

**INDUCTION OF CALLOGENESIS AND SHOOT REGENERATION OF A MEDICINAL PLANT SPECIES
PERISTROPHE BICALYCOLATA (RETZ.) NEES.**

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ABSTRACT

In the present study, protocol for callus induction and regeneration for the medicinal plant species, *Peristrophe bicalyculata* (Retz.)Nees has been developed by using leaf explants. Young apical leaf explant was used for callus induction on MS medium containing BAP and NAA at 2.0 and 0.8 mg^l⁻¹ respectively showed maximum callus induction (80%). The amount of callus responded for shoot formation (81%) was obtained in the MS medium containing BAP (2.0 mg^l⁻¹) and GA₃ (0.3mg^l⁻¹).The elongated shoots were rooted on half strength medium supplemented with IBA (2.0 mg^l⁻¹) and IAA (0.2 mg^l⁻¹) for shoots rooted. Regenerated plantlet were successfully acclimatized and hardened off inside the culture and then transferred to green house with better survival rate.

Keywords: *Peristrophe bicalyculata*, MS medium, multiple shooting, Acclimatization.

1. INTRODUCTION

The world has a very rich biodiversity of woody plants, many of which are medicinally important. Because of the use in medicine, woody plants require rapid and reliable methods of propagation. The conventional methods of propagation such as cuttings, graftings and layering are very slow. The rapid loss of rooting ability with age of woody plants makes them difficult to propagate. So, they require alternative method. Plant propagation by tissue culture is the possible approach to overcome the problem.

Peristrophe bicalyculata (Retz.)Nees belonging to the family Acanthaceae, having lot of medicinal properties. The plant is used on blood pressure, kidney, liver functions and skin related problems. It is also used as an antidote for snake poison when macerated in an infusion of rice, and as an insect repellent (Dwivedi, 2008). The ethanol extract of the plant has been reported to exhibit analgesic, anti-inflammatory and antibacterial properties (Chopra, 1959; Dwivedi, 2002). Although undocumented, the plant is used in South West Nigeria in the treatment of hypertension and other cardiovascular diseases. The essential oil shows tuberculostatic activity *in vitro*. Ayurvedic pharmacopoeia of India recommends the dried root in insomnia and for fear- psychosis in children. Leaves of the plant were used traditionally as analgesic, antipyretic, anti-inflammatory, sedative, stomachic, anticancer, fertility, diuretics and diarrhoea.

To date, there has been no report on *in vitro* regeneration of *P. bicalyculata*. Herein, we described the optimization of culture conditions and plant growth regulators required for callus initiation, shoot regeneration and rooting of plantlets from immature leaflets of *P. bicalyculata*.

2. MATERIALS AND METHODS

Leaf segments from young and healthy branches of *P. bicalyculata* were used as explants. They were collected from pot cultured individuals maintained in a mist chamber. For surface sterilization, the collected immature leaves were washed with tap water twice and then treated with 5 % tween-20 solutions for 5 min followed by rinsing in tap water. To eliminate fungal contamination, explants were further treated with 5 % antibiotics (Amphicillin and Rifampicin) for 30 min followed by 3 rinses in sterile double distilled water. Further, surface sterilization was carried out by dipping the explants in 0.1% HgCl₂ for 3 min followed by 3-4 rinses in sterile double distilled water.

2.1. Media and culture condition

Murashige and Skoog (MS) (1962), medium containing 3 % sucrose solidified with 1 % agar (tissue culture grade, Himedia, India) was used. The pH of the medium was adjusted to 5.6-5.8 prior to the addition of agar before autoclaving at 121^oC for 15 min. All the culture bottles were kept in culture chamber at 25± 2^o C under 16/8 hr (light/dark) photoperiod with a light intensity of 2000 lux

supplied by cool white fluorescent tubes and with 60-65% relative humidity.

2.2. Callus induction medium

The explants were transferred to culture bottles containing 25 ml MS medium supplemented with different concentrations and combinations of BAP and NAA for callus induction.

2.3. Shoot induction medium

MS medium containing different concentrations and combinations of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) and GA₃ at 0.3 mg/l was used for shooting attributes.

2.4. Rooting of elongated shoots and acclimatization

After proper shoot induction, the plantlets were carefully removed from the medium and washed with sterilized double distilled water properly, so as to avoid any trace of medium on roots. *In vitro* regenerated shoots (5-6 cm long) were excised and transferred onto the rooting media containing half strength MS medium supplemented with IBA and IAA for rooting. After proper root formation, these rooted plantlets were transferred to hardening medium composed by garden soil, sand and vermicompost in different proportion and maintained in greenhouse condition to know the survivability rate.

2.5. Statistical Analysis

All the experiment was done at least twice using triplicate. The data was statistically processed and means were compared using Duncan's Multiple Range Test ($P < 0.05$).

3. RESULTS AND DISCUSSION

Calli formation was observed in leaf explants after 25 days. The best response of callus (80%) was observed in the MS medium supplemented with cytokinin BAP (2.0 mg/l) and auxin, NAA (0.8 mg/l) (Table 1). The result is

supported by Thambiraj & Paulsamy (2012). Further studies were carried out for shoot regeneration capacity of the callus. Shoots were initiated from the callus obtained leaf explants. The best result of shooting (81%) was observed on the MS medium fortified with BAP (2.0 mg/l) and GA₃ (0.3 mg/l). The maximum number of multiple shoots 11.77 shoots/callus & shoot length (6.3 cm) were produced in the same concentrations and combinations of growth regulators (Table 2). The superiority of BAP over the other cytokinins on shoot bud production and proliferation of shoots has been reported for several medicinal and aromatic plant species by Jebakumar & Jayabalan, 2000; Hussain & Anis, 2006; Raja *et al.*, 2008; Faisal & Anis, 2003.

Induction of rooting is an important step for *in vitro* plant propagation. Excised shoots were inoculated on MS medium with IBA and IAA for proper root development. The rooting responses were summarized in Table 3. Maximum rooting (78%), number of roots (9.78 roots/shoot) & root length (6.6 cm) was observed on the MS medium supplemented with IBA and IAA at 2.0 & 0.2 mg/l respectively (Table 3). These findings are in agreement with those reported by Sujatha & Reddy, 1998; Ahn *et al.*, 2007; Alam *et al.*, 2010 and Ramanathan *et al.*, 2011.

After the development of roots, the plantlets were taken out from the culture bottles and washed with sterilized distilled water to remove adhering agar medium, so that the chance of contamination could be stopped. Then these juvenile plantlets were transferred to the hardening medium containing garden soil, sand and vermicompost (1:1:1 ratio by volume) where the leaf callus derived plantlets survivability rate was higher 76% (Table 4). Admixture of all these three components may offer conducive environment by providing proper nutrients, adequate aeration and required minerals respectively to the plantlets.

Table 1. Effect of growth regulators on callus induction from leaf explants of the species, *Peristrophe bicalyculata*.

Growth regulators (mg/l)				Days required for callus formation after inoculation	Callus formation (%)
BAP	2,4-D	NAA	Kn	Leaf Explant	Leaf Explant
0.5	0.0	0.0	0.0	18	35.25 ^a ± 1.63
1.0	0.0	0.0	0.0	19	47.76 ^c ± 0.82
1.5	0.0	0.0	0.0	17	56.86 ^e ± 1.63
2.0	0.0	0.0	0.0	20	64.35 ^g ± 2.45
2.5	0.0	0.0	0.0	21	70.53 ⁱ ± 0.82

3.0	0.0	0.0	0.0	19	60.84 ^f ± 0.82
0.0	0.5	0.4	0.0	16	46.57 ^c ± 1.63
0.0	1.0	0.4	0.0	15	54.37 ^e ± 1.63
0.0	1.5	0.4	0.0	20	61.36 ^f ± 0.82
0.0	2.0	0.4	0.0	23	69.58 ⁱ ± 1.63
0.0	2.5	0.4	0.0	16	37.97 ^a ± 1.63
0.5	0.0	0.2	0.0	15	59.00 ^{cd} ± 0.82
1.0	0.0	0.4	0.0	16	67.35 ^e ± 1.63
1.5	0.0	0.6	0.0	21	75.35 ⁱ ± 0.82
2.0	0.0	0.8	0.0	25	80.13 ^j ± 1.63
2.5	0.0	1.0	0.0	21	72.25 ^h ± 1.63
3.0	0.0	1.2	0.0	18	52.87 ^b ± 1.63
0.0	0.3	0.0	0.2	15	46.48 ^c ± 1.63
0.0	0.6	0.0	0.4	17	51.59 ^d ± 0.82
0.0	0.9	0.0	0.6	16	55.32 ^e ± 1.63
0.0	1.2	0.0	0.8	18	60.21 ^f ± 0.82
0.0	1.5	0.0	1.0	14	49.00 ^{cd} ± 0.82

Means in columns followed by different letter (s) are significant to each other at 5% level according to DMRT.

Table 2. Effect of different concentrations of growth regulators on shoot initiation, shoot number and shoot length after the subculturing of leaf derived callus of the species, *Peristrophe bicalyculata*.

Growth regulators (mg/l)				Culture response (%)	No. of shoots/callus	Shoot length (cm)
BAP	NAA	GA ₃	IAA			
0.3	0.0	0.0	0.0	53.23 ^d ± 0.82	3.14 ^{ab} ± 0.82	2.5 ^{ab} ± 0.82
0.3	0.0	0.0	0.0	62.64 ^{hi} ± 0.82	5.74 ^{bcd} ± 0.82	3.8 ^{abc} ± 1.63
0.3	0.0	0.0	0.0	65.78 ^j ± 1.63	7.98 ^{def} ± 1.63	4.0 ^{abc} ± 1.63
0.3	0.0	0.0	0.0	58.37 ^{fg} ± 0.82	4.00 ^{abc} ± 1.63	5.2 ^{bc} ± 0.82
0.3	0.0	0.0	0.0	60.64 ^{gh} ± 1.63	9.38 ^{fgh} ± 1.63	4.6 ^{abc} ± 0.82
0.3	0.0	0.0	0.0	70.53 ^{kl} ± 1.63	9.64 ^{fgh} ± 0.82	5.7 ^c ± 1.63
0.5	0.0	0.3	0.0	69.27 ^k ± 0.82	8.75 ^{hi} ± 1.63	4.3 ^{abc} ± 0.82
1.0	0.0	0.3	0.0	75.00 ^m ± 1.63	9.35 ^{ghi} ± 0.82	3.9 ^{abc} ± 0.82
1.5	0.0	0.3	0.0	77.58 ⁿ ± 0.82	10.17 ⁱ ± 0.82	5.0 ^c ± 1.63
2.0	0.0	0.3	0.0	81.58 ⁿ ± 0.82	11.77 ⁱ ± 0.82	6.3 ^c ± 1.63
2.5	0.0	0.3	0.0	75.96 ^b ± 1.63	9.98 ^{efg} ± 0.82	5.7 ^{abc} ± 0.82
3.0	0.0	0.3	0.0	50.36 ^a ± 1.63	5.32 ^{bcd} ± 0.82	2.5 ^{ab} ± 0.82
0.0	0.5	0.0	0.0	55.26 ^{de} ± 0.82	6.41 ^{ghi} ± 0.82	3.5 ^{abc} ± 1.63
0.0	0.5	0.0	0.0	41.12 ^a ± 0.82	5.67 ^{fgh} ± 0.82	4.9 ^{bc} ± 0.82
0.0	0.5	0.0	0.0	49.11 ^c ± 0.82	6.52 ^{cde} ± 1.63	5.3 ^{bc} ± 1.63
0.0	0.5	0.0	0.0	58.85 ^{fg} ± 1.63	3.43 ^{ab} ± 1.63	4.7 ^{abc} ± 1.63
0.0	0.5	0.0	0.0	64.26 ^{ij} ± 0.82	5.28 ^{bcd} ± 0.82	3.3 ^{abc} ± 0.82
0.0	0.5	0.0	0.0	72.16 ^l ± 1.63	7.47 ^{efg} ± 1.63	2.8 ^{ab} ± 0.82
0.5	0.0	0.0	0.2	43.49 ^d ± 0.82	3.90 ^{def} ± 0.82	2.0 ^{abc} ± 0.82
1.0	0.0	0.0	0.2	28.00 ^k ± 1.63	1.38 ^{ghi} ± 0.82	1.9 ^a ± 0.82
1.5	0.0	0.0	0.2	57.45 ^{ef} ± 1.63	4.15 ^{abc} ± 1.63	3.4 ^{abc} ± 0.82
2.0	0.0	0.0	0.2	62.75 ^{hi} ± 0.82	2.47 ^a ± 0.82	3.5 ^{abc} ± 1.63
2.5	0.0	0.0	0.2	59.32 ^{fg} ± 0.82	3.74 ^{ab} ± 1.63	2.0 ^a ± 0.82
3.0	0.0	0.0	0.2	55.21 ^{ij} ± 1.63	6.00 ^{fgh} ± 0.82	2.5 ^{ab} ± 0.82

Means in columns followed by different letter (s) are significant to each other at 5% level according to DMRT.

Table 3. Effect of different concentrations of growth regulators on root number, rooting percentage and root length after the subculturing of leaf callus derived *in vitro* produced shoots of the species, *Peristrophe bicalyculata*.

Growth regulators (mg/l)			Shoots rooted (%)	No. of roots/shoot	Root length (cm)
IBA	IAA	NAA			
0.5	0.2	0.0	60.30 ^j ± 0.41	6.67 ^{abc} ± 0.82	5.4 ^{a-d} ± 0.82
1.0	0.2	0.0	68.25 ^k ± 0.82	7.39 ^{def} ± 1.63	5.9 ^{ef} ± 0.82
1.5	0.2	0.0	76.29 ^m ± 0.41	8.81 ^f ± 1.63	6.0 ^f ± 1.63
2.0	0.2	0.0	78.65 ^l ± 0.82	9.78 ^{ef} ± 0.41	6.6 ^{ef} ± 1.63
2.5	0.2	0.0	62.54 ^h ± 1.63	4.98 ^{abc} ± 0.82	4.8 ^{de} ± 0.41
3.0	0.2	0.0	48.45 ^g ± 0.82	3.00 ^{ab} ± 0.82	3.2 ^{a-d} ± 0.16
0.5	0.0	0.3	19.65 ^e ± 0.41	2.26 ^{bcd} ± 1.63	1.1 ^{bcd} ± 0.82
1.0	0.0	0.3	25.32 ^d ± 0.82	3.19 ^{ab} ± 1.63	3.9 ^{a-d} ± 0.33
1.5	0.0	0.3	35.54 ^c ± 1.63	4.64 ^{abc} ± 0.82	2.8 ^{abc} ± 0.49
2.0	0.0	0.3	39.24 ^b ± 0.82	4.38 ^{abc} ± 0.33	3.5 ^{a-d} ± 0.41
2.5	0.0	0.3	45.17 ^a ± 0.41	2.59 ^a ± 0.82	2.6 ^{ab} ± 0.82
3.0	0.0	0.3	50.98 ^a ± 0.82	3.38 ^{ab} ± 0.82	2.2 ^a ± 0.16
0.5	0.0	0.0	34.87 ^c ± 0.82	3.29 ^{ab} ± 1.63	3.8 ^{a-d} ± 0.82
1.0	0.0	0.0	45.67 ^f ± 0.82	5.48 ^{bcd} ± 0.49	2.9 ^{abc} ± 0.82
1.5	0.0	0.0	52.54 ^h ± 1.63	6.76 ^{cde} ± 1.63	4.1 ^{bcd} ± 0.82
2.0	0.0	0.0	51.87 ^h ± 0.82	5.53 ^{bcd} ± 0.82	4.5 ^{cde} ± 0.41
2.5	0.0	0.0	48.88 ^g ± 0.41	4.68 ^{abc} ± 0.82	3.2 ^{a-d} ± 0.33
3.0	0.0	0.0	55.34 ⁱ ± 0.82	5.58 ^{bcd} ± 0.82	4.8 ^{de} ± 0.24

Means in columns followed by different letter (s) are significant to each other at 5% level according to DMRT.

Table 4. Effect of different composition of hardening medium on survivability rate of leaf callus derived *in vitro* rooted plantlets of the species, *Peristrophe bicalyculata*.

Hardening medium composition (V/V)	No. of plantlets under hardening	No. of plantlets survived	Survivability (%)
Red soil + sand (1:1)	50	24	42 ^a ± 0.82
Garden soil + sand + vermicompost (1:1:1)	50	41	76 ^d ± 0.41
Decomposed coir waste + perlite + compost (1:1:1)	50	35	71 ^c ± 1.63
Vermicompost + soil (1:1)	50	33	64 ^b ± 0.65
Red soil + sand + vermicompost (1:1:1)	50	25	50 ^a ± 0.82

Means in column followed by different letter (s) are significant to each other at 5% level according to DMRT.

From the above study, it is concluded that multiple shoot and root cultures of *Peristrophe bicalyculata* were established from leaf explants on MS medium supplemented with combination of hormones. This protocol has potential for large-scale micropropagation and application in molecular plant breeding research programs.

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