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# Effect of plant hormones on micropropagation of *Plumbago zeylanica* (Chitrak)–A versatile medicinal plant

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**Abstract**
Chitrak (*Plumbago zeylanica* Linn.) is versatile medicinal Plant. Axillary bud cultures of *P. zeylanica* were used to evaluate effect of different concentrations of BAP; Kinetin; combinations of BAP and IAA; combinations of BAP and IBA in Murashige and Skoog (MS) medium. Axillary buds cultured on these media showed direct shoot regeneration. Bud break was observed 100% in all media used within 6-12 days. Maximum shoot length was observed on MS medium containing 1.5 mg/l Kinetin. Multiple shoots were developed on media containing BAP (0.5 mg/l, 1 mg/l, 2 mg/l, 2.5 mg/l, 3 mg/l); BAP + IAA (1mg/l BAP +1 mg/l IAA, 2mg/l BAP +2 mg/l IAA, 2.5mg/l BAP +2.5 mg/l IAA); BAP + IBA (0.5mg/l BAP +0.5mg/l IBA, 2mg/l BAP +2 mg/l IBA, 2.5mg/l BAP +2.5 mg/l IBA). Callus formation was observed when these *in vitro* regenerated shoots were cultured on MS medium containing 1mg/l IBA + 0.1 mg /l BAP but when these shoots were cultured on MS medium containing 0.5 mg/l IBA, root formation was observed and small plantlets were formed.

**Keywords:** axillary bud, plant hormones, *Plumbago zeylanica* Linn, MS medium

## Introduction
Plants are the traditional source of medicine. World Health Organization (WHO) is boosting developing countries to use herbal remedies which they have been using traditionally since time immemorial. In recent time because of pronounced side effects of allopathic drugs, people are shifting towards herbal cure [1]. *P. zeylanica* Linn. is multivalent medicinal plant. It is used extensively in Ayurveda and Unani systems of medicine. The roots of plants are source of Plumbagin which has various pharmacological activities like antimalarial, antibacterial, anticancer, antifertility action, cardiotonic, antibiotic etc. Coconut oil processed with roots is used as hair tonic [2]. The decoction of root of Chitrak is used in *sikatameha* (urinary ailment) [3]. Poor seed quality, erratic germination, seedling mortality under natural conditions, extensive and destructive harvesting of *P. zeylanica* for its medicinal use, insufficient attempt for replenishment or cultivation have led to the depletion of natural population of *P. zeylanica* [2]. And it is listed as threatened medicinal plant [4, 5]. Hence it is necessary to conserve and propagate it properly. Micropropagation is best method for mass multiplication of threatened plants.
The present study aims to evaluate effect of different plant hormones (BAP, Kinetin, BAP+IAA, BAP+IBA) on axillary bud culture of *P. zeylanica*.

## Materials and Methods
althy shoots of actively growing plants of one to two year old were selected. Leaves were cut leaving small basal portion of petiole without disturbing axillary bud. Nodal segments of 4-5 cm were cut. The explants were first washed thoroughly in running tap water for 30 minutes. Then material was washed with distilled water containing a drop of Tween 20. The material was repeatedly rinsed with sterile distilled water for 4-5 times. The material was surface sterilized by immersing in 70% ethyl alcohol for 1 minute followed by repeated washing with sterile distilled water. Then the material was again surface sterilized by 0.1% mercuric chloride for 8 minutes Explants were rinsed properly with sterile distilled water for 3-4 times [6]. Surface sterilized nodal segments were cut into smaller pieces (0.5-1 cm) and then cultured aseptically in test tubes containing culture medium. Medium used for axillary bud cultures was Murashige and Skoog (MS) medium [7]. MS medium supplemented with different concentrations of BAP; Kinetin; combinations of BAP and IAA; combinations of BAP and IBA; coconut water (15% v/v) were used for axillary bud culture. The *in vitro* regenerated shoots were then cultured on M.S. medium containing 1mg/l IBA + 0.1 mg /l BAP and 0.5 mg/l IBA. The cultures were incubated in tissue culture room at $25\pm2^0$ C with photoperiod of 16 hours.

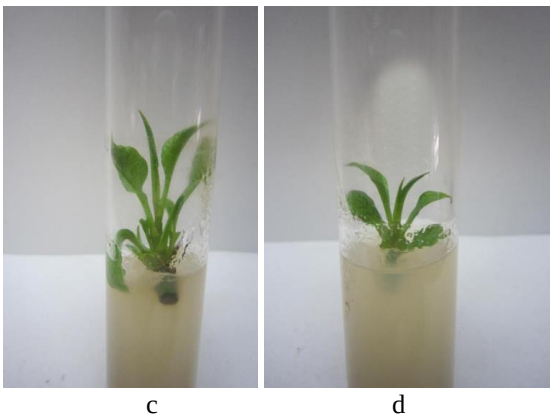
## Results and Discussion
Nodal explants cultured on MS medium supplemented with various concentrations of BAP alone; Kinetin alone; combinations of BAP and IAA; combinations of BAP and IBA; coconut water showed direct shoot regeneration and 100% bud break response within 6-12 days. Kanungo et al (2012) also reported bud break in axillary bud culture of *P. zeylanica* on different combinations of BAP+IAA+IBA within a period of one week [8]. Among the different concentrations of plant hormones tested, maximum shoot length (4cm) on the 30th day of inoculation was observed on MS medium supplemented with kinetin (1.5 mg/l) (Table 1). Maximum (7) leaves on main shoots on the 30th day of inoculation were developed on MS medium supplemented with 1mg/l BAP +1 mg/l IAA (Table 1). Multiple shoots were developed on media containing BAP (0.5 mg/l, 1 mg/l, 2 mg/l, 2.5 mg/l, 3 mg/l); BAP + IAA (1mg/l BAP +1 mg/l IAA, 2mg/l BAP +2 mg/l IAA, 2.5mg/l BAP +2.5 mg/l

IAA); BAP + IBA (0.5mg/l BAP +0.5mg/l IBA, 2mg/l BAP +2 mg/l IBA, 2.5mg/l BAP +2.5 mg/l IBA) (Table 1). Multiple shoots started arising from nodal explants after 3 weeks of incubation. Multiple shoot formation was observed first (on 23rd day of inoculation) on MS medium supplemented with1mg/l BAP. Maximum 5 shoots per explants were found on MS medium supplemented with 0.5mg/l BAP; 1mg/l BAP; 2.5mg/l BAP +2.5 mg/l IAA and 2.5mg/l BAP +2.5 mg/l IBA up to 44th day of inoculation. No multiple shoot formation was found on MS medium supplemented with kinetin and coconut water.

**Table 1:** Effect of different concentrations and combinations of hormones in MS medium on shoot growth from axillary bud culture

| Sr. No. | Concentration of Growth Regulators (mg/l) | | | | Average number of leaves on main shoot (on 30th day of inoculation) | Average shoot length in cm. (on 30th day of inoculation) | No. of shoots developed per explant (up to 44th day of inoculation) |
|---|---|---|---|---|---|---|---|
| | BAP | Kinetin | IAA | IBA | | | |
| 1 | 0.5 | - | - | - | 6 | 2.5 | 5 |
| 2 | 1 | - | - | - | 5.5 | 2.6 | 5 |
| 3 | 1.5 | - | - | - | 4 | 3 | 1 |
| 4 | 2 | - | - | - | 6 | 2.35 | 4 |
| 5 | 2.5 | - | - | - | 5 | 3.1 | 3 |
| 6 | 3 | - | - | - | 4 | 2.2 | 4 |
| 7 | - | 0.5 | - | - | 2.5 | 2.35 | 1 |
| 8 | - | 1 | - | - | 3 | 1.3 | 1 |
| 9 | - | 1.5 | - | - | 5 | 4 | 1 |
| 10 | - | 2 | - | - | 4.5 | 3.4 | 1 |
| 11 | - | 2.5 | - | - | 5 | 2.3 | 1 |
| 12 | - | 3 | - | - | 6 | 2.95 | 1 |
| 13 | 0.5 | - | 0.5 | - | 6 | 2.5 | 1 |
| 14 | 1 | - | 1 | - | 7 | 3.25 | 4 |
| 15 | 1.5 | - | 1.5 | - | 4 | 1 | 1 |
| 16 | 2 | - | 2 | - | 6 | 2.25 | 4 |
| 17 | 2.5 | - | 2.5 | - | 2 | 2.3 | 5 |
| 18 | 3 | - | 3 | - | 2 | 3.3 | 1 |
| 19 | 0.5 | - | - | 0.5 | 6 | 2.75 | 4 |
| 20 | 1 | - | - | 1 | 3 | 2.05 | 1 |
| 21 | 1.5 | - | - | 1.5 | 1 | 0.9 | 1 |
| 22 | 2 | - | - | 2 | 6 | 2.4 | 4 |
| 23 | 2.5 | - | - | 2.5 | 6 | 3.05 | 5 |
| 24 | 3 | - | - | 3 | 6 | 2.4 | 1 |
| 25 | Coconut water (15% v/v) | | | | 3.67 | 2.97 | 1 |

a b



c d

e

**Fig 1:** Shoots obtained through axillary bud culture on 30th day of inoculation on MS medium supplemented with **a.** BAP (0.5 mg/l) **b.** Kinetin (1.5 mg/l) **c.** BAP (1 mg/l) + IAA (1 mg/l) d. BAP (2 mg/l) + IBA (2mg/l) e. coconut water

**Fig 2:** Root formation in regenerated shoot on M.S medium supplemented with IBA (0.5 mg/l)

a b

**Fig 3:** (a and b) Callus formation on MS medium supplemented with IBA (1mg/l) + BAP (0.1 mg /l)

**Fig 4:** Multiple shoots formation

Sivanesan and Jeong (2009) [9], Lubaina *et al* (2011) [10], Ceasar *et al* (2013) [11] reported superiority of BAP in multiple shoot induction than kinetin which is in conformity with result of the present study. The *in vitro* regenerated shoots when cultured on MS medium containing 1mg/l IBA and 0.1 mg /l BAP, callus formation was observed but when these shoot were cultured on M.S medium containing 0.5 mg/l IBA, they showed root formation and small plantlets were formed. Many previous investigators studied micropropagation of *P. zeylanica* using axillary bud culture on M.S. medium supplemented with different plant hormones like BAP, IBA, IAA, NAA, $GA_3$ and TDZ. Rout *et a*l (1999) reported multiple shoot production from nodal explants of *P. zeylanica* on MS medium supplemented with BA [12]. Chaplot *et al* (2006) [2] observed average 36 shoots per node after three subcultures and even more after 6-8 cycles of subcultures using MS medium supplemented with BA (4.4 mg/l) + IAA (1.44 mg/l) [2]. Sivanesan and Jeong (2009) [9] reported maximum number of shoots (8) in axillary bud culture on MS medium supplemented with 1 mg/l BAP [9]. Gbadamosi and Egunyomi (2010) [13] also got multiple shoots when nodal cuttings were cultured on M.S medium supplemented with different concentrations of NAA + BAP [13]. Lubiana *et al* (2011) [10] observed highest shoot multiplication (20.2$\pm$0.32) from nodal explants cultured on MS medium supplemented with BAP 1 mg/l [10]. Maximum multiple shoots (19.56$\pm$0.04) per explant and maximum shoot length 4.98$\pm$ 0.87cm was reported by nodal explants cultured on M.S medium supplemented with BAP (2 mg/l)+ IAA (1.5 mg/l)+ IBA (1 mg/l) by Kanungo *et al* (2012) [8]. Hassan *et al* (2012) [14] also reported multiple shoot formation when nodal explants of *P. zeylanica* were cultured on MS medium containing different concentrations of BAP and BAP+NAA or IAA [14]. Caesar *et al* (2013) used MS medium supplemented with different concentrations of BAP, $GA_3$ and TDZ for micropropagation of *P. zeylanica* from nodal explants. They observed bud break after 10 days of inoculation and found TDZ superior to BAP and $GA_3$ in multiple shoot induction and production of lengthier shoots [11]. The present study illustrates a direct shoot induction efficacy of axillary bud cultures of *P. zeylanica* on MS medium supplemented with different concentrations and combinations of Auxins and Cytokinins.

## Conclusion

The present study provides simplified protocol for 100% bud break and fast micropropagation through direct shoot

regeneration from axillary bud cultured on MS medium supplemented with different concentrations and combinations of Auxins and Cytokinins. BAP is superior to kinetin in multiple shoot induction

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**References**

1. Agrawal SS, Paridhavi M. Herbal Drug Technology. Universities Press (India) Private Limited, Hyderabad, 2007.
2. Chaplot BB, Dave AM, Jasrai YT. A valued medicinal plant-Chitrak (*Plumbago zeylanica* Linn): Successful plant regeneration through various explants and field performance. Plant Tissue Culture & Biotech,2006:16(2):77-84.
3. Chaudhari SS, Chaudhari GS. A review of *Plumbago zeylanica* Linn.- A divine medicinal plant. Int. J. Pharm. Sci. Rev. Res.,2015:30(2):119-127.
4. Parthipan B. Biodiversity of Medicinal plants of the Marunduvalmalai Hills, Kanyakumari District, Tamil Nadu State. In: Ramakrishnan N (editor). Biodiversity in Indian Scenarios, Daya Publishing House, Delhi, 2006.
5. Mittal V, Sharma SK. An *in vitro* antimicrobial activity of callus and root extracts of *Plumbago zeylanica* Linn. in various test microorganisms. International Journal of Pharmaceutical Sciences Review and Research,2010:5(2):1-4.
6. Chaudhari SS, Chaudhari GS. *In vitro* regeneration of *Plumbago zeylanica* Linn. through embryo culture. IOSR Journal of Pharmacy and Biological sciences,2014:9(2):07-10.
7. Khanna VK. Plant Tissue Culture Practice. Kalyani Publishers, Ludhiana, 2003.
8. Kanungo S, Sahoo S, Sahu R. Development of simplified protocol for *in vitro* propagation of valuable medicinal plant *Plumbago zeylanica* Linn. through nodal explants found in Odisha. India. Journal of Medicinal Plant Research,2012:6(13):2627-2632.
9. Sivanesan I, Jeong BR. Micropropagation of *Plumbago zeylanica* L. African Journal of Biotechnology,2009:8(16):3761-3768.
10. Lubiana AS, Nair GM, Murugan K. Shoot multiplication and direct organogenesis of an important medicinal plant *Plumbago zeylanica* L. (Plumbaginaceae). Journal of Research Biology,2011:1(3):424-428.
11. Ceasar AS, M. Ayyanar M, Ignacimuthu S. An Improved Micropropagation Protocol for *Plumbago zeylanica* L. An Important Medicinal Plant. Asian Journal of Biological Sciences,2013:6(4):214-220.
12. Rout GR, Saxena C, Samantaray S, Das P. Rapid Clonal propagation of *Plumbago zeylanica* Linn. Plant Growth Regulation,1999:28:1-4.
13. Gbadamosi IT, Eguyomi A. Micropropagation of *Plumbago zeylanica* L. (Plumbaginaceae) in Ibadan, Southwestern Nigeria. Journal,2010:4(4):293-297.
14. Hassan AKMS, Haque F, Jahan MAA, Roy SK. *In vitro* mass propagation of *Plumbago zeylanica* L. through direct organogenesis. Bangladesh J. Sci. Ind. Res.,2012:47(3):297-302.