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Rapid direct root induction of *Plumbago zeylanica* Linn.

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ABSTRACTS

Plumbago zeylanica Linn. also known as 'Chitrak' belongs to the family Plumbaginaceae. Medicinally the most valued part of the plant Plumbago is its root. The roots have potential pharmacological activities and are widely used medicinally. The aim of the present study was to standardize a protocol for rapid, direct adventitious root induction and proliferation of Plumbago zeylanica, so that well developed roots suitable for medicinal uses are obtained within short time. Roots were directly induced from leaves, internodes and nodal explants on media supplemented with different concentrations and combinations of Plant Growth regulators. Media supplemented with Napthylacetic acid and Kinetin was found to be most effective in direct root induction. The in vitro induced roots were multiplied on suitable media. The roots showed best multiplication on ½ strength M.S. medium supplemented with 0.5mg/L Indole-3- butyric acid. The roots were compared with authenticated root sample via High Performance Thin Layer Chromatography. The components showed good separation on solvent system consisting of Toluene: Glacial acetic acid (9.5: 0.5). The profile of the in vitro induced root sample was found to be matching with that of authenticated root sample.

Key words: *Plumbago zeylanica*, growth regulators, root proliferation, Napthoquinones,

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INTRODUCTION

Plumbago zeylanica Linn. also known as White Leadwort, Chitrak, Chitra is an evergreen perennial shrub belonging to the family Plumbaginaceae. It is found in the tropical regions of the world. The roots are reputed to have a wide spectrum of therapeutic properties in the Ayurvedic system of medicine. They are useful in curing many ailments such as skin diseases, diarrhea, plague and leprosy [1]. The root and its constituents are credited with various potential pharmacological activities like anti-atherogenic[2],hepatoprotective and neuroprotective[3], anticancer[4],antibacterial[5], abortifacient [6], anti-inflammatory [7]. Root contains a number of Napthoquinone derivatives viz., Plumbagin, plumbagic acid glucosides, chitranone, maritinone, elliptinone and isoshinanolone [8-12]. This plant grows quite slowly and it takes long time until the roots are suitable for use [13]. More over conventional methods of propagation have proven to be difficult and inadequate to meet the escalating demand of the plant and its roots in market [14]. *In vitro* root culture serves as an efficient alternative method for rapid and mass scale root production [14]. Adventitious roots induced by *in vitro* methods showed high rate of proliferation and active secondary metabolism [15].This potential of the *in vitro* raised roots was exploited in the present study to induce large scale root multiplication within short time period so as to make the roots available for medicinal use at the earliest.

MATERIALS AND METHODS

Plant Material

Young and healthy branches were collected from one-year-old plant of *Plumbago zeylanica* grown and maintained by the Bappalal Vaidya Botanical Research Centre, Dept. of Biosciences of Veer Narmad South Gujarat University. The green young nodes, internodes, green young leaves with petioles were used as explants. The explants were excised, washed with running water for 30 min. Disinfecting the explants with 70% alcohol for 30-60 sec prior to surface sterilization in 0.1% $HgCl_2$ for 3 min was found to be very effective in reducing contamination. The surfaced sterilized explants were rinsed for 4-5 times in sterile distilled water, trimmed and inoculated on culture media.

Root culture

The culture media composed of Basal Murashige and Skoog's (M.S.) culture media with 30.0 gm /L sucrose, solidified with 0.8%Agar and supplemented with various combinations and concentrations of Plant growth regulators (PGRs) viz. 0.5-2.0mg/L 2,4-D, 0.05-2.0 mg/L of Napthylacetic acid (NAA), Indole acetic acid (IAA), Indole-3- butyric acid (IBA), 0.05-2.0 mg/L Benzylaminopurine (BAP), Kinetin (Kn).The pH of the medium was adjusted to5.7- 5.8 using 0.1 N HCl or 0.1 N NaOH before autoclaving. The media was dispensed in 25x150 mm culture tube, plugged with non-absorbent cotton and sterilized at a pressure of 15 lbs/inch^2 (121°C) for 20 min. All the cultures were maintained at 25 $\pm$ 2^o C with 16-h light/ 8-h dark photoperiods. Each treatment had ten replicates and all experiments were repeated thrice.

After 2-3 weeks, the emerging roots were transferred to full strength or ½ strength MS media supplemented with 0.01- 0.5 mg/L IAA/ IBA. They were harvested after 4 weeks. The phytochemical profile of the *in vitro* raised roots was compared with authentic root sample.

Solvent Extraction and HPTLC

The roots developing from different explants were dried and powdered. 5gms of powdered root were extracted with 50ml methanol under reflux for an hour [16]. The extracts were then co-chromatographed with the extracts of the root sample procured from local market, Surat, indentified and authenticated by experts at Veer Narmad South Gujarat University. 10ul of Sample were applied on 5 x 10 cm pre-coated silica gel 60 F 254 aluminium plate (MERCK) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed WIN CATS software.

Development of chromatogram:

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x 10 cm saturated with solvent system consisting of Toluene: Glacial acetic acid (9.5: 0.5) for 15 min.

Detection of spots:

The air-dried plates were viewed in ultraviolet radiation at 254 nm, 366 nm and also under visual light after spraying with 10% methanolic KOH reagent [17].

RESULTS AND DISCUSSION

All the explants showed direct root regeneration in M.S. media supplemented with different concentrations and combinations of NAA and Kinetin. The leaf explants formed root directly on media containing higher level of NAA and lower level Kinetin. The best result was obtained at 2.0 mg /L NAA + 0.05 mg /L Kn (Fig.1A). 80% of the explants showed response at this composition. The roots were found to be emerging directly within 4-5 days of inoculation with a maximum of 8.22 $\pm$ 0.8 roots per explants reaching an average length of 3.41 $\pm$ 0.3 cm after about 4weeks of inoculation (Table 1).

The intermodal segments formed roots after callus induction. At hormonal combinations of high NAA and low Kn callus formation was observed within 4-5 days of inoculation. The roots were induced after another week in the same media combination. However rate of root regeneration and number of roots formed was low. Best composition was found to be M.S. +1.0mg/LNAA+ 0.01 mg L^{-1}Kn, at which 60% of the explants showed response (Fig 1B, Table 1). The nodal segments showed simultaneous shoot and root induction on media supplemented with combination comprising of higher level of Kn and low level of NAA. The best result was observed at the concentration of 0.1 mg/Lof NAA with 1.5 mg/Lof Kinetin (Fig. 1C). Roots were found to be induced after shoot

induction within one week of inoculation [18]. The roots attained an average length of 3.66 ± 0.3 cm with a maximum number of 11.07 ± 0.2 within 4 weeks of inoculation (Table 2). Callus formation was observed from the nodal explants at a concentration of higher NAA and lower Kn. These *in vitro* raised roots were used as explants and cultured on media supplemented with PGRs favourable root proliferation. Maximum root proliferation was observed on ½ strength M.S. media supplemented with 0.5mg/L IBA (Fig.1D). Earlier reports showed that root cultures were established from the leaf explants of *Plumbago rosea* on Gamborg's medium supplemented with 1.0 mg/Lof NAA with 0.1 mg/Lof Kinetin [16]. In the present work with *Plumbago zeylanica* also NAA and Kn combination was found to be capable of direct root induction. Sivanesan and Jeong [14] reported that combination of Auxins enhanced the number of roots in this plant. However in the present work it was found that only Auxins were not capable of direct adventitious root induction. In *Rauwolfia serpentine* the leaf explants were found to be the best explants for direct root induction [19]. In the present work it was observed that leaf explants showed fast root induction but the number of roots developing from the nodal explants was found to be higher. The roots were compared with authentic root sample via HPTLC. Presence of Napthoquinone was clearly detected in the root extracts. Red fluorescence at UV 366nm after spraying with 10% methanolic KOH, indicated the presence of the Napthoquinone [17]. The chromatograms of the cultured root and the root sample showed identical profile (Fig. 2).

Table- 1: Effect of NAA and Kn on direct root induction of the leaf and intermodal explants of *Plumbago zeylanica* (after 3weeks of inoculation)

Growth regulators (mg /L)	Mean % of root regeneration ± S.E.		Mean No. of root ± S.E.		Mean root length (cm) ± S.E.	
	Leaf	Internodes	Leaf	Internodes	Leaf	Internodes
0.5 NAA + 0.05 Kn	Nil	Nil	Nil	Nil	Nil	Nil
1.0 NAA + 0.05 Kn	Nil	Nil	Nil	Nil	Nil	Nil
1.5 NAA + 0.05 Kn	40.0 ± 2.0	Nil	3.33± 1.6	Nil	2.60 ± 0.6	Nil
2.0 NAA + 0.05 Kn	**80.0 ±0.3**	Nil	**8.22 ± 0.8**	Nil	**3.41 ± 0.3**	Nil
0.5 NAA + 0.1 Kn	30.0 ± 0.0	Nil	3.4 + 0.5	Nil	3.37 ± 0.2	Nil
1.0 NAA + 0.1 Kn	70.0 + 0.7	**60.0 ± 0.0**	3.25 ± 0.6	**2.96 ± 0.3**	3.20 ± 0.8	**2.24 ± 0.4**
1.5 NAA + 0.1 Kn	60.0± 0.3	20.0 ± 0.7	1.50 ± 0.5	2.05 ± 0.1	4.06 ± 0.7	1.70 ± 0.9
2.0 NAA + 0.1 Kn	60.0 + 0.0	40.0 + 0.7	5.40 ± 0.9	1.76 ± 0.5	3.50 ± 0.8	2.0 ± 0.5
0.5 NAA + 0.5 Kn	70.0 ± 0.6	30.0 ± 0.3	2.66 ± 0.3	2.07 ± 0.2	2.60 ± 0.6	2.15 ± 0.3
1.0 NAA + 0.5 Kn	40.0 + 0.7	10.0 ± 0.7	3.40 ± 0.5	1.5 ± 0.3	3.41 ± 0.3	1.85 ± 0.2
1.5 NAA + 0.5 Kn	50.0 +0.3	40.0 ± 0.0	8.22 ± 0.8	1.33 ± 0.4	3.37 ± 0.2	0.93 ± 0.2
2.0 NAA + 0.5 Kn	60.0 +0.0	40.0 ± 0.3	3.25 ± 0.6	1.10 ± 0.2	3.20 ± 0.8	1.46 ± 0.3

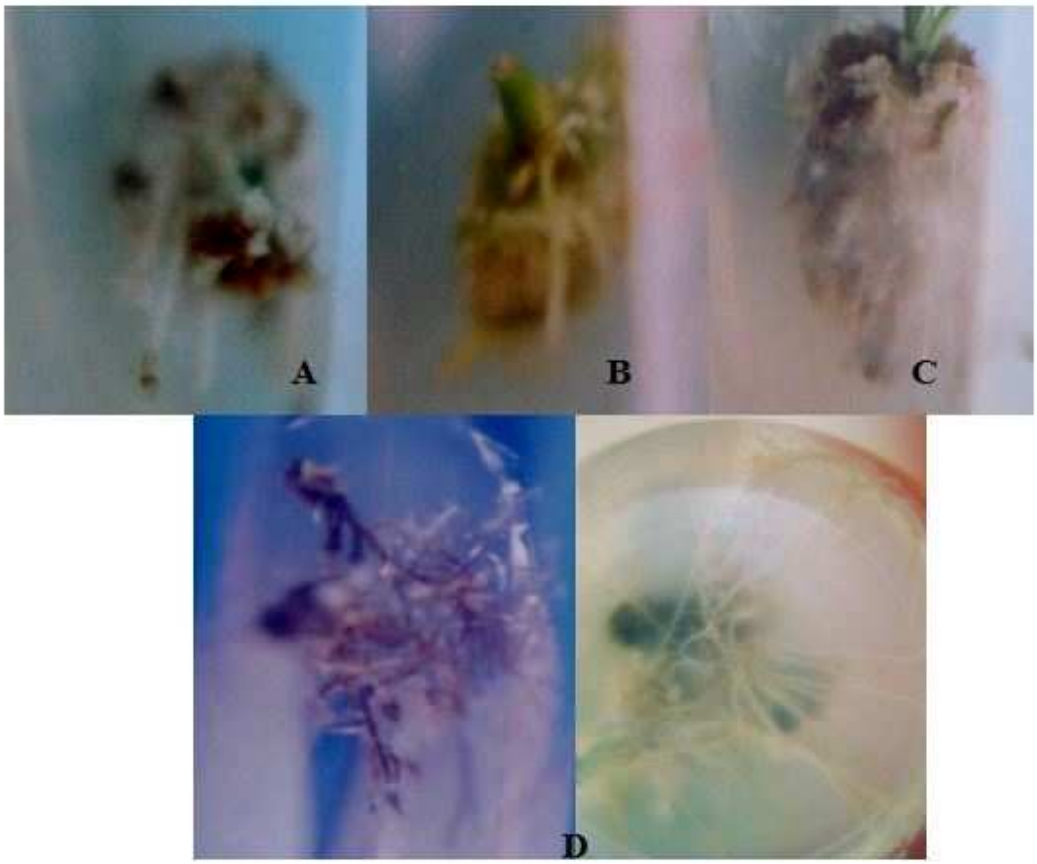
(Values represent means ± standard error of 10 replicates per treatment in three repeatedexperiments).

Table-2: Effect of Kinetin and NAA combination on root induction from the nodal explants of *Plumbago zeylanica* (Data recorded after 3 weeks)

Growth regulators (mg L^{-1})	Mean % of root regeneration ± S.E.	Mean No. of root ± S.E.	Mean root length (cm) ± S.E.
0.1 Kn + 0.05 NAA	20.0 ± 1.3	2.12 ± 0.3	2.3 ± 0.5
0.5 Kn +0.05 NAA	60.0 ± 0.8	2.5 ± 0.2	3.45± 0.3
1.0 Kn + 0.05 NAA	60.0 ± 0.7	3.22±0.4	2.63 ± 0.5
1.5 Kn + 0.05 NAA	70.0 ± 0.5	8.13 ± 0.2	2.5 ± 0.9
2.0 Kn + 0.05 NAA	50.0 ± 0.3	6.05 ± 0.2	2.86 ± 0.8
0.5 Kn + 0.1 NAA	60.0± 0.3	4.56 ± 0.3	3.21 ± 0.6
1.0 Kn + 0.1 NAA	70.0 ± 0.6	3.66 ± 0.7	3.0 ± 0.5
1.5 Kn + 0.1 NAA	**80 ± 0.3**	**11.07 ± 0.2**	**3.66 ± 0.3**
2.0 Kn + 0.1 NAA	80.0 ± 0.7	5.72 ± 0.8	3.1 ± 0.8
1.0 Kn + 0.5 NAA	70.0 ± 0.7	4.32 ± 0.9	2.5 ± 0.4
1.5 Kn + 0.5 NAA	60.0 ± 0.3	4.05 ± 0.7	4.66 ± 0.2
2.0 Kn + 0.5 NAA	50.0 ± 0.7	4.09 ± 0.6	4.0 ± 0.2

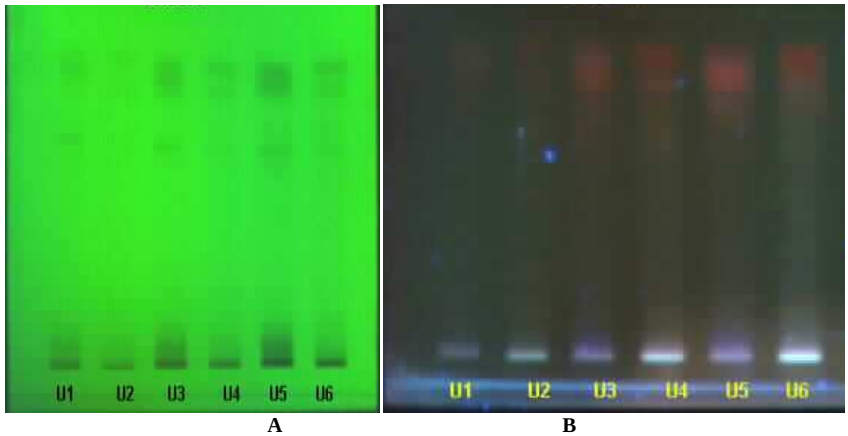
Values represent means ± standard error of 10 replicates per treatment in three

Figure1 (A-D) Direct root induction and multiplication from different explants of *P. zeylanica*



A -Roots directly developing from leaf explants at 2.0 mg/LNAA + 0.05 mg/LKn.
B- Roots developing from intermodal explants at 1.0mg/LNAA+ .01 mg L^{-1}Kn.
C- Simultaneous direct Root and shoot induction from nodal explants at 0.1 mg/Lof NAA +1.5 mg L^{-1} of Kn.
D -Roots cultured at ½ strength M.S. media +0.5mg/L IBA.

Figure 2 (A, B) HPTLC Plates



A **B**

A -U1, U3, U5- 5ul, 10ul and 15ul extract of sample root, U2, U4, U6 – 5ul, 10ul and 15ul extract of cultured root of P. zeylanica at 254nm
B -U1, U3, U5- 5ul, 10ul and 15ul extract of sample root, U2, U4, U6 – 5ul, 10ul and 15ul extract of cultured root of P. zeylanica at 366nm

CONCLUSION

Simple protocol was developed for early root induction from the leaf, internodal and nodal explants using different concentrations and combinations of growth regulators. M.S. Media supplemented with NAA-Kn combinations was found to be the most effective in direct root induction. The *in vitro* raised roots were proliferated on appropriate rooting media for 4weeks. The roots developing from the nodal explants showed maximum proliferation. The phytochemical profile of the roots were analysed and compared with that of authentic root sample through HPTLC. The profile was found to be matching. Further investigation for the characterization of the phytoconstituents is required. This technique provides an excellent alternative method for easy and rapid availability of the medicinally valuable roots of *Plumbago zeylanica*.

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