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Organogenesis from Shoot Tip and Leaf Explants of *Morinda Citrifolia,* L. An Important Medicinal Tree

Gajakosh A.M., M. Jayaraj, G.V. Mathad and P.V. Pattar

Department of Botany, Karnataka University, Dharwad-580003. India

Abstract: Shoot tip and leaf explants of *Morinda citrifolia* were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of 6-Benzyl amino purine (BAP), kinetin (Kn), Indole-acetic-acid, Indole-butyric-acid and Naphthalene acetic acid. Both explants responded for regeneration. High frequency and maximum number of multiple shoots were recorded on MS medium containing 5.0 mg/L (BAP), 2.0 mg/L (Kn) and 0.5 mg/L (IBA) in shoot tip culture. *In vitro* shoots were excised from shoot clumps and transferred to rooting medium containing different concentration of Indole-butyric-acid and Naphthalene acetic acid.

Key words: *Morinda citrifolia* % *In vitro* studies % Multiple shoots % Growth regulators

Abbreviations: MS-Murashige and Skoog (1962) medium; BAP-6-Benzyl amino purine; Kn-Kinetin; 2,4-D-2,4-Dichlorophenoxy acetic acid; IAA-Indole-3-acetic acid; IBA-Indole-3-butyric acid; NAA-Naphthalene acetic acid

INTRODUCTION

Medicinal plants are the source of various chemical substances essential for man kind. The exploitation of tissue culture techniques indeed desirable for their *in vitro* propagation and extraction of important chemical compounds. *Morinda citrifolia* L. is an important medicinal tree species, native of Polynesia. It belongs to the family Rubiaceae which is commonly known as Noni and Indian mulberry generally used in traditional and folk medicines for treatment of cold, influenza[1], diabetes, jaundice, asthma, high blood pressure, cancer[2], hepatitis, tuberculosis and different kinds of ulcers [1, 2]. It is found in tropical and sub tropical countries of world. Certain chemicals are identified in Noni includes sterols, minerals, antraquinones, putative proxeronine, vitamins and alkaloids [3-6]. Hence the present investigation is therefore aims to develop a rapid and high frequency shoot regeneration system from shoot tip and leaf explants of *Morinda citrifolia*, for providing continuous supply of a better source of elite plant to be used as standard material in the field of drug research as well as in manufacturing of drugs.

MATERIALS AND METHOD

Explants of *Morinda citrifolia* were obtained from plants grown in Botanical garden, Karnataka University Dharwad, India. Shoot tip and leaf explants were excised from healthy tree. Explants were washed with running tap water containing few drops of liquid soap. These were then surface sterilized with 0.1% mercuric chloride for 3-5 min and finally rinsed 3-5 times with sterile distilled water to remove the traces of mercuric chloride. To induce multiple shoots, explants were cultured on MS medium supplemented with various growth regulators. The medium containing 3% sucrose (w/v) and the pH was adjusted to 5.5-5.7. It was then solidified with 0.8% (w/v) agar and autoclaved.

RESULTS AND DISCUSSION

After inoculation on MS medium supplemented with different combinations of 6-benzyl amino purine (BAP), kinetin (Kn), Indole-acetic-acid, Indole-butyric-acid and Naphthalene acetic acid;. explants became hypertrophied [7]. and within few days callus was induced. Proliferation of shoot bud took place after 25 days. The leaf explants

Correspoding Author: M. Jayaraj, Department of Botany, Karnataka University, Dharwad-580003. India

Table 1: Morphogenic response of shoot tip explants of *Morinda citrifolia* L. cultured on MS basal medium with Kn and BAP alone and with different combinations

Sl. NO.	Medium	Cytokinin Concentration In mg/L	Frequency of Explants showing response
1	MS+Kn	1.0	2.5
2	MS+Kn	2.0	8.2
3	MS+Kn	5.0	32.6
4	MS+BAP	1.0	6.2
5	MS+BAP	2.0	14.2
6	MS+BAP	5.0	42.1
7	MS+BAP	10.0	35.9
8	MS+Kn+BAP	1.0+1.0	8.6
9	MS+Kn+BAP	1.0+2.0	19.6
10	MS+Kn+BAP	1.0+5.0	38.1
11	MS+Kn+BAP	2.0+1.0	29.8
12	MS+Kn+BAP	2.0+2.0	52.3
13	MS+Kn+BAP	2.0+5.0	69.9
14	MS+Kn+BAP	5.0+1.0	42.6
15	MS+Kn+BAP	5.0+2.0	38.7
16	MS+Kn+BAP	5.0+5.0	22.1

Table 2: Morphogenic response of leaf explants of Morinda citrifolia L. cultured on MS basal medium with different Auxins.

Sl. NO.	Medium	Auxin Concentration In mg/L	Frequency of Explant showing response
1	MS	-	-
2	MS+ IAA	0.5	6.2
3	MS+ IAA	1.0	8.3
4	MS+ IAA	2.0	25.9
5	MS+ IAA	5.0	56.1
6	MS+ IAA	10.0	32.1
7	MS+ IBA	0.5	8.1
8	MS+ IBA	1.0	12.2
9	MS+ IBA	2.0	23.6
10	MS+ IBA	5.0	63.9
11	MS+ IBA	10.0	10.2
12	MS+ NAA	0.5	4.8
13	MS+ NAA	1.0	9.6
14	MS+ NAA	2.0	36.5
15	MS+ NAA	5.0	53.6
16	MS+ NAA	10.0	6.9

showed callus induction after 2 weeks of inoculation. Subculture of this callus on MS medium supplemented with different concentrations of 6-benzyl amino purine (BAP) and kinetin (Kn) induced multiple shoots. The frequency of shoot bud initiation was 69.9% and maximum numbers of shoots were 12 which were regenerated from shoot tip on MS medium containing 5.0 mg/L BAP and 2.0 mg/L Kn. Similarly optimum callus from leaf explants was developed on the MS medium [8]. supplemented with 2.0 mg/L NAA and 2.0 mg/L IBA. Maximum numbers of shoots were regenerated from subculture of callus on MS medium supplemented with 5.0 mg/L BAP and 1.0 mg/L Kn in combination with 1.0 mg/L IBA.

Effect of BAP and Kn on shoot bud induction: When shoot tip were reared with BAP and Kn individually callus formation took place without any further development, but when these are combined in MS medium supplemented with 5.0 mg/L BAP and 2.0 mg/L Kn showed maximum number of multiple shoots with 12 shoots per explants with the frequency of 69.9%.

Individually when tested for callus formation from shoot tip BAP was found to be superior than Kn where as multiple shoots were reported in several plants such as *Phoenix dactylifolia, Anacardium occidentale, Holorrhena antidysentrica, Crataeva magna, Randia dumetorum and Tectona grandis* [9-15].

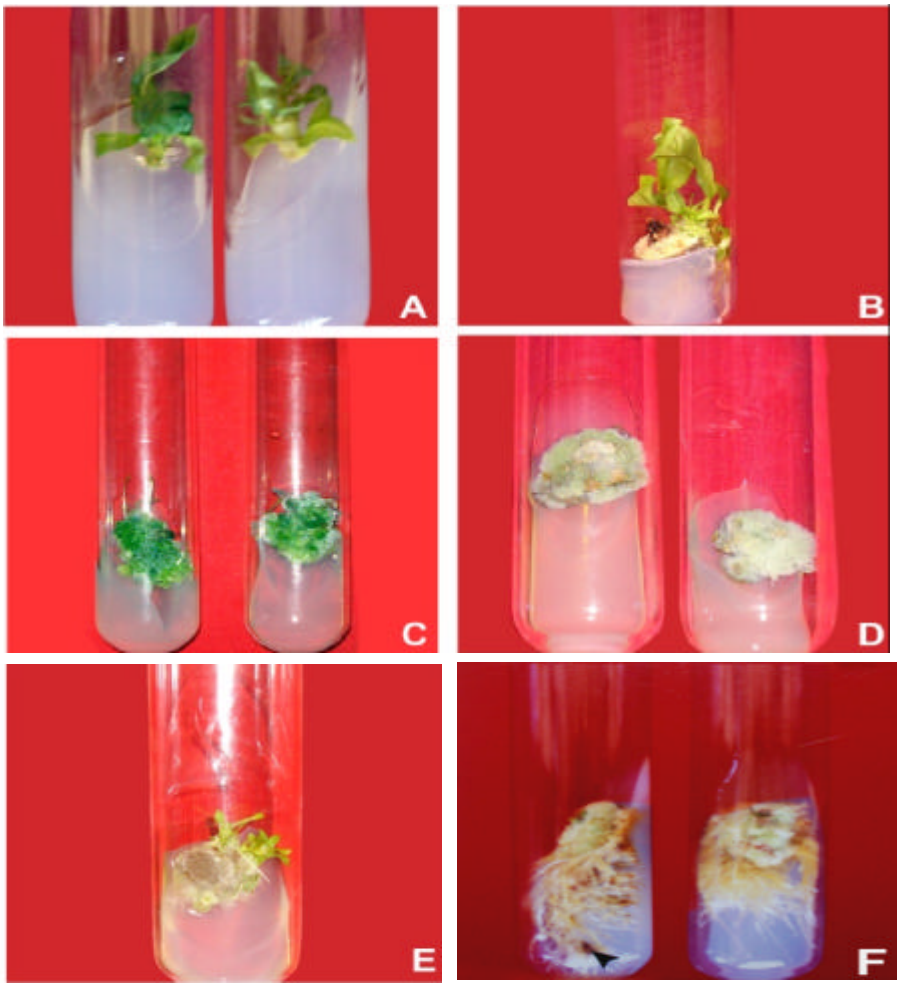

Fig. 1A-F: Photographs showing callus, multiple shoots, roots on MS medium supplemented with different combination of Auxins and Cytoikinins

A. Shoot tip on MS medium+2.0 mg/L BAP+1.0 mg/L IBA; B. Multiple shoots on MS medium+5.0 mg/L BAP+2.0 mg/L Kn; C. Callus induction and multiple shoots on MS medium+5.0 mg/L BAP+1.0 mg/L Kn+1.0 mg/L IBA; D. Callus from Leaf on MS medium+2.0 mg/L NAA+2.0 mg/L IBA; E. Multiple shoots from leaf callus on MS medium+5.0 mg/L BAP+1.0 mg/L Kn+1.0 mg/L IBA; F. Root formation from the secondary callus with 1.0 mg/L IAA+2.0 mg/L IBA/2.0 mg/L NAA.

Effect of different Auxins on root formation: For root induction, micropropagated plantlets were transferred to MS medium supplemented with different concentrations of IBA, NAA and IAA. Individually these Auxins produced thin and short roots. The frequency of root development were low in lower concentration of Auxin, but the frequency of root development were maximum in the medium containing 5.0 mg/L IBA and 2.0 mg/L NAA. However, above 5.0 mg/L IBA concentration, the frequency of root development decreased, whereas in *Ziziphus-spina-christi.* Higher concentration of IBA (10.0 mg/L) induced rooting [16].

Effect of different Auxins on callus formation from leaf Explants: Various concentrations of IAA, IBA and NAA tried for verifying morphogenetic response of young leaf explants. Lower concentrations of IBA and NAA showed the response but friable callus was formed when MS medium supplemented with 2.0 mg/L NAA and 2.0 mg/L IBA. Higher concentrations of IBA (5.0 mg/L) and NAA (5.0 mg/L) induced direct root.

Subculture of Callus for Organogenesis: When the callus was subcultured on MS medium containing IBA and NAA they induced secondary callus, MS medium

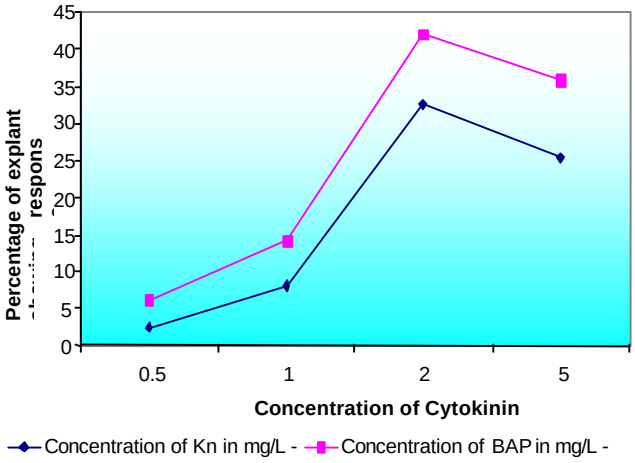

Fig. 2: Morphogenic response of shoot tip explants to Kn and BAP

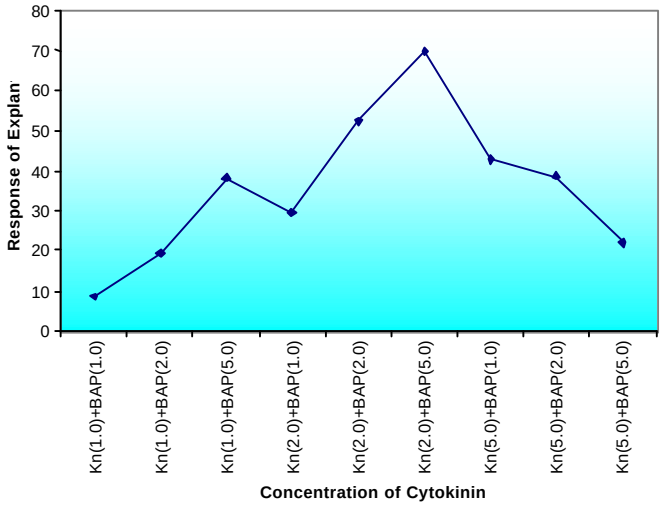

Fig. 3: Morphogenic response of shoot tip explants to different combinations of Kn+BAP

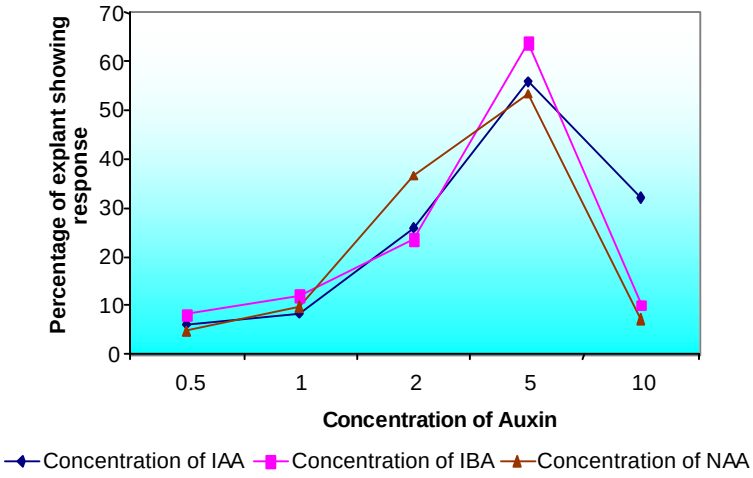

Fig. 4: Morphogenetic response of leaf explants of *Morinda citrifolia* L. cultured on MS basal medium with different Auxins

supplemented with 1.0 mg/L IAA 2.0 mg/L IBA 2.0 mg/L NAA induced secondary callus and then direct root formation is observed. Whereas MS medium supplemented with 5.0 mg/L BAP and 1.0 mg/L Kn in combination with 1.0 mg/L IBA induced multiple shoots with highest frequency. In *Randia dumetorum* multiple shoots were induced on MS medium containing lower concentration of BA (1.0 mg /L) and NAA (1.0 mg/L) [14]. Similarly in case of *Ziziphus-spina-Christi* higher concentration of BAP (5.0 mg/L) in MS medium induced callus where as lower concentration of BAP (1.0 mg/L) in MS medium induced multiple shoots [16].

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