Ann. Bangladesh Agric. (2020) 24 (1) : 89-98
www.doi.org/10.3329/aba.v24i1.51938

ISSN 1025-482X (Print)
2521-5477 (Online)

REGENERATION OF MEDICINAL PLANT *PAEDERIA FOETIDA* THROUGH DIRECT ORGANOGENESIS USING NODAL EXPLANTS

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Abstract

Chinese fever vine *(Paederia foetida* L.), a valuable medicinal plant has been greatly utilized in therapeutic purposes throughout the world. Since conventional propagation techniques of *P. foetida* are very slow, inefficient and cannot cope with the increasing demand, *in-vitro* regeneration through tissue culture could be an alternative means of rapid propagation. Therefore, the efforts were made to develop a suitable protocol through direct organogenesis of *P. foetida*. After surface sterilization, the nodal explants were cultured in Murashigue and Skoog (MS) medium and MS medium supplemented with different concentrations and combinations of plant growth regulators. MS medium supplemented with 6-benzylaminopurine; BAP (2.0 mg L^{-1}) produced the maximum number of shoots; 4.40 ± 0.98 and 5.40±1.12 after 15 and 30 days of culture respectively. The number of shoots gained by 15 days was found to be the highest; 1.20±0.80 at BAP (4.0 mg L^{-1}) followed by 1.00±0.55 at BAP (2.0 mg L^{-1}). Although the combination of BAP + Kinetin (2 mg L^{-1} +2 mg L^{-1}) showed the highest shoot growth (3.40 ± 1.08 cm) by 15 days, sole application of BAP (2.0 mg L^{-1}) or Kn (0.5, 1.0, 2.0 and 3.0 mg L^{-1}) showed similar responses. BAP (2.0 mg L^{-1}) showed the best responses for developing the highest number of leaves; 18.60 ± 2.42 and 29.20 ± 2.73 respectively after 15 and 30 days of culture. Similarly, development of the maximum number of leaves (10.60 ± 0.68) was reported by 15 days at BAP (2.0 mg L^{-1}). Rooting was significantly induced in indole-3-acetic acid (IAA) supplemented to 1/2 strength MS medium as compared to control (only ½ strength MS medium). The best performance of rooting was observed by 0.5 mg L^{-1} IAA which produced average 4.33 roots per shoot after 21 days of culture. The regenerated plants showed similar morphology to the mother plants. Thus, a suitable protocol for successful multiplication of *P. foetida in vitro* was established using nodal explants.

Keywords: Explant, regeneration, organogenesis.

Introduction

Chinese fever vine *(Paederia foetida* L.) belonging to the family Rubiaceae is widely distributed throughout the Asia. The plant has been greatly used as the potential source of traditional medicine specially in Asian sub-continent. It has been very ubiquitous that medicinal plants offer a great role across the varied cultures and civilizations throughout the world as a valuable and safe natural resource. Medicinal plants are being considered as the most important source of life saving drugs for the majority of world's population (Kumar and Gupta, 2008). About

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85% of world's population greatly rely on this natural assets for their primary health care needs (Pesic, 2015). In Asia, along with China and India, Bangladesh is being considered as the natural harbor of medicinal plants and country of which have a long historical background for the therapeutic uses of herbal medicine (Steinmetz, 1961; Blatter *et al.*, 1981; Haque *et al.*, 2014). The medicinal plant *P. foetida* is very good source of secondary metabolites such as iridoid glycosides, sitosterol, stigmasterol, alkaloids, carbohydrates, proteins, amino acids and volatile oils which make attraction to the pharmaceutical companies (Blatter et al., 1981). Due to having popularity to the village practitioners and ayurvedic application, the plant has been treated as one of the potential medicinal herbs in Bangladesh (Steinmetz, 1961; Nandkoni, 2002; Khare, 2007). Various parts of this plant are utilized in traditional medicine in different ways such as juice, poultice and decoction of leaves, roots and fruits are used for the treatment of diarrhoea, stomachache, toothache, gout, dysentery, piles etc. (Blatter *et al.*, 1981; Nandkoni, 2002). Though having lot of importance, it is a matter of concern that the medicinal plant *P. foetida* is going to be endangered in Bangladesh due to loss of natural habitats by deforestation, urbanization, industrialization, agricultural encroachment, environmental pollution and lack of proper conservation strategies. With these concerns and to meet up the continuous demands of pharmaceutical companies which largely depend upon materials procured from naturally occurring stands, it is very imperative to ensure rapid multiplication and conservation of medicinal plant like *P. foetida*. However, increased demands, indiscriminate use and abused harvesting have led to the decreased availability *P. foetida* in our country. Hence, there is a need to increase the propagation of *P. foetida* for the benefit of human beings in a sustainable way. The plant *P. foetida* could be multiplied by seeds and stem cuttings but the process is very slow and inefficient (Aquilar, 2001). Although application of phytohormone is promising in stem cutting, the technique is not suitable for maintaining safe plant parts and further genetic improvement. Since conventional methods of propagation confronting several challenges and are not coping with the increasing demands, it is very imperative to establish a suitable protocol for rapid regeneration of *P. foetida in-vitro*. Plant tissue culture could be the alternative means of rapid clonal propagation which has been widely used for the commercial propagation of a large number of plant species including medicinal plants (George and Sherrington, 1984). Tissue culture has superiority over conventional method of plant propagation because of high multiplication rate (Hussain *et al.*, 2012). Although few efforts were claimed for direct and indirect organogenesis in *P. foetida* (Amin *et al.*, 2003; Srivastava and Srivastava, 2004; Alam *et al.*, 2010; Ghosh *et al.*, 2019), further efforts are needed to reproduce and standardize the protocol to make it more suitable and cost effective that should be helpful for efficient propagation and genetic improvement of the plant. Considering the above facts, the present study was undertaken to develop a suitable protocol for direct organogenesis of *P. foetida in-vitro* using nodal explants with different concentrations and combinations of growth regulators.

Materials and Methods

Sterilization and culture of plant materials

About 1.0 cm of healthy and juvenile nodal explants were collected from donor *P. foetida* plants grown at the Department of Crop Botany, BSMRAU. Then, those explants were thoroughly washed with running tap water for 5 to 6 times, followed by washing with Tween-20 solution for 5 minutes and distilled water for at least 3 times. After that, surface sterilization was done by submitting the explants in 70% ethanol for 5 mins followed by 1% sodium hypochlorite (NaOCl) for 2 mins and 0.1% mercuric chloride ($HgCl_2$) for 2 mins respectively. Then the explants were washed repeatedly with sterile distilled water and allowed for culture in freshly prepared MS (Murashige and Skoog, 1962) medium or medium fortified with different growth regulators for showing shooting responses. For root initiation, ½ strength MS medium and ½ strength MS medium supplemented with different concentrations of indole-3-acetic acid (IAA) were used. A 16 h photoperiod at 25±2 ºC under an illumination of 35-50 $\mu molm^{-2}s^{-1}$ was used to incubate the cultures as described by Thirupathi *et al.* (2013).

Treatment combinations

MS medium or MS medium supplemented with different concentrations and combinations of plant growth regulators *viz.* 6-benzyleaminopurine (BAP) and kinetin (Kn) either sole or in combinations were used to find out the suitable growth regulator or combination of growth regulators for direct organogenesis of *P. foetida* using nodal explants. For the observation of shooting responses, MS medium without growth regulators was treated as control (0.0). MS medium supplemented with BAP (0.5, 1.0, 2.0, 3.0 and 4 mgL^{-1}), Kinetin; Kn (0.5, 1.0, 2.0, 3.0 and 4 mgL^{-1}) and combined application of BAP and Kn (0.5+0.5, 1.0+1.0, 1.5+1.5, 2.0+2.0 and 4.0+3.0 mgL^{-1}) were used as different treatment combinations. For rooting, ½ strength MS medium was used as control (0.0) and ½ strength MS medium supplemented with different concentrations of indole-3-acetic acid, IAA (0.2, 0.5 and 1.0 mgL^{-1}) was used as treatment.

Data collection and analysis

Regular observation was made in growth and shooting pattern of the explants in *in-vitro* and data regarding the number of shoots, shoot length and number of leaves were collected after 15 and 30 days after culture. The number of root initiation from the explant was recorded after 14 days and 21 days of culture. The experiment was set by following Completely Randomized Design (CRD) with 5 replications. The data were analysed by following the Statistix 10 package computer programme. Mean separation using LSD values was calculated by the same programme. Student t-test was also used for showing statistical difference.

Results and Discussion

The present efforts were made to develop a suitable protocol for the rapid multiplication and sustainable utilization of valuable medicinal plant *P. foetida*. The nodal explants of *P. foetida* were cultured in MS medium or MS medium supplemented with different concentrations and combinations of plant growth regulators for showing their performance regarding number of shoots, shoot length, number of leaf and rooting of the developed shoots. The results of that responses are presented and discussed below as following subheads.

Number of shoots regenerated *in-vitro*

The explants cultured in MS medium and MS medium supplemented with different growth regulators showed significant variation in number of shoot proliferation. The highest mean value of number of shoots (4.40±0.98) was observed in MS medium supplemented with BAP (2.0 mg L^{-1}) followed by 3.80±0.37 found in BAP + Kn (2.0+2.0) mg L^{-1} after 15 days of culture (Table 1). The data of which were statiscally similar suggesting that single application of BAP was sufficient for the initiation of new shoots in *P. foetida*. After 30 days of culture, the highest shoot number (5.40±1.12) was also maintained by BAP (2.0 mg L^{-1}) followed by 4.00±0.32 produced at BAP + Kn (2.0+2.0) mg L^{-1}. This might be due to the less efficiency of single application of Kn in shoot initiation in this observation (Table 1) which was supported by the findings of Ashraf *et al.* (2014). The number of shoot differences was found to be the maximum (1.20±0.80) by 15 days at BAP (4.0 mg L^{-1}) followed by (1.00±0.55) at BAP (2.0 mg L^{-1}). It was due to the cause that cytokinin, especially BAP always performed better for direct shoot regeneration (Ashraf *et al.*, 2014). The results of the present study was also consistent to the findings of Behera *et al.* (2017) where 6 shoots per explant were reported as the best performance at BAP (3.0 mg L^{-1}). The findings were also supported by Alam et al.

Table 1. Effect of different concentrations and combinations of plant growth regulators for the initiation of new shoots *in-vitro*

Treatments (mg L^{-1})	No. of shoot after 15 days of culture (means ± SE)	No. of shoot after 30 days of culture (means ± SE)	Differences of no. of shoots (means ± SE)
Control (0.0)	2.00 ± 0.00 ^{ef}	2.00 ± 0.00 ^{de}	0.00 ± 0.00 ^{c}
BAP (0.5)	2.80 ± 0.37 ^{bcde}	3.00 ± 0.55 ^{bcd}	0.20 ± 0.20 ^{c}
BAP (1.0)	3.00 ± 0.32 ^{bcde}	3.20 ± 0.37 ^{bcd}	0.20 ± 0.20 ^{c}
BAP (2.0)	4.40 ± 0.98 ^{a}	5.40 ± 1.12 ^{a}	1.00 ± 0.55 ^{ab}
BAP (3.0)	3.20 ± 0.49 ^{bcd}	3.60 ± 0.51 ^{bc}	0.40 ± 0.24 ^{bc}
BAP (4.0)	2.40 ± 0.24 ^{def}	3.60 ± 0.81 ^{bc}	1.20 ± 0.80 ^{a}
Kn (0.5)	2.20 ± 0.20 ^{def}	2.20 ± 0.20 ^{de}	0.00 ± 0.00 ^{c}
Kn (1.0)	2.20 ± 0.20 ^{def}	2.20 ± 0.20 ^{de}	0.00 ± 0.00 ^{c}
Kn (2.0)	2.00 ± 0.00 ^{ef}	2.00 ± 0.00 ^{de}	0.00 ± 0.00 ^{c}
Kn (3.0)	2.60 ± 0.40 ^{cdef}	2.60 ± 0.40 ^{cde}	0.00 ± 0.00 ^{c}
Kn (4.0)	2.40 ± 0.24 ^{def}	2.60 ± 0.24 ^{cde}	0.20 ± 0.20 ^{c}
BAP + Kn (0.5+0.5)	2.20 ± 0.20 ^{def}	2.20 ± 0.20 ^{de}	0.00 ± 0.00 ^{c}
BAP + Kn (1.0+1.0)	3.20 ± 0.49 ^{bcd}	3.20 ± 0.49 ^{bcd}	0.00 ± 0.00 ^{c}
BAP + Kn (1.5+1.5)	3.60 ± 0.51 ^{abc}	3.60 ± 0.51 ^{bc}	0.00 ± 0.00 ^{c}
BAP + Kn (2.0+2.0)	3.80 ± 0.37 ^{ab}	4.00 ± 0.32 ^{b}	0.20 ± 0.20 ^{c}
BAP + Kn (4.0+3.0)	1.60 ± 0.51 ^{f}	1.60 ± 0.51 ^{e}	0.00 ± 0.00 ^{c}
LSD	1.17	1.39	0.76

Data are means ± SE of 5 replications. Data with same letter did not show significant difference at 5% level of significance (P<0.05)

(2010) where, BAP was found to be a good promoter of shoot organogenesis of *P. foetida in-vitro*. The similar role of BAP has also been reported by other investigators using different plant species (Tiwari *et al.*, 2000; Martin, 2003; Agarwala *et al.*, 2010). Contrary, Amin *et al.* (2003) found lower concentration of BAP (1.0 mg L^{-1}) was effective for axillary shoot proliferation of *P. foetida*. Thus, the effects of plant growth regulators were little bit conflicting on axillary shoot proliferation in *P. foetida* and this was likely to be the presence of genotypic, morphological, physiological and seasonal variation of plant parts used as explants (Arab *et al.*, 2014). In comparison with BAP, the performance of Kn was not effective when used as single agent for direct shoot regeneration and the results of which was very consistent to the reports of Begum *et al*. (2002) and Ashraf *et al.* (2014). Therefore, findings of the present effort suggested that the sole application of BAP (4.0 mg L^{-1}) was suitable for direct shoot organogenesis of *P. foetida* using nodal explants.

Observation of shoot growth

The significant differences of the growth of the shoots were observed by the application of growth regulators. Although single application of Kn (0.5, 1.0, 2.0 and 3.0 mg L^{-1}) showed very good efficacy for increasing shoot growth after 15 days of culture, the highest mean value (4.22±0.59 cm) was recorded in MS medium supplemented with Kn (2.0 mg L^{-1}) followed by 4.04±0.13 cm at Kn (3.0 mg L^{-1}) (Table 2). After 30 days of culture, the highest mean value (6.72±0.48 cm) was recorded by Kn (3.0 mg L^{-1}) followed by 6.64±0.80 cm gained at Kn (2.0 mg L^{-1}) (Table 2). The mean value of the shoot growth difference by 15 days was found to be the maximum (3.40±1.08 cm) when BAP and Kn were used in combination at 2.0 mg L^{-1} of each. This was due to the synergistic effects of the combined application of BAP and Kn. The findings showed conformity to the results of Ashraf et al. (2014) where they found combined application of BAP and Kn maintained the highest contribution for shoot elongation of medicinal plant *Chlorophytum borivilianum*. The sole application of Kn (3.0 mg L^{-1}) or BAP (2.0 mg L^{-1}) also provided more or less similar responses to the combined application of BAP and Kn at 2.0 mg L^{-1} of each (Table 2). The finding was supported by Behera et al. (2017) where single application of 3.0 mg L^{-1} BAP produced average shoot length 3.0 cm after 30 days of culture. In another observation, Mahmood and Hauser (2015) reported that rose nodes produced the tallest shoots from the medium containing 1.0 mg L^{-1} BAP indicating the sufficient role of single cytokinin for increasing shoot length *in-vitro*. With these observation, our findings suggested that although single application of cytokinin showed good responses for shoot elongation, the combined application of BAP and Kn was needed for getting the best performance in *P. foetida*.

Initiation of leaf

The initiation and multiplication of new leaves were greatly regulated by the exogenous application of plant growth regulators. The number of leaves was counted after15 and 30 days of culture and significant differences of leaf numbers were recorded. After 15 day of culture, the maximum number of leaves (18.60±*2.42*) was recorded in MS medium supplemented with BAP (2.0 mg L^{-1}) followed by 18.40 ± 1.47 at the combination of BAP + Kn (1.5+1.5) mg L^{-1} (Table 3). The number

Table 2. Effect of different concentrations and combinations of plant growth regulators on shoot growth *in-vitro*

Treatments (mg L^{-1})	Average shoot length (cm) at 15 days of culture (means ± SE)	Average shoot length (cm) at 30 days of culture (means ± SE)	Differences of shoot length (cm) (means ± SE)
Control (0.0)	2.74 ± 0.58 ^{cde}	3.60 ± 0.75 ^{def}	0.86 ± 0.19 ^{de}
BAP (0.5)	3.12 ± 0.32 ^{bcd}	5.00 ± 0.26 ^{abcd}	1.88 ± 0.17 ^{bcde}
BAP (1.0)	2.14 ± 0.55 ^{def}	3.12 ± 0.73 ^{ef}	0.98 ± 0.42 ^{cde}
BAP (2.0)	1.62 ± 0.29 ^{fg}	4.24 ±0.39 ^{bcde}	2.62 ± 0.34 ^{ab}
BAP (3.0)	1.54 ± 0.60 ^{fg}	3.06 ± 0.65 ^{ef}	1.52 ± 0.61 ^{bcde}
BAP (4.0)	0.82 ± 0.21 ^{gh}	2.24 ± 0.76 ^{fg}	1.42 ± 0.61 ^{bcde}
Kn (0.5)	3.80 ± 0.25 ^{abc}	6.10 ± 0.24 ^{ab}	2.30 ± 0.30 ^{abcd}
Kn (1.0)	3.64 ± 0.37 ^{abc}	5.96 ± 0.45 ^{abc}	2.32 ± 0.41 ^{abcd}
Kn (2.0)	4.22 ± 0.59 ^{a}	6.64 ± 0.80 ^{a}	2.42 ± 0.76 ^{abc}
Kn (3.0)	4.04 ± 0.13 ^{ab}	6.72 ± 0.48 ^{a}	2.68 ± 0.45 ^{ab}
Kn (4.0)	2.74 ± 0.51 ^{cde}	4.46 ± 0.90 ^{bcde}	1.72 ± 0.54 ^{bcde}
BAP + Kn (0.5+0.5)	2.42 ± 0.25 ^{def}	5.04 ± 0.67 ^{abcd}	2.62 ± 0.58 ^{ab}
BAP + Kn (1.0+1.0)	1.92 ± 0.40 ^{ef}	3.98 ± 0.92 ^{def}	2.06 ± 0.65 ^{abcde}
BAP + Kn (1.5+1.5)	2.16 ± 0.09 ^{def}	4.14 ± 0.09 ^{cde}	1.98 ± 0.15 ^{abcde}
BAP + Kn (2.0+2.0)	1.8 ± 0.17 ^{efg}	5.20 ± 1.06 ^{abcd}	3.40 ± 1.08 ^{a}
BAP + Kn (4.0+3.0)	0.34 ± 0.12 ^{h}	0.94 ± 0.60 ^{g}	0.60 ± 0.50 ^{e}
LSD	1.08	1.87	1.52

Data are means ± SE of 5 replications. Data with same letter did not show significant difference at 5% level of significance ($P<0.05$).

of leaves produced by those treatments were statistically similar. Similarly, the highest number of leaves (29.20 ± 2.73) was recorded at BAP (2.0 mg L^{-1}) followed by 24.40 ± 1.94 at the combination of BAP + Kn (1.5 mg L^{-1} + 1.5 mg L^{-1}) after 30 days of culture (Table 3). The highest number of leaves (10.60±0.68) was gained by 15 days at BAP (2.0 mg L^{-1}) followed by 8.00±3.56 at BAP (4.0 mg L^{-1}) and 7.20±0.58 at BAP (0.5 mg L^{-1}) (Table 3). The results suggested that along with shooting responses, BAP (2.0 mg L^{-1}) showed tremendous impacts on the development of new leaf (Table 3). Although no report was claimed on the effect of growth regulators in *P. foetida* leaf development, investigations were made in other plant species. For instance, Mahmood and Hauser (2015) found that BAP (1.0 mg L^{-1}) with MS medium was very synergistic for producing new leaves in rose suggesting the role of BAP in leaf initiation which was consistent to our findings where BAP (2.0 mg L^{-1}) showed the prominent effect in leaf initiation from nodal explants of *P. foetida*.

Table 3. Effect of different concentrations and combinations of plant growth regulators for the initiation of leaf *in-vitro*

Treatments (mg L^{-1})	No. of leaves after 15 days of culture (means ± SE)	No. of leaves after 30 days of culture (means ± SE)	Differences of no. of leaves (means ± SE)
Control (0.0)	9.40 ± 1.63 ^{b}	11.60 ± 1.75 ^{cd}	2.20 ± 0.73 ^{c}
BAP (0.5)	9.20 ± 0.86 ^{b}	16.40 ± 1.29 ^{bc}	7.20 ± 0.58 ^{ab}
BAP (1.0)	12.40 ± 1.60 ^{b}	16.80 ± 2.25 ^{bc}	4.40 ± 1.94 ^{bc}
BAP (2.0)	18.60 ± 2.42 ^{a}	29.20 ± 2.73 ^{a}	10.60 ± 0.68 ^{a}
BAP (3.0)	11.60 ± 2.60 ^{b}	17.60 ± 2.56 ^{b}	6.00 ± 2.37 ^{abc}
BAP (4.0)	9.20 ± 2.60 ^{b}	17.20 ± 4.32 ^{bc}	8.00 ± 3.56 ^{ab}
Kn (0.5)	8.80 ± 0.66 ^{bc}	15.20 ± 0.80 ^{bc}	6.40 ± 0.75 ^{abc}
Kn (1.0)	8.20 ± 1.53 ^{bc}	13.60 ± 1.33 ^{bcd}	5.40 ± 0.51 ^{bc}
Kn (2.0)	7.80 ± 0.66 ^{bc}	12.00 ± 0.63 ^{bcd}	4.20 ± 0.66 ^{bc}
Kn (3.0)	9.60 ± 1.63 ^{b}	15.20 ± 1.36 ^{bc}	5.60 ± 0.81 ^{bc}
Kn (4.0)	9.80 ± 0.80 ^{b}	14.00 ± 1.79 ^{bcd}	4.20 ± 1.11 ^{bc}
BAP + Kn (0.5+0.5)	10.00 ± 0.89 ^{b}	15.60 ± 1.17 ^{bc}	5.60 ± 1.17 ^{bc}
BAP + Kn (1.0+1.0)	10.00 ± 1.90 ^{b}	15.20 ± 2.15 ^{bc}	5.20 ± 1.36 ^{bc}
BAP + Kn (1.5+1.5)	18.40 ± 1.47 ^{a}	24.40 ± 1.94 ^{a}	6.00 ± 0.63 ^{abc}
BAP + Kn (2.0+2.0)	9.20 ± 1.85 ^{b}	16.00 ± 0.63 ^{bc}	6.80 ± 1.62 ^{abc}
BAP + Kn (4.0+3.0)	4.40 ± 1.60 ^{c}	8.80 ± 2.94 ^{d}	4.40 ± 3.12 ^{bc}
LSD	4.71	5.87	4.61

Data are means ± SE of 5 replications. Data with same letter did not show significant difference at 5% level of significance ($P<0.05$).

Effect of IAA on the rooting of *P. foetida*

Since earlier report indicated that rooting in *P. foetida* was induced by the sole application of either IAA or IBA or NAA *in-vitro* (Amin *et al.*, 2003). Hence, for showing rooting performance, we used only single rooting inducer; IAA in ½ strength MS medium at different concentrations. Here, number of root initiation from the explants was recorded after 14 days and 21 days of culture. As compared to control, rooting induction was significantly increased by the application of IAA at all the concentrations used. Although there was no significant difference was observed by different concentrations of IAA, the highest number of root initiation was recorded as 3.67 and 4.33 by IAA (0.5 mg L^{-1}) after 14 and 21 days of culture respectively (Fig. 1 and 2). Previous investigations reported 3.50 and 2.00 roots per shoot by the application of IBA at 0.1 mgL^{-1} and 0.5 mg L^{-1} respectively (Amin *et al.*, 2003; Alam *et al.*, 2010). In our findings, the highest number of roots per shoot (4.33) was reported by ½ strength MS medium supplemented with IAA (0.5 mg L^{-1}) after 21 days of culture. The data of which were consistent to those findings, although more rooting was observed in our observation. Therefore, by this study, it was obvious that IAA (0.5 mg L^{-1}) is very suitable for enhancing

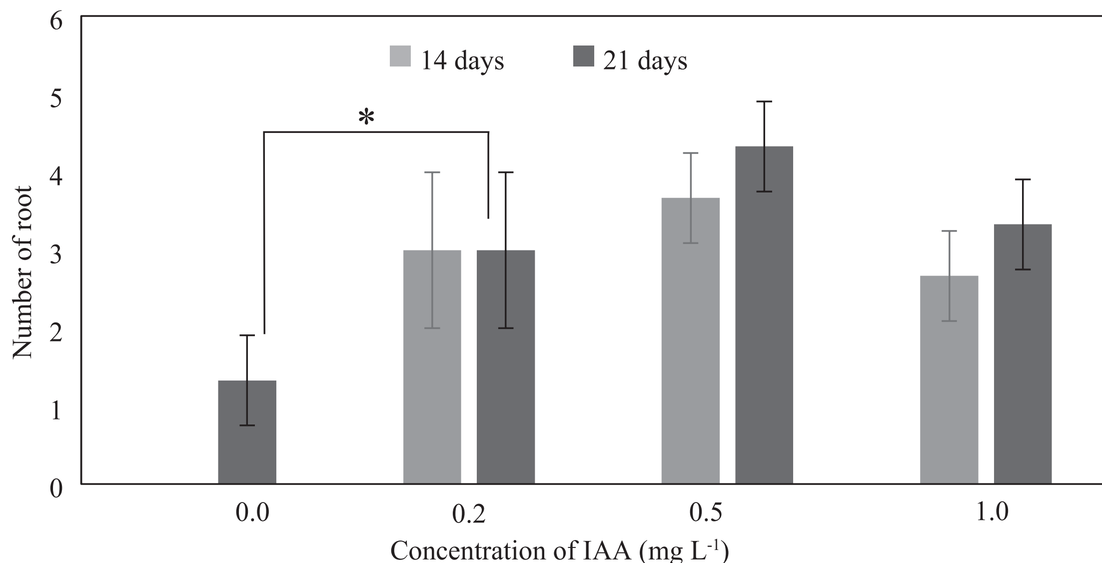

Fig. 1. Effect of plant growth regulator (IAA) on the rooting of *P. foetida.* Regenerated shoots were supposed to ½ strength MS medium or ½ strength MS medium supplemented with different concentrations of IAA (0.2, 0.5 and 1.0 mg L^{-1}). The number of shoots were monitored after 14 days and 21 days of culture. Error bars indicate standard deviation. Asterisk indicates significant difference (P<0.05, t-test).

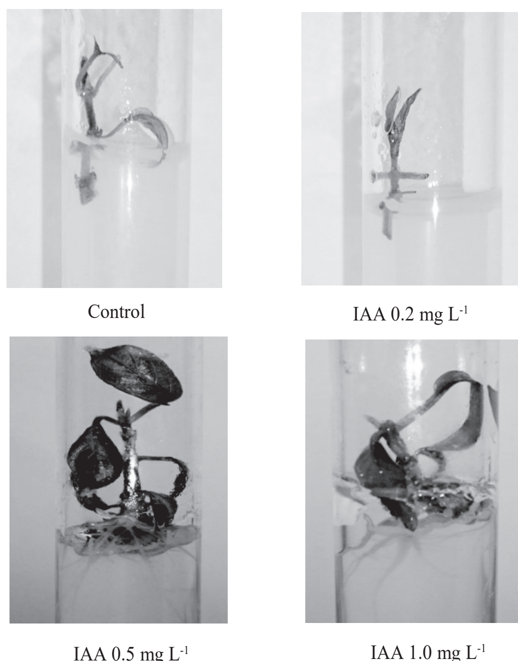

Fig. 2. Effects of IAA on the rooting of shoots regenerated from direct organogenesis after 21 days of culture.

rooting in *P. foetida in-vitro*. After that, the rooted plantlets were gently separated from the agar medium and individually transferred to plastic pots filled with sand, soil and vermicompost for few days for acclimatization. Then the acclimatized plants were transferred to the large pots and allowed to further growth at ambient condition which were found to be successfully established.

Conclusions

Based on the findings of the present efforts, it can be concluded that MS medium supplemented with BAP (4.0 mg L^{-1}) was found to be very effective for the regeneration of new shoots. Combined application of BAP + Kn (2 mg L^{-1} +2 mg L^{-1}) with MS medium showed the best responses for enhancing shoot length. MS medium supplemented with BAP

(2.0 mg L^{-1}) showed the best efficacy for the development of new leaf. MS medium (1/2 strength) supplemented with IAA (0.5 mg L^{-1}) showed the best performance for initiation of roots. Although the protocol which we claimed here likely to be useful for the large scale multiplication of medicinal plant *P. foetida*, further efforts by engaging other plant parts and growth regulators should be needed to find out the best *in-vitro* regeneration strategy of *P. foetida*.

Acknowledgements

The authors acknowledge Research Management Wing (RMW), Bangabandhu Sheikh Mujibur Rahman Agricultural University and Ministry of Science and Technology, People's Republic of Bangladesh for financial supports for successful accomplishment of the research.

References

Agarwala, B., F. S. Azam, M. A. Khatun, F. Rahman and M. Rahmatullah. 2010. Simultaneous shoot regeneration and rhizogenesis of *Wedelia chinensis* for *in-vitro* clonal propagation. *American-Eurasian Journal of Sustainable Agriculture*. 4(1): 65–70.

Alam, M. A., F. S. Azam, M. M. Karim, F. Rehana, N. Sharmin, M. A. Kalpana and M. A. Khatun. 2010. In vitro regeneration of *Paederia foetida*: a widely used medicinal vine in Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*. 4(2): 164–169.

Amin, M. N., M. M. Rahman and M. S. Manik. 2003. *In-vitro* clonal propagation of *Paederia foetida* L. A medicinal plant of Bangladesh. *Plant Tissue Culture*. 13(2): 117–123.

Aquilar N. 2001. *P. foetida* L. In: Van Valkenburg, JL, Bunyapraphatsara, N (Eds), Plant Resource of South-East Asia. Medicinal and poisonous plants. Blackhuys Publisher, Ledien, The Netherlands.

Arab, M. M., A. Yadollahi, A. Shojaeiyan, S. Shokri, and S. M. Ghojah. 2014. Effects of nutrient media, different cytokinin types and their concentrations on *in-vitro* multiplication of G×N15 (hybrid of almond× peach) vegetative rootstock. *Journal of Genetic Engineering and Biotechnology*. 12(2): 81–87.

Ashraf, M. F., M. A. Aziz, N. Kemat and I. Ismail. 2014. Effect of cytokinin types, concentrations and their interaction on *in-vitro* shoot regeneration of *Chlorophytum borivilianum* Sant. & Fernandez. *Electronic Journal of Biotechnology*. 17(6). http://dx.doi.org/10.1016/j.ejbt.2014.08.004

Begum, F., M. N. Amin, and M. A. K. Azad. 2002. *In-vitro* rapid clonal propagation of *Ocimum basilicum* L. *Plant Tissue Culture*. 12: 27–35.

Behera, B., S. Behera, P. K. Jena, D. P. Barik, and S. K. Naik. 2017. Adventitious Shoot organogenesis and plant regeneration from internode explants of *Paederia foetida* L.: a valuable medicinal plant. *Biosciences Biotechnology Research Asia*. 14(3): 893–900.

Blatter, E., J. F. Caius and K. S. Mhaskar . 1981. *Indian Medicinal Plants*. 2: 1297-1299.

George, E. F. and P. D. Sherrington. 1984. In *Plant propagation by tissue culture*. Exegetics Ltd. 39–71.

Ghosh, T. K., S. H. Binto, J. U. Ahmed, S. M. Z. Al-meraj and M. Z. Alam. 2019. Indirect shoot organogenesis of a valuable medicinal plant *P. foetida* L. using nodal explants. *Fundamental and Applied Agriculture.* 4(2): 806–814. Doi: 10.5455/faa.24912.

Haque, M., L. Bari, M. Hasan, M. Sultana and S. Reza. 2014. A survey on medicinal plants used by the folk medicinal practitioners in Tangail Sadar Upazilla, Tangail, Bangladesh. *Journal of Environmental Science and Natural Resources*. 7:35–39. doi: 10.3329/jesnr.v7i1.22141.

Hussain, A., I. A. Qarshi, H. Nazir and I. Ullah. 2012. Plant tissue culture: current status and opportunities. *In Recent advances in plant in vitro culture*. *IntechOpen*.1: 1–28

Khare, C. P. 2007. *Indian Medicinal Plants*. 459 P.

Kumar, J. and P. K. Gupta. 2008. Molecular approaches for improvement of medicinal and aromatic plants. *Plant Biotechnology Reports*. 2(2): 93.

Mahmood, S. and B. Hauser. 2015. Influence of cytokinins on the shoot proliferation and subsequent rooting in rose. *Asian Journal of Agricultural Research*. 9(5): 259–267.

Martin, K. P. 2003. Plant regeneration through somatic embryogenesis on *Holostemma ada-kodien*, a rare medicinal plant. *Plant Cell, Tissue and Organ Culture*. 72(1): 79–82.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*. 15(3): 473–497.

Pesic, M. 2015. Development of natural product drugs in a sustainable manner. Brief for United Nations Global Sustainable Development Report.

Nandkoni, K. M. 2002. Indian materia medica. Bombay Popular Prakashan, Mumbai 892 P.

Sen, S and R. Chakraborty. 2015. Toward the integration and advancement of herbal medicine: a focus on traditional Indian medicine," *Botanics: Targets and Therapy*. 5: 33–44.

Srivastava, S. K. and N. Srivastava. 2004. *In-Vitro* multiplication of *Paedaria foetida* L-a rare medicinal plant. *Journal of plant Biochemistry and Biotechnology*. 13(1): 89–91.

Steinmetz, E. F. 1961. *Paederia foetida*. *Pharmaceutical Biology*. 1(4): 133–144.

Thirupathi, M., Srinivas, D. and K. J. Reddy. 2013. High frequency of multiple shoots inducation in *P. foetida* (L), a rare medical plant. *Plant Journal*. 1(5): 60–65.

Tiwari, K. N., N. C. Sharma, V. Tivari and B. D. Singh. 2000. Micropropagation of *Centella asiatica* (L.), a valuable medicinal herb. *Plant Cell, Tissue and Organ Culture*. 63(3): 179–185.