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In vitro shoot proliferation and plant regeneration of *Plumbago indica* L. (Ractochita), a rare medicinal shrub of Bangladesh

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Abstract

A protocol was established for *in vitro* shoot proliferation and plant regeneration of a rare medicinal shrub of Bangladesh, *Plumbago indica* L. (Plumbaginaceae) using shoot tip and nodal explants. Best shoot induction was observed on MS medium supplemented with 0.5 mg/l BAP, in which 92% of nodal explants responded to produce maximum number (42.8 ± 1.18) of shoots per explants. *In vitro* raised shoots rooted on half strength of MS medium with 0.5 mg/l IAA. For acclimatization and transplantation, the plantlets in the rooting culture tubes were kept in normal room temperature for 7 days before transplanting them in pots containing soil where plantlets were reared for three weeks. The survival rate of regenerated plantlets was 82%.

Key words : *Plumbago indica,* Medicinal plant, Shoot proliferation, Regeneration, Acclimatization

Introduction

Medicinal plants are important source of traditional and synthetic medicines containing different types of organic compounds having therapeutic properties. Approximately 80% of people in developing countries still rely on traditional medicines for their primary health care. This usually involves the use of plant extracts (Vieira and Skorupa, 1993). Many medicinal plant species are disappearing at an alarming rate, as a result of rapid agricultural and urban development, deforestation and indiscriminate collection. The tissue culture technique is very efficient in rapid mass propagation and conservation of these important medicinal plants (Fay, 1992; Lakshmi and Mythili, 2003; Sagare *et al.,* 2000).

Plumbago indica L. commonly known as 'Ractochita' belongs to the family - Plumbaginaceae, is a rare medicinal herb of Bangladesh (Bhadra *et al.*, 2009). Its root, rootbark and milky juice of whole plant are used for medicinal purposes by the village people particularly of tribal areas as it contains two important alkaloids namely, napthoquinone and plumbagin (Ghani, 2003). Roots are used for procuring abortion and the milky juice is used for treatment of scabies, leucoderma etc. (Anonymous,1989).

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This plant species has become rare in Bangladesh and needs to be propagated rapidly to meet up the medicinal demand and also for conservation purposes (Bhadra *et al.,* 2009). Propagation through seed is very difficult due to poor quality, lower germination rate and less seedling survivability under natural field conditions (Chaplot *et al.,* 2005). 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. It is important, therefore to develop an efficient micropropagation technique for *Plumbago indica* L. for rapidly disseminate superior clones. Many important medicinal herbs throughout the world have been successfully propagated *in vitro* by organogenesis (Chen *et al.,* 2001; Chueh *et al.,* 2001; Erdei *et al.,* 1981; Hatano *et al.,*1986; Hiraoka and Oyanagi, 1988; Huang *et al.,* 2000; Matsumoto *et al.*,1986; Nishioka, 1988; Shoyama *et al.,* 1983; Tsay *et al.,* 1989). There have been few reports to date on *in vitro* mass propagation of *Plumbago indica* L. using shoot tip and nodal

explants (Bhadra *et al.,* 2009; Gopalakrishnan *et al.,* 2009; Kumar and Bhavanandan, 1988; Yogananth and Basu, 2009). Bhadra *et al.* (2009) reported that leaf and nodal segments of two months old field grown seedlings of *Plumbago indica* L. were cultured on agar solidified MS supplemented with different concentrations and combinations of NAA, IAA, 2,4-D and picloram and BAP and Kn; the nodal segments produced either multiple shoot buds or callus of different nature depending on the combinations of plant growth regulators. Gopalakrishnan *et al.* (2009) and Kumar and Bhavanandan (1988) observed that multiple shoots were found by using different concentration of cytokinin with auxins through indirect organogenesis using leaf explants and nodal segments and Yogananth and Basu (2009) observed multiple shoots by using different culture media through direct organogenesis from nodal segments. The present study was therefore undertaken to develop a protocol for *in vitro* shoot proliferation and plant regeneration of rare medicinal herb of Bangladesh, *Plumbago indica* L. using shoot tip and nodal explants.

Materials and Methods

Plumbago indica L. grown at Medicinal Plants Garden of Bangladesh Agricultural University, Mymensingh was used as a source of explants. Shoot tip and nodal explants with a single axillary bud of *Plumbago indica* L. were cultured on MS medium (Murashige and Skoog, 1962) following normal *in vitro* culture procedures (Bhadra *et al.*, 2009; Gopalakrishnan *et al*., 2009; Yogananth and Basu, 2009) for adventitious shoot regeneration. 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^{O}C for 20 min. The cultures were incubated for a 16 h photoperiod at 24 ± 2^O 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 alone were incorporated onto 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 onto 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^O C temperatures for a 12 h photoperiod under 1500 lux/m^2 sun light for acclimation. Established plants were transplanted in earthen pots containing soil under natural conditions and the survival rate was recorded.

Results and Discussion

Shoot tip and nodal explants of *Plumbago indica* L. 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 42.8 ± 1.18 when the explants were cultured in MS with 0.5 mg/l BAP (Table I, Fig. 1b). Both the explants responded in the same medium but highest numbers of micro shoots were observed to be induced from nodal explants (Fig. 1c) on the same medium. 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). Bhadra *et al*. (2009) reported that leaf and nodal segments of two months old field grown seedlings of *Plumbago indica* L. were cultured on agar solidified MS supplemented with different concentrations and combinations of NAA, IAA, 2,4-D and picloram and BAP and Kn; the nodal segments produced either multiple shoot buds or callus of different nature depending on the combinations of plant growth regulators. A similar phenomenon was observed in *Plumbago indica* L. by other researchers (Gopalakrishnan *et al.*, 2009; Kumar and Bhavanandan, 1988; Yogananth and Basu, 2009).

Table I: Effect of growth regulators in MS on morphogenic response of *Plumbago indica* L. 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			66.2±2.87	16.8± 0.59	63.2±0.51	26.4± 0.82
0.3			72.4±1.96	22.4± 0.77	72.6±2.14	34.6± 0.72
0.5			**78.6±2.16**	**36.8± 0.59**	**88.2±1.84**	**42.8± 1.18**
1.0			63.4±1.57	27.6± 0.45	71.4±2.38	31.6± 0.72
1.5			57.6±2.16	22.4± 0.72	67.6±2.16	24.4± 0.66
2.0			34.8±2.58	15.8±0.76	33.6±1.84	19.4±0.82
0.5	0.1		61.4±2.87	26.2± 0.59	68.6±1.70	30.6± 1.14
1.0	0.2		42.6±0.87	15.6±0.77	43.6±0.51	24.6±0.91
1.5	0.5		28.2±1.66	14.0± 0.63	41.2±2.47	18.4± 0.66
2.0	0.5		22.2±1.96	11.4± 0.45	32.2±0.66	12.8± 0.95
0.5		0.1	48.8±1.77	25.0± 0.39	56.8±2.14	28.2± 0.76
1.0		0.2	26.6±1.66	20.2± 0.65	47.6±2.10	22.2± 0.51
1.5		0.5	21.0±1.14	13.4± 0.45	32.6±1.63	16.4± 0.91
2.0		0.5	16.2±0.86	10.4± 0.59	18.4±0.84	11.4± 0.76

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

82.6% regenerated shoots rooted (Fig. 1d) when cultured individually on root induction medium consisted of half-strength MS medium with 0.5 mg/l IAA (Table II). Use of auxins singly or in combination for rooting was also reported by different authors (Gbadamosi and Egunyomi, 2010; Mallikadevi *et al.,* 2008; Hassan and Khatun, 2010; Selvakumar *et al.,* 2001; Rout *et al.,* 1999; Verma *et al.,* 2002).

Table II: Effect of auxin(s) on root induction in regenerated shoots of *Plumbago indica* L. on half strength MS

Growth regulators (mg/l)			% of shoots producing	No. of roots/shoot
IAA	IBA	NAA	roots (±SE)	(±SE)
0.5			**82.6±1.08**	**16.4±0.96**
0.75			77.2±1.16	12.8±0.63
1.0			66.2±1.46	10.2±0.59
1.5			61.0±0.10	08.6±0.72
	0.5		76.8±1.85	06.2±0.76
	0.75		64.2±1.53	05.0±0.63
	1.0		62.0±0.71	04.8±0.65
	1.5		59.4±1.08	03.2±0.71
		0.5	65.2±1.16	06.8±0.65
		0.75	60.4±0.75	05.6±0.96
		1.0	58.6±0.93	04.6±0.72
		1.5	52.2±1.53	03.2±0.71

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

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 *Plumbago indica* L. 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±2^{o}C) 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. 1e).

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

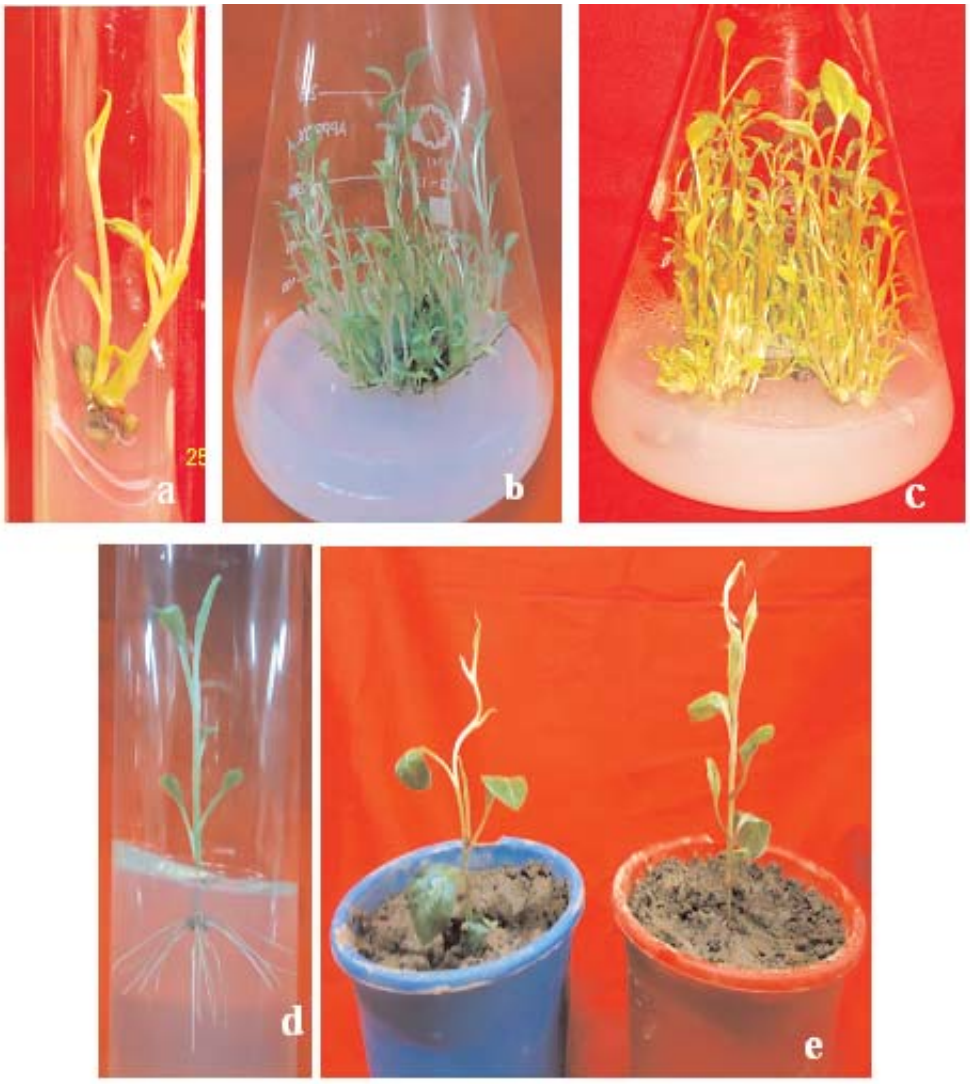

Fig 1: *In vitro* regeneration of *Plumbago indica* L. from nodal explants

(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) Development and multiplication of shoots from nodal explants on MS + 0.5 mg/l BAP after nine weeks of culture.
(d) Rooting of *in vitro* regenerated shoots cultured on half strength MS + 0.5 mg/l IAA in third weeks of culture.
(e) Acclimatized regenerated plants of two months old.

appears to be readily adaptable for large scale clonal propagation and plantation of *Plumbago indica* L. plants.

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