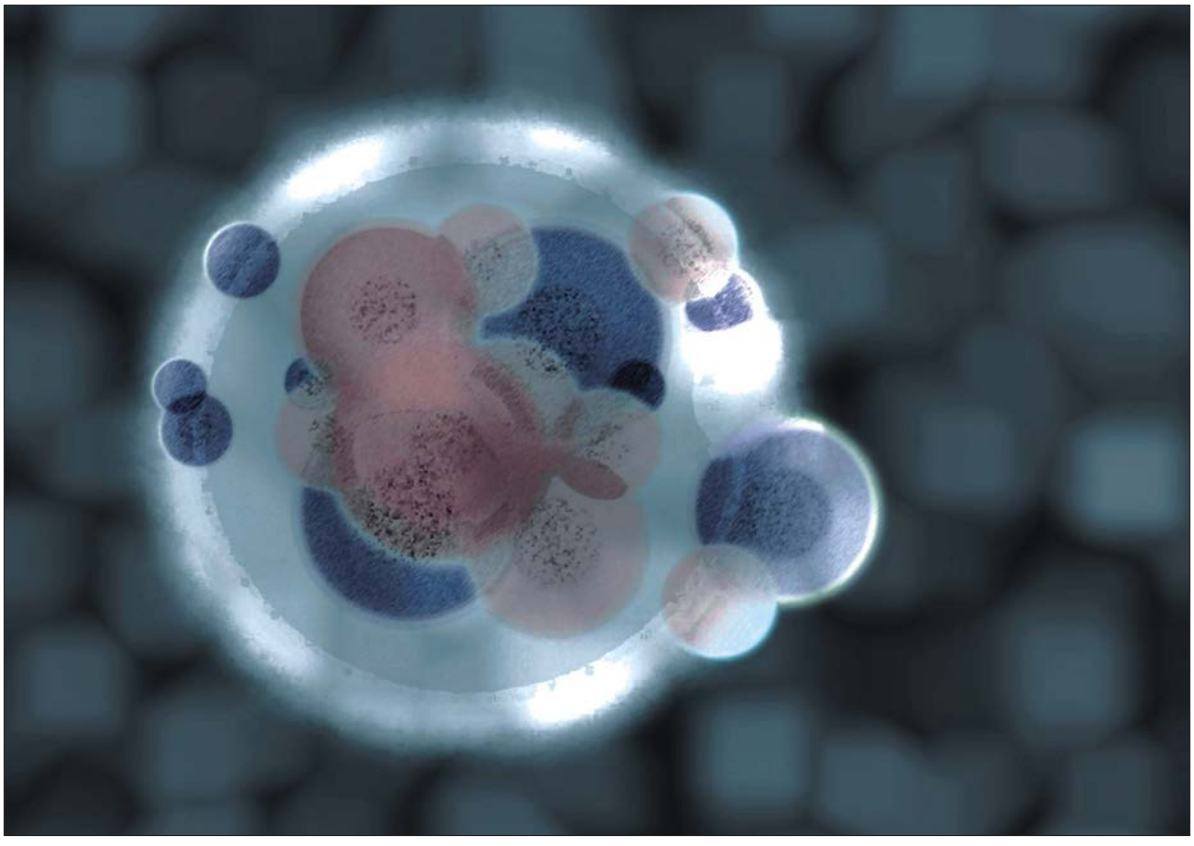

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Callus Induction and Indirect Regeneration in *Peltophorum pterocarpum* (DC.) Backer ex K. Heyne

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Abstract: The present study was conducted to induce callus from different *in vitro* grown seedling explants viz., cotyledon, nodal segment and leaf segment and to develop shoot buds from callus. At first, all the explants were cultured on MS medium supplement with different concentrations and combinations of BAP, KIN, NAA and 2,4-D. Among all the explants, cotyledon showed best performance for callus induction. For this explant, the highest callusing (93.33%) and the highest fresh weight of callus (1.38 g) was observed in MS+2.0 mg L^{-1} BAP+0.5 mgL^{-1}NAA media composition. Among all the concentrations and combinations, higher concentration of cytokinin (BAP) with lower concentration of auxin (NAA) was proved to be the best for callus induction. Calli, which were obtained from cotyledon and leaf segment in MS+2.0 mg L^{-1} BAP+0.5 mg L^{-1} NAA were subcultured in MS media having different concentrations and combinations of BAP and KIN with NAA. It was found that MS medium supplemented with KIN-NAA combination was the best for development of shoot buds from calli.

Key words: *Peltophorum pterocarpum*, callus, auxin, cytokinin

INTRODUCTION

Peltophorum pterocarpum (DC.) Backer ex K. Heyne is a multipurpose tree. As it's natural mode of propagation through seed is very low, Salah Uddin *et al.*[1] developed techniques for *in vitro* clonal propagation of this plant. In the present investigation, attempts were made for *in vitro* adventitious callus induction and regeneration of shoots via callus in *Peltophorum pterocarpum*.

From the literature survey, it has been concluded that sufficient works has not been done on woody ornamental plants of leguminosae family for their improvement through *in vitro* technique. But recently, some reports are emerging on callus induction from woody species e.g., *Albizia lebbeck*[2,3], *Aegle marmelos*[4], *Lagerstroemia speciosa* L.[5], *Dalbergia sissoo*[6], *Eucalyptus marinata*[7] and some other plants. *In vitro* morphogenetic response in *Peltophorum pterocarpum* following phytohormones and gamma irradiation has been reported by Hossain and Hossain[8]. The present investigation was undertaken to develop a standard protocol for *in vitro* production of callus and regeneration of shoots via callus by using different *in vitro* grown seedling explants viz., cotyledon, nodal segment and leaf segment.

MATERIALS AND METHODS

Seeds of *Peltophorum pterocarpum* were used to raise axenic seedlings. Cotyledons, nodal segments and leaf segments collected from young seedlings were used for conducting different experiments in the present investigation. All the seeds were collected from the campus of Rajshahi University, Bangladesh. The experiments were conducted during the years of 2000-2002.

Aseptic seed germination and raising of *in vitro* seedlings were done by methods described by Salah Uddin *et al.*[1]. Cotyledons were excised from *in vitro* grown seedling and one or two segments were inoculated into each culture tube. Explants with 1-2 nodes were excised from seedling and inoculated into each culture tube and leaves were also excised from the shoots. All the explants were inoculated in MS media[9] supplemented with different concentrations and combinations of Auxins (NAA; 2,4-D) and Cytokinins (BAP, KIN). After callus formation, calli derived from different explants were cut into small size and again subcultured in axuin and cytokinin hormonal combinations. Then the shoot buds were developed from different calli within 3-5 weeks. All the media were

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supplemented with 30 mg L^{-1} sugar, jelled with 5 mg L^{-1} T.C. agar supllied by North Carolina Biologica Supply Co. and autoclaved at 121°C for 20 min under 15 lbs psi pressure. The pH of the media was adjusted to 5.5. The cultures were kept in a growth camber under 16 h light period at 27±2°C temperature. In each treatment, 10-15 explants were inoculated.

RESULTS AND DISCUSSION

Most of the explants responded to induce callus in different degrees. *Peltophorum pterocarpum* showed callus induction in cotyledons, which were collected from *in vitro* grown 5 days old seedlings. Among all the concentrations and combinations, maximum callusing response from cotyledon was observed to be 93.33% in the medium MS + 2.0 mg L^{-1} BAP+0.5 mg L^{-1} NAA. The highest fresh weight of callus was also obtained in the same media composition (2.0 mg L^{-1} BAP+0.5 mg L^{-1} NAA) and the weight was 1.38 g. The lowest response was observed as 8.33% in MS+0.5 mg L^{-1} 2,4-D. The lowest fresh weight of callus (0.51 g) was also obtained in the same media composition (Table 1). Most of the calli derived from cotyledons were light green in colour.

Nodal segments from *in vitro* grown seedlings of 15 days old (after germination) were cultured on MS media supplemented with different concentrations and combinations of auxins and cytokinins. Nodal segments were placed on MS media horizontally and callusing started from the two cut ends of the explants. Among all the concentrations and combinations, maximum callusing (80%) from nodal segment was observed in MS medium having 2.0 mg L^{-1} BAP+0.5 mg L^{-1} NAA (Fig. 1A). The highest fresh weight of callus was also obtained in the same media composition and the weight was 1.25 g. The lowest response of callus induction was observed in MS+0.1 mg L^{-1} 2,4-D and the lowest fresh weight of callus was also obtained in the same media composition. Most of the calli derived from nodal segments were light ash in colour and sticky in nature.

Immature leaf segments from *in vitro* grown seedlings of 15 day old (after germination) were cultured on MS media supplemented with different concentrations and combinations of auxins and cytokinins. Among all the hormonal treatments, maximum callusing was observed in MS media supplemented with 2.0 mg L^{-1} BAP+0.5 mg L^{-1} NAA which was 86.66%. The highest fresh weight of callus was also observed in the same media composition and the weight was 1.27 g. The lowest callus induction was observed in 3.0 mg L^{-1} 2,4-D supplemented MS medium, which was 8.33%. The lowest fresh weight of callus was 0.5 g and was obtained in the same media composition. The highest percentage of calli derived from leaf segments were light green in colour and some of the calli were creamy in colour. The nature of calli was hard or friable.

Among all the hormonal combinations, BAP with NAA were proved to be the best for induction of callus. The second highest callus induction was found in MS

Table 1: Effect of different concentrations and combinations of BAP, NAA, KIN and 2,4-D in MS medium on callus induction from *in vitro* grown plant parts viz., cotyledon, nodal segment and leaf segment

Supplements (mg L^{-1})	Cotyledon			Nodal segment			Leaf segment		
BAP-NAA-KIN-2,4-D	% of explants Induced callus	Callus color and type	Mean fresh weight of callus (g)	% of explants induced callus	Callus color and type	Mean fresh weight of callus (g)	% of explants induced callus	Callus color and type	Mean fresh weight of callus (g)
1.0-00-00-00	13.33	LG and Friable	0.89	6.66	BW and sticky	0.71	13.33	LG and Friable	0.73
2.0-00-00-00	46.66	LG and Hard	0.99	26.66	LA and sticky	0.90	40.00	LG and Hard	0.81
3.0-00-00-00	40.00	LG and Hard	0.78	13.33	LA and sticky	0.83	40.00	LG and Friable	0.80
1.0-0.2-00-00	46.66	LG and Friable	1.13	13.33	BW and sticky	0.89	46.66	LG and Friable	0.98
1.0-0.5-00-00	60.00	LG and Hard	1.21	33.33	LA and sticky	1.21	53.33	LG and Hard	1.24
1.0-1.0-00-00	53.33	LG and Hard	1.07	26.66	LA and sticky	0.99	46.66	LG and Friable	1.01
2.0-0.2-00-00	86.66	LG and Hard	1.22	26.66	CR and sticky	0.99	80.00	LG and Friable	1.13
2.0-0.5-00-00	93.33	LG and Hard	1.38	60.00	LA and sticky	1.25	86.66	LG and Hard	1.27
2.0-1.0-00-00	80.00	LG and Hard	1.27	53.33	LA and sticky	1.03	80.00	LG and Hard	1.25
00-0.2-1.0-00	6.66	LG and Friable	0.77	--	--	--	--	--	--
00-0.5-1.0-00	13.33	LG and Hard	0.80	13.33	CR and sticky	0.68	13.33	LG and Hard	0.80
00-1.0-1.0-00	6.66	LG and Friable	0.78	--	--	--	6.66	LG and Friable	0.72
00-0.2-2.0-00	26.66	LG and Hard	0.98	20.00	LA and sticky	0.95	20.00	LG and Friable	0.90
00-0.5-2.0-00	33.33	LG and Hard	1.04	26.66	LA and sticky	1.11	26.66	LG and Hard	0.99
00-1.0-2.0-00	20.00	LG and Friable	0.83	13.33	BW and sticky	0.90	13.33	LG and Friable	0.83
00-00-00-0.1	--	--	--	--	--	--	--	--	--
00-00-00-0.5	8.33	BW and Hard	0.51	--	--	--	--	--	--
00-00-00-1.0	25.00	BW and Hard	0.63	8.33	CR and Hard	0.37	16.66	CR and Hard	0.85
00-00-00-2.0	33.33	LG and Hard	0.90	25.00	LA and sticky	0.46	25.00	CR and Hard	0.79
00-00-00-0.3	33.33	LG and Hard	0.87	25.00	LA and sticky	0.41	8.33	LG and Hard	0.51
00-00-00-4.0	--	--	--	--	--	--	--	--	--

BW = Brownish White, LA = Light Ash, CR = Creamy, LG = Light Green, (Data collected after 30 days of subculture. Fifteen *in vitro* grown explants were cultured for each treatment)

Table 2: Effect of different concentrations and combinations of BAP and KIN with NAA on development of shoot buds from callus derived from cotyledon and leaf segment

Growth regulators of pre culture (mg L^{-1})	Growth regulators of subculture (mg L^{-1})	Mean number of shoot buds per culture	
		Cotyledon derived callus	Leaf segment derived callus
2.0 BAP+0.5 NAA	KIN+NAA		
	0.5+0.2	5.08	4.83
	0.5+0.5	4.66	4.33
	0.5+1.0	--	--
	1.0+0.2	6.08	4.66
	1.0+0.5	5.41	4.08
	1.0+1.0	--	--
	2.0+0.2	11.75	10.08
	2.0+0.5	11.33	9.66
	2.0+1.0	8.59	7.15
	3.0+0.2	9.62	8.52
	3.0+0.5	9.40	7.30
	3.0+1.0	7.21	5.41
2.0 BAP+0.5 NAA	BAP+NAA		
	0.5+0.2	--	--
	0.5+0.5	3.33	3.25
	0.5+1.0	--	--
	1.0+0.2	3.91	3.16
	1.0+0.5	3.66	--
	1.0+1.0	--	--
	2.0+0.2	7.91	5.75
	2.0+0.5	6.35	5.50
	2.0+1.0	5.41	--
	3.0+0.2	6.25	3.91
	3.0+0.5	5.25	2.0
	3.0+1.0	--	--

(Data collected after 42 days of culture. Twelve test tubes were inoculated in each treatment)

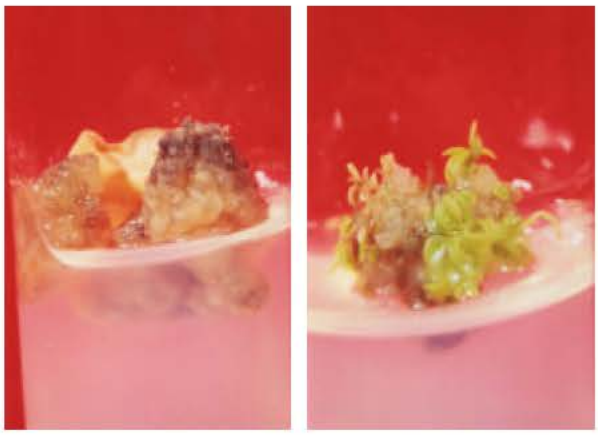

Fig. 1A: Callus formation from nodal segment explant in MS+2.0 mg L^{-1} BAP+0.5 mg L^{-1} NAA
B: Soot regeneration from cotyledon derived callus in MS+ 2.0 mg L^{-1} KIN+0.5 mg L^{-1} NAA

media supplemented with different concentrations of BAP alone. 2,4-D in different concentrations was proved not much suitable for callus induction. Among all the explants, cotyledons were proved to be the best for callus induction.

Calli which were obtained from cotyledon and leaf segment of *in vitro* grown seedling in the standardized hormonal composition (2.0 mg L^{-1} BAP+0.5 mg L^{-1} NAA), were used as explants for indirect regeneration. Calli were subcultured on MS media having different concentrations of KIN and BAP with NAA. Two types of responses were noticed. In some cases, adventitious buds were not formed whereas in some other media compositions, adventitious buds developed. Calli derived from cotyledon and leaf segments gave rise to shoots in higher concentrations of BAP and KIN with lower concentrations of NAA. But in lower concentrations of BAP and KIN with higher concentrations of NAA, explants did not respond well.

Cotyledon derived callus showed the highest number (11.75) of shoot buds per explant and it was observed in MS + 2.0 mg L^{-1} KIN + 0.5 mg L^{-1} NAA (Fig. 1B). For leaf segment derived callus, the highest number of shoot buds per explant (10.08) was also observed in MS + 2.0 mg L^{-1} KIN + 0.2 mg L^{-1} NAA media composition (Table 2).

MS medium containing higher concentration of cytokinin with lower concentration of auxin was found to be the best in respect of callusing response. The present results corroborate with that of Nagmani and Venketswaran[10] and Reza[3]. In the present investigation, it was found that MS medium supplemented with KIN-NAA combination was best for regeneration.

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