**In Vitro Organogenesis in Phyllanthus amarus Schum. and Thonn.**

Rajagopal Chitra* • E. Vadivel

Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-3, Tamil Nadu, India

*Corresponding author: * chitra.varadharaj@gmail.com

**ABSTRACT**

A procedure is outlined for organogenesis of *Phyllanthus amarus* using nodal segments and shoot tips. Multiple shoots were induced from both explants on Murashige and Skoog medium (MS) supplemented with benzyl amino purine (BAP) and kinetin (Kin). MS + BAP (4.0 mg l⁻¹) + Kin (4 mg l⁻¹) resulted in maximum multiple shoots from shoot and nodal segments. Among the explants, nodal segments produced more shoots (5.94 shoots/explant) than shoot tips (5.22 shoots/explant). The shoot tip-derived plants (95%) showed a higher survival percentage than nodal segments (88%). The addition of gibberellic acid (GA₃) to the medium enhanced shoot length. Sub-culture of multiple shoots onto MS medium supplemented with BAP (2.0 mg l⁻¹) and GA₃ (0.5 mg l⁻¹) resulted in a 7-fold increase in shoots. Rooting (87%) of the shoots was best achieved in ½ MS + IBA (0.5 mg l⁻¹) + IAA (0.5 mg l⁻¹). The plantlets were hardened in a mixture of sand and vermiculite (1: 1) and successfully established in natural soil, where they grew and matured normally.

**Keywords:** bhumyamalaki, mass production, micropropagation, nodal segment, shoot tip

**INTRODUCTION**

*Phyllanthus amarus* Schum. and Thonn. (Euphorbiaceae), known popularly in the Indian system of medicine as “Bhumyamalaki”, has been traditionally used in the treatment of a variety of ailments including hepatic disorders (Bharatiy 1992; Unander 1998). The plant is being used as one of the important ingredients in many indigenous polyherbal formulations and other ayurvedic preparations. This plant is a favorite choice of rural people because of its immense medicinal properties: antidote, against liver diseases, antiviral, antioxidant, hepatoprotective, and anti-inflammatory properties and strong inhibitory effect against neurogenic disorders (Kiemer et al. 2003; Chattopadhyay et al. 2006).

The conventional method of propagation of these species is through seeds. However, poor germination potential restricts their multiplication. Micropropagation offers an alternative method for cloning these plants (Unander 1991; Santos et al. 1994). This research describes the in vitro propagation of *P. amarus* from nodal segments and shoot tips and successful establishment of plantlets in soil.

**MATERIALS AND METHODS**

Two month-old plants of *P. amarus* raised from seeds and maintained in the greenhouse of the Medicinal Plant Conservatory of the Botanical Garden, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore were used as the source of explants. Excised nodal segments (1.0-1.5 cm) were initially washed with teepol for 2 min and then under running tap water for 3 min. This was followed by treatment with 0.1% mercuric chloride for 3 min. After 4-5 washes with sterile distilled water, explants were inoculated on basal medium consisting of Murashige and Skoog (1962) salts and vitamins, 3% sucrose and 0.8% agar. Basal medium was supplemented with various concentrations of kinetin (Kin; 1.0-5.0 mg l⁻¹), benzyl amino purine (BAP; 1.0-5.0 mg l⁻¹) and gibberellic acid (GA₃; 0.5 mg l⁻¹) for multiple shoot formation, indole-3-acetic acid (IAA; 0.25-1.25 mg l⁻¹) and indole-3-butyric acid (IBA; 0.25-1.25 mg l⁻¹) for rooting.

The medium was buffered to pH 5.8 and dispensed in 25 × 150 mm culture tubes before autoclaving at 121°C for 15 min. All cultures were maintained at 25 ± 2°C, under a 16-h photoperiod provided by cool white fluorescent light (35 μE m⁻² s⁻¹) with 70%
relative humidity. Each experiment was performed in triplicate with a total of 20 explants inoculated per treatment.

Axillary shoots, which developed after 30 days of culture, were dissected out individually for further multiplication and this process was repeated continuously every 20 days. Finally, cluster shoots developed and were transferred to basal medium supplemented with 1.0 mg l⁻¹ GA₃ for elongation before transferring to ½-strength MS medium containing IBA/IAA for rooting. The rooted plantlets were transferred to plastic pots containing sand and vermiculite (1: 1), humidity being maintained by covering with plastic bags. The survival percentage was determined after 20 days in pots. Statistical parameters like mean, standard error and critical difference for all the observations were assessed by adopting standard methods of analysis as suggested by Panse and Sukhatme (1978).

RESULTS AND DISCUSSION

Multiplication and of axillary shoots was achieved on MS medium with BAP and Kin (3.0 mg l⁻¹ each) (Table 1) and elongation with 2.0 mg l⁻¹ BAP and 0.5 mg l⁻¹ GA₃ (Table 2). Of these, BAP in combination with both GA₃ and Kin was the most effective in inducing multiple shoots (Fig. 1B) from both shoot tips and nodal segments. These results indicate that BAP, a cytokinin, played an important role in induction of multiple shoot formation and was very effective in shoot proliferation. However, BAP at higher concentrations not only reduced the number of shoots formed but also resulted in their stunted growth.

When Kin was added to the culture medium along with BAP, a remarkable effect was seen in the induction of multiple shoots. This was in agreement with the reports suggested by Vincent et al. (1992), who found that use of Kin with BAP in the culture medium enhanced multiple shoot induction in *Kaempferia galanga*. Similar effects have already been documented in *Gymnema elegans*, where Kin in the culture medium enhanced bud break in explants (Komalavalli and Rao 1997). Anilkumar et al. (2005) reported that maximum multiple shoots in *Ocimum basilicum* L. were obtained in MS medium supplemented with 5 mg l⁻¹ BAP and 10 mg l⁻¹ Kin.

GA₃ is known to have stimulatory effect on stem elongation in different plants. The same effect was seen in the present study when GA₃ was supplemented to the MS basal medium at a lower concentration responded well to elongation (an average length of 1.83 cm) (Table 2). This was supported by the findings of Pattanaik and Chand (1996) in *Ocimum sanctum* and Chitra et al. (2009) in *Phyllanthus amarus*.

IBA and IAA at a lower concentration (0.5 mg l⁻¹) gave good rooting response (93.63%). Ray and Jha (2002) reported that rooting was highest in *Withania somnifera* microshoots with 0.5 mg l⁻¹ IBA inducing 80% rooting.

<table>
<thead>
<tr>
<th>BAP (mg l⁻¹)</th>
<th>Survival %</th>
<th>Days taken for rooting</th>
<th>No. of shoots per explant</th>
<th>Length of shoots (cm)</th>
<th>Survival %</th>
<th>Days taken for rooting</th>
<th>No. of shoots per explant</th>
<th>Length of shoots (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>23.33</td>
<td>39.00</td>
<td>2.77</td>
<td>1.15</td>
<td>33.32</td>
<td>36.88</td>
<td>1.49</td>
<td>1.06</td>
</tr>
<tr>
<td>2.0</td>
<td>75.00</td>
<td>31.50</td>
<td>4.38</td>
<td>1.52</td>
<td>65.59</td>
<td>31.80</td>
<td>2.94</td>
<td>1.28</td>
</tr>
<tr>
<td>3.0</td>
<td>95.00</td>
<td>30.02</td>
<td>6.05</td>
<td>1.73</td>
<td>88.88</td>
<td>29.55</td>
<td>5.94</td>
<td>1.56</td>
</tr>
<tr>
<td>4.0</td>
<td>70.55</td>
<td>33.55</td>
<td>5.0</td>
<td>1.40</td>
<td>55.55</td>
<td>33.11</td>
<td>5.05</td>
<td>1.33</td>
</tr>
<tr>
<td>5.0</td>
<td>50.00</td>
<td>37.88</td>
<td>3.83</td>
<td>1.26</td>
<td>38.59</td>
<td>34.44</td>
<td>2.38</td>
<td>1.18</td>
</tr>
</tbody>
</table>

Table 2 Effect of BAP and GA₃ on multiple shoots development of *P. amarus*.

<table>
<thead>
<tr>
<th>Concentration of growth regulators (mg l⁻¹)</th>
<th>IAA (mg l⁻¹)</th>
<th>BAP (mg l⁻¹)</th>
<th>Rooting percentage</th>
<th>Days taken for rooting</th>
<th>No. of roots per explant</th>
<th>Length of root (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.25</td>
<td>78.63</td>
<td>21.20</td>
<td>3.34</td>
<td>3.43</td>
<td>4.34</td>
</tr>
<tr>
<td>0.50</td>
<td>0.50</td>
<td>93.63</td>
<td>15.31</td>
<td>5.77</td>
<td>5.37</td>
<td>5.37</td>
</tr>
<tr>
<td>0.75</td>
<td>0.75</td>
<td>89.44</td>
<td>18.30</td>
<td>4.69</td>
<td>2.67</td>
<td>2.67</td>
</tr>
<tr>
<td>1.00</td>
<td>1.00</td>
<td>83.69</td>
<td>21.21</td>
<td>2.03</td>
<td>2.40</td>
<td>2.40</td>
</tr>
<tr>
<td>1.25</td>
<td>1.25</td>
<td>74.13</td>
<td>20.21</td>
<td>2.88</td>
<td>2.10</td>
<td>2.10</td>
</tr>
</tbody>
</table>

Table 3 Effect of IAA and IBA on rhizogenesis of *P. amarus*.

The rooted plantlets were transferred to a hardening chamber. Two types of media viz., sand, soil and leaf mould (1: 1: 1) and sand and vermiculite (1: 1) were attempted. Survival and establishment was higher in the latter medium (Table 4). Within 25 days, plants established better and were transferred to pots with soil, sand, and farmyard manure from where they were planted in the field (Table 4). In conclusion, a protocol for the successful multiplication of *P. amarus* through *in vitro* routes is useful for conservation as well as biotechnological improvement of this pharmacologically important plant species.
ACKNOWLEDGEMENTS

We are grateful to Tamil Nadu State Council for Science and Technology (TNSCST) for the financial assistance.

REFERENCES


Bharatiya VB (1992) Selected Medicinal Plants of India, Tata Press, Bombay, pp 253-257


Panse VG, Sukhatme PV (1978) Statistical Methods for Agricultural Workers, ICAR, New Delhi, pp 134-192


Thimmann KV (1977) Hormone Action in the Whole Life of Plant, University of Massachusetts Press, Amherst, 355 pp

