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Propagation of *Plumbago indica* L. (Plumbaginaceae) through direct organogenesis and induction of callus

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Abstract
Plumbago indica L. is a perennial medicinal herb belongs to Family Plumbaginaceae. This plant has many therapeutic uses in wide array of diseases. Plumbagin is the major bioactive compound in this plant. Tissue culture technology offers an alternative means of vegetative propagation. This research was carried out on callus induction and plantlet regeneration of *P. indica*. Nodal explants grown in MS medium supplemented with 1.5 mg/L BAP produced the longest shoots (1.82 ± 0.3 cm) and maximum multiple shoot induction (5.20 ± 0.4) was observed in MS medium supplemented with 2.0 mg/L BAP. Half strength MS medium supplemented with 0.4 mg/L IBA was the best for root induction (9.4 ± 1.1). Best callus induction from leaf disc explants was observed in MS medium supplemented with 3.0 mg/L BAP, 1.5 mg/L Kn and 1.0 mg/L NAA. Regenerated plantlets were transferred to sterilized potting mixture (compost: coir dust: top soil: sand 1:1:1:1) for acclimatization but low survival percentage (25%) was observed.

Keywords: BAP, IBA, Kn, NAA, Nodal explants, Plumbagin, *Plumbago indica*

1. Introduction
Plumbago indica L. (Family: Plumbaginaceae) is an important medicinal plant that is widely distributed in tropical countries [1]. This plant is originated in Sikkim and Khasi hills of India and migrated to other neighboring countries. Tuberous root of this plant is widely used in Ayurveda, Siddha, Unani, and Homeopathy and also in unmodified ethnic preparations of rural folks [2]. *P. indica* contains several phytochemicals such as naphtha quinones, binaphtho quinones, flavonoids and phytosterols which has high medicinal importance [3]. Plumbagin is the most important naphtha quinone mainly found in the roots and has wide array of pharmacological activities [4]. In natural habitats, this plant is under severe threat due to over exploitation by local communities for traditional medical purposes and also due to non-availability of proper cultivation system. *P. indica* is imported to Sri Lanka mainly from India. This plant is propagated vegetatively by stem cuttings or root tubers but has low regeneration rate due to the low productivity and less viability of seeds [5]. To solve all these drow-backs with large scale production of plantlets for cultivation could be the best option to conserve this valuable medicinal plants growing in natural environment. Therefore, the present study was designed with the objective of developing a suitable tissue culture protocol for mass propagation of *P. indica* through leaf, inter nodal and nodal explants.

2. Materials and methods
P. indica plants were collected and authenticated using specimens from National Herbarium at Royal Botanical Garden, Peradeniya, Sri Lanka. Mother stock was maintained to obtain explants in the shade house and maintained free from pathogens or stress.
Murashige and skoog (MS) [6] medium was used as the basal medium in all experiments. The pH of the medium was adjusted to 5.8. Plant tissue culture agar (14.0 g/L) was added as the solidifying agent. Cultures were maintained at $24 \pm 1\ ^0$C in 16 h photoperiod. There were at least 15 replicates in each treatment unless otherwise stated. Growth regulator free MS medium was used as the control. Completely Randomized Design (CRD) was used in all experiments. "MINITAB", version 15 was used for the statistical analysis.

2.1 Determination of best growth regulator combination for callus induction
*P. indica*inter nodal segments (stem between first and second node) were used as explants. Explants were surface sterilized and cultured on MS medium supplemented with 2, 4 –D (0.5 – 1.5 mg/L) and BAP (0.1 – 0.2 mg/L). Callus development, texture and color of the callus were observed weekly over a period of six weeks.

2.2 *In vitro* callus and shoot induction from different explant
Leaf discs were obtained from first and second fully opened leaves. Nodal segments obtained from first and second node from meristem and inter nodal segments (stem between first and second node 1.0 – 1.5 cm) and leaf discs obtained by cutting the leaf into 80.0 mm2 pieces along the mid rib were

surface sterilized. Explants were cultured on MS basal medium supplemented with 3.0 mg/L BAP, 1.5 mg/L Kn and 1.0 mg/L NAA. Observations were taken weekly over a period of eight weeks.

2.3 Direct shoot induction from nodal segments of *P. indica*

Surface sterilized nodal segments were placed on MS medium supplemented with different concentrations of BAP (1.0 – 3.0 mg/L). Percentage shoot induction, mean number of shoots per explant and mean shoot length were measured after four weeks of incubation in 16 h photoperiod.

2.4 *In vitro* root induction from in vitro grown shoots

One month old in vitro propagated, P. indica shoots were separated from clusters and then transferred in to half strength MS medium supplemented with different concentrations of IBA (0.2 – 0.6 mg/L). Root induction was observed after eight weeks and numbers of roots per shoot and percentage of rooting response was recorded.

2.5 Acclimatization of in vitro propagated plantlets

Two months old in vitro grown plantlets were removed from the medium carefully without damaging to the root system. All traces of agar from roots was removed and dipped in 0.1 mg per 100.0 mL fungicide solution (Carbendazim®) for 15 - 20 minutes. Plantlets were transferred in to disposable cups containing a sterilized potting mixture (compost: coir dust: top soil: sand 1:1:1:1). Plants were kept under shade for seven days and watered daily. Then plants were exposed to morning sun light for sun bathing for 2 h daily. After one month plants were gradually exposed to natural environment completely. Survival percentage of tissue cultured P. indica plantlets was recorded.

3. Results and discussion

3.1 Determination of best growth regulator combination for callus induction

There was no callus induction in growth regulator free MS medium. At low (0.1 mg/L) BAP level explants turned in to yellow after 5 weeks. Callus produced in the presence of 2,4-D at 0.5 and 1.0 mg/L alone showed pale green color, yet turned into brown after 4 to 5 weeks. High BAP level (0.2 mg/L) induced friable callus in the presence of low levels (0.5 and 1.0 mg/L) of 2,4-D (Fig 1.A and B). Callus induction was initiated along the cut surface and were not transulant, thus can be considered as vegetative calli (Table 1). In the present study callus induction was only observed in the presence of low levels (0.5 and 1.0 mg/L) of 2,4-D and combination of low levels (0.5 and 1.0 mg/L) of 2,4-D with high level (0.2 mg/L) of BAP. It is reported that callus induction was highest in MS medium supplemented with 1.0 mg/L 2,4- D and 0.1 mg/L BAP[4]. In contrast to that, in the present study, no callus formation was observed in the presence of 1.0 mg/L 2,4- D and 0.1 mg/L BAP. These differences may be due to the physiological state of mother plant.

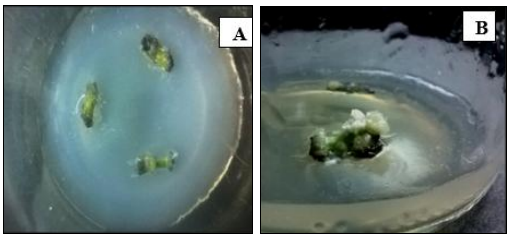

Fig 1: Callus development from inter nodal segments: A) Light green color callus development B) White color callus development after three weeks of incubation.

Table1: Callus induction from inter nodal explants of *P. indica*.

PGR concentration (mg/L)		Callus	induction	Callus type	Colour
2,4-D	BAP				
0.0		0.0	-	-	-
0.5		0.0	+	Hard	Green
1.0		0.0	+	Hard	Green
1.5		0.0	-	-	-
0.0		0.1	-	-	-
0.5		0.1	-	-	-
1.0		0.1	-	-	-
1.5		0.1	-	-	-
0.0		0.2	-	-	-
0.5		0.2	+	Friable	White
1.0		0.2	+	Friable	White
1.5		0.2	-	-	-

3.2 *In vitro* callus and shoot induction from different explants

Brown hard calli were induced from leaf disc explants within two months in the presence of 3.0 mg/L BAP, 1.5 mg/L Kn and 1.0 mg/L NAA (Fig. 2A). Shoots were induced from nodal segments within one month (Fig. 2B). Light green hard calli were induced from inter nodalexplants third week after inoculation (Fig. 2C).

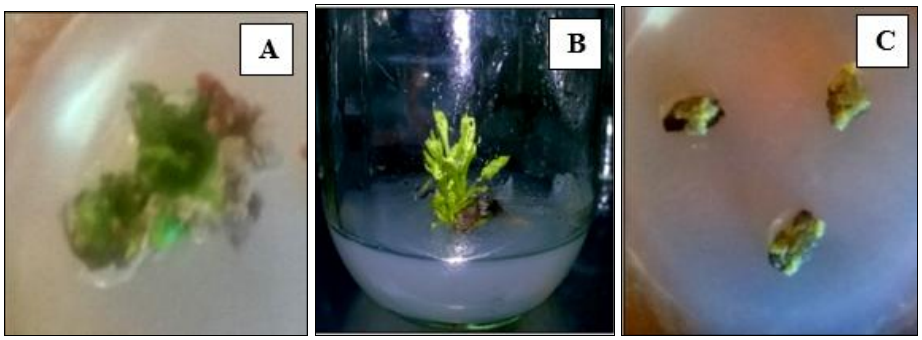

Fig 2: Explants cultured in MS medium supplemented with 3.0 mg/L BAP, 1.5 mg/L Kn and 1.0 mg/L NAA : A) Callus induction from leaf disc B) Shoot induction from nodal segments C) Callus induction from inter nodal segments.

Leaves, nodes and inter nodal explants respond differently in MS medium supplemented with 3.0 mg/L BAP, 1.5 mg/L Kn and 1.0 mg/L NAA. However leaf disc did not show any response [7]. In contrast to that, brown and hard callus were induced from leaf discs explants within two months in the presence of same levels of plant growth regulators (Fig. 2A). Brown color of the callus might be due to the presence of high level of phytochemicals accumulated in the cells. According to Bhadra *et al*., (2009), leaf disc explants did not produce any callus in MS medium supplemented with 3.0 mg/L BAP, 1.5 mg/L Kn and 1.0 mg/L NAA even after 25 – 30 days of incubation. In the present study too no callus formation was observed within 25 – 30 days from leaf discs, however they started to induce callus after six weeks indicating that callus induction from leaf disc explants in that medium is a slow process.

3.3Direct shoot induction from nodal segments of *P. indica*.

Best growth regulator concentrations for highest multiple shoot induction was observed in the presence of 2.0 mg/L BAP (5.20 $\pm$ 0.40) while highest shoot length (1.82 $\pm$ 0.26 cm) was observed in MS medium supplemented with 1.5 mg/L BAP (Table 2). When direct shoots induce from nodal segments, a significant difference was observed between shoot lengths in MS medium supplemented with different concentrations (1.0, 1.5, 2.0, 2.5, 3.0 mg/L) of BAP. Highest mean number of shoots (5.2 $\pm$0.4) and highest percentage of multiple shoot production (95%) was observed in the presence of 2.0 mg/L BAP in MS medium which confirms the results of Jindaprasert *et al* (2010). It was observed that presence of BAP in the medium enhances shoot induction. Control (no added BAP) also showed 100% positive response, yet control and higher BAP concentrations (3.0 mg/L) did not produce multiple shoots but showed shoot elongation, indicating high level of BAP reduces the shoot elongation (Table 2). Multiple shoot produced grew longer within 4 weeks.

Table 2: Influence of various concentrations of BAP in MS medium on *in vitro* multiple shoot induction from *P. indica*nodal segments after 4 weeks

BAP concentration mg/L	Mean number of shoots +SD	Mean shoot length+SD
0.0	1.00 + 0.00	1.40 + 0.31
1.0	1.20 + 0.44	1.64 + 0.38
1.5	1.40 + 0.54	1.82 + 0.26
2.0	5.20 + 0.40	1.66 + 0.47
2.5	1.20 + 0.44	1.38 + 0.24
3.0	1.00 + 0.00	1.14 + 0.28
LSD 5%	0.05	0.05

Note. SD = Standard Deviation; LSD = Least significant difference

3.4 *In vitro* root induction from *in vitro* grown shoots

Half strength MS medium supplemented with 0.4 mg/L IBA found to be the best growth regulator concentration for *in vitro* root induction from *in vitro* propagated shoots, with 100% response with mean of 9.4±1.14 roots per shoot. Growth regulator free half strength MS medium (Control) also showed a 60 % positive response but mean number of roots (1.2 ± 1.3) was significantly low when compared to the presence of IBA. It was observed that increased levels (over 0.5 mg/L) of IBA retarded the root induction (Table 3). It is reported the best root induction (11.76 ± 0.3) in half strength MS medium supplemented with 0.4 mg/L IBA and growth regulator free half strength MS medium also showed 40 % root induction response [8]. Present study also confirms the results of previous study. Thus, it could be suggested that half strength MS medium supplemented with 0.4 mg/L IBA is the most suitable growth regulator concentration for *in vitro* root induction from *in vitro* grown shoots of *P. indica*.

Table 3: Influence of various concentrations of IBA in half strength MS medium on *in vitro* root induction of *P. indica*

IBA concentration mg/L	% of response	Number of roots/Shoot + SD
0.0	60	1.20 + 1.30
0.2	80	2.80 + 1.78
0.3	80	3.00 + 2.55
0.4	100	9.40 + 1.14

0.5	100	7.40 ± 1.34
0.6	80	3.60 ± 2.70
LSD 5%	0.02	0.02

Note. SD = Standard Deviation; LSD = Least significant difference

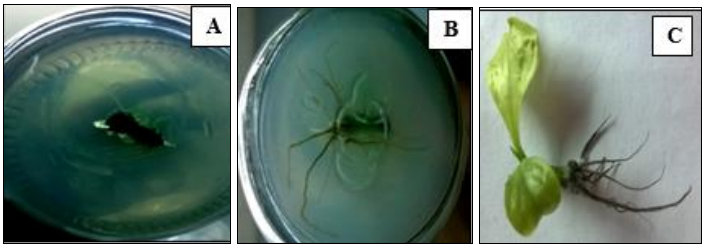

Fig 3: Shoot induction of *P. indica* nodal explants on MS medium supplemented with BAP after four weeks A) Multiple shoot induction in the presence of 2.0 mg/L BAP B) Shoot induction in the presence of 1.5 mg/L BAP.

3.5 Acclimatization of in vitro propagated plantlets

Only one potting mixture (compost: coir dust: top soil: sand 1:1:1:1) was used to acclimatize rooted plantlets. Survival rate of the plantlets were only 25% (Fig 4). Although survival percentage was low, remaining plantlets were healthy and shoot elongation occurred with increasing number of leaves. Low rate of survival observed in this study may be due to inappropriate acclimatization process, thus improving conditions in acclimatization medium could increase the survival ability of tissue cultured plantlets. Most of the plantlets died due to microbial infections in roots which is caused by high water retention ability of the mixture. Although plantlets were dipped in fungicide solution (0.1 g Carbendazim® in 100 mL of sterile distilled water) before planted in the potting mixture, the concentration of fungicide used for dipping plantlets may not be strong enough to avoid fungal growth, thus it might be possible to increase survival rate by increasing the Carbendazim concentration or dipping period.

Fig 4: Acclimatized *P. indica* plant after two month

4. Conclusion

Base on the findings of this study, immature plant parts (nodal, inter nodal and leaf) could be used as explant to induce shoots and callus of *P. indica* plants. MS basal medium supplemented with 3.0 mg/LBAP, 1.5 mg/L Kn, 1.0 mg/LNAA could be used to induce callus from immature leaf and inter nodal explants. This medium also could be induced shoots from nodal explants. MS basal medium supplemented with 1.5 mg/LBAP is the best growth regulator concentration for *in vitro* shoot induction from nodal explants. Growth regulator free MS basal medium (control) also has the ability to induce shoots from nodal explants. Half strength MS basal medium supplemented with 0.4 mg/LIBA is ideal for *in vitro* root induction.

5. Acknowledgement

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6. References

1. Dassanayake MD, Clayton WD. A revised handbook to the flora of Ceylon. Oxford & IBN publishing, New delhi, India, 1997, 386.
2. Jose B, Dhanya BP, Silja PK, Krishnan PN, Satheesh kumar K. *Plumbagorosea* L. – A review on tissue culture and pharmacological research. International Journal of Pharmaceutical Sciences Review and Research**.** 2014; 25(1):246-256.
3. Ganesan K, Gani SB. Ethnomedical and pharmacological potentials of *Plumbagozeylanica* L. - A Review. American Journal of Phytomedicine and Clinical therapeutics. 2013; 3:313-337.
4. Jindaprasert A, Samappito S, Springob K, Schmidt J, Gulder T, Deeknamkul W, *et al*. *In vitro* plant callus and root cultures of *Plumbagoindica*and their biosynthetic potential for plumbagin. King Mongkuts Agro-Indian Journal. 2010; 2(1):53-65.
5. Lenora RDK, Drarmadasa RM, Abeysinghe DC, Arawwawala LDAM. Investigation of Plumbagin content in *Plumbagoindica* Linn. Grown under different growing system. Pharmacologia. 2012; 3(2):57-60.
6. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue culture. Physiologiaplantarum. 1962; 15(3):473-497.
7. Bhadra SK, Akhter T, Hossain MM. *In vitro* micro-propagation of *Plumbagoindica*L. through induction of direct and indirect organogenesis, Journal of Plant Tissue Culture and Biotechnology. 2009; 19(2):169-175.
8. Kumar SG, Joseph LH, Thangavel K. *In vitro* propagation of *Plumbagorosea*L. Journal of Applied Biology and Biotechnology. 2014; 2(2):1-7.