Int. J. Mendel, Vol. 31 (3-4), 115-118, 2014 **MICROPROPAGATION**

EFFECT OF DIFFERENT GROWTH REGULATORS ON *in vitro* MULTIPLICATION OF *Plumbago zeylanica* Linn.

Amrita, I.N. Mishra and Sandhya Jha

Key words : *Plumbago*, micropropagation, *in vitro*

Plumbago zeylanica Linn is an important medicinal plant. A micropropagation technique is described for the multiplication of *Plumbago zeylanica* through shoot proliferation. Nodal explants with axillary buds were used to initiate the culture. Four different types of sterilizations were carried out. Treatment of explants with fungicides 1% (Care) for 10 minutes followed by treatment with 0.2% streptomycin and 0.1% mercuric chloride respectively was found to be the best with 78.57% survival. The sterilized explants were then inoculated in MS medium supplemented with BAP (1-3mg/l), NAA (0.25, 0.5, 1mg/l) & IAA (0.10 mg/l). Multiple shoot differentiation was influenced by the conc. of BAP with best results on MS medium with 1mg/l BAP+0.5mg/l NAA. After 4 weeks of shoot multiplication the microshoots were transferred to the rooting medium– 1/2MS+0.25, 2 mg/l IBA +2% sucrose (w/v), ½ MS+1BAP+5IBA+2% sucrose (w/v) and 1/2MS+1BAP+5NAA+2% sucrose (wfv) respectively. Rooting was observed in 1/2MS+0.25 IBA+2% sucrose (w/v) after 43 days.

Abbreviations : BAP – 6-Benzylaminopurine; 2,4-D – 2,4-Dichlorophenoxyacetic acid; HgCl2 – Mercuric Chloride; IAA – Indole-3-acetic acid; IBA – Indole-3-butyric acid; 2IP - 2-Isopentenyladenine; Kn–Kinetin (furfuryl amino purine); MS – Murashige and Skoog's medium; NAA – Napthalene acetic acid; TDZ – Thidiazuron

INTRODUCTION

Plumbago zeylanica is an important medicinal plant, native to warm temperate to the tropical regions of India (Rout *et al*., 1999). The shrubs of *Plumbago* are evergreen, densely branched and are of about 2m in height. It flowers during August-September and fruiting occurs in winter. An alkaloid plumbagin, is present in the roots of *Plumbago* which has medicinal uses (Das and Rout, 2002). Plumbagin stimulates the secretion of sweat (sudorific), urine and bile. It is used in the treatment of rheumatism, piles, diarrhoea, anasarca and skin diseases (Anonymous, 1989). In Siddha medicine it is used in the treatment of cancers of uterine cervix, scrotum, penis, breast, oral cavity and lungs. It is highly effective against insect pests and also shows resistance to antibiotics (streptomycin/rifampicin) in *E.coli* and *S.aureus. Plumbago zeylanica* is widely used in both traditional and commercial medicine. Due to its high medicinal value *P. zeylanica* is continuously collected from the wild and over exploited. Seeds of *Plumbago* have poor seed quality, low generation rate and less seedling survivability, thus propagation of *Plumbago* through seeds under natural field conditions is very difficult (Chaplot *et al*., 2005). Thus for preventing the extinction of the plant, alternative methods of propagation are required. With the help of micropropagation technique one can generate many plants from a single plant at a time, i.e., rapid mass propagation within a small space.The aim of the present study was to establish a successful protocol for micropropagation of *P. zeylanica* on different concentrations of growth regulators.

MATERIALS AND METHODS

Plant material

Shoots (20-30 cm long) of *Plumbago zeylanica* were collected from the central nursery of Forest Research Institute (FRI), Dehradun. Nodal segments with single axillary bud were used as explants.

Culture medium

Murashige and Skoog (MS) medium was used for culture establishment supplemented with 3% sucrose (as a carbohydrate source) and different concentrations of various hormones. The medium was gelled with 0.7% bacteriological agar. The pH of medium was adjusted to 5.6 with the help of NaOH or HCl prior to adding agar. The culture medium was autoclaved at 15 lb pressure and 121°C temperature for 15 minutes. Nodal explants were inoculated on MS media supplemented with BAP (1- 3mg/l), NAA (0.25, 0.5, 1mg/l) & IAA (0.10mg/l). All cultures were incubated at 25 ± 2°c under a 16 h photoperiod with a light intensity of 30 m mol/m^2/s by 40 W cool white fluorescent tubes. The cultures were maintained by regular subculture on fresh medium with BAP (1, 3 mg/l) and NAA (0.5, 1 mg/l).

Induction of rooting and acclimatization

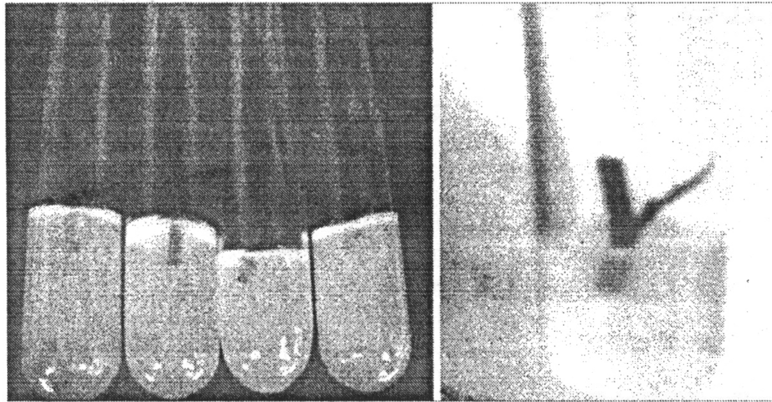
In vitro rooting

In vitro multiplied shoots of *P. zeylanica* were separated into propagules of 3-5 shoots each and cultured on the appropriate medium for *in vitro* root induction. A half strength MS medium containing 2% (20g/l) sucrose, supplemented with IBA (0.25, 2.0 and 5.0 mg/l) and NAA was used as root induction medium.

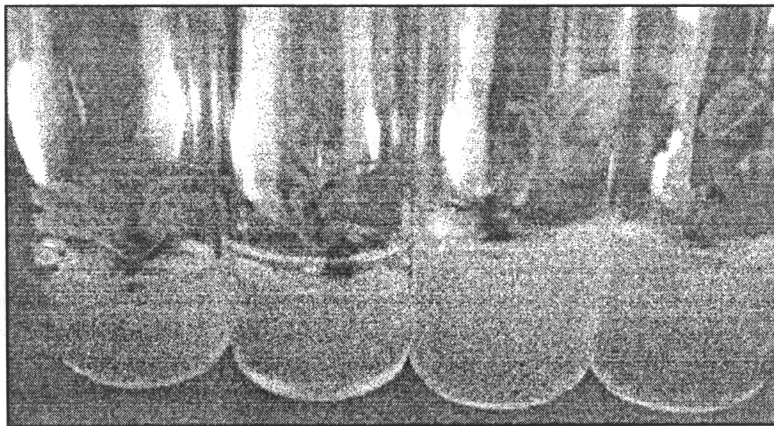
Ex vitro rooting

In vitro multiplied shoots were washed with distilled water to remove the medium completely and then dipped in 70% ethanol for few seconds. The bottom of shoots were then immersed in IBA 4000 ppm(w/w) and then were potted in autoclaved vermiculite. The pots were then kept in the mist chamber.

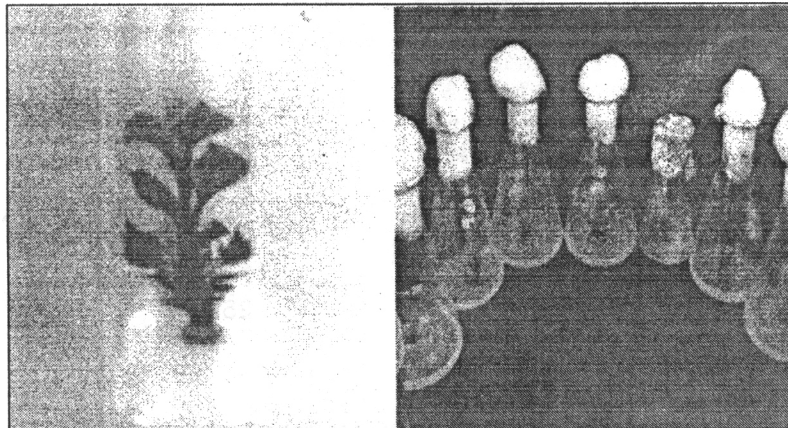
Department of Botany, L.N. Mithila University, Darbhanga - 846 004



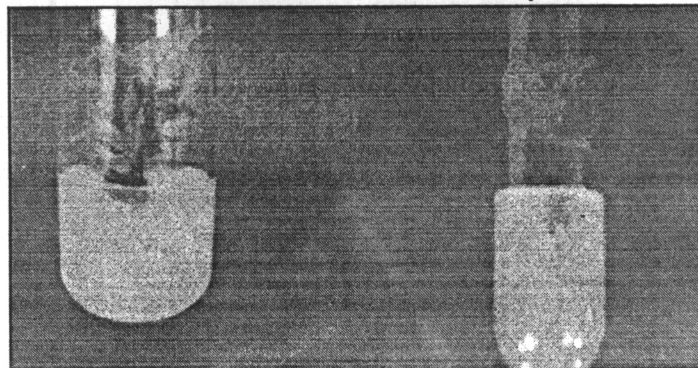
Inoculated explants **Bud Induction**



After four week of inoculation



Subcultured microshoots **Multiplication**



***In-vitro* rooting**

RESULT AND DISCUSSION

TABLE-1 : EFFECT OF DIFFERENT STERILIZATIONS

Sl. No.	Treatment	Total no. of explant inoculated	Total no. of contaminated tubes	No. of Survived tubes	Survival % age	Remarks
1	$HgCl_2$ 0.1% for 10 mins.	19	19	0	0	8 cultures contaminated after 6 days, 3 more after 15 days & rest 8 after 27 days.
2	$HgCl_2$ 0.1% for 10 mins. +Fungicide 1%	22	11	11	50	8 explants contaminated after 11 days, 5 more after 21 days.
3	$HgCl_2$ 0.1% for 10 mins.+Fungicide 1% + Ethanol(70%) dip	10	6	4	40	2 cultures contaminated after 14 days & rest 4 after 19 days.
4	$HgCl_2$ 0.1% for 10 mins. + Fungicide 1% + 0.2 % Streptomycin for 5 mins.	28	6	22	78.57	1 culture contaminated after 7 days, rest 5 after 21 days.

In 1st Sterilization method 8 cultures contaminated only in 6 days and no plant survived after 27 days, survival % was 0%. In 2nd sterilization method 8 explants contaminated after 11 days and 5 more in 21 days. The survival % was 50%. In case of 3rd sterilization procedure 2 cultures were contaminated after 14 days and other 4 after 19 days, 40% explants survived. In 4th sterilization process only 1 culture contaminated after 7 days and 5 more after 21 days, the survival % was 78.57%. Thus the best sterilization procedure for *Pumbago zeylanica* is the 4th one.

TABLE-2 : EFFECT OF DIFFERENT CONCENTRATIONS OF GROWTH REGULATORS

Sl. No.	No. of explant inoculated	Hormones(mg/ml)			No. of bud break	Avg. Shoot no.	Shoot length (cm)	Shooting %
		BAP	NAA	IAA				
1	19	2	0.25	-	10	0.52	0.5-1	47.36
2	15	1	-	-	14	0.93	1-3	73.33
3	8	1	-	0.10	3	0.37	1-2	37.5
4	15	3	1	-	19	1.26	2-2.5	93.33
5	15	1	0.5	-	25	1.66	2-3.5	100

Nodal explants were inoculated on MS media supplemented with BAP (1 - 3mg/l), NAA (0.25, 0.5, 1mg/l) & IAA (0.10mg/l). The first bud break was observed on MS 2BAP+0.25NAA, 1BAP, 1BAP+0.10IAA, 3BAP+1NAA, 1BAP+0.5NAA after 6 days, 8 days, 8 days, 7 days and 7 days of culturing respectively. Maximum number of bud break was observed on MS 1BAP+0.5NAA. Average shoot no. was observed to be 0.52, 0.93, 0.37,1.26, 1.66 on MS 2BAP+0.25NAA, 1BAP, 1BAP+0.10IAA, 3BAP+1NAA, 1BAP+0.5NAA respectively. Shooting % was recorded as 47.36%, 73.33%, 37.5%, 93.33%,100 on MS 2BAP+0.25NAA, 1BAP, 1BAP+0.10IAA, 3BAP+1NAA, 1BAP+0.5NAA respectively. Similar effects of BAP and NAA on shoot multiplication were recorded by Gbadamosi *et al*. (2010) during their work on micropropagation of *Plumbago zeylanica* L.

TABLE-3 : SUBCULTURING

Sl. No.	Day of subculturing after first culture	No. of cultured plants subcultured	Concentration of growth hormone used during subculture
1	22 days	5 culture	1BAP+0.5NAA
2	28 days	2 culture	1BAP+0.5NAA
3	26 days	2 culture	1BAP+0.5NAA
4	36 days	4 culture	1BAP+ 1NAA
5	26 days	8 culture	1BAP+1NAA
6	28 days	2 culture	1BAP+ 1NAA
7	28 days	6 culture	3BAP+1NAA

5 plants out of the 15 culture of 1BAP were subcultured after 22 days of first culture in MS medium with 1BAP+0.5NAA, 2 plants were subcultured from the same culture after 28 days. 2 more plants were subcultured on MS with 1BAP+0.5NAA from 15 cultures of MS with BAP+0.5NAA after 26 days. 4 plants were subcultured after 36 days of first culture from the 7 culture of MS with 1BAP+0.10IAA in 1BAP+1NAA. 8 plants were subcultured after 26 days from the 15 cultures of MS with 1BAP+0.5NAA on MS with 1BAP+1NAA and 2 more tubes were subcultured from 15 tubes of 1BAP+0.5NAA on same MS medium. 6 cultures were subcultured after 28 days on MS with 3BAP+1NAA from the 15 culture tubes of 3BAP+1NAA.

TABLE- 4 : ROOTING

Sl. No.	No. of cultures rooted	Concentration of growth hormones used (mg/l).			Days to rooting	No. of roots
		BAP	IBA	NAA		
1	3	-	2	-	No rooting	0
2	3	-	0.25	-	43 days	8
3	4	1	5	-	No rooting	0
4	2	1	-	5	No rooting	0
Ex vitro rooting						
1	2	Vermiculite				

For rooting, ex *vitro* and *in vitro* methods were developed. For *in vitro* rooting, microshoots were transferred on half strength MS medium with 2% (w/v) sucrose and 2 & 0.25 mg/l IBA respectively in first two cases, in third case half MS+1 BAP+5 IBA+2%(w/v) sucrose was used and in fourth case half MS+1BAP+5NAA+2%(w/v) sucrose was used. Only the microshoots in 1/2MS+0.25IBA+2%(w/v) sucrose showed rooting after 43 days. Number of roots was 8. *Ex vitro* rooting was done with application of IBA 4000ppm(w/v) in vermiculite.

This micropropagation technique leads one to infer as to how it may be helpful in obtaining quite a large number of plantlets in a short time. This may largely enable *P. zeylanica* to overcome its exploitation, and pharmaceutical industries would be able to manufacture herbal drugs from this valuable plant.

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