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In vitro propagation of emetic nut *Randia dumetorum* (Lamb.)

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An efficient protocol for *in vitro* shoot multiplication of *Randia dumetorum* (Emetic nut) has been developed. The seeds of *R. dumetorum* were germinated *in vitro* in MS medium in 5 weeks. Subsequent propagation using shoot tip as an explant was carried out in MS medium along with different concentrations and combinations of BAP (0.5-2.0) and NAA (0.0-2.0). Maximum shoot multiplication was obtained (12.7 shoots per shoot tip) in MS medium containing 1mg/L BAP and 1mg/L NAA. Micropropagated shoots were rooted in ½ MS medium supplemented with 1 mg/l IBA. This is the first report of *in vitro* plant propagation of *R. dumetorum*. *In vitro* grown plantlets showed a survival rate of 70% after 2 months of transplantation to natural environment.

Keywords: Endangered species, *In vitro* propagation, *Randia dumetorum*, Shoot tip, Tribes

Randia dumetorum Lamb, (Emetic Nut; Rubiaceae) is a thorny shrub or small tree. In Bangladesh it is mainly distributed in the humid and temperate climatic zones of hilly regions. Plants are mainly used for medicinal purpose. The fruit contain a number of neutral and acid di- and tri-terpenoids, saponin, glycosides, essential oils and acid resin. The fruit are used as emetic, diaphoretic, antispasmodic and are also effective in bronchitis and asthma. The seeds contain lead and bark contains two coumarin glycosides. The bark is sedative, nervine and used to relieve pain of bruises and febrile bone aches. Bark also acts as astringent and is useful in diarrhoea and dysentery[1]. The fruit are also used for washing by the tribes[2]. Indiscriminate collection of this plant from its natural habitat is leading to depletion of its resources and now it has become an endangered species. A rapid *in-vitro* propagation of this species is urgently required for its conservation and to meet the demand. Natural and conventional methods of propagation are very slow. *In vitro* propagation methods of higher species plants have been described[3-7]. It is established that shoot tip or bud of higher plants is suitable for rapid micropropagation[3]. In the present communication a protocol for efficient and reliable *in vitro* propagation of plants through shoot multiplication using seeds of *Randia dumetorum* and then subsequent multiplication using shoot tip culture is reported.

Matured seeds were collected from a naturally growing plant population at hilly forest of Chittagong Hill Tract. Seeds were washed by detergent (Tween 20) and surface sterilized with 0.1% (w/v) aqueous $HgCl_2$ solution for 10 min and then rinsed 5-6 times with sterile distilled water. Seeds were aseptically cultured in 40×150-mm glass bottle containing 25 ml of the semi-solidified MS medium[8-10] supplemented with (mg/l) inositol 100; nicotinic acid 0.5; pyridoxine HCl 0.5; thiamin HCl 0.1; 3% sucrose and 0.8% (w/v) agar without any phyto-hormones. Seed were germinated and the epicotyls from 15 day-old aseptically germinated seedlings were cultured in the same medium to obtain sufficient explants for multiplication. Shoot tips (2-3 cm long) from the *in vitro* grown plantlets were used as explants for multiplication. The shoot tips were cultured in semi-solidified MS basal medium[8] supplemented with different concentrations and combinations of BAP (0.5, 1, 1.5 and 2 mg/l) and NAA (0.0, 0.5, 1, 1.5 and 2 mg/l). One culture was maintained in MS medium without any hormonal supplement (Control). For

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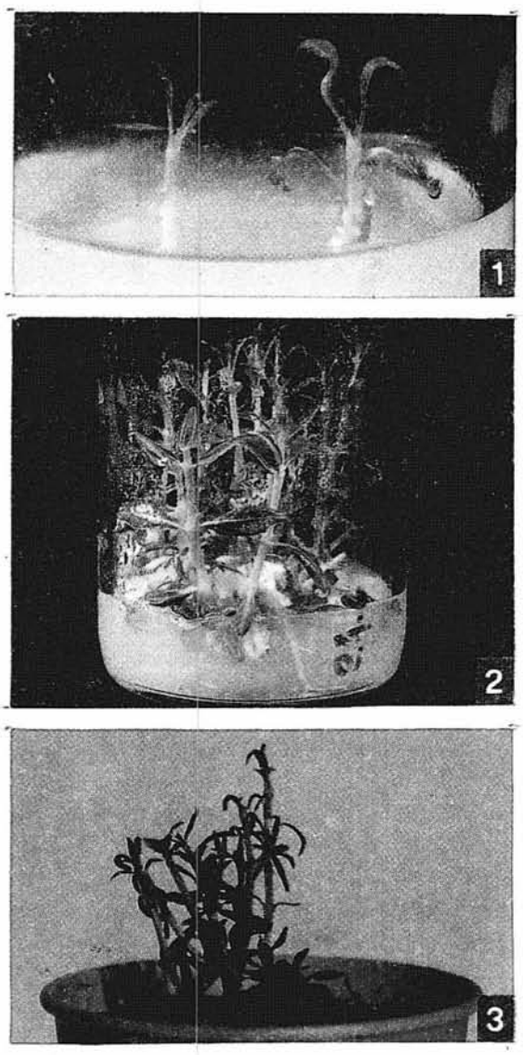
rooting of the multiplied shoots measuring 4-6 cm. long, were sub-cultured in half strength MS medium supplemented with IBA or NAA in different concentrations (0.5, 1, 1.5 and 2 mg/l) with appropriate controls. *p*H of all the media was adjusted to 5.8 using 0.1 *N* HCl or 0.1 *N* NaOH before autoclaving. Media were solidified using 0.8% (w/v) agar. Cultures were incubated under 12 hr photoperiod (cool-white, fluorescent light, 50-μ mol $m^{-2}s^{-1}$) at 25°±2°C with 78% RH. Eight-week-old rooted plantlets were transferred to pots and covered with polythene bags for one week to acclimatize the plantlets to natural environment. Then they were transferred to net house for hardening.

Different experiments were conducted to find out the optimum culture conditions for direct shoot regeneration and subsequent plantlet formation. Mature seeds were germinated *in vitro* in MS medium without any growth regulators. The culture of epicotyls provided sufficient shoot tips to be used as explants for subsequent multiplication (Fig. 1). Among different combinations of cytokinin (BAP) and auxin (NAA) used in MS medium for regeneration from shoot tips, the number of shoots regenerated per explant was maximum 12.7 shoots at 1mg/l BAP with 1mg/l NAA (Fig. 2). In contrast, the number of shoots formed per culture in control was only 2.5. It was also found that higher concentrations of BAP and NAA were less effective on shoot multiplication but produced fast growing callus (Table 1).

The cultured shoot cuttings produced roots at the cut ends without callus formation at the base in MS medium supplemented with different concentrations of IBA or NAA (Table 2). Best rooting (95%) was observed in MS medium supplemented with 1mg/l IBA, whereas in the control medium no rooting was observed. *In vitro* grown plantlets showed a survival rate of 70% after 2 months of transplantation to natural environment. (Table 3; Fig. 3).

The genus Randia (Rubiaceae) has been studied by the various authors. Three new species of South American Randia (Rubiaceae, Gardenieae) have been described[11]. A new triterpenoid saponins, Senen and two ilexoside were isolated from the methanolic extract of the leaves of *R. formos*[12]. Diuretic and urolithiatic activities of the aqueous extract of the fruit of *R. echinocarpa* have also been reported[13]. One new genus and four new species of Randia of eriophyoid from Thailand were described[14]. Scientists have been working on taxonomical, morphological, phylogenitical and phytochemical aspects of *Randia*. The present research attempt is the first work on *R. dumetorum* (Emetic Nut; Rubiaceae) for conservation and rapid propagation through *in vitro* culture.

In the present investigation, it was observed that multiple plant regeneration from the seedlings explants *R. dumetorum* is possible. Direct plant multiplication rate from *in vitro* derived shoot tips depended on proper growth regulator formulation i.e., auxin-cytokinin ratio. However, higher concentrations of



Figs 1-3—(1)— Initiation of shoot tip culture of *R. dumetorum in vitro* on agar solidified MS medium; (2)—Elongation and proliferation of shoots on MS+ 1 mg/l BAP+ 1mg/l NAA after 4 weeks of shoot tip culture and (3)—Hardening of plants in natural environment

Table 1 — Effects of BAP and NAA on direct shoot multiplication rate and shoot length after 4 weeks of shoot tip culture

[Values are mean ± SE from 5 replications in each treatment]

Conc. of growth regulators		Intensity of callus growth	No. of shoots per explants
BAP	NAA		
Control		–	2.5 ± 0.22
0.5	0.0	+	3.1 ± 0.32
0.5	0.5	+	4.0 ± 0.42
0.5	1.0	+	2.9 ± 0.35
0.5	1.5	+	2.56 ± 0.36
0.5	2.0	+	2.1 ± 0.16
1.0	0.0	+	5.2 ± 0.24
1.0	0.5	+	8.4 ± 0.45
1.0	1.0	+	12.7 ± 0.48
1.0	1.5	+	10.3 ± 0.42
1.0	2.0	++	6.8 ± 0.40
1.5	0.0	++	4.9 ± 0.38
1.5	0.5	++	5.2 ± 0.34
1.5	1.0	++	8.5 ± 0.47
1.5	1.5	++	5.0 ± 0.22
1.5	2.0	+++	2.4 ± 0.23
2.0	0.0	++	3.1 ± 0.26
2.0	0.5	++	4.2 ± 0.30
2.0	1.0	+++	4.7 ± 0.33
2.0	1.5	+++	4.4 ± 0.35
2.0	2.0	+++	3.8 ± 0.46

(+) Poor, (++) Moderate, (+++) Massive callus formation
Control = without any hormonal supplement

Table 2 — Effect of auxin (IBA or NAA) on root induction from shoots of *R. domatium* after 4 weeks of culture

[Values are mean ± SE from 5 replications in each treatment]

Auxins	Conc. of growth regulator	% of cutting rooted	No. of roots per cutting	Average length of the root (cm)
	Control	—	—	—
IBA	0.5	65	2.2 ± 0.53	2.4±0.16
	1.0	95	3.1±0.0	2.8±0.40
	1.5	90	3.1±0.22	2.6±0.63
	2.0	82	2.6±0.15	2.5±0.18
	Control	—	—	—
NAA	0.5	57	1.8±0.18	1.9±0.40
	1.0	85	2.2±0.47	3.0±0.16
	1.5	86	2.5±0.12	2.8±0.22
	2.0	89	2.1±0.32	2.4±0.31

(-) no callusing; (+) slight callusing.
Control = without any hormonal supplement

hormonal supplement showed inhibitory effect on shoot formation as they induced rapid callus formation during shoot multiplication. This is the first report that the tissue culture technique is being applied to *R. dumetorum*. This work provides primary information and methodology for rapid propagation, *in vitro* and *ex situ* conservation of this endangered and valuable medicinal plant.

Table 3 — Plants survival rate

Number of plants regenerated	Number of plants survived after transfer to soil	Survival rate (%)
40	28	70.00
44	31	70.45
39	28	71.79
40	28	70.00
42	29	69.04

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