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Callus induction and plant regeneration of *paederia foetida* L., a widely used medicinal vine in Bangladesh

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Abstract

An efficient protocol was established for *in vitro* plant regeneration of *Paederia foetada* L. (Family. Rubiaceae), a widely used medicinal vine of Bangladesh through callus culture in using nodal segment. Yellowish green nodular callus was observed from nodal segments on MS basal medium supplemented with 1.5 mg/L BAP + 0.5 mg/L NAA within three weeks. Large number of shoots (14.4±1.29) were obtained when the callus was sub cultured on MS medium with 0.5 mg/L BAP. *In vitro* raised shoots rooted on half strength MS medium with 0.5 mg/L IBA. The survival rate of plantlets was found to be 85%. Regenerated plants were morphologically uniform having normal leaf shape and growth.

Keywords: : *Paederia foetida;* Medicinal plant; Callus culture; Shoot proliferation; Regeneration; Acclimatization

Introduction

Paederia foetida L. commonly known as 'Gondhabadali' belongs to the family - Rubiaceae, is a perennial climbing vine (Fig.1a) that is used by traditional medicinal practitioners in Bangladesh for treatment of rheumatism, intestinal disorders and liver inflammation (Alam *et al*., 2010). It is also traditionally used for stomach ailments by Garo (Mia *et al.,* 2009) and Santal tribes (Hanif *et al*., 2008) in Bangladesh.

The various plant parts are utilized in traditional medicine of Bangladesh in different ways. Leaf juice is astringent and given to children for treatment of diarrhoea; poultice of leaves are used to relieve distention due to flatulence, in herpes infections and during retention of urine; decoction of leaves are used to dissolve vesicle calculi and acts as diuretic; leaves and roots are also regarded as tonic and stomachache and given to sick and convalescing patients; fruit is specific against toothache (Ghani, 2003). Leaves possess tonic and astringent properties; they are sold in Calcutta markets in a fresh condition and are used in soups and other food preparations for invalids and convalescents, particularly those suffering from bowel complaints (Anonymous, 1966). It is also reported to be used in gout, diarrhea, dysentery, piles, inflammation of the liver and as an emetic (Blatter *et al*.,1981; Nandkoni, 2002). Different pharmacological reports exist for this species which includes anti-inflammatory effect (Srivastava *et al*.,1973; De, *et al*.,1994), relief in gastro-intestinal disorder by helminthic infections (Roychoudhury *et al*.,1970); and antidiarrhoeal effects (Afroz *et al*., 2006). Fresh leaves reportedly have antioxidant properties (Osman *et al*.,2009).

Paederia foetida L., which has great medicinal value is facing danger of extinction; for its conservation, *in vitro* multiplication may prove one of the best technique (Srivastava and Srivastava, 2004). 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 to develop an efficient micropropagation technique for Paederia *foetda* L. to rapidly disseminate superior clones once they are identified. Tissue culture technique can play an important role in the clonal propagation of elite clones and germplasm conservation of this medicinal herb. There have been few reports to date on *in vitro propagation of Paederia foetida* L. (Alam *et al*., 2010; Amin *et al*., 2003; Srivastava and Srivastava, 2004), and these researchers observed that multiple shoots were found by using different concentration of cytokinin

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with auxins through direct organogenesis from shoot tip and nodal explants. But the present study was therefore undertaken to develop a protocol for *in vitro* propagation through indirect organosis by callus culture of this widely used medicinal vine in Bangladesh.

Materials and methods

The experiment was conducted at Biological Research Division of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. Healthy and profusely growing vine of *Paederia foetida* L. was collected from BCSIR Campus, Dhaka and used as source of explants. Shoot tips 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 8 min, followed by five rinses with sterile distilled water in front of a 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 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* rooting. All media were supplemented with 30 g/L sucrose, 7 g/L agar (Difco) and dispensed into 15 x 150 mm culture tubes and 250 mL conical flasks. The pH of the media was adjusted to 5.8 before autoclaving 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 tips 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 to select the best cytokinin for the response of callus and 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 callus induction and 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 with different concentrations and combinations of NAA, IBA and IAA.

Table I. Effect of different concentrations and combinations of growth regulators on MS for the adventitious shoot regeneration from the nodal callus of *Paederia foetida* L.

Growth regulators (mg/l)			% of explants producing callus with shoots (±SE)	Mean no. of shoots/culture (±SE)	Mean length of shoots (cm) (±SE)
BAP	NAA	IAA			
0.1			46.8±2.06	8.8±0.37	3.2±0.20
0.3			72.6±2.09	9.6±0.51	4.6±0.40
0.5			84.2±0.80	14.4±1.29	6.8±0.37
1.0			73.8±1.36	9.4±0.51	4.2±0.20
1.5			66.8±2.06	7.6±0.40	3.6±0.40
2.0			54.4±0.75	5.8±0.37	3.2±0.20
0.5	0.1		61.2±1.02	9.2±1.74	5.2±0.58
1.0	0.2		72.6±2.09	8.6±0.51	3.8±0.37
1.5	0.5		88.2±1.36	7.4±1.74	3.2±0.37
2.0	0.5		76.2±1.66	6.0±0.71	2.6±0.24
0.5		0.1	54.0±1.41	7.4±0.75	3.6±0.37
1.0		0.2	48.8±1.59	6.8±0.37	3.4±0.51
1.5		0.5	43.8±1.28	5.4±0.75	2.6±0.51
2.0		0.5	34.4±1.60	4.8±0.37	2.2±0.37

Data were recorded after two months of inoculation. Results are mean ± SE of three experiments with 15 replications.

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 under a 12 h photoperiod (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

Within seven to fifteen days of culture callus was formed at the cut surface of nodal explant when cultured on MS medium supplemented with 0.5 - 2.0 mg/L BAP either alone or in combination with 0.1- 0.5 mg/L NAA or IAA (Table I). Maximum (88.2%) callus was observed on MS medium with 1.5 mg/L BAP + 0.5 mg/L NAA after three weeks of culture and in this combination yellowish green compact and nodular callus developed (Table I, Fig.1b). Initiation of shoot buds was observed from the same callus when sub cultured on MS + 0.5 mg/L BAP after six weeks (Fig.1c), Shoot differentiation and profuse shoot formation was found to be best from callus on the same medium after ten weeks of culture (Fig.1d). However, BAP with NAA was found to be more effective than BAP alone and BAP with IAA for callus induction (Table I). According to Preece *et al*. (1991), callus forms frequently at the basal cut ends of nodal explants on cytokinin-enriched medium in species exhibiting strong apical dominance.

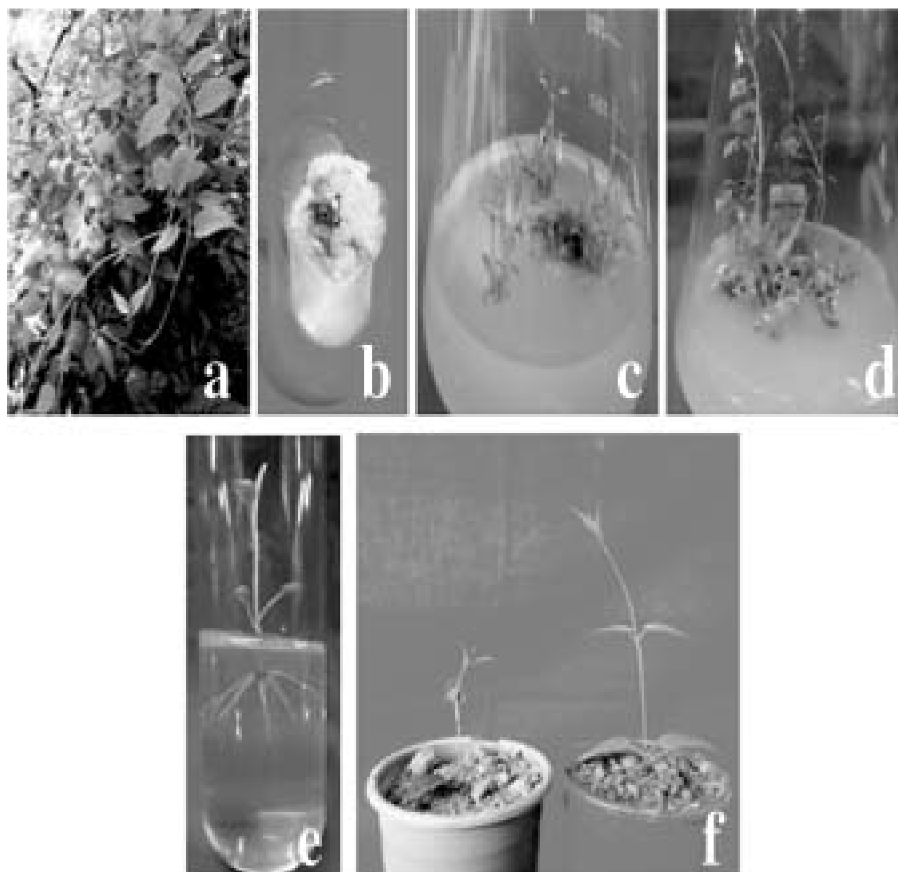

Fig. 1. *In vitro* plant regeneration from callus culture of *Paederia foetada* L.

a. A mature plant from where explants were taken.

b. Yellowish green nodular callus expansion on the MS + 1.5 mg/L BAP + 0.5 mg/L NAA after three weeks of culture.

c. Initiation of shoot buds from the same callus on MS + 0.5 mg/L BAP after six weeks of culture.

d. Shoot differentiation from callus on the same medium after ten weeks of culture.

e. Rooting of *in vitro* regenerated shoots in half strength MS + 0.5 mg/L IBA in eight weeks.

f. Acclimatized regenerated plants of three months old.

The highest number of shoots was 14.4±1.29 per culture and length of shoot was 6.8±0.37 developed on MS with 0.5 mg/L BAP (Table I, Fig.1e). There were differences in regeneration frequencies, number of shoots per culture and length of shoots per culture in different combination. As stated by Martin (2002) the high morphogenic efficiency of nodal segments derived callus may be due to the presence of some internal components from the pre-existing axillary buds that are essential for induction of caulogenesis. Shoot buds developed and elongated from callus culture on the same medium. This continued in two subsequent subcultures made up of identical constituents at an interval of 15 days. Shoot regeneration via a callus phase was the simplest way to induce somaclonal variation and thus pave the way for improvement of the species (Thorpe *et al.*,1991). Such indirect organogenesis was reported in many medicinal plant species including *Abrus precatorius* (Biswas *et al.,* 2007), *Asparagus cooperi* (Ghosh and Sen, 1989), *Ananas comosus* (Akbar *et al*., 2003), *Bixa orellana* (Khan *et al*., 2002), *Gloriosa superba* (Sivakumer *et al*., 2003), *Holostema adakodien* (Martin, 2002), P*hellodendron amurense* (Azad *et al.,* 2005), *Plumbago zeylanica* (Das and Rout, 2002), *Rotula aquatica* (Martin, 2003) and *Scoparia dulcis* (Hassan *et al.,* 2008).

Rooting in regenerated shoots of *Paederia foetida* L. was achieved at 81.2 percent when the excised shoots were cultured individually on root induction medium consisting of half-strength MS medium with 0.5 mg/L IBA (Table II). Roots initiated by third weeks of culture. In this medium the highest percent (81.2) was responded and maximum number (13.6±0.24) of roots and length of roots (3.5±0.22) were formed at the cut end of microshoots within four weeks of culture. Use of auxins singly or in combination for rooting was also reported by different authors (Baskaran and Jayabalan, 2005; Hassan and Roy 2005; Hassan and Khatun, 2010; Sahoo and Chand 1998; Sivakumar and Krishnamurthy, 2000).

After eight weeks the rooted shoots (Fig.1e) were transferred to pots. None of the plantlets survived when directly transferred from rooting medium to the pot under natural conditions. About 85 percent of the transplanted plantlets of *Paedaria foetada* L. survived if the plantlets 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 (2000 lux/m^2) in a chamber with 80 percent humidity. During this period of acclimation shoots elongated, leaves expanded and turned deep green and healthier (Fig.1f).

After three weeks, plants were transferred to an open place and gradually acclimated to outdoor conditions, where 85 percent of plants were survived. The technique described here appears to be a promising method of propagation of *Paedaria foetada* L. As the potentiality of shoot multiplication from callus continued for a long time, regenerates may

Table II. Effect of half strength MS medium with different concentrations of auxin(s) on root formation in regenerated shoots of *Paederia foetida* L.

Growth regulators (mg/l)			% of shoots producing roots (±SE)	No. of roots/shoot (±SE)	Average length (cm) of roots (±SE)
BAP	NAA	IAA			
0.5			81.2±0.86	13.6±0.24	3.5±0.22
0.75			67.2±1.53	11.8±0.37	2.3±0.20
1.0			63.2±1.46	9.2±0.37	2.2±0.29
	0.5		71.0±0.10	9.6±0.51	2.3±0.20
	0.75		57.8±1.85	9.2±0.37	2.2±0.19
	1.0		54.2±1.53	8.0±0.71	2.0±0.20
0.5	0.5		59.4±1.08	8.2±0.37	1.9±0.10
1.0	1.0		52.0±0.71	7.8±0.37	1.6±0.19
0.5		0.5	55.2±1.16	7.8±0.58	1.6±0.16
1.0		1.0	51.4±0.75	6.6±0.51	1.2±0.24
0.5	0.5	0.5	52.6±0.93	6.2±0.73	1.4±0.16
1.0	1.0	1.0	44.4±1.63	5.6±0.51	1.2±0.19

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

be characterized by somaclonal variation. Several species of *Amaranthus* (Bennici *et al.*, 1997), *Ananas comosus* (Akbar *et al*. 2003), *Cuphea* (Millam *et al.*, 1997), *Dubosia* (Lin and Griffin, 1992) and *Salvia* (Liu *et al.*, 2000) produced regenerates through callus-mediated adventitious shoot differentiation. Such regenerates may prove to be a potential source of somaclonal variants, giving birth to traits agronomic importance. The regenerated plants of Paedaria foetada L. are currently being screened for agronomically useful genetic variants.

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