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Regeneration of Multiple Shoots from Different Explants viz. Shoot Tip, Nodal Segment and Cotyledonary Node of *in vitro* Grown Seedlings of *Peltophorum pterocarpum* (DC.) Backer ex K. Heyne

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Abstract: The present study was conducted to establish a protocol for rapid multiplication of shoots from different *in vitro* grown explants viz. shoot tip, nodal segment and cotyledonary node of *Peltophorum pterocarpum*. All the explants were cultured on MS media containing different concentrations and combinations of BAP, KIN and NAA. Among different hormonal concentrations and combinations, KIN+NAA showed the best result and cotyledonary node explants gave best shoot multiplication. The highest number (4.50 %) of multiple shoots was observed in MS media containing 2.0 mg L^{-1} KIN+0.5 mg L^{-1} NAA. In some cases, BAP also showed better result. The regenerated shoots were transferred on MS media having IBA for adventitious root initiation. Then the plantlets with elongated shoots and root system were considered ready to transfer to soil.

Key words: BAP, KIN, multiple shoot, NAA, *Peltophorum pterocarpum*

INTRODUCTION

Peltophorum pterocarpum (DC.) Backer ex K. Heyne, is a woody ornamental plant of leguminosae family. It is a unique, umbrella shaped and first class flowering and shade tree. It is planted as an ornamental tree along the roadsides, gardens, parks etc. Its native range is Southeastern Asia through Malaysia to Northern Australia. It is widely cultivated in tropical areas[1]. It is found throughout Andaman, Sri Lanka, South and North America, area from Malaysia to Australia. The tree has a fast rate of growth and is good windbreaker. It is also a first-class shade tree. The heart wood is hard and strong. It is suitable for house furniture, paneling, cabinet work and as firewood. It's pod contain edible pulp which is eaten by cattle and leaves can be used as fodder. Normally the plant spreads by seeds. But it is a very slow process in nature. Due to the unfavorable condition of nature, both seed and seedling become harmed. Moreover, this particular plant has considerable seed dormancy. There is no vegetative propagation of this plant. Tissue culture propagation could offer a valuable alternative and a reliable procedure for large scale propagation of it. *In vitro* multiple shoot regeneration may give higher rate of propagation within very short time and space.

According to the available literature, it is found that sufficient works has not been done on woody ornamental plants of leguminosae family for their improvement through *in vitro* technique.

Direct multiple shoot production from different parts of the seedling has been reported in *Albizia lebbek, Albizia odorastissima, Delonix regia* and some other legume plants. Micropropagation of *Delonix regia* through immature embryo derived shoot tips[2] and micropropagation of *Caesalpinia pulcherrima* through nodal bud culture of mature tree[3] has already been published. *In vitro* morphogenetic response in *Peltophorum pterocarpum* following phytohormones and gamma irradiation has been reported by Hossain and Hossain[4]. Beside this, no other reports are available so far, on micropropagation of *Peltophorum pterocarpum*. The present study was undertaken with on aim of establishing an efficient protocol for *in vitro* plant regeneration from shoot tips, nodal segments and cotyledonary nodes of *in vitro* grown seedlings.

MATERIALS AND METHODS

Seeds of *P. pterocarpum* were used to raise axenic seedlings. Shoot tips, nodal segments and cotyledonary

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nodes of those axenic seedlings were used for conducting different experiments in the present investigation. All the seeds were collected from the campus of Rajshahi University, Bangladesh.

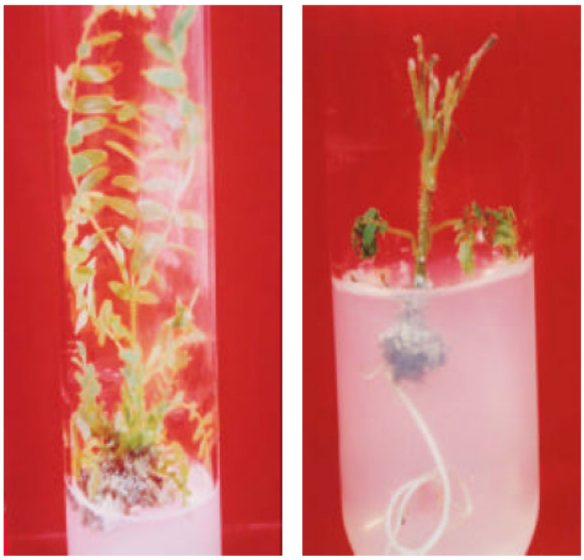
For aseptic seed germination and raising of *in vitro* grown seedling, the collected seeds of *P. pterocarpum* were taken in a conical flask and thoroughly washed under running tap water for 3-5 h to soften the seed coats and to reduce the level of microorganism. Then the seeds were taken in a conical flask containing distilled water with few drops of disinfectant viz. savlon and tween-80 (wetting agent). The seeds were treated for about 12-15 min with constant shaking. Then the seeds were washed thoroughly by distilled water about 5-6 times to ensure the removal of all chemicals even trace of it. The primary sterilized seeds were then treated with 0.1% $HgCl_2$ (w/v) by gentle shaking for 5 min. Then the sterilized seeds were washed about 5-6 times with sterile distilled water immediately to remove all traces of $HgCl_2$. The sterilization procedure was done in front of the running laminar airflow cabinet.

The seeds were perforated with the help of a sterile needle and implanted on semisolid agar jelled MS[5] media. Seedlings raised in axenic culture in the test tubes were the sources of seedling explants. Shoot tips were excised from *in vitro* grown seedling and one explant was inoculated into each culture tube. Explants with 1-2 nodes were excised from seedling and inoculated into each culture tube. Cotyledonary nodes were excised with a fine sterile scalpel. Half of each cotyledon was cut at that time. All the explants were cultured singly in MS media supplemented with various concentrations and combinations of auxins (NAA, IBA) and cytokinins (BAP, KIN). All the media were supplemented with 30g L^{-1} sugar, jelled with 5g L^{-1} agar 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 chamber under 15 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 shoots in different degrees. Data were collected after 21 days of subculture. Fifteen test tubes were sub cultured for each treatment.

A number of treatments were tried to regenerate shoots from shoot tip explants that were excised from aseptically grown seedlings cultured *in vitro*. A number of explants responded to regenerate multiple shoots in different concentrations of BAP and KIN alone or in different combination of BAP and KIN with NAA.



A B

Fig. 1: Multiple shoot formation from nodal segment explant in MS+2.0 mg L^{-1} KIN+ 0.5 mg L^{-1} NAA. B. Adventitious root formation in MS+2.0 mg L^{-1} IBA

The highest percentage of shoot tip explant regenerated shoots was 80.00% in MS media having 4.0 mg L^{-1} BAP after 21 days of subculture, the highest mean number of shoots per explant were recorded 3.71 in this particular media composition (Table 1). The mean length of the longest shoots were also recorded as 3.60 cm in the same media composition. The lowest percentage of shoot tip regenerated shoots was 13.33% in MS media supplemented with 0.2 mg L^{-1} KIN, 0.5 mg L^{-1} KIN, 1.0 mg L^{-1} NAA and 1.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA. The lowest mean number of shoots were also recorded in these media compositions.

In nodal segment explants, the highest percentage of cultures that regenerated shoots was 73.33% in MS media supplemented with 2.0 mg L^{-1} KIN + 0.5 mg L^{-1} NAA (Fig. 1). The highest mean number of shoots per culture was recorded 4.0 and the mean length of the longest shoots was recorded 3.70 cm in the same media composition. The lowest percentage of nodal segments regenerating shoots was 6.66% in 1.0 mg L^{-1} BAP supplemented media. The lowest mean number of shoots per culture and the lowest mean length of the longest shoots per culture were also recorded in the same media composition.

In cotyledonary node explants, the highest percentage of cultures that regenerated shoots was 86.66% in MS media supplemented with 2.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA. The highest mean number of shoots per explant (4.50) and the highest mean length of the longest shoots (3.90 cm) were also recorded in this media composition.

Table 1: Effect of different concentrations and combinations of BAP, KIN and NAA in MS medium on direct regeneration of shoots from different *in vitro* grown plant parts viz. shoot tip, nodal segment and cotyledonary node. (Fifteen test tubes were inoculated for each treatment)

	Results after 21 days of culture								
	Shoot tip			Nodal segment			Cotyledonary node		
Supplements (mg L^{-1}) BAP+KIN+NAA	% of explants regenerated directly	Mean No. of shoots per explant	Mean length of the longest shoots (cm)	% of explants regenerated directly	Mean No. of shoots per explant	Mean length of the longest shoots (cm)	% of explants regenerated directly	Mean No. of shoots per explant	Mean length of the longest shoots (cm)
0.2+00+00	20.00	1.35	2.61	--	--	--	--	--	--
0.5+00+00	26.66	3.10	2.70	--	--	--	--	--	--
1.0+00+00	26.66	3.40	2.90	6.66	1.00	1.34	2.66	2.60	2.60
2.0+00+00	53.33	3.58	3.10	20.00	2.10	1.55	33.33	2.75	2.90
3.0+00+00	60.00	3.59	3.50	26.66	2.50	2.00	40.00	2.90	2.61
4.0+00+00	80.00*	3.61*	3.60*	53.33	3.00	2.50	60.00	3.70	2.95
5.0+00+00	53.33	3.50	3.05	33.33	2.80	2.10	33.33	2.80	2.75
00+0.2+00	13.33	1.32	2.50	--	--	--	--	--	--
00+0.5+00	13.33	1.34	2.50	13.33	2.50	2.30	20.00	2.60	2.50
00+1.0+00	53.33	3.00	2.75	33.33	2.80	2.50	33.33	2.85	2.65
00+2.0+00	73.33	3.40	2.90	40.00	3.50	2.75	53.33	3.66	2.65
00+3.0+00	60.00	3.10	3.60	60.00	3.80	3.70	66.66	3.95	3.80
00+4.0+00	26.66	2.80	2.70	33.33	2.90	2.90	40.00	2.90	3.00
00+5.0+00	--	--	--	--	--	--	--	--	--
1.0+00+0.2	20.00	1.40	2.55	--	--	--	--	--	--
1.0+00+0.5	33.33	2.70	2.70	20.00	2.55	2.70	26.66	2.75	2.80
1.0+00+1.0	13.33	1.30	2.48	6.66	1.25	2.40	13.33	2.50	2.40
2.0+00+0.2	53.33	3.58	2.70	33.33	3.50	2.75	40.00	3.60	2.90
2.0+00+0.5	60.00	3.70	3.10	53.33	3.75	3.00	73.33	4.20	3.85
2.0+00+1.0	46.66	3.15	2.60	26.66	3.15	2.50	33.33	3.40	3.50
3.0+00+0.2	33.33	3.50	2.55	33.33	3.40	2.50	33.33	3.55	2.60
3.0+00+0.5	46.66	2.00	3.00	46.66	2.10	2.40	53.33	4.00	2.65
3.0+00+1.0	33.33	1.70	2.80	--	--	--	--	--	--
00+1.0+0.2	--	--	--	--	--	--	--	--	--
00+1.0+0.5	26.66	2.90	2.90	20.00	2.70	2.95	26.66	2.80	2.96
00+1.0+1.0	20.00	2.75	2.75	13.33	2.50	2.60	20.00	2.55	2.75
00+2.0+0.2	73.33	3.95	3.00	60.00	3.90	3.20	53.33	3.95	3.35
00+2.0+0.5	73.33	3.98	3.50	73.33**	4.00**	3.70**	86.66***	4.50***	3.90***
00+2.0+1.0	53.33	3.15	2.80	53.33	3.50	3.10	66.66	3.90	3.75
00+3.0+0.2	53.33	3.40	2.60	53.33	3.45	3.00	53.33	3.65	3.20
00+3.0+0.5	60.00	3.50	3.25	60.00	3.90	3.50	66.66	4.20	3.75
00+3.0+1.0	46.66	3.20	2.90	46.66	3.25	3.25	60.00	4.00	3.50

*Highest number for shoot tip explant, ** Highest number for nodal segment explant, *** Highest number for cotyledonary node explant.

Table 2: Effect of different concentrations of IBA in MS medium on root induction in regenerated shoots (Twelve test tubes were inoculated for each concentration).

Supplements (mg L^{-1}) IBA	No. of shoots subcultured	No. of shoots to which root initiated	% of shoots to which root initiated	Time taken to initiate rooting (days)	Mean length of of the longest roots (cm)	Degree of rooting
0.05	12	4	33.33	20	2.72	+
0.10	12	5	41.66	15	3.50	+
0.20	12	9	75.00	12	3.71	++
0.50	12	10	83.33	7	4.25	+++
1.0	12	11	91.66*	6	4.62	+++
2.0	12	12	75.00	14	3.85	++

+ Poor rooting, ++ Moderately good rooting, +++ Satisfactory rooting ,* Highest % of shoots to which root initiated

The lowest percentage of shoot regenerating explants was recorded to be 13.33% in 1.0 mg L^{-1} BAP+1.0 mg L^{-1} NAA supplemented MS media (Table 1). The lowest mean number of shoots per explant and the lowest mean length of the longest shoots were also observed in the same media composition.

The regenerated shoots were very carefully rescued from the culture tubes, placed on a sterile petridish and cut from the basal end of the shoots. Then each of the shoots was cultured on freshly prepared rooting medium containing MS salts and different concentrations of IBA for adventitious root initiation. Among different concentrations, MS+1.0 mg L^{-1} IBA showed the best result, where root initiated after 6 days of culture and 91.66% of root induction was recorded in this media. The highest mean length of longest roots (4.62 cm) was also recorded in the same media composition (Table 2).

The plantlets with elongated shoots and root system were considered ready to transfer to soil. The plantlets were transplanted to small polythene bags or plastic pots containing garden soil and compost in the ratio of 2:1. The soil substance was treated with 0.1% Agrosen (fungicide) solution.

In the present investigation it was observed that KIN+NAA combination in MS media is more effective for shoot multiplication from both nodal segment and cotyledonary node explants. It was also found that BAP alone in MS media was more effective for shoot multiplication from shoot tip explants.

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