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Rapid clonal propagation of an endangered medicinal plant *Plumbago zeylanica* Linn.

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Abstract

The Present investigation has given a method for *in vitro* micropropagation of a valued endangered medicinal plant Plumbago *zeylanica* Linn. (Plumbaginaceae) called chitrak. In this study we used nodal part of *P .zeylanica* as explant. Explants were cultured on MS media (Murashige and Skoog) supplemented with different concentrations of Growth hormones like BAP (0.2-1.0mg/l) NAA (0.2-1.0mg/l) BAP+NAA (0.2-1.0mg/l) . The excellent shoot formation followed by 1mg/l BAP+ 1mg/l NAA in this combination was observed as 100% and maximum shoot length was recorded (5.38±0.99 cm.). Regenerated shoots were rooted on half strength MS medium supplemented with 1 IBA (0.2-1.0) mg/l and IAA (0.2-1.0) mg/l. But the maximum number roots (7.00±0.98) were produced when the medium supplemented with IAA similarly maximum root length (6.99±0.89cm) contributed by IBA at 1.0 mg/l on MS medium.

Keywords: *In vitro*, micropropagation, Plumbago *zeylanica,* medicinal plant.

Introduction

Plumbago *zeylanica* (known vernacularly as Chitraka, *Chitramulamu*, *Tellachitramulamu*, *Agnichela*, *Agnimaala* or by its trade or popular names of "Lead wort-white flowered" and "Ceylon Lead wort") of the Plumbaginaceae, is distributed in tropical region of india (Anonymous 1989) and subtropical countries of the world. This plant is a spreading or subscandent, herbaceous, suffrutescent plant, 1 - 2 m in length. (K. Madhava Chetty et al. 2006). The root contains medicinally important compound known as 'Plumbagin' is present in all the variety of Plumbago *zeylanica* to a maximum of about 0.91%. Leaves and stems contain a little Plumbagin, fixed oil and volatile oil. This active chemical is vesicant, has properties of vitamin K, and is antibiotic on several human pathogent (Burkill, 1985). Properties of Chitraka are carminative, disperses contusion, anthelmintic, anti-inflammatory and antiphlogistic. Leaves minty, pungent and toxic. Roots are bitter in tast (Teshome K et al.).

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Chitrak has been used for arthritis, loose motions, dyspepsia, piles, gynacological practices and obesity. It promotes appetite, helps digestion also used for diarrhea, dysentery, and peptic ulcers, as diuretic, for abscesses, ascites, liver problems, coryza, hoarseness and sore throat. In Ethoipia, it is used for a variety of skin diseases. *P. zeylanica* extract has antifertility (Bhargava, 1984). Antitumer (Kavimani et al.,1996). antiplasmodial, antimicrobial, antihyperglycemic, hypolipidaemic and antiatherosclerotic activities Plumbagin showed anticancer, and antibiotic, antibacterial antifungal, (Krishnaswamy and Purushothaman, 1980) and antifungal activities. (R. Jeyachandran et al. 2008) and also has a stimulant action on the nervous system (Chopra, et al., 1996). (Didry et al. 1994). The plant is continuously collected from the wild and over exploited from ancient times. In view of this, there is a need for alternative means of clonal propagation with a view to preventing the plant from extinction. Biotechnological tools such as *In-Vitro* micropropagation can play an important role in the conservation of endangered medicinal plant as well as for rapid multiplication and quality improvement of valuable medicinally important plant species. Plumbago *zeylanica* is an important medicinal plant used in ayurveda since ancient times. In the present study we have observed the effect of variable

concentration of auxin and cytokinin on the regeneration potential. (S. Kumar Mishra et al.)

Material and Methods

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). General techniques involve the preparation of nutrient media, sterilization, inoculation, maintenance of culture.

The nodal explants were collected from healthy growing plant. Excision was carried out when the stem was green single node cutting from stem were standardized to about 1.0-1.5 cm length and all leaves were detached. Explants were then wash thoroughly in running tap water for 20 minutes to remove dust, durt and unnecessary particle adhering to them. Kept it in water with a small amount of bavistine (fungicide) for an hour. There after explants were then washed with distilled water for 30 minutes followed by a wash with shop solution for 20 min. and then it was immediately followed by 5 times rinse in distilled water to remove shop. After this explants was surface sterilized with 0.1% mercuric chloride treatment were given to explants for 3 min followed by 4 rinsed in sterile distilled water then the explants were cultured on ms media supplemented with G.H., 3% of sucrose, 6% of agar. The pH of the media was adjusted to 5.6-5.8 with 0.1 N NaOH or 0.1 N HCl before autoclaving at 15 lb pressure at 121°C for 15 minutes. The cultures were incubated under 16 hours of photo period (2000 Lux) cool, white fluorescent light at 25±2°C. The surface sterilization and inoculation procedure were carried out inside laminar air flow. Various concentrations of plant growth regulators is used for culture initiation and multiplication. The explants with bud proliferation cultures were transferred to fresh MS media for shoot multiplication, the cultures were maintained by regular subculture on fresh medium with the same composition. Then it is transferred into rooting media for root induction. Triplicates were used for each treatment.

Results and Discussion

Different concentrations of growth hormones tested like auxin (IAA, IBA, NAA) and cytokinin (BAP), the combination and their concentration were mentioned in table. The result was observed that the best shoot proliferation was found when MS supplemented with different concentrations of BAP (0.2-1.0mg/l) and NAA (0.2- 1.0) was used, after two weeks of incubation. Multiple shoots start arising from the nodal explants after 2 weeks of incubation. The present study exemplifies a positive modification of shoot induction efficacy on MS with combination of auxin and cytokinins (BAP, NAA) was producing maximum number of shoots and longer shoot length. Excision and culture of the nodal segments from *in vitro* derived shoots facilitated the development of increased number of shoots. The highest percentage of shoots proliferation was found in MS combination 1.0 BAP and 0.5 NAA. The maximum shoot length (5.38±0.99 cm.). was observed on the same proliferation medium within two weeks of incubation. For rooting, regenerated shoots were transferred in to rooting media. The plant growth regulators not only control the shoot bud formation but also influence the root induction. The effect of IAA and IBA on root induction was carried out. Root formations have been recorded within 7 days in MS media supplemented with 1.0 mg/L IAA and 1.0 mg/l IBA. Among them maximum number of roots (7.00±0.98) and root length (5.21±0.58) of the plant were noted in the MS medium containing 1.0 mg/l of IAA and 1.0 mg/l of IBA with number of root (5.81±0.61) and root length (6.99±0.89cm) respectively.

In the present study we developed a simple and efficient protocol for direct regeneration from nodal explants of Plumbago *zeylanica*. *In-vitro* propagation of plants holds tremendous potential for the production of high-quality plant-based medicines (Murch *et al*., 2000). The protocol can be exploited for commercial propagation and conservation of potential endangered medicinal plant resources. Micropropagation has many advantages over conventional methods of vegetative propagation, which suffer from several limitations (Nehra *et al*., 1994).

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Table 1: Different growth regulators for shooting response of *P. zeylanica*

MS media / Growth Regulators	Con. Mg/l	Shoot formation %	Shoot length cm.
KN	0.2	20	0.58+1.09
	0.4	20	0.65+00
	0.6	25	0.83+0.5
	0.8	50	1.63+00
	1.0	80	3.17+0.12
BAP	0.2	30	0.87+0.3
	0.4	50	1.56+0.13
	0.6	70	2.65+0.61
	0.8	85	3.47+0.89
	1.0	90	4.95+0.9
BAP+NAA	1.0+0.2	50	2.84+0.99
	1.0+0.4	60	3.35+0.99
	1.0+0.6	70	4.35+18
	1.0+0.8	90	2.25+0.89
	0.2+1.0	40	2.38+0.25
	0.4+1.0	50	4.25+0.99
	0.6+1.0	60	3.75+0.99
	0.8+1.0	70	4.90+0.89
	1.0+1.0	95	5.38+0.99

MS = Murashige and Skoog salt base (Murashige and Skoog, 1962); NAA = 1-napthalene acetic acid; BAP = Benzyl aminopurine; KN =Kinetin, mg/L = milligram per litter. The data is based on 3 replicate cultures, results were recorded after 15 days and are presented as mean ±SD= Standard deviation.

Table2: Effect of growth regulators on *in-vitro* rooting of *P. zeylanica*

Growthregulators	Conc.(mg/l)	Average no. of roots	Root length (cm)
IAA	0.2	2.80±0.69	1.50±0.51
	0.4	3.40±0.98	2.10±0.96
	0.6	4.40±0.98	3.00±1.21
	0.8	6.00±0.98	4.01±0.51
	1.0	7.00±0.98	5.21±0.58
IBA	0.2	1.81±0.62	2.11±0.85
	0.4	2.63±0.62	3.00±1.21
	0.6	4.12±0.62	4.50±0.96
	0.8	5.01±0.62	5.23±0.89
	1.0	5.81±0.62	6.99±0.89

IAA= indol-3acitic acid, IBA= indol-3 butyric acid, cm= centimeter, mg/l=milligram per litter. The data is based on 3 replicate cultures are presented as mean ±SD= Standard deviation.

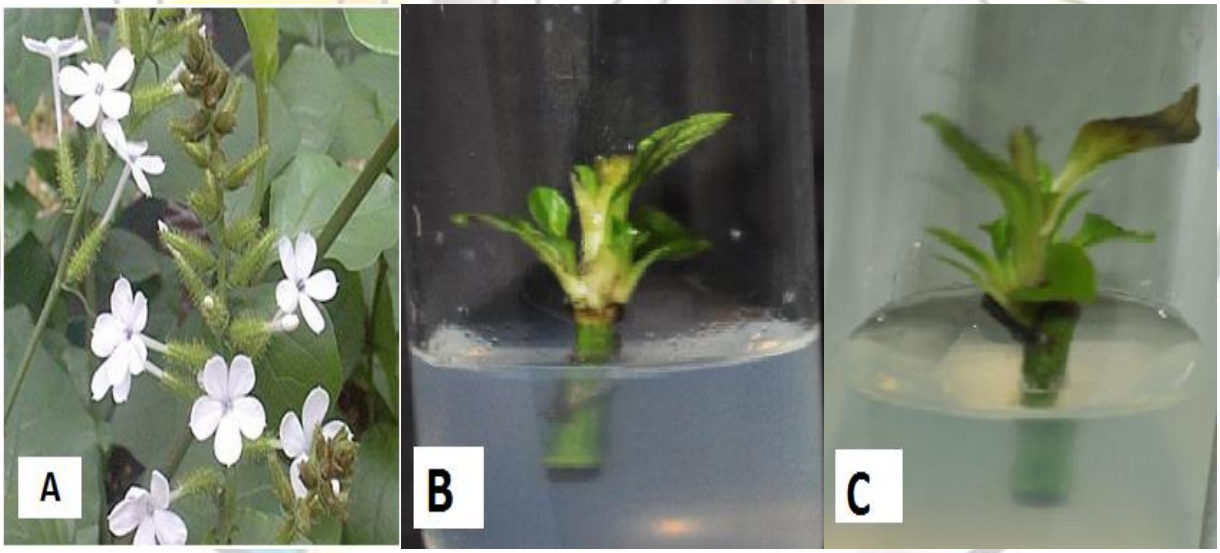

Fig. 1: A. *Plumbago zeylanica* (mature flowering plant), B & C shoot bud induction.

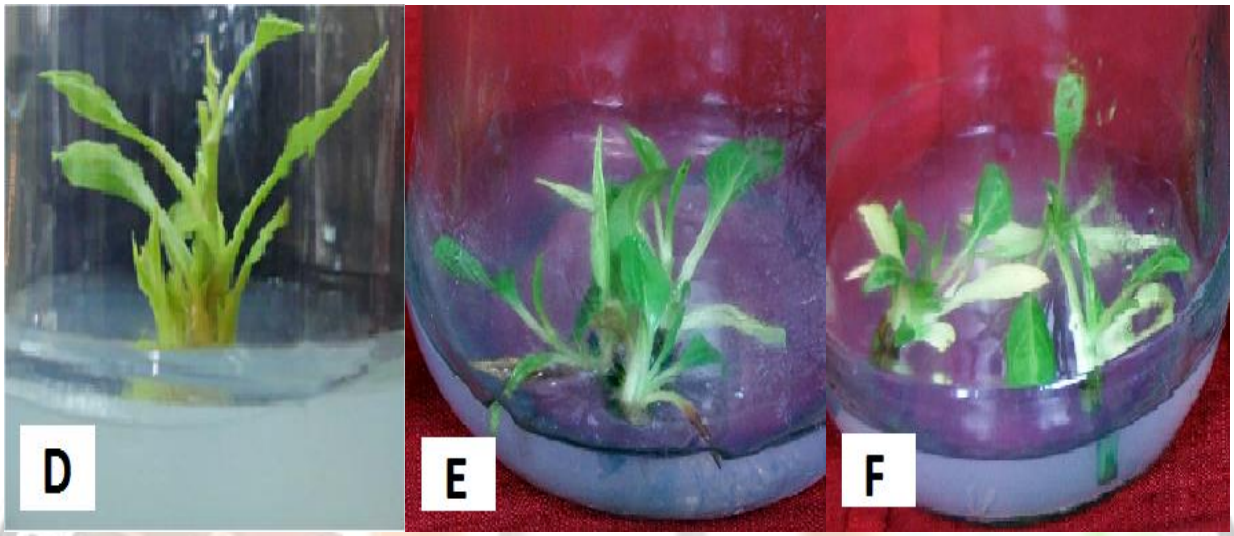

Fig. 2: D, E & F shoot multiplication

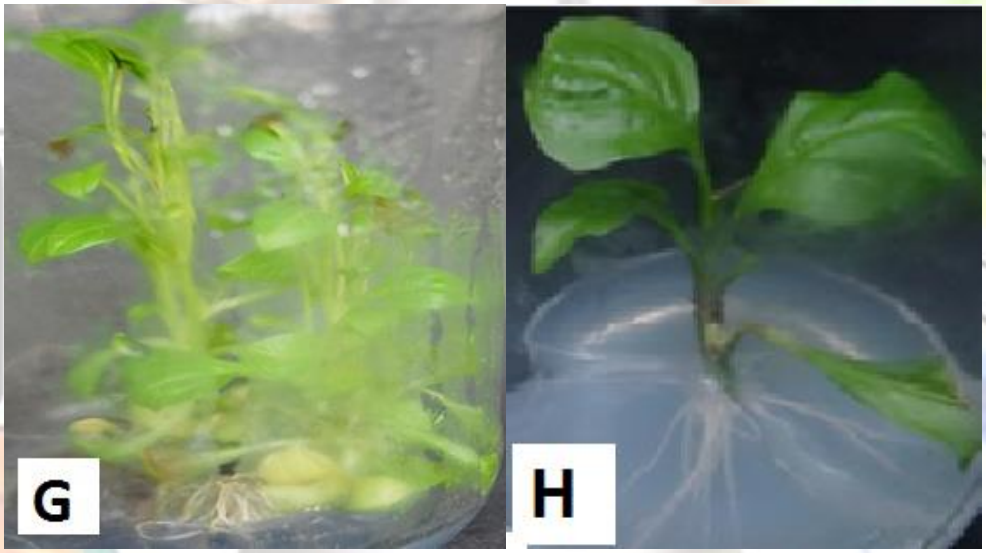

Fig. 3: G & H root induction