Short Communication

Micropropagation of *Phyla nodiflora* (L.) Greene: An important medicinal plant

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Abstract

Nodal explants of Phyla nodiflora (L.) Greene, were cultured on Murashige and Skoog (MS) medium supplemented with different combination and concentration of cytokinins and auxins for multiple shoots regeneration. The maximum numbers of shoots were found in MS medium supplemented with 2.5 mgl^{-1}6-benzylaminopurine (BA) and 0.5 mgl^{-1} kinetin (KN). Elongated shoots were transferred to rooting medium containing quarter strength or half strength or full strength MS medium supplemented with Indole acetic acid (IAA) or Indole-3-butyric acid (IBA). Highly efficient roots were promoted on half strength MS medium supplemented with 1.0 mgl^{-1}Indole-3-butyric acid (IBA). Rooted plantlets were successfully acclimatized and established in soil. This protocol could be very useful for mass cultivation of phyla nodiflora (L.). In present study, we have established higher frequency of shoot regeneration from nodal explants of phyla nodiflora (L.).

Keywords*: Phyla nodiflora; Nodal explants; MS medium, Rooting; Hardening*

Phyla nodiflora (L.) Greene (= *Lippia nodiflora* (L.) Mihex) (Verbenaceae) is distributed in South Africa and Central America (Terblanche and Kornelin, 1996). It is a runner plant with scanty roots and possess a number of ethanobotanical uses in diseases like adenopathy, chronic indolent ulcers, etc (Kirtikar and Basu; 1975).

Alkaloids reported from *phyla nodiflora* (L.) showed significant analgesic, anti-inflammatory, and anti pyretic activities (Costa *et al.,* 89; Forestieri *et al.,* 1996). Halleridone and Hallerone compounds isolated from *P. nodiflora* (Ravikanth *et al*., 2000) are used for anti-cancer, anti-tumor, anti-malarial, anti-fungal and cytotoxic activities (Nishino *et al.,* 1988). Micropropagation of *Lippia junelliana* (Mold.) Tronc (Julianai or Juliani *et al.,* 1999) and *Lippia alba* (Gupta *et al*., 2001) was reported. The present study aims to develop the micropropagation of *phyla nodiflora*. For this purpose the axiliary node explants were excised from 2 years old *P. nodiflora*, grown in the Botanical Garden of Bharathidasan University, Tiruchirappalli and washed thoroughly in tap water for 10 min, then in 5% (v/v) detergent solution "Teepol" (Reckitt Benckiser, India) for 2 min and washed thoroughly several times with distilled water. The surface sterilization was done with 70% (v/v) alcohol for 10 seconds and washed with sterile distilled water for three times, followed by 2% (v/v) sodium hypochlorite for 2 min and thorough wash with sterile distilled water for 2 times and followed by aqueous mercuric chloride solution 0.1% (w/v) for 3 min and thoroughly washed with sterile distilled water in the sterile condition.

MS medium (1962) supplemented with sucrose (3% w/v), myo-inositol (100 mgl^{-1}, w/v), benzyl adenine (BA-0.5 mgl^{-1} - 3.0 mgl^{-1}) either individually or in combination with 6-Furfurylaminopurine (KN-0.5 mgl^{-1} - 3.0 mgl^{-1}), indole acetic acid (IAA- 0.4 -1.4 mgl^{-1}), indole butyric acid (IBA-0.4 -1.4 mgl^{-1}), α-naphthalene acetic acid (NAA-0.5 mgl^{-1}) was used for shoot induction, multiplication and root induction. The pH of the medium was adjusted to 5.8 before gelling with agar (0.8% w/v) and autoclaved at 1.06 kg/sq cm and 121°C for 15 min. The explants were inoculated vertically onto culture medium 15 ml in culture (25 X 150 mm) and subcultured every 4 weeks unless otherwise stated. The cultures were maintained in a culture room at 25 ± 2ºC under a photoperiod with light inten-

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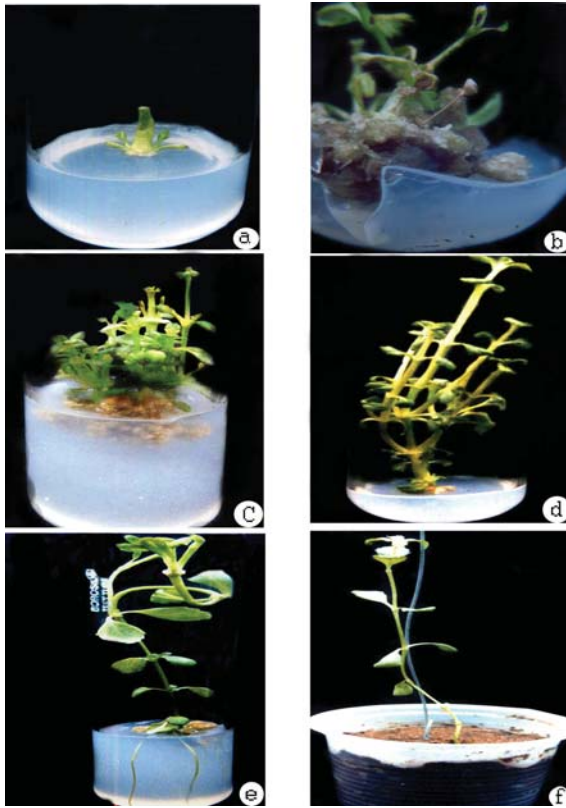

Plate 1. a and b shoot bud iniation, c. Multiple shoot formation, d. Elongation of multipe shoots, e. Rooting, f. Hardening.

sity of 35 μ $\sum m^{-2}s^{-1}$ from Philips cool white fluorescent tubes with 55-60% relative humidity. Each treatment replicated five and was repeated twice. Treatment of different strength of MS and auxins a root response and root length had mean ± standard error. All the treatment comparly were statistically by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1976).

The result showed that the axillary nodal explants cultured on MS basal medium without growth regulators induced of two shoots as well as 6.0 cm shoot length. All concentrations of BA (or) KN promoted shoot bud initiation in node explants of *P. nodiflora*. BA was the most efficient cytokinin for the axillary bud initiation and subsequent proliferation of axillary buds (Plate 1a and b). The higher number of shoots was produced in 2.5 mgl^{-1} BA (12.5 shoots/ node) (Fig. 2a, Plate 1c). MS medium supplemented with KN (0.5 mgl^{-1}) showed the higher number of shoots (9.3 shoots/ explant) as well as longer shoot length (Plate 1d and Fig. 2a). Excision of the node segments from these *in vitro* shoots and its culture facilitated the development of more than 10 shoots/explant. During subculture, basal axillary buds of the developed axillary buds also underwent initiation.

MS medium supplemented with BA and IBA was most effective for shoot regeneration (Fig. 1b) BA at

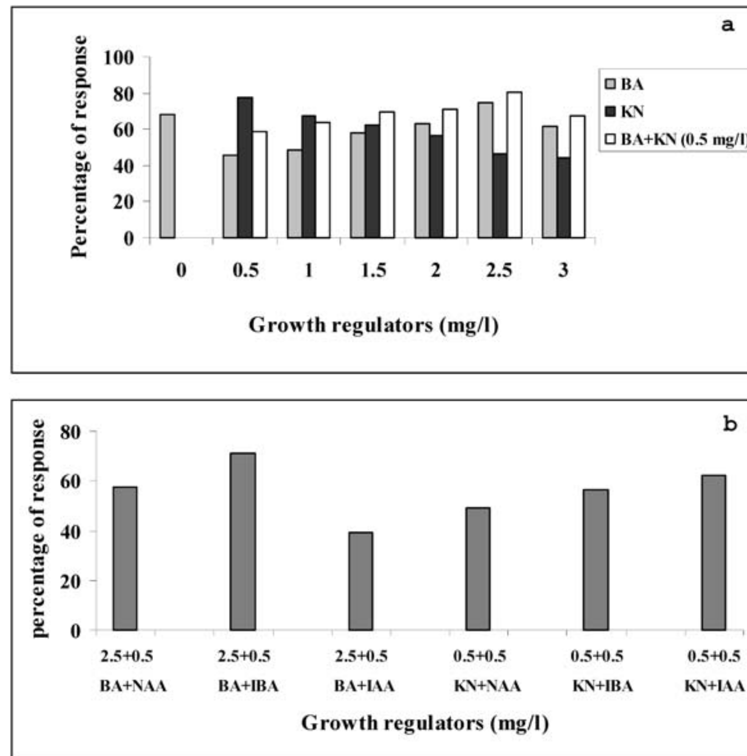

Figure 1. a and b indicates percentage of response on node explants of *P. nodiflora.* 0 = control -free growth regulators, The data recorded after four week culture.

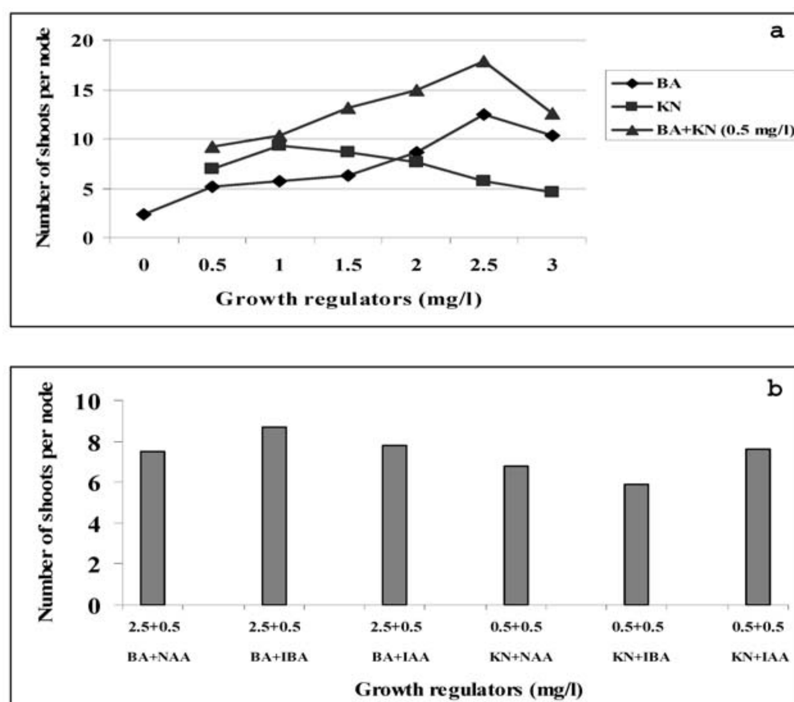

Figure 2. a and b indicates number of shoots on node explants of *P. nodiflora*. 0 = control -free growth regulators. The data recorded after four week culture.

2.5 mgl^{-1} in combination with IBA 0.5 mgl^{-1} was most effective for axillary bud multiplication, which developed a mean of 8.7 shoots per node explants (Fig. 2b).

MS medium supplemented with different combinations and concentrations of BA and KN was also used (Fig. 1a). When the concentration of BA was raised to 2.5 mgl^{-1} and combined with KN 0.5 mgl^{-1}, a higher number of multiple shoots were formed (Fig. 2a). During the present study, the micropropagated shoots exhibited leaf and shoot tip abscission. Shoot tip abscission occurred at the node region below the shoot tip.

Three different strengths (full strength, half strength and quarter strength) of MS medium without auxins (control) and/or supplemented with IAA (0.4-1.4 mgl^{-1}) and IBA (0.4-1.4 mgl^{-1}) were used for root induction. Root was not developed on different strength of MS basal (control) medium. However, highest root induction was observed on half-strength MS basal medium supplemented with IBA 1.0 mgl^{-1} resulted in 72.7% root initiation (Table 1 and Plate 1e). The root lengths were varied in all MS basal strength with IAA or IBA concentrations (Table 1). Full-strength MS medium supplemented with IAA or IBA were developed higher basal callus than half-strength and quarter-strength with IAA or IBA. The rooted plantlets were successfully transferred to hardening and well established in field condition (Plate 1f). The survival rate was 92% and plants showed normal growth with similar phenotype of mother plants.

All concentrations of BA (or) KN promoted shoot bud initiation in node explants of *P. nodiflora*. Ragava Swamy *et al. (*1992) reported that BA was the most efficient cytokinin for the axillary bud initiation and subsequent proliferation of axillary buds. A similar result was observed by Baskaran and Jeyabalan, 2005. Gupta *et al.* (2001) reported that BA showed the higher number of shoots rather than combination of either KN or NAA in *Lippia alba*. In *Ceropegia jainii* and *C. bulbosa*, BA alone is reported as most effective (Patil, 1998). MS medium supplemented with KN showed the higher number of shoots as well as longer shoot length. Similar results were reported in *Ceropegia candelabrum* (Beena *et al.,* 2003). MS medium supplemented with BA and IBA was most effective for shoot regeneration. Similar results were reported in *Holostemma annulare* (Sudha *et al*., 1998), *Hemidesmus indicus* (Sreekumar *et al*., 2000) and *Holostemma ada-kodien*, (Martin, 2002).

A synergistic effect of BA and KN in promoting the shoot initiation has been studied earlier reported (Emmannel *et al*., 2000). The micropropagated shoots exhibited leaf and shoot tip abscission. Similar phenomenon has also been reported during the *in vitro*

Table 1. The effect of growth regulators on root induction from *Phyla nodiflora* (L.) after 35 days culture.

Growth regulator (mg/l)	Rooting response (%) Explants/Mean ± S.E	Root length / explant Mean ± S.E (cm)
MS Free medium	-	-
MS + IAA		
0.4	-	-
0.6	54.6 ± 0.54^{g}	1.6 ± 0.27^{d}
0.8	49.3 ± 0.72^{h}	2.0 ± 0.47^{c}
1.0	67.3 ± 2.37^{b}	2.0 ± 0.00^{c}
1.2	65.6 ± 1.18^{c}	3.33 ± 0.27^{a}
1.4	47.0 ± 2.05^{i}	1.3 ± 0.28^{c}
MS + IBA		
0.4	57.3 ± 1.96^{f}	1.3 ± 0.27^{d}
0.6	60.8 ± 2.75^{e}	1.6 ± 0.27^{d}
0.8	64.0 ± 0.94^{d}	2.3 ± 0.28^{b}
1.0	69.7 ± 0.72^{a}	2.0 ± 0.00^{c}
1.2	57.3 ± 1.44^{f}	2.0 ± 0.28^{c}
1.4	-	-
½ MS + IAA		
0.4	57.0 ± 1.24^{h}	1.66 ± 0.94^{gh}
0.6	60.3 ± 0.72^{f}	1.77 ± 0.36^{fg}
0.8	54.8 ± 3.80^{ij}	1.33 ± 0.27^{i}
1.0	62.3 ± 1.90^{de}	2.00 ± 0.47^{c}
1.2	59.3 ± 1.96^{fg}	1.50 ± 0.28^{b}
1.4	45.3 ± 2.37^{k}	1.83 ± 0.39^{f}
½ MS + IBA		
0.4	66.3 ± 0.72^{e}	2.7 ± 0.27^{a}
0.6	62.7 ± 0.54^{d}	2.3 ± 0.35^{c}
0.8	69.3 ± 1.08^{b}	2.2 ± 0.48^{d}
1.0	72.7 ± 0.72^{a}	2.5 ± 0.53^{b}
1.2	62.3 ± 0.98^{de}	1.0 ± 1.52^{ij}
1.4	55.0 ± 1.24^{i}	2.5 ± 0.53^{h}
¼ MS + IAA		
0.4	48.0 ± 1.70^{k}	1.0 ± 0.00^{i}
0.6	56.6 ± 1.30^{h}	1.2 ± 0.27^{b}
0.8	62.6 ± 1.90^{f}	1.6 ± 0.26^{fg}
1.0	63.3 ± 0.7^{0de}	1.3 ± 0.27^{h}
1.2	43.6 ± 1.50^{l}	1.5 ± 0.28^{g}
1.4	50.3 ± 2.20^{j}	1.0 ± 0.00^{i}
¼ MS + IBA		
0.4	59.0 ± 1.44^{g}	2.6 ± 0.27^{d}
0.6	67.0 ± 1.24^{bc}	3.0 ± 0.47^{b}
0.8	67.3 ± 0.98^{b}	1.7 ± 0.48^{f}
1.0	69.6 ± 1.00^{a}	3.4 ± 0.53^{a}
1.2	64.3 ± 1.00^{d}	2.7 ± 0.48^{c}
1.4	54.3 ± 1.80^{j}	2.5 ± 0.54^{e}

The data were recorded of after 4 weeks culture. Mean followed by the different letters in each column are significantly different at the $P < 0.05$ Level Means were compared using Duncan's multiple range test (DMRT). IAA: Indole acetic acid; IBA: Indole butyric acid; MS: Murashige and Skoog medium.

multiplication on *Hemidesmus indicus* (Patnaik and Debata, 1996), and *Gymnema sylvestre* (Komalavalli and Rao, 2000). The highest root induction was observed on half-strength MS basal medium supplemented with auxins. The root lengths were varied in all MS basal strength with IAA or IBA concentrations. Similar results were observed in *Madhuca longifolia* (Rout and Das, 1993), *Gymnema sylvestre* (Komalavali and Rao, 2000) and *Eclipta alba* (Baskaran and Jeyabalan, 2005).

The present study was established for reproducible and higher frequency of shoot regeneration in *P. nodiflora* by node culture. This protocol can be used for continuous and rapid multiplication of *P. nodiflora*. These *in vitro* raised plants did not show any morphological abnormality when compared to original plants.

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