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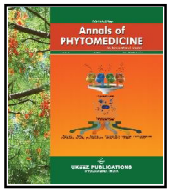

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An efficient protocol for callus induction in *Poeciloneuron indicum* Bedd.: An endemic medicinal plant of Western Ghats

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Abstract

Poeciloneuron indicum Bedd. is an endemic plant of Clusiaceae, useful as both in medicine as well as in timber. Among the tested culture medias, MS and WPM are proved to be potential media to induce callus 95% of callusing was observed in MS medium supplemented with 1.5 ppm of TDZ alone, followed by 94% of callusing with 1.5 ppm of TDZ and 0.5 ppm of 2, 4-D in combination. WPM was less potent compared to MS media and resulted with 65% of callusing which was supplemented with 1.5 ppm of 2,4-D and 0.5 ppm of TDZ, followed by 59% callusing when supplemented with TDZ alone. Leaf explants were categorized in to L1 (brown young leaves), L2 (pale green leaves) and L3 (green) among which, L2 has shown better callusing, followed by L1, however no response was observed with L3. Compared to leaf and young stem, leaf explants has shown better response with greater amount of callusing in the above-mentioned growth hormone. Callus observed was compact and hard, faded white to pale brownish in colour. This is the first report for callus induction on the selected plant species.

1. Introduction

The genus, Poeciloneuron of Clusiacea, formerly has only two species, *viz.*, *Poeciloneuron pauciloflorum* Bedd. and *Poeciloneuron indicum* Bedd. endemic to Western Ghats and medicinally important. Medicinal plants being exploited by humans since a long time and are globally valuable sources of herbal products, and they are disappearing at a high speed (Chen *et al*., 2016). Since most of them are not used to have a good adopted method of cultivation and harvesting there is an urgency in conserving them (Priyadarshini *et al.*, 2019). Generally, plants can be conserved by many methods but much higher multiplication rates can be obtained by tissue culture methods when compared to conventional methods (Mercier and Kerbauy, 1995). Present study is a first step towards such conservation strategies, and hence could be further elaborated in micropropagation technique which can be the future interest of establishing rare plants of restricted regions in more efficient manner. Different parts of the plant are used in treating dysentery, diarrhea and decoction of root is used as oral contraceptive. Extreme exploitation of plant may damage the plant in local regions of its endemic regions of Western Ghats by people in and around Western Ghats for their benefits. The wood of the tree was known in the usage of railway steeper before the introduction of concrete steeper because of its good strength. The plant propagates through seeds which have anomaly of twin seedling (Subramanian *et al.*, 2005), which is reported to have survival risks. Different techniques for conservation of plants have been practiced worldwide, the most important being tissue culture. Commonly called 'Baliga' (Same has been deposited as herbarium with accession number UOMBOT22P174 at DOS in Botany, University of Mysore) found abundant in Agumbe region of Kuduremukh forests of Western Ghats. Callus can be the efficient form through which propagation can be made; the present attempt was successfully made towards establishing protocol for callus production using different parts of the plant as explants.

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2. Materials and Methods

Media such as Murashige and Skoog media (1962), Woody plant media (Lloyd and McCrown , 1980), Nitsch and Nitsch media (Nitsch and Nitsch, 1969) and Whites media (White *et al*., 1983) were used for culturing which were prepared by using ready media by Hi-media were supplemented with 3% sucrose for carbon source and 0.7% agar and 0.5 mg/l of PVP or activated charcoal for preventing browning of media were homogenized after adjusting the pH to 5.8 using 0.1N NaOH and 1N HCl, homogenised media were poured to culture vessels which were sterilized in pressure cooker for 15 min. in 15000 psi. Culture vessels were kept under UV light after cooling for inoculation.

Media were supplemented with varied concentrations of auxins and cytokinin from 0-5 mg/l individually and in combinations. Auxins used in present study are 2,4-D, IAA and IBA whereas cytokinins used were kinetin, BAP and TDZ. Plant growth regulators were added to media before adjusting the pH.

Plant materials were collected from Kuduremukh region of Western Ghats. Fresh young leaves and stem selected as explants were washed thoroughly in running tap water for about 10 minutes followed by distilled water for 2-to-3 min. Explants were then washed with approximately 100 ml of water containing 0.5 g of Bavistin (0.5%) by subjecting to rotary shaker for 10 to 15 min. Traces of Bavistin were removed by washing 3 to 4 times in distilled water which were

further surface sterilized using 0.1% mercuric chloride solution for about 20 to 40 sec depending on the tenderness of the explant since young materials tends to denature when exposed to mercuric chloride.

Explants were transferred to presterilized laminar air flow chamber by swabbing it with 70% ethanol, followed by UV light expose for 30 min.

Explants were cut into appropriate size using fresh sterilized blade and were wounded with cuts which increase point of contact with media for callus initiation. Explants were incubated after inoculation in incubation chamber with controlled temperature of 25 ± 2°C. Photoperiod was restricted to 8 hours followed by 16 hours of dark in total of 24 h of a day. Observations were made regularly and responses were tabulated. Regular observations were made on rate of contamination, callus initiation, curling and growth. Initiated callus were checked for the weight and fresh weight was recorded using digital balance after removing it from culture media and rinsing it in distilled water until excess media were removed and further dried by keeping then in an oven at 40°C for 48 h (Bano *et al*., 2022) and weight of dried callus were recorded (Table 2).

3. Results

Many different media suggested in different works on tissue culture was given a try to get a better protocol for callus initiation from different explants of the selected plant, responses of different explants for different combinations of plant growth hormones in different media such as Woody plant media, Murashige and Skoog media, whites media and Nistch media are tabulated in Table 1 and Table 2. MS media resulted in better callus quantity compared to WPM as listed in Table 2 where callus growth and Weight were more in MS media with 1.5 ppm TDZ alone with gradual increase in weight as callus becomes older and maximum yield attained during 70 -90th day of callusing with 2.09 g wet weight which gave the dry weight of 0.97 g.

Among the selected explants, leaf explant responded better compared to stem with comparatively early curling and callusing which can be seen in Figure I. Among all, the works on Clusiaceae members it is prominent that leaf discs are better in callusing than stem which might be because all of them are tree which tends to have more rigidity compared to leaf explants. Young stem explants were reported with callusing by 24th day of inoculation whereas curling in leaf explants started in 6th day of inoculation, followed by initiation of callusing by 17th day of inoculation.

Among leaf explants of L1, L2 and L3; L2 was better in callusing with respect to its callus initiation time and callus mass production. L1 was younger and more expected to give best callusing but failed to achieve the same even it showed the curling in initial days of inoculation, *i.e*., 3rd day from the date of inoculation, since it secreted more of phenolics content and caused browning of media. Browning of medium was comparatively high with L2 explants which were managed with appropriate concentration of activated charcoal and PVP. Browning of media is a common problem in tissue culture of woody plant. Very little or no callus was observed with L3 explants; contamination rates (fungal) were also high for L3 explants when compared with other selected explants.

Contamination was more of fungal. Bacterial contaminations were very rare for which chloramphenicol antibiotic of 0.5 mg/l in media were used. Fungal contaminations were controlled by using Bavistin fungicide during surface sterilization in 1 g/100 ml concentration which was best in reducing fungal contaminations when compared to other tested concentrations.

Callus obtained were of different textures and varied with different growth hormones and in combinations of growth hormones. Some were friable, some were soft and some were rigid and compact which even resulted in getting good yield of dried callus which was further used for phytochemical analysis and analysing different activities. Callus which was compact seemed to have less of watery content compared to soft and friable callus, and hence could get better yield after drying them in different time intervals as represented in Table 2. MS media failed to induce callusing with 2,4-D alone in lower concentration, whereas it was efficient in inducing callusing with 2 ppm of 2,4-D alone with 78. 5 %. MS media supplemented with 2,4-D along with kinetin also achieved the callusing in varied concentrations as combination which resulted in 82% callusing with 1.5 ppm of 2,4-D and 0.5 ppm of kinetin. Different concentrations of kinetin also resulted in callusing with maximum of 78% when used alone with 2ppm concentration. 2,4-D and TDZ in combination resulted in second highest callusing with 94% when used in 0.5 ppm and 1.5 ppm, respectively. Lower concentrations of kinetin with TDZ in MS media could not induce any callus but higher concentrations were better in callusing among which 0.5 ppm of kinetin with 1.5 ppm of TDZ was reported with 84% of callusing. Among all the tested combinations of growth hormones, TDZ alone with 2 ppm resulted highest induction percentage 95% callusing. WPM supplemented with different concentrations of 2,4-D, kinetin and TDZ alone and in combinations could not yield better callus compared to Murashige and Skoog media although some concentrations resulted in callusing among which, TDZ and 2,4-D in 0.5 ppm and 1.5 ppm concentration, respectively, was best with 65 % callusing, followed by 59% callusing with 2 ppm of TDZ alone. Nitsch media and Whites media failed to induce better callusing compared to MS media and WPM although best callusing in Nitsch media was with 62% when supplemented with 1ppm of 2,4-D along with 1ppm of kinetin, whereas best callusing in Whites media was with 58% when supplemented with 2 ppm of kinetin alone.

Table 1: Effect of different growth hormones and culture media on callus induction of *P. indicum*

Plant growth regulator			Murashige and Skoog medium			Woody plant medium			Nitsch medium			White's medium		
2,4-D (mg/l)	K (mg/l)	TDZ (mg/l)	Callus induction in %	Callus growth	Callus texture	Callus induction in %	Callus growth	Callus texture	Callus induction	Callus growth	Callus texture	Callus induction	Callus growth	Callus texture
0.50	0.00	0.00	0.00	NC	-	0.0	NC	-	0.0	NC	-	0.0	NC	-
0.75	0.00	0.00	0.00	NC	-	0.0	NC	-	0.0	NC	-	0.0	NC	-
1.00	0.00	0.00	0.00	NC	-	0.0	NC	-	48	NC	-	0.0	NC	-

1.50	0.00	0.00	60	+	Compact	0.0	NC	-	0.0	NC	-	0.0	NC	-
2.00	0.00	0.00	78.5	+	Compact	0.0	NC	-	0.0	NC	-	0.0	NC	-
0.50	0.50	0.00	0.00	NC	-	0.0	NC	-	0.0	NC	-	0.0	NC	-
0.75	0.25	0.00	0.00	NC	-	0.0	NC	-	0.0	NC	-	0.0	NC	-
1.00	1.00	0.00	80.0	++	Friable	50.2	+	Soft	62	++	Soft	0.0	NC	-
1.50	0.50	0.00	82.0	++	Friable	0.0	NC	-	0.0	NC	-	0.0	NC	-
0.50	1.50	0.00	73.0	++	Compact	0.0	NC	-	0.0	NC	-	0.0	NC	-
0.00	0.50	0.00	0.00	NC	-	0.0	NC	-	0.0	NC	-	0.0	NC	-
0.00	1.00	0.00	63.0	+	Soft	0.0	NC	-	39	+	Compact	49	++	Compact
0.00	1.50	0.00	75.0	+	Soft	38.0	+	Friable	0.0	NC	-	37	+	Compact
0.00	2.00	0.00	78.0	++	Compact	0.0	NC	-	0.0	NC	-	58	++	Compact
0.00	0.00	0.50	0.00	NC	-	0.0	NC	-	0.0	NC	-	0.0	NC	-
0.50	0.00	0.50	85.0	++	Compact	0.0	NC	-	0.0	NC	-	0.0	NC	-
0.50	0.00	1.00	82.0	++	Compact	41.0	+	Compact	0.0	NC	-	0.0	NC	-
0.50	0.00	1.50	94.0	+++	Compact	0.0	NC	-	0.0	NC	-	0.0	NC	-
1.50	0.00	0.50	83.3	++	Compact	65.0	++	Compact	0.0	NC	-	0.0	NC	-
0.00	0.00	0.50	0.00	NC	-	0.0	NC	-	0.0	NC	-	0.0	NC	-
0.00	0.50	0.50	0.00	NC	-	0.0	NC	-	0.0	NC	-	0.0	NC	-
0.00	0.50	1.00	58.0	+	Friable	0.0	NC	-	0.0	NC	-	0.0	NC	-
0.00	0.50	1.50	84.0	++	Compact	29.8	+	Friable	0.0	NC	-	0.0	NC	-
0.00	1.50	0.50	73.2	++	Compact	0.0	NC	-	0.0	NC	-	0.0	NC	-
0.00	0.00	0.50	0.00	NC	-	0.0	NC	-	0.0	NC	-	0.0	NC	-
0.00	0.00	1.00	76.0	+	Soft	0.0	NC	-	0.0	NC	-	0.0	NC	-
0.00	0.00	1.50	95.0	+++	Compact	0.0	NC	-	0.0	NC	-	0.0	NC	-
0.00	0.00	2.00	82.0	+++	Compact	59.0	++	Compact	0.0	NC	-	0.0	NC	-

NC (No Callus), + (Less Response), ++ (Moderate Response), +++ (Good Response)

Table 2: Comparison of fresh and dry weight of callus on different media for different time intervals

Media	Plant growth hormone (ppm)	Days of culture					
		30-40		50-60		70-90	
		Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
MS	1.5 ppm of TDZ	1.03	0.24	1.75	0.89	2.09	0.97
WPM	1.5 ppm of 2,4-D +0.5 ppm of TDZ	0.9	0.17	1.3	0.23	1.52	0.58

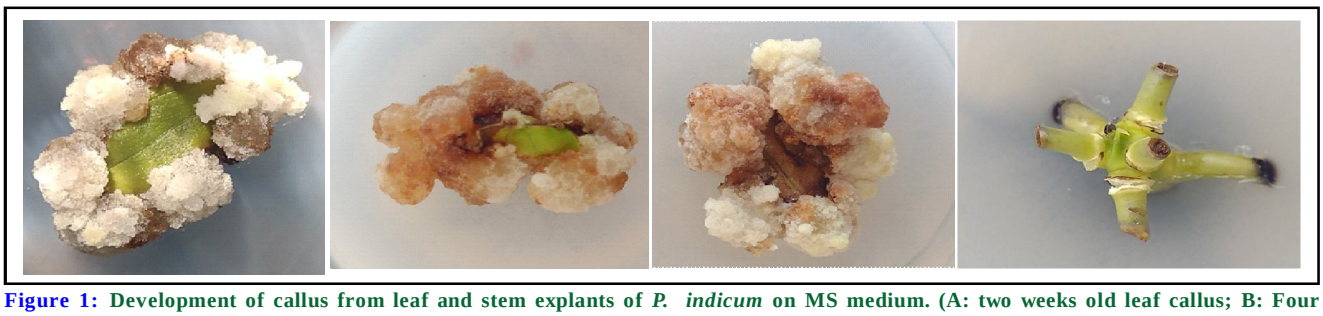

Figure 1: Development of callus from leaf and stem explants of *P. indicum* on MS medium. (A: two weeks old leaf callus; B: Four weeks old leaf callus; C: Six weeks old leaf callus; D: Two weeks old callus initiated from stem nodules)

4. Discussion

Few works on members of family Clusiaceae have already been done and standardised the protocols which were preferred while carrying out present study which required little modifications and proven that MS media and Woody plant media were more suitable in being potent as observed in callusing which was supported with the works of Neondo (2014), Saini *et al.* (2014) and Suwaneree *et al.* (2019). Both stem and leaf explants of *Musua ferrea*, stem explants of *Garcinia schamburgkiana* and leaf explants of *Garcinia schomburgkiana* are also reported with compact callus as reported by Saini *et al*, (2014) and Suwaneree *et al*. (2019), respectively. *Mammaea americana* (apricot tree) is also a member of family Clusiaceae whose callasogenesis was achieved using leaf segments on various media in which best results were observed by Ferreira *et al*. (2014) on MS media containing 2.0 mg L-1 2,4-D + 2.0 mg L-1 BAP. Suwanseree *et al*. (2019) concluded their work on four of Garcinia species such as, Garcinia mangostana, *Garcinia schomburgkiana*, *Garcinia cowa*, and *Garcinia celebica* that all the four are capable of callusing with treatments of TDZ over MS media which supports the present work results. As observed in the present study, it is clear that kinetin, 2, 4-D and TDZ alone and in particular combinations listed in Table 1 are having better callusing where few are similar with the other studies of Clusiaceae members as listed before.

5. Conclusion

P. indicum as any other tree was known for its higher phenolic exudates release and was the main drawback of the work since it cannot yield or response callus as many of the members of the family do. Callusing was considered best with respect to the percentage of callusing in which it was observed with highest callusing when supplemented with TDZ. Among various media used, callus was best on MS media. Leaf explant is best in callusing among which L2 gave higher response rate and lower contamination.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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