Rapid Micropropagation of *Plumbago zeylanica* L. An Important Medicinal Plant

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Abstract: An effective protocol for *in vitro* shoots multiplication and plant regeneration of *Plumbago zeylanica* L. was reported here. A rapid shoot proliferation was observed on the nodal explants of *P. zeylanica* in MS medium supplemented with 1.0mg/L BA and 1.0 mg/L GA_3. The highest length of shoot (5.88±0.44) was achieved after 1 week of incubation. Regenerated shoots were rooted on half strength MS medium supplemented with 1.0mg/L BA and 0.5 mg/L IAA. The rooted plantlets were successfully established in soil with 100 percent survival rate.
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1. Introduction

The use of medicinal plants is increasing worldwide. According to the World Health Organization (WHO), approximately 80% of the world's population currently uses herbal medicines directly as teas, decocts or extracts with easily accessible liquids such as water, milk, or alcohol (Julsing *et al*., 2007). *In-vitro* propagation of plants holds tremendous potential for the production of high-quality plant-based medicines (Murch *et al*., 2000).

Micropropagation has many advantages over conventional methods of vegetative propagation, which suffer from several limitations (Nehra *et al*., 1994). *Plumbago zeylanica* L. belongs to the family plumbaginaceae. Its roots are the main source of plumbagin, an alkaloid used as anticancer drug (Krishnasamy and Purushothaman, 1980; Jayaraman, 1987; Lin *et al*., 2003). It is also used as an irritant of the skin, in the treatment of rheumatism, piles, diarrhea and anasarca (Anonymous, 1989). Recently, antioxidant activity (Tilak *et al*., 2004) and anti allergic activity (Dai *et al*., 2004) reported from its stem and roots. There are number of reports elucidating the chemical and pharmacological properties of *P. zeylanica*. However, only limited work has been done on *in vitro* studies of this plant (Sivanesan, 2007). This study describes an effective protocol of direct regeneration from nodal explants of *P. zeylanica.*

2. Materials and Method

The Materials and Methods of Plant tissue culture were the standard methods as described in Plant Cell, Tissue and Organ Culture Fundamental methods (Gamborg and Phillips, 2004)

The explants viz. nodal segment was excised from plant and washed with running tap water. It is then soaked in 2% Tween 20 solution for 5 min and with (0.1%) mercuric chloride for 2 minutes followed by washing with distilled water 5 times for surface sterilization. The sterilized explants were cultured on MS basal medium (Murashige & Skoog, 1962) supplemented with 3% of sucrose, 6% of agar and growth hormones BA (0.5 -1.5 mg/L) and GA3 (05-1.5 mg/L). It is then transferred to a half strength rooting media supplemented with BA (0.5-1.5 mg/L) and IAA (0.5-1.5 mg/L). The pH of the media was adjusted to 5.6-5.8 before autoclaving at 121°C for 15 minutes. The cultures were incubated under 16 hours of photo period (2000 Lux) at 25±2°C for 3 weeks. Plantlets with the developed roots were removed from culture tube, washed with running tap water and successfully transferred to pots.

3. Results

The shoots rapidly emerged on MS media containing BA and GA_3. The maximum length of shoots (5.88±0.44) was observer on MS medium containing 1 mg/L BA and 1 mg/L GA_3. The highest percentage of shoot induction was also observed in the same combination (Table 1). The maximum of 3-

4 shoots were emmerged on the medium containing BA (1.0) + GA_3 (1.5) with 87.5 percentage when compared to other combination. Some authors have also reported combination of BA and GA_3 for achieving multiple shoot formation (Sarker *et al* 2003; Sakila *et al*., 2007). Elongeted shoots were 4-5.8 cm long and subjected to roorting experiment. Regenerated shoots were transfered to half strength MS medium without growth regulators and found no root formation which is in correlation with report by Sivanesan, 2007. Shoots rooted on half strength MS media supplemented with 1 mg/L BA and 0.5 mg/L IAA within 7 days. Adventitious rooting was also observed on the same media. The role of BA and IAA in shoot and root formation have also been recorded in many other plant species (Marta *et al*., 2009; Taware *et al*., 2010). The formation of callus was also observed from cut ends of microshoots, this callus formation reduced the formation of roots. The result clearly shows that optimum concentration of IAA and BA would be a good inducer of adventitious rooting in *P. zeylanica*. Rooted plantlets were cleaned with tap water to remove agar traces. It is hardened and placed in the earth ware pots containing sterilized sand, soil and manure (1:1:1). Potted plantlets were slowly acclimatized and shifted to green house with 100 % survival rate, which was an improved over 90% survival obtained by Rout *et at*. (1999).

Table 1: Effect of BA and GA_3 on shoot initiation and elongation of *Plumbago zeylanica.*

Plant growth regulators (mg/L)	**Shoot elongation mean ± S.D.**	**Response**	**Culture Establishment (%)**
BA (0.5) + GA_3 (0.5)	1.83±0.42	Single shoot	75.0%
BA (0.5) + GA_3 (1.0)	2.83±0.40	Single shoot	87.5%
BA (0.5) + GA_3 (1.5)	4.00±0.38	Single shoot	87.5%
BA (1.0) + GA_3 (0.5)	4.12±0.20	Multiple shoot	87.5%
BA (1.0) + GA_3 (1.0)	5.88±0.44	Multiple shoot	60.0%
BA (1.0) + GA_3 (1.5)	3.98±0.22	Multiple shoot	75.0%
BA (1.5) + GA_3 (0.5)	2.93±0.13	Single shoot	87.5%
BA (1.5) + GA_3 (1.0)	1.82±0.41	Single shoot	100.0%

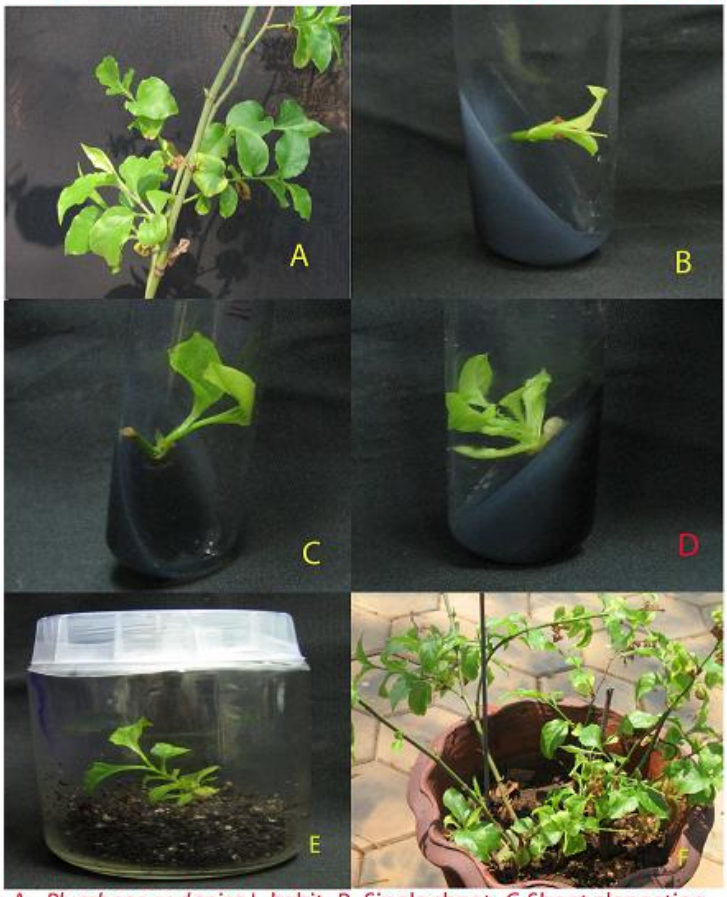
- The data is based on 15 replicate cultures, the experiment was repeated thrice, SD= Standard deviation, mg/L = milligram per litter.

Table 2: Effect of plant growth regulators on rooting medium after 30 days.

Plant growth regulators (mg/L)	**Mean of root length ± S.D.**	**Response**	**Culture Establishment (%)**
BA (0.5) + IAA (0.5)	-	-	-
BA (0.5) + IAA (1.0)	-	-	-
BA (0.5) + IAA (1.5)	3.41 ± 0.84	Single rooting	87.5
BA (1.0) + IAA (0.5)	4.81 ± 0.70	Profuse rooting	100
BA (1.0) + IAA (1.0)	3.24 ± 0.96	Single rooting	87.5
BA (1.0) + IAA (1.5)	-	-	-
BA (1.5) + IAA (0.5)	-	-	-
BA (1.5) + IAA (1.0)	-	-	-
BA (1.5) + IAA (1.5)	-	-	-

* The data is based on 15 replicate cultures, the experiment was repeated thrice, SD= Standard deviation, mg/L = milligram per litter.

Figure 1. (In vitro studies on *Plumbago zeylanica* L.)



A - *Plumbago zeylanica* L. habit, B -Single shoot , C-Shoot elongation
D - Multiple Shoots, E - Rooting of *Plumbago zeylanica* L.
F - Hardening of *Plumbago zeylanica* L.

4. Discussion

The addition of GA_3 was found to favor shoot elongation (Deore and Johnson, 2008; Najaf-Abadi and Hamidoghli, 2009). It promotes cell division and elongation in the sub apical zone of the shoots (George 1993). Furthermore, application of GA_3 in elongating regenerated shoots has also been described in many other plant species (Baburaj et al., 1987; Sujatha and Reddy 1998). Along with GA_3 the BA at the concentration of 1 mg/L showed promising results. The activity of BA compared to other cytokinins is also reported in many such plants i.e., *Gymnema sylvestre* (Komalavalli *et al*., 2000), *Holostemma annulare* (Sudha *et al*., 2000), *Hyptis suaveolens* (John Britto *et al*., 2001a), *Anisomeles indica* (John Britto *et al.*, 2001b), *Zehneria scabra (*Anand and Jeyachandran 2004), *Smilax zeylanica* (Hassan and Roy 2004) and *Phyllanthus urinaria (*Kalidass and Mohan, 2009). Similarly, in our experiment combination of GA_3 with BA was found effective during shoot initiation from the nodal explant.

Root formation was induced in the *in vitro* regenerated shoots by culturing them on half strength of MS medium with 1 mg/L BA and 0.5 mg/L IAA. IAA was found to be most effective at different concentrations tested for producing roots on the shoot cuttings. These results are in agreement with other studies (Reddy et al. 2001; Sudha et al. 2005; Mederos-Molina 2006). The percentage of shoots forming roots and days to rooting significantly varied with different concentrations and combination of IAA & BA. In contrast, Rout *et al.*1999 and Sivanesan, 2007 have reported root induction from

multiple shoots of *Plumbago zeylanica* in half strength MS media supplemented with NAA. In conclusion, the protocol standardized through this study demonstrates a rapid and effective method of *in vitro* shoots multiplication and plant regeneration of *P. zeylanica*.

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