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Callus mediated shoot proliferation from internode explant of *Paederia foetida* L.

Nupur Naik[1], B. R. Behera[1], S. K. Naik and D. P. Barik[Ψ]

Department of Botany, Ravenshaw University, Cuttack-753 003, Odisha, India

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ABSTRACT

The present study describes the establishment of callus culture and subsequent plant regeneration using *in vitro* internode explants of *Paederia foetida.* Four different types of explants were evaluated for callus culture, of which *in vitro* internode explant was found to be the most suitable. MS basal medium supplemented with 15 different combinations of growth regulators were assessed for callus initiation and proliferation. MS supplemented with 1.5 mg/l N^6-benzyladenine (BA) and 0.1 mg/l naphthalene acetic acid (NAA) was found to be the most suitable for establishment of callus culture irrespective of explants used. The highest callus induction (100% and 93.75%) was observed in *in vitro* internode and *in vivo* internode explants respectively. The maximum percentage as well as number of shoot regeneration (33.3%; 2) was observed from the callus derived from *in vitro* internode explants inoculated on MS fortified with 1.0 mg/l BA and 0.5mg/l indole 3-butyric acid (IBA) medium.

1. Introduction

Paederia foetida L. commonly known as Gandhaprasarini (Hindi) and Pasaruni (Odiya), belongs to the family *Rubiaceae*, is an important medicinal plant. It is native to eastern and southern Asia (Wagner *et al.,* 1999). It is also found in India (Himalayas from Dehradun eastwards upto an altitude of 1800m and also in Assam, Bihar, Odisha and Andhra Pradesh), China and Philippine Island. In Peninsular Malaysia, it grows wild in open places, scrambling over trees and bushes.

The plant is well known for its use in Ayurvedic medicines of India (Mishra *et al.,* 2004). It is also widely used for the treatment of asthma, rheumatism, bowel problems, diarrhea, diabetes and seminal weakness (Blatter *et al.,* 1981; Nandkoni, 2002). It is considered as an antispasmodic, diaphoretic, expectoretant and stomachic. The leaves are boiled, mashed and applied to abdomen for urinary retention. The leaves are eaten to aid digestion and to expel gas. Fresh leaves are reported to have antioxidant properties (Osman *et al.,* 2009). Bark decoction is used as emnetic, piles and liver inflammation, decoction of whole plant is used for abdominal pain, abscesses and arthritis. Roots has been claimed to be an amollient and a caraminative. The plant has anti-inflammatory effect (Srivastava *et al.,* 1973; De *et al.,* 1994), relief from gastro-intestinal disorder by helminthic infections (Roychoudhury *et al.,* 1970); antidiarrheal effects (Afroz *et al.,* 2006). The fruit is used for toothache (Ghani, 1998). *Paederia foetida* also contains many useful chemical constituents such as iridoid glucoside, asperuloside, scandoside and peaderoside. The plant also contains alkaloids, b-paederin and essential oils. The leaves and stems contain ursolic acid, epifriedelinol, friedelin, sitosterol, stigmasterol and campestrol. Embelin is isolated from aerial parts. Leaves contain mixture of fatty acids-capric, myristic, arachnidic and palmitic acids (Alam *et al.,* 2010).

Conventional propagation of *Paederia foetida* is primarily through seeds and stem cuttings is inadequate to meet commercial demands. Besides, the plant is also facing danger of extinction (Srivastava and Srivastava, 2004). So, development of an *in vitro* plant regeneration protocol is

[Ψ] *Corresponding author; Email:* barikdp@yahoo.com
[1] Both the authors have equal contribution

essential to conserve the plant. An efficient *in vitro* propagation method may play an important role in rapid multiplication and germplasm conservation of this medicinally important herb. To our knowledge, during the past years, there have been a few reports regarding *in vitro* clonal propagation of *Paederia foetida* L. using different methods of plant tissue culture (Srivastava *et al.,* 1973; Alam *et al.,* 2010). However, an efficient callus mediated organogenesis from internode or leaf has not yet been reported for this plant species. Therefore, our objective was to develop a rapid, effective and reproducible callus mediated plant regeneration protocol for *Paederia foetida* using internode or leaf explant.

2. Materials and methods

Healthy plants were selected from the medicinal plant garden, Department of Botany, Ravenshaw University, Cuttack, Odisha. Young leaves, internodes and nodal explants were collected from healthy plants of *Paederia foetida*. Explants were washed under running tap water for 30 min followed by 8 min treatment with 5% (v/v) aqueous solution of Teepol (Reckitt Benckiser Ltd., HP, India) and 5 min rinse with double distilled water. Then the explants were surface sterilized with 0.1% (w/v) aqueous solution of $HgCl_2$ for 3 min (leaf explants) and 5 min (internode & node explants). Finally, explants were washed thoroughly 5 times with sterilized double distilled water.

Sterilized mature leaf and internode explants (*in vivo* explants) were inoculated on Murashige and Skoog's (1962) (MS) medium alone or MS supplemented with a range of growth regulators i.e. BA (1.0- 4.0 mg/l) + NAA (0.1- 2.0 mg/l) for callus initiation and proliferation. Mature nodal explants were used to develop *in vitro* shoots. These established shoots were used as the explant source for *in vitro* leaf and internode (*in vitro* explants). The *in vitro* leaf and internode explants were inoculated on the same callus initiation and proliferation medium as mentioned earlier. The calli after establishment were repeatedly sub-cultured on the same medium at an interval of 20 days. The pH of all the media were adjusted to 5.8 ± 0.1. Irrespective of media, 0.8% agar (Himedia, India) and 0.3% sucrose were used.

Green, semi hard calli were transferred to MS medium augmented with BA or Kin 1.0-4.0 mg/l and combinations of BA (1.0 mg/l) and IBA (0.1 -1.5 mg/l) for shoot regeneration. *In vitro* generated shoots were excised and transferred to rooting media comprising of one fourth strength MS (¼ MS), half strength MS (½ MS) and MS supplemented with IBA 0.05-2.0 mg/l.

The culture were maintained at 25 ± 1^0C under 16 h photoperiod of 35 µmol/ m^2s^1 photon flux density provided by cool white fluorescent tubes (Philips, India) with relative humidity 55-60%. Photographs were taken on Sony digital (7.2 megapixel) camera. Mean percentage of explants forming callus, mean % of callus showing shoot bud formation, mean number of shoots / callus were calculated. Mean data pooled from a total 4 no of replications each comprising of 4 culture tubes containing 2 explants per tube for callus experiments whereas, for shoot regeneration mean data pooled from a total 3 no of replications each comprising of 5 culture tubes containing 1 explant per tube. For rooting experiments mean data pooled from a total 3 no of replications each comprising of 4 culture tubes containing 1 explant per tube. Data were analyzed using analysis of variance (ANOVA) for a completely randomized design (CRD). Duncan's New Multiple Range Test (DMRT) was used to separate the means for significant effect (Gomez and Gomez, 1984).

3. Results and discussion

MS medium devoid of growth regulators failed to initiate callus. Calli were successfully initiated from irrespective of the explants (*in vivo* and *in vitro* leaf and internode explants) cultured on MS media supplemented with cytokinin (BA) + auxin (NAA). The response of *in vivo* and *in vitro* explants for callus initiation and proliferation was different in different hormonal combination (Table 1). Callus initiation was on the margins of the cut ends of the explants proceeding towards the center. The callus proliferation frequency % was higher in *in vitro* explants than *in vivo* explants. Least days required for callus initiation was recorded as 21 and 18 days for *in vivo* as well as *in vitro* leaf and internode explants respectively. Similar types of observations on different explant types on callus induction were observed in *Tylophora indica* (Thomas, 2007) and *Centella asiatica* (Mohapatra *et al.,* 2008). Different tissues may have different levels of endogenous hormones, and therefore, the type of explants source in most of the cases have a crucial impact on the callus initiation and its regeneration success (Das *et al.,* 2013).

The maximum callus induction frequency was found to be different in different explants type. Optimal medium for callus induction was found to be MS + 1.5 mg/l BA + 0.1 mg/l NAA. Of the four different explants evaluated the *in vitro* internode explants showed maximum (100%) callus induction followed by *in vivo* internode (93.75%) (Fig 1A, B). Both the source of leaf explants (*in vivo* leaf, 84.37 %; *in vitro* leaf, 90.6 %) showed inferior response for callus initiation in compare to internode explants (Table 1). Combination of BA and NAA are also found suitable for callus induction in *Adhatoda vasica* (Azad and Amin, 1998), *Cucurma longa* (Salvi *et al*, 2006), *Ophiorrhiza prostate*

Table1
Effect of various concentrations and combinations of growth regulators for callus formation of different explants of *Peaderia foetida* L.

MS + Growth regulators (mg/l)		% of callus proliferation				Average days for callus initiation			
BA	NAA	LEAF		INTERNODE		LEAF		INTERNODE	
		In vivo	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*
MS		0	0	0	0	0	0	0	0
1.0	0.1	78.12	84.37	90.62	96.87	21	21	18	18
	0.5	81.25	81.25	84.37	87.5	21	21	18	18
	1.0	62.5	59.3	84.37	81.25	38	28	27	18
	2.0	62.5	62.5	81.25	84.37	43	32	32	28
1.5	0.1	84.37	90.6	93.75	100	21	21	18	18
	0.5	78.12	87.5	90.62	96.87	21	21	18	18
2.0	0.1	78.12	81.25	84.37	96.87	28	21	20	18
	0.5	59.37	62.5	81.25	87.5	26	21	21	18
3.0	0.1	59.37	56.25	75	81.25	32	28	28	24
	0.5	53.12	56.25	78.12	87.5	32	32	28	28
4.0	0.1	62.5	68.75	75	71.87	32	32	28	28
	0.5	65.62	68.75	81.25	84.37	32	32	28	28
CD at 5%		1.32	1.21	1.41	1.36				

Mean data pooled from a total 4 no of replications each comprising of 4 culture tubes containing 2 explants per tube (4 x 4 x 2 = 32). CD at 5%, Duncan's New Multiple Range Test

(Beegum *et al,* 2007) and *Rauwolfia serpentine* (Salma *et al*, 2008).

Greenish calli were observed in most of the combination of BA and NAA. However, white friable callus was recorded when a comparatively high concentration of NAA (2.0 mg/l) was used along with 1.0 mg/l BA. Shoot regeneration was found only in green callus. MS basal medium fortified with kinetin resulted in no shoot bud differentiation from callus. Regeneration of only a single shoot was observed in all the concentration of BA. Four to six shoot buds appeared on green calli after 20 days of transfer to the optimum shoot regeneration medium (1.0 mg/ l BA + 0.5 mg/l IBA) (Fig. 1 C). Of the shoot buds only one or two shoot bud(s) elongated to shoots. The highest shoot regeneration (33.3%) with 2 shoots / callus was observed on calli derived from *in vitro* internode explants inoculated on shoot regeneration medium at day 45 of culture (Table 2, Fig. 1 D). Combination of BA and IBA was also recorded to have optimum shoot regeneration capacity in *Aristolochia indica* (Soniya and Sujitha, 2006), *Centella asiatica* (Hossain *et al.,* 2000; Martin, 2004; Mohapatra *et al.,* 2008), *Ocimum basilicum* (Sahoo *et al.,* 1997), and *Vitex trifolia* (Hiregoudar

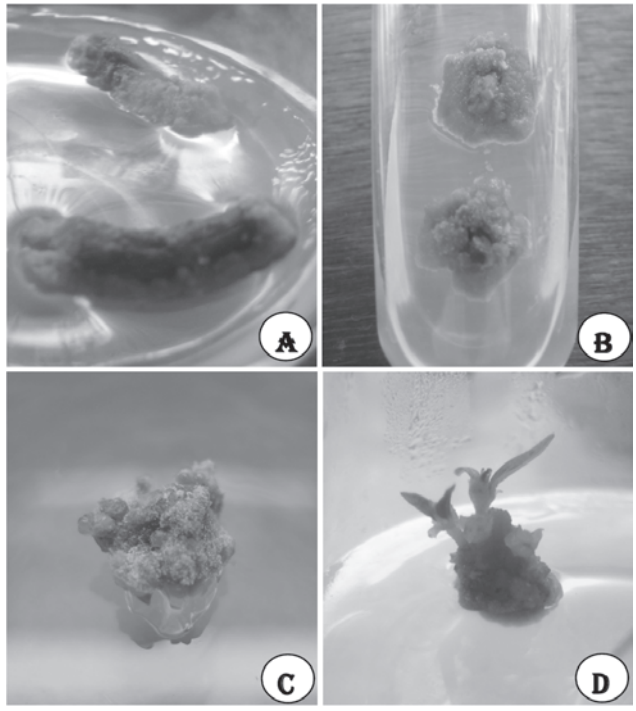

Fig. 1 (A) *In vivo* internode and **(B)** *In vitro* internode derived callus on MS + 1.5 mg/l BA + 0.1 mg/l NAA at day 40 **(C)** Shoot bud formation from *in vitro* internode derived callus on MS + 1.0 mg/ l BA + 0.5 mg/l IBA at day 20 **(D)** Shoot elongation on MS + 1.0 mg/l BA + 0.5 mg/l IBA at day 45.

Table 2

Effect of different concentrations of cytokinin alone or in combination with auxin on shoot regeneration from *in vitro* internode derived callus of *Paederia foetida*.

MS + Growth regulators (mg/l)			Cultures responded (%)	Average number of shoots/callus	Average shoot length (cm)
BA	IBA	Kin			
	MS		-	-	-
1.0	-	-	26.0[e]	1	1.9
2.0	-	-	25.3[e]	1	2.1
3.0	-	-	26.0[e]	1	2.3
4.0	-	-	19.3[f]	1	1.8
1.0	0.1	-	28.6[d]	1	2.1
1.0	0.5	-	33.3[a]	2	2.1
1.0	1.0	-	31.8[b]	2	2.3
1.0	1.5	-	30.3[c]	1.5	2.3
—	—	1.0	—	—	—
—	—	2.0	—	—	—
—	—	3.0	—	—	—
—	—	4.0	—	—	—

— = Nil/ No response

Mean data pooled from a total 3 no of replications each comprising of 5 culture tubes containing 1 explant per tube (3 x 5 x 1 = 15). Mean values within column with same superscripts are not significantly different ($p < 0.05$; Duncan's New Multiple Range Test)

et al., 2006). Rooting experiment is under progress and results of rooting experiment are yet to be documented.

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