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Adventitious Shoot Organogenesis and Plant Regeneration from Internode Explants of *Paederia foetida* L.: A Valuable Medicinal Plant

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A plant regeneration protocol via adventitious shoot organogenesis from internode explants of *Paederia foetida* (Skunk vine) is reported here for the first time. Three explants (leaf, mature internode and internode derived from axenic shoot cultures) were tested for shoot organogenesis. Leaf explants failed to induce adventitious shoots whereas axenic internode explant was found to be superior to mature internode explants for the induction of adventitious shoots. Axenic internode explants cultured on MS medium supplemented with 3.0 mg/l BAP showed maximum (86.7 %; 10.4 shoots per explant) adventitious shoot organogenesis. The regenerated shoots were best rooted (90 %; 14 roots per shoot) on half-strength MS medium. Eighty percent of the rooted shoots were successfully acclimatized in soil: sand (1:1) mixture. All these acclimatized plants were successfully transferred to larger pots containing garden soil and subsequently established in the field.

Keywords: Direct adventitious organogenesis, Medicinal plant, Internode, *Paederia foetida*.

Paederia foetida L. is an extensive twining, herbaceous, perennial vine belonging to family Rubiaceace[1]. The plant is considered to be native of both temperate and tropical Asia, from India to Japan and South East Asia[2,3]. The plant has unique characteristics of foetid smell due to the presence of methyl mercaptan[4,5]. It is well known for its folkloric medicinal values[6,7,8]. Besides, the plant is used as leafy vegetable food[9,10,11,12]. In India mostly the plant is used in dual purpose of food and medicine[8]. In Odisha (India) people take rice with cooked *P. foetida* leaf to cure different aliment including rheumatism and gout[7,8]. In the states of West Bengal and Arunachal Pradesh the leaf is used as juice or soup to treat bowel problems including diarrhoea and dysentery[8]. All most all the parts of the plants including leaf, root, bark and fruit have medicinal value[5,10,13,14]. The leaf of the plant has anti-diarrhoeal, hepatoprotective, anti-arthritic, anti-inflammatory properties. The pharmaceutical importance of *P. foetida* is mainly due to the presence of different secondary metabolites including iridoid glycosides, triterpenoids and β-sitosterol etc[8]. A number of herbal formulations with *P. foetida* as a components are also available in the market of India[15].

Anthropogenic activities including unsustainable harvesting by local vaidyas from wild, deforestation, urbanization, forest fires etc. have posed threats to this medicinally important plant species[16]. Unfortunately, this plant has been enlisted as a vulnerable plant in different states of India including Odisha due to the above mentioned reasons[16,17]. The conventional propagation is through seeds and stem cuttings[18] and is not adequate to meet the demand of the plant. Therefore *in vitro* propagation methods through plant tissue culture can be an alternate way of producing large scale plantlets of this medicinally important plant species. Thus purpose of the present study was

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to develop an efficient *in vitro* plant regeneration system mediated by direct shoot organogenesis from different plant parts of *P. foetida*. To the best of our knowledge, no reports are available on adventitious shoot organogenesis of this vulnerable medicinal plant. Here, we for the first time report an efficient *in vitro* system for inducing direct adventitious shoot from internode explant of *P. foetida*.

MATERIALS AND METHODS

Explant Sources

Young and healthy shoots of a two year old *Paederia foetida* maintained in the Department of Botany, Ravenshaw University were collected. Leaves and internode segments were excised from the young shoots. Both these explants were washed separately under running tap water for about 30 min. They were then washed thoroughly in 5% (v/v) aqueous solution of Teepol (Reckitt Benckiser Ltd. HP, India) for 8 min followed by 5 rinses with double distilled water. The explants were then surface sterilized inside a laminar air flow chamber with 0.1% (w/v) aqueous solution of mercuric chloride ($HgCl_2$, Hi-Media, Mumbai, India) for 4 min (leaf) and 5 min for (internode) followed by 5 rinses with sterile double distilled water. Surface sterilized leaf segments (3 - 4 mm^2) and internode segments (1.5 cm) were used for adventitious shoot regeneration.

Another experiment was carried out to compare the efficacy of axenic internode segments with that of the mature internode for adventitious shoot regeneration. Axenic shoot cultures were established on Murashige and Skoog's (1962)[19] (MS) medium supplemented with 3.0 mg/l N^6-benzylaminopurine (BAP) using mature nodal segments of *P. foetida*. The *in vitro* regenerated primary shoots were cut into single internode pieces (1.0 -1.5 cm) and used as explants. Due to poor response of mature leaves, the axenic leaf segments were not used in this experiment.

Direct Adventitious Shoot Regeneration

All the explants (mature leaf, mature and axenic internode) were cultured on MS medium supplemented with BAP (1.0-5.0 mg/l), Kinetin (KIN; 1.0-5.0 mg/l), Metatopolin (mT; 1.0-5.0 mg/l) and Thidiazuron (TDZ; 0.1-1.0 mg/l) individually. At the same time BAP (1.0-3.5 mg/l) and KIN (1.0-4.0 mg/l) in combination with different concentrations of 1-Naphthalene acetic acid (NAA; 0.1, 0.2, 0.5 mg/l) were also tested to check the influence of NAA, if any, on the shoot regeneration. Further, optimal level of BAP was employed in combination with adenine sulphate (ADS; 25-75 mg/l) for shoot regeneration experiment. Adventitious shoot organogenesis response of different position (abaxial and adaxial side) of leaf in contact with the medium was also studied.

All the culture media were augmented with 30.0 g/l sucrose as carbon source and 0.8 g/l agar as gelling agent. The pH of the media was adjusted to 5.8 ± 0.1 before autoclaving. The culture vessels containing the media were autoclaved at 121°C and 15 pound per square inch pressure for 17 minutes. The cultures were maintained at 25 ± 1°C under 16 h photoperiod.

Rooting and Acclimatization

The healthy elongated shoots were excised and transferred to different rooting medium for development of complete plantlets. Rooting medium including MS, ½ MS, ¼ MS, 1/8 MS and ½ MS supplemented with different concentrations of NAA (0.01 and 0.05 mg/l) and indole-3-butyric acid (IBA) (0.01 and 0.05 mg/l) were tested. The cultures were maintained as per the conditions described for adventitious shoot proliferation earlier. The rooting media were supplemented with 3 % sucrose and gelled with 0.6% agar. Traces of agar were removed from plantlets with well developed roots by washing the plantlets carefully under gentle stream of tap water. They were then potted to small thermocol cups (7.5 cm dia) containing autoclaved soil: sand (1:1) and were covered with polyethylene bags to maintain the humidity. These plantlets were kept under the same culture condition in the culture room as described earlier. Holes were punched in the polyethylene bags after three to four days to reduce the humidity and the bags were completely removed after 10-12 days of plantation. Sterile distilled water was used for watering the plantlets once in two days. After three weeks, plants were transferred to larger pots containing garden soil and kept out door for further hardening. After 1 month they were transferred to field.

Statistical Analysis

For adventitious shoot proliferation

experiments from leaf explants, mature and axenic internodal explants, 5 culture vessels with two explants each were used. Ten culture tubes containing one explant each were taken for rooting experiment. All these experiments were repeated thrice. Data were recorded regarding the frequency of shoot proliferation, number of shoots per explant, shoot length, frequency of rooting, roots per shoot and root length. The analysis of variance (ANOVA) was carried out to analyze the data and significant differences between the means were assessed by Duncan's New Multiple Range Tests[20] at $P \leq 0.05$.

RESULTS

Direct Adventitious Shoot Organogenesis

All the explants *i.e.* leaf, mature as well as axenic internodes failed to regenerate adventitious shoot on growth regulator free MS basal medium. Mature internode explant was more responsive than the leaf explant for the induction of adventitious shoots. Leaf explants failed to regenerate shoot irrespective of the media tested. Leaf explants cultured on MS medium augmented with BAP or KIN or mT or TDZ exhibited callusing and remained devoid of any shoot regeneration. Callusing was also observed from leaf explants when cultured on MS supplemented with every combination of BAP and NAA. Position of leaf (abaxial or adaxial) in contact with the medium had no effect on shoot organogenesis (Data not shown). The percentage and number of adventitious shoot regeneration from mature internode explants was influenced by the concentrations and /or combination of growth regulators tested (Table 1). The frequency of adventitious shoot regeneration from mature internode explants varied depending upon the plant growth regulators used (Table 1). A maximum of 70 % of mature internode explants exhibited adventitious shoot organogenesis with an average of 6.0 shoots/ explant on MS medium supplemented with 3.0 mg/l BAP. On this medium shoot buds were initiated within 10-14 days of culture of explants and attended an average height of 3.0 cm after 30 days of culture (Fig. 1a, b).

A separate experiment was carried out to compare the effectiveness of axenic internode segments with that of the mature internode for adventitious shoot regeneration. Due to inability of mature leaf segments for adventitious organogenesis, axenic leaf segments were eliminated from this experiment. Overall the axenic internode explants derived from established shoot culture were found to be more responsive than mature internode explants for shoot organogenesis (Table 1). Of the different plant growth regulators either alone or in combination tested, MS medium augmented with 3.0 mg/l BAP showed the best results. About 86.7 % of the axenic internode explants showed adventitious shoot initiation within 7-10 days of culture (Fig. 1c). Overall 10.4 shoots/ explant with an average length of 4.1 cm were recorded within 30 days of culture (Fig. 1d, e). KIN, TDZ and mT alone were less effective for adventitious shoot regeneration than BAP (Fig. 1f, g). However, BAP in combination with different concentrations of NAA (0.1-0.5 mg/l) and ADS (25-75 mg/l) failed to enhance the shoot regeneration frequency as well as number of shoots/explant (Table 1; Fig. 1h). Both the sources of explants showed direct adventitious shoot regeneration accompanied by a small amount of callus formation.

Rooting of Shoots

Well elongated *in vitro* regenerated shoots showed different response to the rooting media tested (Table 2). Optimal rooting was observed on half-strength MS medium devoid of any growth regulators, where 90 % of the regenerated shoots exhibited rooting with an average number of 14 roots /shoot and average root length of 3.0 cm (Table 2, Fig.1i). Inclusion of auxins including NAA and IBA to the rooting medium was detrimental for rooting. In fact augmentation of NAA led to basal callusing and failed to induce rooting. However, comparatively low level of rooting (56.7 %) was observed on half strength MS containing 0.01 mg/l IBA (Table 2).

Acclimatization of Plantlets

The plantlets were successfully acclimatized in small thermocol cups containing a mixture of autoclaved sand and soil (1:1) with a survival percentage of eighty (Fig. 1j). The plantlets were then transferred to larger pots where cent-percent of these plants were survived. These acclimatized plants, after one month, were subsequently transferred to field condition with zero mortality.

Table 1. Response of plant growth regulators on direct adventitious shoot formation from internode explants of *Paederia foetida* L

Basal medium						Shoot regeneration (%)		Mean number of shoots/ explant		Average shoot length (cm)	
BAP (mg/l)	KIN (mg/l)	TDZ (mg/l)	mT (mg/l)	NAA (mg/l)	ADS (mg/l)	M	A	M	A	M	A
0.0	0.0	0.0	0.0	0.0	0.0	0.0^{o}	0.0^{r}	0.0^{v}	0.0^{z}	0.0^{r}	0.0^{q}
1.0						26.7^{j}	33.3^{klm}	2.0^{fghijklmn}	2.1 ^{nopqrstu}	1.5^{fghi}	1.7^{efghij}
2.0						30.0^{i}	40.0^{ijk}	2.5^{efghij}	2.9^{hijklmn}	1.5^{fghi}	1.9^{efgh}
2.5						46.7^{cd}	63.3^{cde}	4.3^{b}	5.8^{b}	2.0^{bcdef}	2.0^{defg}
3.0						70.0^{a}	86.7^{a}	6.0^{a}	10.4^{a}	3.0^{a}	4.1^{a}
3.5						63.3^{b}	70.0^{c}	4.2^{bc}	5.2^{bc}	1.8^{defg}	3.0^{bcd}
4.0						46.7^{cd}	53.3^{fg}	4.0^{bcd}	4.4^{cde}	1.5^{fghi}	2.4^{cdef}
5.0						43.3^{de}	46.7^{ghi}	2.5^{efghij}	3.6^{defgh}	1.0^{ijklmn}	2.0^{defg}
	1.0					30.0^{i}	36.7^{jkl}	1.2^{nopqrs}	1.5^{qrstuvwx}	1.0^{ijklmn}	1.4^{fghijklm}
	2.0					33.3^{h}	40.0^{ijk}	1.6^{jklmnopq}	1.9^{opqrstuvw}	1.0^{ijklmn}	1.2^{ghijklmn}
	3.0					36.7^{fg}	46.7^{ghi}	1.9^{ghijklmno}	1.9^{opqrstuvw}	1.2^{ghijkl}	1.4^{fghijklm}
	4.0					40.0^{ef}	53.3^{fg}	2.6^{efghi}	2.8^{hijklmno}	1.3^{ghijk}	1.8^{efghi}
	5.0					30.0^{i}	36.7^{jkl}	1.1^{nopqrst}	1.5^{qrstuvwx}	1.0^{ijklmn}	1.4^{fghijklm}
		0.1				30.0^{i}	40.0^{ijk}	2.2^{fghijklm}	3.0^{hijklm}	2.0^{bcdef}	1.4^{fghijklm}
		0.3				50.0^{c}	70.0^{c}	3.3^{bcde}	4.3^{cdef}	2.4^{abcd}	3.3^{abc}
		0.5				36.7^{fg}	63.3^{cde}	2.7^{efgh}	3.2^{ghijk}	2.3^{bcde}	2.5^{cde}
		0.8				30.0^{i}	60.0^{def}	2.0^{fghijklmn}	2.5^{hijklmnopq}	1.7^{efgh}	2.0^{defg}
		1.0				20.0^{l}	50.0^{gh}	1.7^{ijklmnop}	2.0^{nopqrstuv}	1.0^{ijklmn}	1.5^{efghijkl}
			1.0			6.7^{n}	16.7^{opqr}	1.0^{opqrst}	2.4^{jklmnopqr}	0.5^{mnopq}	1.7^{efghij}
			2.0			30.0^{i}	50.0^{gh}	2.4^{ijk}	3.3^{fghij}	1.8^{defg}	3.0^{bcd}
			3.0			23.3^{k}	40.0^{ijk}	2.3^{ijkl}	3.0^{hijklm}	1.5^{fghi}	2.5^{cde}
			4.0			20.0^{l}	33.3^{klm}	2.0^{fghijklmn}	2.5^{hijklmnopq}	1.5^{fghi}	2.0^{defg}
			5.0			20.0^{l}	26.7^{mno}	1.5^{klmnopqr}	2.0^{nopqrstuv}	1.0^{ijklmn}	1.2^{ghijklmn}
1.0				0.1		23.3^{k}	33.3^{klm}	2.0^{fghijklmn}	2.5^{hijklmnopq}	1.4^{fghij}	1.9^{efgh}
1.0				0.2		20.0^{l}	30.0^{lmn}	1.7 ^{ijklmnop}	2.0^{nopqrstuv}	1.0^{ijklmn}	1.7^{efghij}
1.0				0.5		16.7^{m}	23.3^{op}	1.0^{opqrstu}	1.0^{rstuvwxy}	0.6^{lmnop}	1.0^{ghijklmno}
2.0				0.1		26.7^{j}	36.7^{jkl}	2.5^{efghij}	2.7^{hijklmnop}	2.0^{bcdef}	1.6^{efghijk}
2.0				0.2		20.0^{l}	30.0^{lmn}	2.0^{fghijklmn}	2.2^{klmnopqrst}	1.5^{fghi}	1.0^{ghijklmno}
2.0				0.5		20.0^{l}	30.0^{lmn}	2.0^{fghijklmn}	2.0^{nopqrstuv}	1.0^{ijklmn}	1.5^{efghijkl}
2.5				0.1		36.7^{fg}	60.0^{def}	2.7^{efgh}	3.0^{hijklm}	2.7^{ab}	2.4^{cdef}
2.5				0.2		33.3^{h}	50.0^{gh}	3.0^{def}	2.7^{hijklmnop}	2.4^{abcd}	2.0^{defg}
2.5				0.5		26.7^{j}	40.0^{ijk}	1.5^{klmnopqr}	1.5^{qrstuvwx}	1.0^{ijklmn}	1.0^{ghijklmno}
3.0				0.1		63.3^{b}	83.3^{ab}	3.0^{def}	5.2^{bc}	2.5^{abc}	3.0^{bcd}
3.0				0.2		50.0^{c}	66.7^{cd}	2.0^{fghijklmn}	4.0^{defg}	2.0^{bcdef}	2.0^{defg}
3.0				0.5		40.0^{ef}	60.0^{def}	1.5^{klmnopqr}	3.0^{hijklm}	1.0^{ijklmn}	1.5^{efghijkl}
3.5				0.1		40.0^{ef}	70.0^{c}	1.5^{klmnopqr}	4.5^{cd}	1.1^{hijklm}	3.7^{ab}
3.5				0.2		26.7^{j}	63.3^{cde}	1.1^{nopqrst}	3.1^{ghijkl}	1.0^{ijklmn}	3.0^{bcd}
3.5				0.5		20.0^{l}	46.7^{ghi}	1.0^{opqrstu}	3.0^{hijklm}	0.5^{mnopq}	2.5^{cde}
	1.0			0.1		30.0^{i}	36.7^{jkl}	1.2^{nopqrs}	1.5^{qrstuvwx}	1.0^{ijklmn}	1.0^{ghijklmno}
	1.0			0.2		26.7^{j}	30.0^{lmn}	1.0^{opqrstu}	1.0^{rstuvwxy}	0.8^{jklmno}	1.0^{ghijklmno}
	1.0			0.5		CWR/0.0^{o}	26.7^{mno}	0.0^{v}	1.0^{rstuvwxy}	0.0^{r}	0.5^{mnop}
	2.0			0.1		26.7^{j}	46.7^{ghi}	2.0^{fghijklmn}	2.0^{nopqrstuv}	1.5^{fghi}	2.0^{defg}
	2.0			0.2		16.7^{m}	40.0^{ijk}	1.0^{opqrstu}	1.5^{qrstuvwx}	1.0^{ijklmn}	1.0^{ghijklmno}
	2.0			0.5		CWR/0.0^{o}	20.0^{opq}	0.0^{v}	1.5^{qrstuvwx}	0.0^{r}	1.0^{ghijklmno}
	3.0			0.1		30.0^{i}	40.0^{ijk}	1.6^{jklmnopq}	2.1^{nopqrstu}	1.3^{ghijk}	1.5^{efghijkl}
	3.0			0.2		23.3^{k}	36.7^{jkl}	1.0^{opqrstu}	1.5^{qrstuvwx}	1.0^{ijklmn}	1.2^{ghijklmn}
	3.0			0.5		CWR/0.0^{o}	30.0^{lmn}	0.0^{v}	2.0^{nopqrstuv}	0.0^{r}	1.5^{efghijkl}
	4.0			0.1		36.7^{fg}	50.0^{gh}	2.9^{efg}	3.5^{efghi}	2.3^{bcde}	2.5^{cde}

	4.0	0.2		30.0^{i}	43.3^{hij}	2.0^{fghijklmn}	2.3^{jklmnopqrs}	1.3^{ghijk}	1.5^{efghijkl}
	4.0	0.5		CWR/0.0^{o}	33.3^{klm}	0.0^{v}	2.8^{hijklmno}	0.0^{r}	2.0^{defg}
3.0			25	36.7^{fg}	53.3^{fg}	1.5^{klmnopqr}	3.0^{hijklm}	1.3^{ghijk}	2.5^{cde}
3.0			50	33.3^{h}	46.7^{ghi}	1.2^{nopqrs}	2.5^{hijklmnopq}	1.3^{ghijk}	2.0^{defg}
3.0			75	26.7^{j}	40.0^{ijk}	1.0^{opqrstu}	2.0^{nopqrstuv}	1.0^{ijklmn}	1.5^{efghijkl}

M-Mature internode explant, A-Axenic internode explant, CWR- Callus with rooting; Values within a column followed by same letters are not significantly different ($P \leq 0.05$; DMRT)

Table 2. Rooting of *in vitro* derived shoots

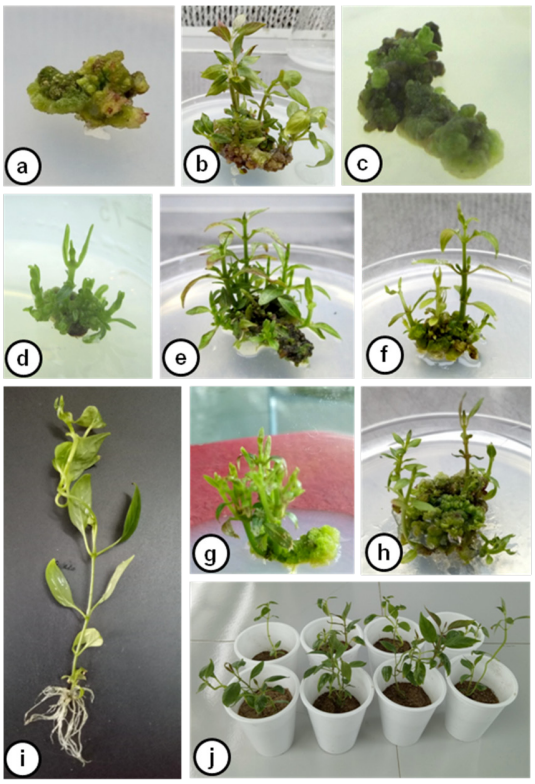
Media	Rooting (%)	Number of roots/explant	Root length (cm)
MS	70.0^{c}	8.0^{cdef}	1.5^{bcde}
½ MS	90.0^{a}	14.0^{a}	3.0^{a}
¼ MS	80.0^{b}	12.0^{ab}	2.5^{ab}
1/8 MS	60.0^{d}	10.5^{bc}	2.0^{abc}
½ MS + IBA (0.01 mg/l)	56.7^{de}	10.0^{bcd}	1.8^{bcd}
½MS + IBA (0.05 mg/l)	50.00^{ef}	9.0^{bcde}	1.5^{bcde}
½MS + NAA (0.01 mg/l)	Callusing	-	-
½MS + NAA (0.05 mg/l)	Callusing	-	-

Values within a column followed by same letters are not significantly different ($P \leq 0.05$; DMRT)

DISCUSSION

Plant regeneration mediated by adventitious shoot formation is a reliable technique for clonal propagation as this method usually does not produce somaclonal variations[21]. Further such plant regeneration protocol is a prerequisite for development of genetic transformation methods in plants[22,23]. During our attempt to develop an efficient plant regeneration protocol for *P. foetida* through adventitious shoot organogenesis, the influence of type and source of explants on direct adventitious shoot organogenesis was investigated. Out of the two types of explants tested (leaf and internode) for the purpose, only the internodal explants responded positively. However, between the internode explants of two different sources i.e. mature and axenic internode explants derived from established shoot cultures, the later showed better results for shoot organogenesis. This type of significant difference in the frequency and number of shoot regeneration potential of the different explants has also been recorded in a number of medicinal plant species, which may be due to different physiological state, age and cellular differentiation among the constituent cells[24,25]. Internode explants have also been effectively used in direct adventitious shoot organogenesis in a number of medicinal plants including *Verbena officinalis*[26], *Murraya koeingii*[27], *Piper crocatum*[28]. Similar to our results internode explants were found to be superior to leaf explants in *Ophiorrhiza prostrata*[25]. However, both leaf and internode explants derived from mature plant of *Dianthus caryophyllus* were found to be effective for adventitious shoot organogenesis[29]. Contrary to our results, use of either mature or juvenile/ axenic leaf explants for adventitious organogenesis have also been reported in medicinal plants including *Solanum aculeatissimum*[30] and *Potentilla fulagens*[31].

Growth regulators free MS medium failed to exhibit shoot organogenesis from all the explants tested. *In vitro* organogenesis depends upon the supplementation of exogenous plant growth regulators (cytokinin and/or auxin) to the basal medium[32]. Thus addition of plant growth regulators to the basal medium was essential for adventitious shoot organogenesis in *P. foetida*. In this study, one of the important factors influencing adventitious shoot organogenesis was the type, ratio and concentrations of plant growth regulators



(a) Initiation of shoot buds from mature internode explant on MS + 3.0 mg/l BAP after 14 days of culture;
(b) Elongation of adventitious shoots on MS supplemented with 3.0 mg/l BAP after 30 days of culture;
(c) Initiation of shoot buds from axenic internode explant after 10 days of culture on MS + 3.0 mg/l BAP;
(d) Multiple adventitious shoot organogenesis from axenic internode explant on MS + BAP (3.0 mg/l);
(e) Elongation of adventitious shoots on MS medium augmented with 3.0 mg/l BAP after 30 days of culture;
(f) Shoot organogenesis from axenic internode explant on MS + mT (2.0 mg/l);
(g) Regeneration of adventitious shoots from axenic internode on MS + 0.3 mg/l TDZ;
(h) Adventitious shoot regeneration from axenic internode on MS + BAP (3.0 mg/l) + NAA (0.2 mg/l) medium;
(i) Rooting of *in vitro* formed shoot on ½ MS medium after 21 days of culture;
(j) Acclimatized plantlets in thermocol cups containing sand: soil (1:1).

Fig. 1. Adventitious shoot organogenesis from different internode (mature and axenic) explants of *P. foetida*.

used in the culture medium. It was observed that basal medium supplemented with different cytokinin BAP, KIN, mT and TDZ individually was sufficient for direct adventitious shoot organogenesis in *Paederia foetida*. Among the tested cytokinin BAP showed comparatively higher percentage of regeneration, number of shoots per explant and average shoot length compared to other cytokinins. Similar results of BAP being the best for adventitious shoot organogenesis have been reported by Akbas *et al.* (2011)[33] in *Hypericum spectabile*, Rahaman *et al.* (2011)[34] in *Solanum surattense* and Chauhan *et al*. (2012)[35] in *Garcinia indica*.

It was observed in a number of cases including *Asteracantha longifolia*[36] and *Tanacetum cinerariifolium*[37] additions of auxins like NAA, to the basal medium supplemented with BAP had synergistic effect on shoot organogenesis. However, in our case of adventitious shoot organogenesis of *P. foetida* was in agreement with other medicinal plants e.g. *Solanum surattense*[34] and *Aralia elata*[38] where combination of NAA + BAP failed to supersede the effect of singular supplementation of BAP.

Joshi *et al.* (2011)[27] reported addition of ADS to the optimum medium was beneficial for adventitious organogenesis in *Murraya koeingii*. However, in the present study, augmentation of different concentrations of ADS to the optimum regeneration medium (MS + 3.0 mg/l BAP) failed to enhance the frequency of shoot regeneration and the number of shoots per internode explant. Combination of BAP and ADS was also not beneficial for organogenesis in *Anethum graveolens*[39].

In earlier micropropagation reports of *P. foetida*, the rooting was best seen on different concentrations of IBA (0.1 mg/l; Amin *et al.,* 2003)[40], (0.5 mg/l; Alam *et al.,* 2010)[41], (2.4 µM; Thirupathi *et al.,* 2013)[3]. However, in our study half-strength MS devoid any of the growth regulators was best suited for rooting of *in vitro* regenerated shoots. Further, inclusion of IBA even at lower concentrations (0.01 and 0.05 mg/l) was unfavourable for rooting of *in vitro* regenerated *P. foetida* shoots, result are in accordance with previous study on a medicinal plant, *Spilanthes acmella*, which have reported that half-strength MS medium was enough for

rooting of adventitiously regenerated shoots[42]. Acclimatization of regenerated plants to the outside environment with negligible mortality is the last step and most vital for the success of plant tissue culture. We had successfully acclimatized the regenerated plantlets in field and no morphological variation was detected in the field established plants when compared with that of the mother plant.

CONCLUSION

In conclusion, a simple and efficient *in vitro* plant regeneration protocol of *P. foetida via* adventitious shoot organogenesis was successfully developed. This protocol can be applied for large scale plant regeneration aiming at its commercial utilization and germplasm conservation as well as genetic transformation studies of *P. foetida*.

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