ORIGINAL ARTICLES

In Vitro Regeneration of *Paederia foetida*: a Widely Used Medicinal Vine in Bangladesh


1Department of Biotechnology & Genetic Engineering, University of Development Alternative, Dhanmondi, Dhaka, Bangladesh.
2Dept. of Pharmacy, Lincoln College, Mayang Plaza, Block A, No 1, Jalan SS 26/2, Taman Mayang Jaya, 47301, Petaling Jaya, Selangor Darul Ehsan, Kuala Lumpur, Malaysia.

**ABSTRACT**

*Paederia foetida* L. (Family Rubiaceae) is a perennial climbing vine that is used by traditional medical practitioners in Bangladesh for treatment of rheumatism, intestinal disorders and liver inflammation. This herb contains iridoid glycosides, sitosterol, stigmasterol, alkaloids and volatile oils. Others have reported that this species has anti-inflammatory, anti-diarrheal activities, anti-oxidant effect. Since the plant has commercial demand in Bangladesh, we established a clonal propagation method to rescue it from extinction and large scale propagation as well as to promote conservation. The excised nodes from juvenile shoot apices were implanted on MS (Murashige and Skoog) medium fortified with different concentration of 6-benzylaminopurine (BAP) following surface sterilization with 0.1% HgCl₂. Highest percentage (84%) of explants proliferated on MS supplemented with BAP at 1.0 mg/l, having an average of 2.04 shoots per explant with an average length of 1.60 cm. Addition of indole-3-acetic acid (0.5 mg/l) with BAP (1.0 mg/l) showed regenerated shoots of 1.65 cm. Multiple shoots (2.53 shoots/explant) resulted as a cluster when the media was prepared with [BAP (1.0 mg/l) + kinetin (0.5 mg/l)] to observe the synergism of auxin and cytokinin. Micro-shoots, when excised and cultured on ½ MS medium enriched with indole-3-butyric acid (0.3 mg/l) showed the best root induction percentage (85%), where average root length was observed to be 1.85 cm. In *vitro* raised plantlets were transplanted to earthen pot media after proper hardening through gradual exposure to relative humidity and sunlight and acclimatization. The survival percentage was 90%. After 4 weeks of observation, each plant was observed to be successfully growing. This method for clonal propagation can play a role for producing plantlets in large quantities and conservation of this medicinal herb to fill the requirements of traditional medical practitioners in Bangladesh.

**Key words:** *Paederia foetida*, medicinal plant, regeneration, BAP

**Introduction**

Medicinal plant species are used by traditional medicinal healers all over the world including Bangladesh. Use of plants for therapeutic purpose has a strong and long historical background within Bangladesh. Cultivation of medicinal plants is a common phenomenon in the country. However, it has been thus far mostly limited to homestead areas. But in recent times, different pharmaceutical companies and herbal industries are creating high demand for medicinal herbs. They and also different non-government organizations (NGOs) are
executing awareness programs to cultivate medicinal plants as a cash crop. Farmers in different region of Bangladesh have already initiated commercial cultivation of medicinal plants and benefiting through this cultivation. To meet the demand of plantlets for plantation, clonal propagation or micropropagation can become a favorable source for obtaining sufficient plantlets. The method of in vitro propagation often serves to both the researchers and commercial cultivators by producing plantlets on a large scale.

*Paederia foetida* (Family: Rubiaceae; “Gandhabadali” in Bangla) is one the potential herb in Bangladesh used for various medicinal practices. The major classes of chemical constituents present in this plant are iridoid glycosides, sitosterol, stigmasterol, alkaloids, carbohydrates, proteins, amino acids and volatile oils (Blatter *et al.*, 1981; Nandkoni, 2002; Steinmetz, 1961; Khare, 2007). This species is reported to have ethnomedicinal uses both in Bangladesh (Hannan *et al.*, 2008; Reza *et al.*, 2008) and India (Hynniewta and Kumar, 2008). It is 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 diarrhea; 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 vesical 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. It is also used in the preparation of Dasmularishta, an Ayurvedic formulation (Ghani, 1998). 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 antidiarrheal effects (Afroz *et al.*, 2006). Fresh leaves reportedly have antioxidant properties (Osman *et al.*, 2009).

The objective of this study was to develop an in vitro method of clonal propagation for large-scale production of this species and for different transformation studies in vitro.

**Material and methods**

**Explant Source and Preparation:**

Healthy and juvenile shoots of 1-15 cm were collected from donor *Paederia foetida* plants (2 years old), grown in the Garden of Medicinal Plants at the Department of Biotechnology and Genetic Engineering, University of Development Alternative and were used in the study. Leaves were trimmed and single nodes were excised with a sharp knife. The single node explants were surface sterilized in a three step procedure: washing for 30 minutes in running tap water; immersion in Tween-80 for 2 minutes, followed by three times washing with distilled water; and finally surface sterilization was performed with 0.1% HgCl₂ for 3 minutes, followed by three times washing with sterile distilled water.

**Culture of Establishment:**

All the explants were inoculated to establish the culture on basal MS (Murashige and Skoog) (Murashige and Skoog, 1962) medium containing different concentrations of 6-benzylaminopurine (BAP) alone and in combination with Kinetin (Kn) indole-3-acetic acid (IAA), sucrose (3%) and agar (0.7%) (see Result section for further details). The pH of the medium was adjusted to 5.8 before autoclaving and media was sterilized by autoclaving for 20 min at 121°C and 1.05 kg/cm².

Cultures were kept for 30 days in a growth chamber maintained at 26 ± 2°C with a photoperiod of 16/8 h under an illumination of 40-50 μmol m⁻²s⁻¹ provided by cool white fluorescence lamps.

**In vitro plantlet development:**

When in vitro shoots were regenerated from the above media composition the micro-shoots were excised and cultured in ½ MS containing different concentrations of Indole-3-butyric acid (IBA, see in Result section) for root induction.

**Establishment into soil:**

The in vitro developed plantlets were acclimatized in plastic pots containing garden soil and cow dung at the ratio of 2:1 after proper hardening through gradual exposure to sunlight and relative humidity.
**Data Assembly and Scrutiny:**

Weekly growth observations were made from 1st to 4th week of inoculation after setup of each treatment and experimental data were recorded. The parameters were:

a. Percentage response (%).

b. Average length of shoot in cm and number of leaves/shoot.

c. Average number of roots/shoot.

Recorded data were analyzed as mean ± SE.

**Results and discussion**

The performance of the culture medium was evaluated at the culture establishment stage by estimating the percentage of response through bud sprouting (Table-1). All the tested BAP concentrations showed shoot initiation. Among them, the best performing composition of the medium was MS + BAP (1.0 mg/l) (Figure-A). The rate of sprouting was 84% and highest length of shoot was 1.60 cm having 4-6 leaves within 4 weeks (Figure-B). When concentration of this cytokinin was increased up to 6.0 mg/l, it drastically reduced the shoot length and halted the proliferation with callogenetic part at the bases. The successfully regenerated micro-shoots were cultured for 30 days and then used as explants source for the next set of experiments.

Combinations of cytokinins were used to examine their effect on shoot multiplication. A maximum 2.53 shoot per explants was obtained when MS medium was supplemented with [BAP (1.0 mg/l) and Kn (0.5 mg/l)] (Table-1). This combination was found to be most effective for multiplication with an average of 5-6 leaves per explants (Figure-C). Calluses were observed at the base of micro-shoots when MS medium was supplemented with higher cytokinin level. This callus induction seemed to be responsible for reduced shoot length and vigor.

Results obtained from the combination of BAP and IAA showed homogeneity similar to the previous test. Highest proliferated shoot was raised at 1.65 cm when explants were cultured in MS + [BAP (1.0 mg/l) + IAA (0.5 mg/l)], having 4-6 leaves per shoot (Table-1). Multiplication rate was increased (2.14 shoots/explants) in the higher concentrations of both BAP (2.0 mg/l) and IAA (1.0 mg/l) within 2 weeks. Shoot growth and newly flushed leaves was obtained from all the treatments of auxin and cytokinin combination. When, the concentrations of both BAP and Kn exceeded over 2.0 mg/l and 1.0 mg/l, it decreased the shoot growth and formed very highly callogenetic part at the bases of explants (not shown in the table).

When the *in vitro* developed micro-shoots were cultured in root induction medium, successful rooting was observed (Table-2). Maximum rooting was obtained in ½ MS + IBA (0.3mg/l) after three weeks with an average of 4.2 roots/shoot (Figure-D). The rooted plantlets were established into plastic pots containing garden soil. The plantlets grew with healthy leaves and more than 90% survival rate was observed after eight weeks (Figure-E).

![Table 1: Effect of plant growth regulators on Paederia foetida.](image)

<table>
<thead>
<tr>
<th>MS + Hormone (mg/l)</th>
<th>Number of Inoculation</th>
<th>Percentage of Response (%)</th>
<th>Average Length of Shoot (cm) (mean±se)</th>
<th>Average no of shoot/explants (mean ±se)</th>
<th>Average no of leaves/explants (mean±se)</th>
<th>Growth vigor</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>25</td>
<td>72</td>
<td>1.10±0.12</td>
<td>1.56±0.12</td>
<td>3.50±0.44</td>
<td>++</td>
</tr>
<tr>
<td>1.0</td>
<td>24</td>
<td>84</td>
<td>1.60±0.12</td>
<td>2.04±0.22</td>
<td>5.96±0.50</td>
<td>+++</td>
</tr>
<tr>
<td>1.5</td>
<td>34</td>
<td>79</td>
<td>1.64±0.69</td>
<td>1.75±0.15</td>
<td>5.40±0.52</td>
<td>+++</td>
</tr>
<tr>
<td>2.0</td>
<td>33</td>
<td>63</td>
<td>1.05±0.11</td>
<td>1.95±0.18</td>
<td>4.56±0.36</td>
<td>+</td>
</tr>
<tr>
<td>4.0</td>
<td>33</td>
<td>57</td>
<td>0.91±0.21</td>
<td>2.21±0.21</td>
<td>3.81±0.40</td>
<td>+</td>
</tr>
<tr>
<td>6.0</td>
<td>36</td>
<td>31</td>
<td>0.71±0.10</td>
<td>1.81±0.17</td>
<td>3.28±0.41</td>
<td>+</td>
</tr>
<tr>
<td>BAP + Kn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5+0.25</td>
<td>40</td>
<td>77</td>
<td>1.11±0.09</td>
<td>0.95±0.17</td>
<td>3.93±0.22</td>
<td>+</td>
</tr>
<tr>
<td>1.0+0.5</td>
<td>31</td>
<td>90</td>
<td>1.27±0.10</td>
<td>2.53±0.15</td>
<td>4.00±0.32</td>
<td>++</td>
</tr>
<tr>
<td>1.5+0.5</td>
<td>32</td>
<td>78</td>
<td>1.16±0.09</td>
<td>1.72±0.14</td>
<td>5.41±0.27</td>
<td>++</td>
</tr>
<tr>
<td>2.0+1.0</td>
<td>45</td>
<td>69</td>
<td>0.93±0.09</td>
<td>1.71±0.12</td>
<td>4.85±0.30</td>
<td>+</td>
</tr>
<tr>
<td>BAP + IAA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5+0.25</td>
<td>38</td>
<td>68</td>
<td>1.24±0.07</td>
<td>1.54±0.12</td>
<td>3.15±0.36</td>
<td>++</td>
</tr>
<tr>
<td>1.0+0.5</td>
<td>32</td>
<td>87</td>
<td>1.65±0.09</td>
<td>1.58±0.12</td>
<td>3.31±0.51</td>
<td>++</td>
</tr>
<tr>
<td>1.5+0.5</td>
<td>31</td>
<td>84</td>
<td>1.49±0.06</td>
<td>1.64±0.10</td>
<td>5.21±0.25</td>
<td>++</td>
</tr>
<tr>
<td>2.0+1.0</td>
<td>39</td>
<td>69</td>
<td>1.06±0.09</td>
<td>2.14±0.23</td>
<td>3.35±0.17</td>
<td>++</td>
</tr>
</tbody>
</table>
Table 2: Effect of IBA on root induction in *P. foetida*

<table>
<thead>
<tr>
<th>½ MS + IBA (mg/l)</th>
<th>No of shoots inoculated</th>
<th>Percentage of response (%)</th>
<th>Average no of roots/shoot (mean±se)</th>
<th>Average length of root (cm) (mean±se)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>18</td>
<td>63</td>
<td>2.71±0.18</td>
<td>0.94±0.28</td>
</tr>
<tr>
<td>0.2</td>
<td>20</td>
<td>71</td>
<td>2.93±0.18</td>
<td>1.19±0.13</td>
</tr>
<tr>
<td>0.3</td>
<td>21</td>
<td>85</td>
<td>4.20±0.36</td>
<td>1.80±0.12</td>
</tr>
<tr>
<td>0.4</td>
<td>20</td>
<td>61</td>
<td>2.60±0.19</td>
<td>1.08±0.31</td>
</tr>
<tr>
<td>0.5</td>
<td>21</td>
<td>56</td>
<td>2.11±0.15</td>
<td>0.96±0.16</td>
</tr>
</tbody>
</table>

Fig. (A-E): A. *In vitro* shoot initiation in MS + BAP (1.0 mg/l) within 2 weeks; B. Successfully regenerated shoot with healthy leaves in the same medium; C. Multiple shoot induction in MS + [BAP (1.0 mg/l) + Kn (0.5 mg/l)]; D. Root induction was observed in ½ MS + IBA (0.3 mg/l); E. *In vitro* raised plantlets were grown successfully into soil.

Discussion:

Cytokinins are always preferred to establish *in vitro* culture and used for direct shoot regeneration. In the present study, we also found that BAP had a good effect on shoot proliferation in this herbaceous vine. Martine *et al.* (2003) and Agarwala *et al.* (2010) also reported that effect of single cytokinin (BAP) was effective for direct shoot regeneration in *Wedelia chinensis*. Our finding was in agreement with Tiwari *et al.* (2000) because they found that BAP had a good effect on explants of medicinal herb *Centella asiatica* for shoot generation *in vitro*. The role of BAP in bud breaking has been recorded for other medicinal plants such as *Artemisia annua* (Usha and Swamy, 1998), *Tagetes erecta* L. (Misra and Dutta, 1999), and *Wedelia calendulacea* (Emmanuel *et al.*, 2000).

Shoot multiplication was observed by the present authors which could be a good method for *in vitro* multiplication of *P. foetida*. Anilkumar and Nair (2004) reported the effectiveness of combination of BAP and Kinetin for multiple shoot proliferation in *Capsicum* spp. In addition, Sanatombi and Sharma (2007) also found that high level of multiple cytokinins exhibited shoot multiplication. Azam *et al.* (2009) also found the combination of BAP and Kn to be beneficial in mass propagation of trees. However, in the present study, it was observed that higher degree of cytokinin reduced the average number of multiple shoot formation.
Though a combination of auxin and cytokinin has always been found to be a suitable media composition for multiple shoot proliferation and elongation, it was not observed in our present study. Amin et al. (2003) found that combination of BAP and IAA was good for increased shoot length and multiplication which did not harmonize with our present test. However, Rathore et al. (2008) observed that incorporation of auxin with BAP was not better for bud proliferation, a finding confirmed by the present study.

Use of IBA as single auxin to induce rooting is the preferred method in tissue culture. In our present study, we observed IBA as a good root inducer which is supported by the report of Amin et al. (2003) in the same species. This phenomenon was also supported by Dhaka and Kothari (2005).

Conclusion:

This technique, therefore, presents an efficient system for in vitro clonal propagation and conservation of this widely used medicinal herb.

Acknowledgements

Portions of this paper were previously presented in the International Biotechnology Symposium held at Dalian, China in 2008.

References


