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In vitro plant regeneration of *Coccinea cordifolia* (Linn.) Cogn., an anti-diabetic medicinal plant

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Abstract

An efficient protocol was developed for *in vitro* plant regeneration of a popularly used anti-diabetic medicinal plant, *Coccinea cordifolia* (Linn.) Cogn.(Cucurbitaceae) through direct organogenesis using shoot tip and nodal explants. Best shoot induction was observed on MS basal medium supplemented with 0.5 mg/l BAP, in which 88.2% of nodal explants responded to produce maximum number (6.2 ± 0.58) of shoots per culture. *In vitro* raised shoots rooted on half strength MS medium with 0.5 mg/l IBA. The survival rate of regenerated plantlets was 85%.

Key words : *Coccinea cordifolia,* Medicinal plant, Regeneration, Organogenesis, Shoot proliferation, Mass propagation, Acclimatization

Introduction

Coccinea cordifolia (Linn.) Cogn. commonly known as 'Telakucha' belongs to the family - Cucurbitaceae, a luxuriantly growing tendril climber with cordate, broadly dentate leaves, unisexual funnel-shaped white flowers and smooth oblong fruits, grows commonly in jungles and on hedges all over Bangladesh (Ghani, 2003). Various parts of the plant posses hypoglycemic properties, Ethanolic and aqueous extracts of sun-dried and de-fatted root powder and orally hypoglycemic in rabbits; comparable to tolbutamide and popularly used in the treatment of diabetes; leaves are useful in diabetes in human patients; infusion of the plant parts is also used in anoerexia, epilepsy, catarrh, asthma, fever, dropsy and gonorrhoea (Anonymous, 1989; Ghani, 2003). Leaves are externally used in skin eruptions; plant juice cures ear pain (Ghani, 2003).

In recent years, there has been an increased interest in *in vitro* culture techniques which offer a viable tool for mass multiplication and germplasm conservation of rare, endangered and threatened medicinal plants (Ajithkumar and Seeni 1998; Prakash *et al.* 1999). Commercial exploitation and elimination of natural habits consequent to urbanization has led to gradual extinction of several medicinal plants. Micropropagation is an effective approach to conserve such germplasm. *In vitro* propagation has proven as a potential technology for mass scale production of medicinal plant species (Hassan and Roy, 2005; Lui and Li, 2001; Martin 2002, 2003). It is important, therefore, to develop an efficient micropropagation technique for *Coccinea cordifolia* (Linn.) Cogn. for rapidly disseminate superior clones. There is no report on the establishment of micropropagation protocol for *Coccinea cordifolia* (Linn.) Cogn. using shoot tip and nodal explants. The present study was therefore, undertaken to develop a protocol for *in vitro* plant regeneration of a popularly used anti-diabetic medicinal plant, *Coccinea cordifolia* (Linn.) Cogn. through direct organogenesis using shoot tip and nodal explants.

Materials and Methods

Coccinea cordifolia (Linn.) Cogn. grown at Medicinal Plants Garden of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, was used as a source of explants. Shoot tip and nodal explants with a single axillary bud were used for this purpose. The explants were washed thoroughly under running tap water, pre-soaked in liquid detergent for about 30 min, wiped with cotton and dipped in 70% (v/v) ethanol for 1 min. They were then surface-sterilized with 0.1% (w/v) mercuric chloride for 5 min, followed by five times rinse with sterile distilled water under laminar air flow cabinet. The surface-sterilized explants were sized to 1-1.5 cm length containing a single node with an axillary bud or a

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shoot tip with an apical bud. The explants were placed vertically on the culture medium. The new shoots induced from the *in vitro* cultures were further used as an explants for adventitious shoot regeneration.

MS (Murashige and Skoog 1962) basal medium was used for shoot proliferation and adventitious shoot regeneration and half strength MS was used for *in vitro* root induction. All media were supplemented with 30 g/l sucrose, 7 g/l agar (Difco) and dispensed into 15x150 mm culture tubes and 250 ml conical flasks. The pH of the media was adjusted to 5.8 before autoclaving at 1.9 kg/cm^2 pressure at 121°C for 20 min. The cultures were incubated for a 16 h photoperiod at 24 ± 2°C under 1200 lux/m^2 fluorescent light.

Shoot proliferation from shoot tip and nodal explants was obtained in two separate sets of experiments. In the first experiment 0.1-2.0 mg/l BAP and 0.1-2.0 mg/l Kn were incorporated into MS media to select the best cytokinin for the response of shoot induction. In the second set, combination of BAP (0.5-2.0 mg/l) with NAA (0.1-0.5 mg/l) and BAP (0.5-2.0 mg/l) with IAA (0.1-0.5 mg/l) were assessed for shoot multiplication. Number of new shoot proliferation of each culture was recorded after every week of inoculation.

For *in vitro* rooting, individual shoots (3-5 cm) were excised from the proliferated shoot cultures and implanted on half strength MS media with different concentrations and combinations of NAA, IBA and IAA.

The rooted plants were taken out from the culture tubes, washed to remove agar gel adhered to the roots and transplanted to plastic pots with soil and compost (1: 1) for hardening. The plantlets were kept in a polychamber at 80% relative humidity, 32 ± 2°C temperatures for a 12 h photoperiod under 1500 lux/m^2 sun light for acclimation. Established plants were transplanted in earthen pots under natural conditions and the survival rate was recorded.

Results and Discussion

Shoot tip and nodal explants of *Coccinea cordifolia* (Linn.) Cogn. were cultured on MS media supplemented with various concentration of BAP alone and with NAA or IAA for multiple shoot regeneration. The explants were found to be swollen and they produced two to three shoots within three weeks after inoculation (Fig. 1a) on MS media containing BAP alone but the number of shoots increased up to 6.2 ± 0.58 when the explants were cultured in MS with 0.5 mg/l BAP (Table I, Fig. 1b). Both the explants responded in the

Table I: Effect of different growth regulators (BAP, NAA and IAA) in MS on morphogenic response of *Coccinea cordifolia* (Linn.) Cogn. shoot tips and nodal explants

Growth regulators (mg/l)			shoot tips		nodal explants	
BAP	NAA	IAA	% of explants forming shoots	Mean No. of Shoot/explant	% of explants forming shoots	Mean No. of Shoot/explant
0.1			53.4±0.87	2.0±0.77	58.6±1.70	3.4±0.91
0.3			62.6±1.66	3.2±0.63	67.4±0.51	4.6±0.66
0.5			72.4±2.89	4.8± 0.92	88.2±2.80	6.2± 0.58
1.0			63.4±1.57	3.6± 0.45	71.4±2.38	4.6± 0.72
1.5			57.6±2.16	2.4± 0.72	67.6±2.16	3.4± 1.18
2.0			34.8±2.58	1.8±0.76	33.6±1.84	2.4±0.82
0.5	0.1		61.4±2.87	3.2± 0.59	68.6±1.70	4.6± 1.14
1.0	0.2		42.6±0.87	2.6±0.77	43.6±0.51	3.6±0.91
1.5	0.5		28.2±1.66	2.0± 0.63	41.2±2.47	2.4± 0.66
2.0	0.5		22.2±1.96	1.4± 0.45	32.2±0.66	1.8± 0.95
0.5		0.1	48.8±1.77	3.0± 0.39	56.8±2.14	4.2± 0.76
1.0		0.2	26.6±1.66	2.6± 0.65	47.6±2.10	3.2± 0.51
1.5		0.5	21.0±1.14	2.2± 0.45	32.6±1.63	2.4± 0.91
2.0		0.5	18.4±1.96	1.6± 0.76	24.2±2.47	1.8± 0.76

Results are mean ± SE of three experiments with 15 replications.

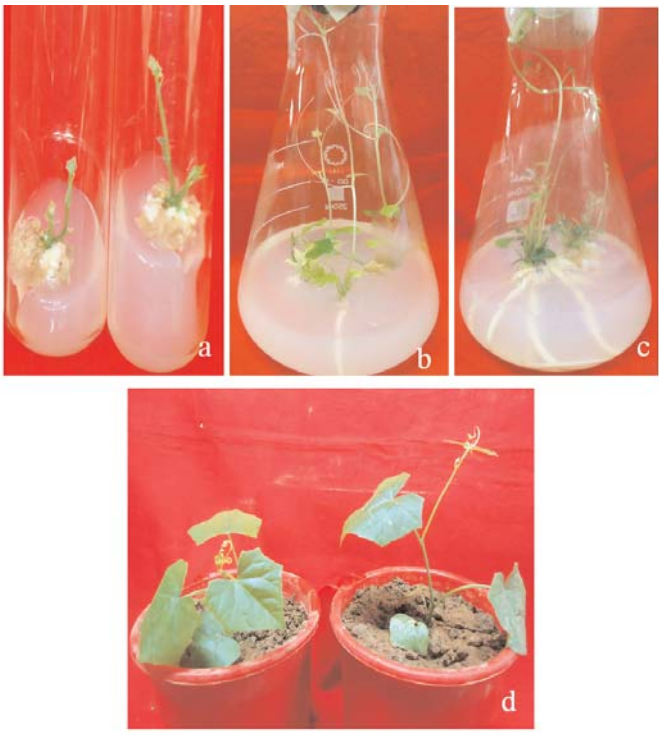

Fig 1: *In vitro* regeneration of *Coccinea cordifolia* (Linn.) Cogn. from nodal explants

(a) Induction of shoots from nodal explants on MS + 0.5 mg/l BAP in three weeks of culture.
(b) Development and multiplication of shoots from nodal explants on MS + 0.5 mg/l BAP after six weeks of culture.
(c) Rooting of *in vitro* regenerated shoots cultured on half strength MS + 0.5 mg/l IBA in third weeks of culture.
(d) Acclimatized regenerated plants of three months old.

same medium but highest numbers of micro shoots were observed to be induced from nodal explants (Fig. 1b). Combinations of BAP with NAA or IAA were not found to be suitable than BAP alone for shoot induction (Table I) and combinations of Kn with NAA or IAA were also not found to be suitable for shoot induction (Data were not shown). In different medicinal plant, it was also observed that multiple shoots were found by using different concentration of cytokinin with auxins by other researchers (Faisal *et al.,* 2003; Mallikadevi *et al.,* 2008; Sahoo and Debata, 1998; Usha *et al.*,2007; Wawrosch *et al.*, 2001).

82.4% regenerated shoots rooted (Fig. 1c) when cultured individually on root induction medium consisted of half-strength MS medium with 0.5 mg/l IBA (Table II). Use of auxins singly or in combination for rooting was also reported by different authors (Baskaran and Jayabalan 2005; Bhadra *et al.,* 2009; Hassan and Khatun, 2010; Sivakumar and Krishnamurthy, 2000).

After four weeks the rooted shoots were transferred to pots. None of the plantlets were survived when directly transferred from rooting medium to the pot under natural conditions. About 85 percent of the transplanted plants of *Coccinea cordifolia* (Linn.) Cogn. survived if the plants in the rooting culture tubes were kept in normal room temperature for seven days before transplantation in pots and reared for three weeks. The plantlets were reared under semi-controlled temperature (30±2ºC) and light (1500 lux) in a chamber with 80 percent humidity. During this period of acclimation shoots elongated, leaves expanded and turned deep green and healthier (Fig. 1d).

After three weeks, plants were transferred to an open place and gradually acclimated to outdoor conditions, where 85 percent plants were survived. The technique described here

Table II : Effect of different auxins (IBA, NAA and IAA) on root induction in regenerated shoots of *Coccinea cordifolia* (Linn.) Cogn. on half strength MS

Growth regulators (mg/l)			% of shoots producing roots (±SE)	No. of roots/shoot (±SE)
BAP	NAA	IAA		
0.5			82.4±1.16	8.8±0.65
0.75			67.2±1.53	6.8±0.65
1.0			63.2±1.46	5.2±0.76
1.5			61.0±0.10	4.6±0.72
	0.5		77.8±1.85	5.2±0.76
	0.75		64.2±1.53	4.0±0.63
	1.0		62.0±0.71	3.8±0.59
	1.5		59.4±1.08	2.2±0.71
		0.5	65.2±1.16	4.8±0.65
		0.75	61.4±0.75	3.6±0.96
		1.0	62.6±0.93	2.6±0.72
		1.5	48.2±0.71	2.2±0.63

Data were recorded after four weeks of culture. Results are mean ± SE of 15 replications.

appears to be readily adaptable for large scale plant regeneration and plantation for sustainable use.

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