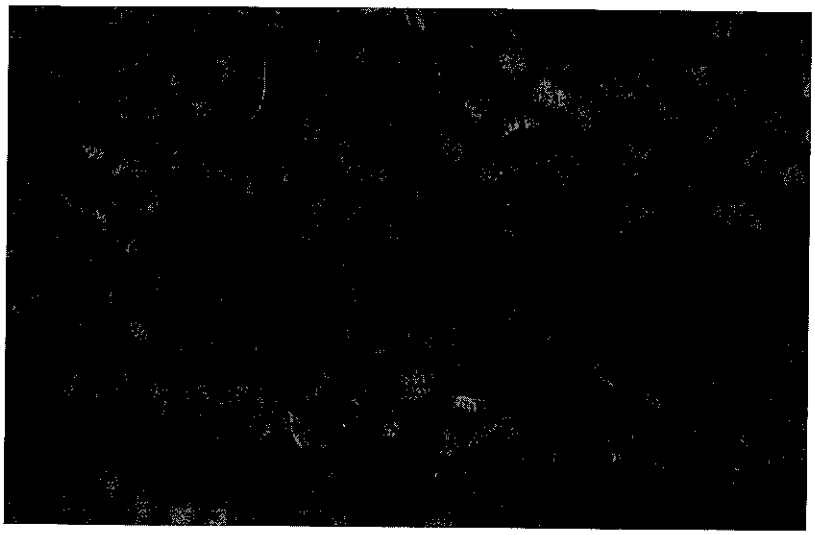

Figure 6. View of the nursery showing hardened curry leaf plants ready for field transfer.

Both *in vitro* and *ex vitro* rooted plantlets were transferred to polythene bags in a shed and kept there for 5–6 weeks before transfer to the field (Figures 5 and 6). 500 plants were hardened in total, 451 of which were transferred to field.

Conclusion

A protocol was developed for micropropagation of curry leaf tree from nodal explants. It potentially can fulfill the demand for uniform planting material of elite trees. The *ex vitro* rooting of shoots grown *in vitro* increases the efficiency of micropropagation and reduces the cost and time required from culture initiation to plant development.

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