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De novo shoot regeneration from root cultures of *Garcinia indica* Choiss

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Roots of plantlets of *Garcinia indica* when cultured for long time on half strength MS medium supplemented with BAP (0.44-2.22 μ*M*) showed production of *de novo* shoots. Roots attached to mother plant showed more number of shoots, while excised root segments produced lesser shoots. Shoots (0.5-0.8 cm) were transferred to elongation medium consisting of Woody Plant Medium (WPM) supplemented with BAP (4.44-22.69 μ*M*), IAA (5.71 μ*M*) and kinetin (4.65 μ*M*). It was observed that shoot length increased to1-2 cm. WPM medium supplemented with NAA (2.69-10.74 μ*M*) and IBA (4.90 μ*M*) induced rooting within 20-25 days. Using the present protocol, 20-25 plantlets could be regenerated from single root explant within 3 to 4 months. The protocol has potential for large scale production of elite plants.

Keywords: *Garcinia indica*, Guttifereae, Roots, Shoots, Organogenesis

Garcinia indica Choiss. belonging to the family Clusiaceae (Guttiferae) is of interest due to presence of hydroxy citric acid (HCA^-) in its fruit rind. HCA exhibits anti-lipidogenic activity in humans by inhibiting the action of citrate cleavage enzyme[1-4] ultimately reducing cholesterol formation. Many antiobesity drug products available in the market contains extract of *Garcinia indica*.

'Kokum butter' prepared from the fruit is a remedy for dysentery and diarrhea. Fruits act as demulcent and emollient[5-6]. Fruit rind extracts also have antifungal and antioxidant properties[7]. Along with this, yellow-pigmented garcinol exerts anti-inflammatory effects and is a neuroprotectant[8]. Finally, the fruit rind of *Garcinia indica* is traditionally used in curry preparation in India.

Garcinia indica plants are distributed in Western Ghats of Maharashtra, India from 0 to 800 meters altitude. It is a polygamodioceous[9], slow growing moderate sized tree with drooping branches[10].

Being polygamodioceous, differentiation between male and female can be made only after flowering which takes approximately 7-8 yr. Because of this cultivation of *Garcinia indica* is limited.

Garcinia indica plants can be propagated by seed germination, shoot cutting and grafting. It has been observed that occasionally mature trees of *G. indica* in nature shows outgrowth of shoots from its roots. This suggests that the root tissue has potential for shoot regeneration. Considering this property of roots of mature plant, following experiment was conducted.

In the present study, an attempt was made to regenerate *de novo* shoots from root explants collected from embryogenically derived plants of *Garcinia indica.*

Materials and Methods

Shoot induction—Somatic embryo derived plantlets (8 months old) served as the explants source[11]. Three experiments were conducted so as to check the effect of mother plant on *de novo* shoot differentiation from root explant. Experiment A (Expt A)-Somatic embryo derived plantlets were incubated for 60 days on defined medium; Experiment B (Expt B)-Roots of somatic embryo derived plantlets showing bulging were separated from mother plant and inoculated on defined medium; and Experiment C (Expt C)-Root segment cut from somatic embryo derived plantlet were used as an explant.

Half strength MS medium[12] supplemented with 6-benzyl amino purine (BAP) (0.44-2.22 μ*M*) containing 2% of sucrose (Himedia, Mumbai, India) and 0.8% of agar (Qualigens, Mumbai, India) were used in all the experiments. *p*H of the medium was adjusted to 5.7-5.8 with 1N NaOH. The growth regulators were incorporated into the medium before

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autoclaving. The medium was autoclaved at 120°C for 20 min at 15 psi. Each treatment was replicated 20 times with 3 sets of experiments. The cultures were incubated at 25° ± 1°C under 16/8 h photoperiod with 35 $\mu E^{-2}m^{-2}s^{-1}$ illumination provided by white cool fluorescent tubes. The frequency of shoot formation on root explants was scored at 15 days intervals for 60 days.

For histological studies, root explants were fixed in glacial acetic acid and absolute ethanol (1:3) for 24 h and then stored at 4°C in 70% ethanol till further use. Fixed tissues were dehydrated through different grades of water:ethanol:tertiary butyl alcohol (TBA) series[13] and then embedded in paraffin wax (melting point 59°-60°C). Serial sections were cut (10 µm), stained with hematoxylene (1% w/v)-eosin (1% w/v), mounted in DPX [2 chloro N (4 methoxy 6 methyl–1,3,5-triazin-2-yl amino carbonyl) benzene sulfonamide] (Qualigens, India) and observed microscopically.

Elongation—Cultures were maintained on the respective medium for 60 days by subculturing in fresh medium at 20 days interval. Regenerated shoots (0.5-1 cm long) height were harvested and transferred to Woody Plant Medium[14] (WPM) containing BAP (4.44-22.69 µ*M*), indole-3-acetic acid (IAA; 5.71 µ*M*) and kinetin (4.65 µ*M*) for elongation.

Rooting—After 45 days, the elongated shoots were transferred for rooting to WPM basal medium along with α-naphthalene acetic acid (NAA; 2.69-10.74 µ*M*) and indole-3-butyric acid (IBA; 4.90 µ*M*). Phytagel (0.25%) was used as gelling agent.

Well-developed plantlets (approximately 4-5 cm) at 4 leaf stage were transferred in pot (7 cm × 8 cm) having mixture of sterilized sand and soil (1:1) with single plantlet per pot and shifted to greenhouse having temperature 25°±2°C with 80% relative humidity for acclimatization. After 3-5 months plants were transferred to pits of size 50 cm^3 and filled with garden soil and farmyard manure (1:1).

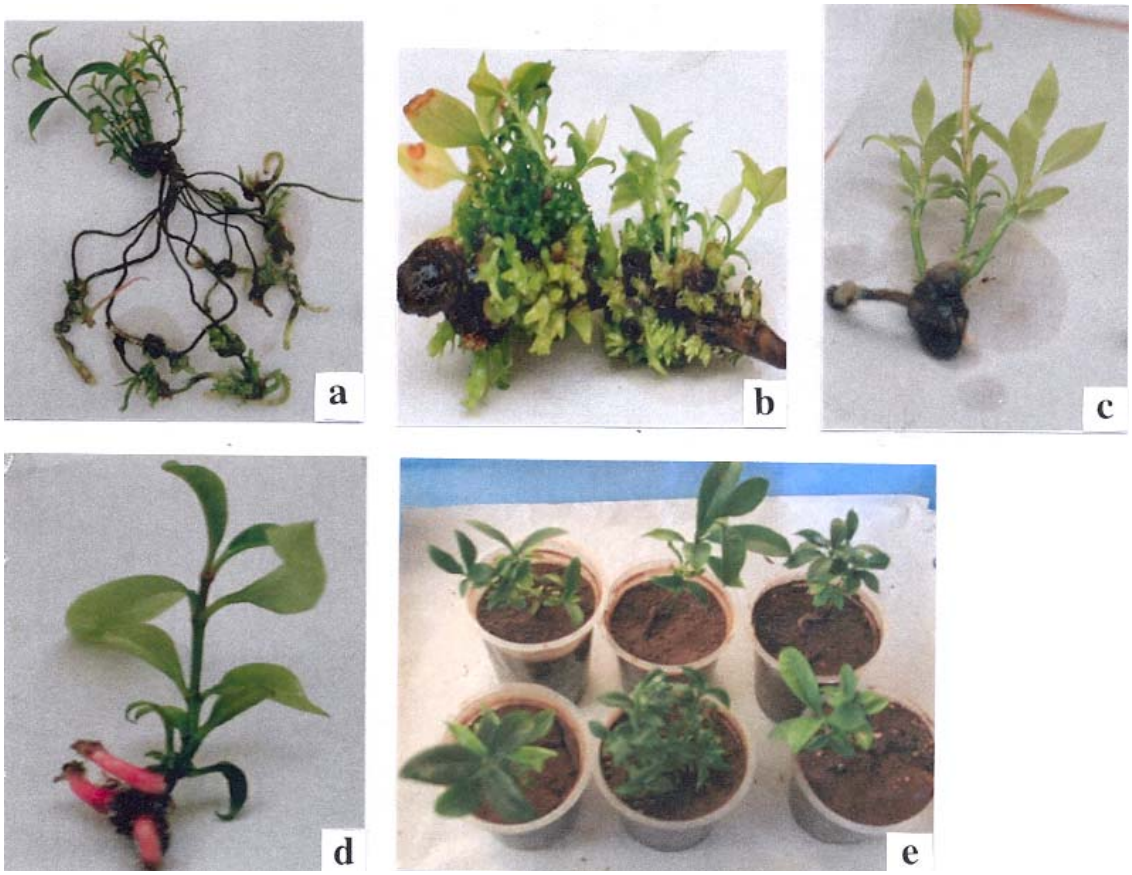

Fig 1—(a and b) Initiation of shoot organogenesis on roots of somatic embryo grown plantlets; (c) Elongated shoots; (d) Rooting of shoots; (e) Hardened plants in greenhouse

Statistical analysis—Data was subjected to ANOVA test by using Agrobase 99 software.

Results and Discussion

In Experiment A and B, shoot regenerated on root explants on half strength MS medium containing BAP (0.44-2.22 μ*M*) after 15-20 days (Fig. 1 a, b). Shoot bud differentiation always occurred at the distal region of roots in bunch of 4 to 20 shoots/bunch. Histological examination indicated that shoot regeneration originated from root cortical tissue (Fig. 2b). Root growth was seen with the initiation of multiple shoot primordial from cortex (Fig. 2c). Some of the cells from cortex became meristematic and produced shoot primordia, which were pushed outside the epidermal layer (Fig. 2d).

In Expt A, concentration of BAP was directly proportional to number of shoots produced per root explant. Maximum number of shoots 29.3 per explant was obtained on medium containing BAP (2.22 μ*M;* Table 1).

In Expt B, reduced concentration of BAP promoted shoot production. Maximum shoots 19.50 per explant

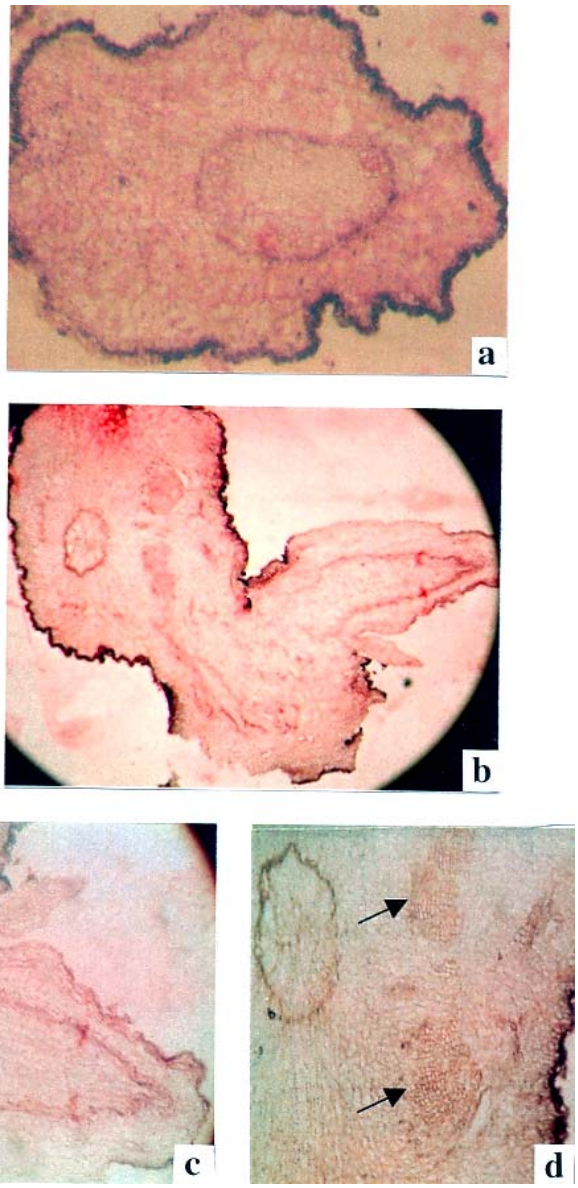

Fig 2—Histological studies- (a) T. S .of a typical root of *Garcinia indica*; (b) Initiation of shoots from cortical region of elongated root; (c) shoot growth from cortical region of root; (d) Section with meristamatic zone at cortical regions of root

Table 1—Number of shoots produced per root after 60 days of incubation on ½ MS basal media containing BAP

MS half strength + BAP (conc.)	Average number of shoots per root		
	Expt A	Expt B	Expt C
0.44 μ*M*	9.75	19.50*	0.35
0.88 μ*M*	21.30	17.14	0.59
1.33 μ*M*	22.76	13.01	1.31
1.78 μ*M*	13.31	9.22	0.88
2.22 μ*M*	29.27**	10.88	0.32
LSD <0.05	10.44	10.48	10.48

Basal medium:1/2 strength MS + sucrose (2%) + agar (0.85%)
Significant at *5% and **1% level

Table 2—Effect of supplementation of different growth hormone in the medium* on elongation of shoots
[Values are mean ± SE of 3 experiments]

BAP (μ*M*)	IAA (μ*M*)	Kinetin (μ*M*)	Mean length of shoots (in cm)
4.44	5.71	4.65	1.59 ± 1.22
8.87	,,	,,	1.04 ± 1.23
13.30	,,	,,	2.15 ± 1.10
17.80	,,	,,	0.79 ± 1.91
22.19	,,	,,	2.89 ± 0.98

*Basal medium: WPM + sucrose (2%) + agar (0.85%)

Table 3—Effect of auxin composition in the medium on rooting
[Values are mean ± SE of 3 Wxperiments]

NAA (μM)	IBA (μM)	% rooting
2.69	4.90	35 ± 1.35
5.37	,,	77 ± 1.06
10.74	,,	23 ± 1.83

Basal medium: WPM + sucrose (2%) + Phytagel (0.25%)

were observed in the medium containing BAP (0.44 μ*M*) while higher concentration of BAP had adverse effect. However, in Expt C, 1-2 shoots per explant were observed irrespective of BAP concentration (Table 1).

In all the experiments increase in shoot numbers were observed till 60 days of culture on all medium having different combinations of growth hormones. After 60 days shoots height ranged from 0.5-0.8 cm. However, the shoots induced in Expt A were superior than Expt B. All the shoots produced on excised root segments turned brown after 40-45 days.

Shoots from 60 days old cultures measuring about 0.5-1 cm were transferred to elongation medium containing WPM basal medium + BAP (4.44-22.19 μ*M*) + IAA (5.71 μ*M*) + kinetin (4.65 μ*M*). Maximum shoot elongation (2.9 cm) was observed on BAP (22.19 μ*M*) (Table 2; Fig 1c). Such elongated shoots were shifted to rooting medium after 45 days of incubation. Maximum rooting (77%) was obtained on the medium supplemented with NAA (5.37 μ*M*) and IBA (4.90 μ*M*; Table 3) in 20-25 days (Fig. 1d).

The plantlets transferred to pots in greenhouse showed 90% survival (Fig. 1e), while under open field in pits 72% of plants survived (Fig. 1e).

Shoots developed from root segments of same plant have been suggested to be genetically uniform[15]. Moreover, root explants are advantageous over the other explants in terms of their easy manipulation and high regeneration potentials[16]. In *Brassica* species shoot differentiation has been reported from root explants, seedling root explants and isolated root segments[17]. In *Citrus aurantifolia*[18] root developed from nodal cuttings shows shoot differentiation on liquid medium. In most of these studies physiological status of mother explants influenced the morphogenetic response.

In vitro plant regeneration using root segments of *Solanum melongena*[19] has been reported. Method for large-scale propagation of *Garcinia indica* by somatic embryogenesis has already been developed[11]. A protocol for *in vitro* grafting for restoring rooting competence in *G. indica* has been standardized[20], while with the use of seed segments as explant direct shoot proliferation has been obtained[21].

In the present study, addition of BAP in the medium showed bud differentiation from root explants. Similar effect of BAP on bud differentiation from root explants has been observed in many species like *Citrus mitis*[22], *Populas tramula*[23].

It appears that in addition to external supply of cytokinines some other factors supplied by mother plant are responsible for shift in the morphogenetic pattern from root to shoot. The effect seems to be regulated by intrinsic factors, which may get produced in the tissue during prolonged culturing on the medium[24].

In conclusion, the study showed that root explants of *Garcinia indica* could be efficiently used for direct shoot regeneration and large-scale multiplication of this important tree species. Genetic uniformity of parent plant material and *in vitro* regenerated plants needs further investigation. Results also showed that type of explant and concentration of cytokines in the medium were crucial for plant regeneration from root explants. Using this protocol, 20-25 plants/root could be produced within 3 to 4 months.

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