



RESEARCH ARTICLE

COMPARATIVE STUDIES OF EFFECT OF SOME PLANT GROWTH REGULATORS AND COCONUT WATER ON CALLUS INDUCTION IN *TINOSPORA CORDIFOLIA* (willd)–A MEDICINAL PLANT

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ABSTRACT

This investigation was carried out to study the effect of some Plant Growth Regulators and Coconut Water on callus Induction using vegetative parts such as Nodal explants in *Tinospora cordifolia* (Wild.) Miers family: *Tinospora cordifolia* (Wild) an important medicinal plant. Callus induction was carried out using different concentrations of Auxins (2, 4-D, IAA, NAA), (1.0–7.0 mg/L) and cytokinins (BAP and kinetin), (2mg/L) alone and in combinations with 5-15% (v/v) Coconut Water. Among the different concentrations of Coconut Water tried, MS medium with NAA+ Kinetin (6.0+2.0 mg/L) along with 5% (v/v) Coconut Water and MS medium with 2,4-D + Kinetin (6.0+2.0 mg/L) along with 10% (v/v) Coconut Water showed best callus response (96.6%) in nodal explants. Whereas nodal explants showed the next best callusing response (93.33%) with 10% (v/v) Coconut Water on MS medium with NAA + BAP (4.0+2.0mg/L) and (7.0+2.0mg/L) respectively. Similarly with IAA + kinetin (7.0+2.0mg/L) + 10% (v/v) Coconut Water, response was 96.6%. The Sub-culturing time required was found to be longer in medium containing Coconut Water.

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INTRODUCTION

Tinospora cordifolia (willd.) Miers (Menispermaceae), perennial climber, distributed throughout tropical India, South Asia, Indonesia, Philippines, Thailand, Myanmar, China and in Srilanka. It possesses anti stress, anti diabetic, anti ulcer, anti-oxidative, radio protective, hepatoprotective, immunomodulator, and learning and memory enhancing properties (Krishna *et al*, 2009; Panchabhai *et al*, 2008; Upadyay *et al*, 2010). The medicated oil of the plant is effectively used to reduce the pain and edema, in gout and skin diseases. It is a natural blood purifier and is very useful for skin problems like acne, psoriasis and eczema. The stem of *Tinospora cordifolia* is bitter, stomachic, diuretic (Nayampalli *et al*, 1982) and stem consists of constituents of several ayurvedic preparations used in general debility, dyspepsia, fever and urinary diseases.

The root possess anti-stress (Patil *et al*, 1997) anti-leprotic (Singh, 2005) and anti-malarial activities (Nayampalli 1982; Wadood, 1992). The root and stem of *Tinospora cordifolia* are prescribed in combination with other drugs as an anti-dote to snake bite and scorpion sting (Nadkarni *et al*, 1976; Kirtikar *et al*, 1975; Zhao *et al*, 1991). Dry barks of *Tinospora cordifolia* has anti-inflammatory (Pendse *et al*, 1977) anti-arthritis (Jana *et al*, 1999), antidiabetic (Gulati *et al*, 1980) anti-allergic (Stanely *et al*, 2000). Neuroprotective (Nayampalli *et al*, 1986). antioxidant (Rawal *et al*, 2004) antineoplastic (Stanely *et al*, 2001) chemopreventive (Jagetia *et al*, 2006) radioprotective (Chaudhary *et al*, 2008) antipyretic (Goel *et al*, 2004) and

antifertility (Vedavathy *et al*, 1991), properties. A variety of constituents such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides have been isolated from *Tinospora cordifolia* plant. *Tinospora cordifolia* is generally propagated by seeds which has poor viability and low percentage of germination. Multiplication by vegetative propagation takes long time hence Plant Tissue Culture is a tool which can be used for micropropagation, conservation and source for secondary metabolite production.

One of the biotechnological approach among them is callus induction, which can be used for production of secondary metabolites (Georgiev *et al*, 2009; Ogita *et al*, 2009; Berkov *et al*, 2009). The objective of this study is to find innovative and economical method for establishment of callus cultures which can be used for production of secondary metabolites.

MATERIALS AND METHODS

Isolation of Coconut Water

The coconut water is prepared by collecting the liquid endosperm from dehusked immature coconuts. Extract of water from each fruit was checked separately to ascertain that it is not fermented before addition to the bulk. Coconut Water from all the fruits was heated at 80-100 °C for 10 minutes with continuous stirring to precipitate out the proteins, fats and other materials. The coconut water was cooled and filtered to remove the precipitated proteins and the filtrate is distributed as aliquots of 100 to 150ml in each flask and stored at -20 °C for future use (George, 1993).

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Table 1 Effect of Auxins and cytokinins in MS medium on Callus induction in nodal explants

MS Media +Plant Growth Regulators	Concentration in mg/L	No. of Tubes inoculated	Callus Response
2,4-D	1	60	20±1.15
	2	60	16.66±0.33
	3	60	20±1.00
	4	60	3.33±0.33
	5	60	6.66±0.00
	6	60	3.33±1.20
	7	60	3.33±0.33
NAA	1	60	10±0.00
	2	60	3.33±0.33
	3	60	0±0.00
	4	60	0±0.00
	5	60	10±0.00
	6	60	16.66±0.67
	7	60	3.33±0.33
IAA	1	60	26.66±0.67
	2	60	13.33±0.33
	3	60	36.66±1.20
	4	60	40±1.15
	5	60	43.33±1.33
	6	60	33.33±0.88
	7	60	16.66±0.67
BAP	1	60	13.33±0.33
	2	60	20±0.00
	3	60	6.66±0.33
	4	60	13.33±1.33
	5	60	26.66±0.33
	6	60	16.66±0.67
	7	60	16.66±0.67
KINETIN	1	60	10±0.00
	2	60	6.66±0.58
	3	60	36.66±1.20
	4	60	66.66±1.33
	5	60	33.33±1.33
	6	60	43.33±1.33
	7	60	36.66±0.33

The values represent the means (Mean±SE). Mean values within column followed by the significantly different by Duncan's multiple range test (P>0.05)

Explant Source and Preparation

Tinospora cordifolia plants were collected locally and authenticated by Dept of Botany, in Yogi Vemana University, vemanapuram, Kadapa, YSR District of Andhra Pradesh. For callus initiation nodal explants 1.5-2cm were excised from 5-6 months old plants and washed under running tap water for 20 min and then with Tween-20. Further sterilization was carried out under laminar air flow cabinet. The explants were treated with 1% sodium hypochlorite for 10 mins and rinsed 3 times with sterile distill water containing a few drops of Tween-20. This was followed by treatment with 95% ethanol for 30 seconds, and by a treatment of 0.1% HgCl₂ for 2 min and rinsing with distill water washes for three times.

All the explants were placed on basal MS medium (Murashige-Skoog, 1962) fortified with different concentration of phytohormones as Kinetin (Kn), 6-benzylaminopurine (BAP), NAA, and IAA and 2,4-Dichlorophenoxyacetic acid alone (1.0–7.0 mg/L) and NAA, IAA, 2,4-D (1.0–7.0 mg/L) in combination with BAP and kinetin (2.0mg/L) and along with Coconut Water (5-15%), sucrose (3%) agar (0.8%), pH of the media was adjusted to 5.7±0.02 prior to autoclaving. Media was sterilized by autoclaving for 15 min at 121 °C.

Data Recording and Analysis

The percentage of the explants producing calli were determined after 3 weeks of culture. The influence of Coconut Water and Plant Growth regulators were recorded on the basis of visual observation, represented as the percentage

of callus formed, their SD, Average Mean of number of responded cultures was recorded.

Culture Condition and Sub-culture

Callus was sub-cultured and maintained onto the same callus inducing medium after every two weeks duration. Large calli were cut into 2– 4 mm diameter pieces during transfer and sub-culture. Cultures were incubated at temperature 25 ± 2 °C.

RESULTS

In this investigation it was observed that the nodal explants on MS medium containing auxins showed poor callus response. A maximum response of 20% was observed on MS medium with 1 & 2mg/L, 2,4-D, which was also seen in MS medium with 6mg/L, NAA 16.66%. The highest callus induction among auxins used was observed on MS medium with 5mg/L, IAA 43.33% (Table 1). In case of MS medium containing cytokinins, best response of callus induction was observed on MS medium with 4mg/L, kinetin 66.66% as shown in the Fig. 1A-B followed by 26.66% response in medium containing 5mg/L BAP (Table 1). A combination of auxins and cytokinins in MS medium showed a varied response. Callus induction was poor in medium containing 3mg/L, 2, 4-D and 2mg/L, BAP (13.33%). The best response was observed in MS medium containing 5mg/L, IAA and 2mg/L, BAP (Fig. 1C-D) and similar response was observed on medium with higher concentration of 7mg/L, IAA and 2mg/L, kinetin 83.33% (Table 2 and Fig. 1E-F). There was a rapid improvement in callus induction, maximum 96.66% response in MS medium supplemented with 5% (v/v) Coconut Water and with 6mg/L, NAA and 2mg/L, Kinetin (Table 3 and Fig. 2A-B). On

Table 2 Effect of combinations of Auxins and cytokinins in MS medium on Callus inoculated in nodal explants

MS media + Plant Growth Regulators	Concentration in mg/L	No. of Tubes inoculated	Callus Response
2,4-D + BAP	1.0+2.0	60	16.66±0.88
	2.0+2.0	60	30±1.00
	3.0+2.0	60	13.33±0.33
	4.0+2.0	60	23.33±0.33
	5.0+2.0	60	20±0.00
	6.0+2.0	60	33.33±0.67
	7.0+2.0	60	33.33±0.88
NAA+BAP	1.0+2.0	60	13.33±0.33
	2.0+2.0	60	16.66±0.67
	3.0+2.0	60	43.33±1.33
	4.0+2.0	60	40±1.15
	5.0+2.0	60	23.33±1.45
	6.0+2.0	60	36.66±0.88
	7.0+2.0	60	50±2.52
IAA+BAP	1.0+2.0	60	30±1.53
	2.0+2.0	60	36.66±1.45
	3.0+2.0	60	46.66±2.03
	4.0+2.0	60	53.33±1.86
	5.0+2.0	60	83.33±0.67
	6.0+2.0	60	66.66±1.76
	7.0+2.0	60	70±0.00
2,4-D + Kinetin	1.0+2.0	60	40±2.00
	2.0+2.0	60	23.33±0.33
	3.0+2.0	60	46.66±1.67
	4.0+2.0	60	53.33±1.33
	5.0+2.0	60	40±0.58
	6.0+2.0	60	70±1.53
	7.0+2.0	60	70±0.33
NAA+ Kinetin	1.0+2.0	60	36.66±0.88
	2.0+2.0	60	30±0.58
	3.0+2.0	60	16.66±0.33
	4.0+2.0	60	60±1.53
	5.0+2.0	60	66.66±1.76
	6.0+2.0	60	73.33±1.33
	7.0+2.0	60	70±0.58
IAA+ Kinetin	1.0+2.0	60	30±1.53
	2.0+2.0	60	20±0.58
	3.0+2.0	60	16.66±1.67
	4.0+2.0	60	53.33±1.45
	5.0+2.0	60	43.33±1.33
	6.0+2.0	60	66.66±2.85
	7.0+2.0	60	83.33±0.33

The values represent the means (Mean±SE). Mean values within column followed by the significantly different by Duncan's multiple range test (P>0.05)

medium containing 10% (v/v) Coconut Water, Callus induction improved widely in all the media combinations the highest response was observed in MS medium with 7mg/L IAA and 2mg/L kinetin (Table 4 and Fig. 2C-D) followed by 6mg/L, 2,4-D and 2mg/L, Kinetin 96.66% (Table 4 and Fig. 2E-F) and the lowest response (40%) of callus induction was seen in MS medium with 2,4,-D and BAP with 2mg/L each concentration of growth regulators (Fig. 2G-H).

DISCUSSION

Van Overbeek (1941) used Coconut water for first time for development of embryos of *Datura stromium*. Lin and Staba (1961) reported that Coconut milk gave significantly improved callus in Peppermint and spearmint tissue cultures. It was observed in this study too that addition of Coconut Water an organic additive increased the callus response in the nodal explants in medium containing 5 or 10% (v/v) Coconut Water Table 3-4. Coconut Water contains a wide diversity of biologically active compounds such as phytohormones which includes mostly, auxins, cytokinins, gibberellins, and their natural inhibitors and regulators which include ethylene, abscisic acid, phenols and flavonols. Auxin like substances (Dix and Van Staden, 1982) zeatin and several unidentified ones (Zwar and Bruce, 1970); Nodal explants showed good callus response in MS medium with Kinetin in our findings as

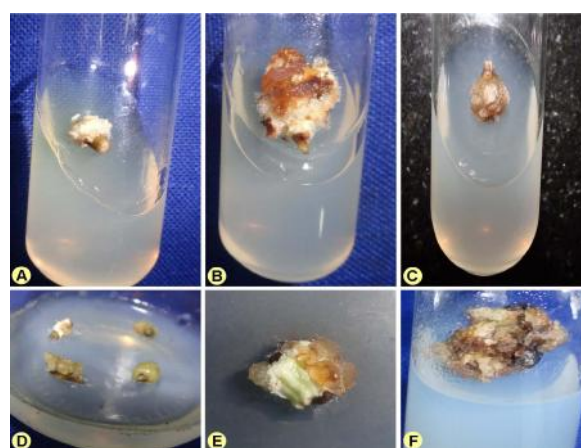


Figure 1 Callus induction from nodal explants in MS medium supplemented with cytokinin (Kinetin) alone and cytokinins (Kinetin & BAP) in combination with auxin IAA: A-B. Kinetin, 4mg/L, (66.66%) A. Callus induction, B. Proliferation of callus; C-D. IAA 5mg/L and BAP 2mg/L (83.33%), C. Initiation of callus, D. Proliferation of callus, E-F. IAA, 7mg/L + kinetin, 2mg/L (83.3%) E. Callus initiation, F. Callus proliferation.

compared to earlier reports (Aditi singh *et al*, 2009). It was also observed that a higher concentration of auxin and cytokinin and 5–10% (v/v) Coconut Water in MS medium induced good caulogenic response (66.6%) in nodal explants as compared to earlier attempt (Khanapurkar *et al*, 2012).

Table 3 Effect of combinations of Auxins and cytokinins in MS medium along with 5%(v/v) Coconut Water on Callus inoculated in nodal explants

MS media + Plant Growth Regulators + 5% (v/v) Coconut Water	Concentration in mg/L	No. of Tubes inoculataed	Callus Response
2,4-D + BAP	1.0+2.0	60	36.66±1.86
	2.0+2.0	60	33.33±0.33
	3.0+2.0	60	50±1.53
	4.0+2.0	60	56.66±0.88
	5.0+2.0	60	46.66±0.67
	6.0+2.0	60	66.66±1.76
	7.0+2.0	60	66.66±1.45
	1.0+2.0	60	70±0.58
NAA+BAP	2.0+2.0	60	50±1.53
	3.0+2.0	60	43.33±2.60
	4.0+2.0	60	83.33±0.67
	5.0+2.0	60	73.33±1.20
	6.0+2.0	60	33.33±0.88
	7.0+2.0	60	13.33±0.58
	1.0+2.0	60	43.33±0.33
	2.0+2.0	60	53.33±1.45
IAA+BAP	3.0+2.0	60	63.33±1.33
	4.0+2.0	60	13.33±0.33
	5.0+2.0	60	3.33±0.33
	6.0+2.0	60	26.66±0.67
	7.0+2.0	60	36.66±2.00
	1.0+2.0	60	40±1.00
	2.0+2.0	60	36.66±1.20
	3.0+2.0	60	16.66±0.33
2,4-D + Kinetin	4.0+2.0	60	43.33±1.45
	5.0+2.0	60	63.33±1.45
	6.0+2.0	60	70±0.00
	7.0+2.0	60	76.66±1.33
	1.0+2.0	60	50±1.15
	2.0+2.0	60	60±1.15
	3.0+2.0	60	70±0.00
	4.0+2.0	60	43.33±1.20
NAA+Kinetin	5.0+2.0	60	86.66±0.67
	6.0+2.0	60	96.66±0.33
	7.0+2.0	60	50±1.53
	1.0+2.0	60	43.33±1.45
	2.0+2.0	60	23.33±0.67
	3.0+2.0	60	50±1.00
	4.0+2.0	60	70±2.08
	5.0+2.0	60	50±1.00
IAA+Kinetin	6.0+2.0	60	60±0.58
	7.0+2.0	60	66.66±0.33

The values represent the means (Mean±SE). Mean values within column followed by the significantly different by Duncan's multiple range test (P>0.05)

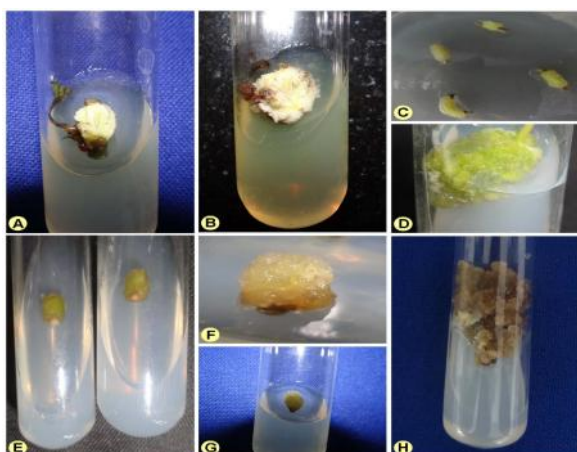


Figure 2 Callus induction from nodal explants in MS medium supplemented with cytokinin (Kinetin) in combination with auxin IAA & NAA along with 5-10% (v/v) Coconut Water: **A-B.** 5% Coconut Water (v/v) and 6mg/L, NAA and 2mg/L, Kinetin (96.66%) **A.** Callus induction, **B.** Proliferation of callus, **C-D.** 10% Coconut Water (v/v) IAA, 7mg/L and kinetin, 2mg/L (96.66%), **C.** Swollen nodal explants, **D.** Proliferation of callus, **E-F.** 10% Coconut Water (v/v) and 2,4-D, 6mg/L and Kinetin, 2mg/L (96.6%), **E.** Swelling of nodal explants, **F.** Proliferation of callus, **G-H.** 10% Coconut Water (v/v) and 2,4-D (2mg/L) and BAP (2mg/L), **G.** Initiation of callus, **H.** Proliferation of callus

Use of coconut water as a supplement for induction of callus has been reported in *Murraya koenigii* by Nazra *et al* (2011) and *Ipomoea batatas* by Patrick (2011) but not well established in *Tinospora cordifolia*.

CONCLUSION

Since *Tinospora cordifolia* has a wide range of medicinal use, mass collection of this plant for bioactive compounds extraction in pharmaceutical shall lead to depletion of this species.

This plant is generally propagated by seeds where viability is poor and has very low germination percentage, while vegetative propagation which takes long time period to grow. Hence development of an economical protocol using callus for growing large scale cultivation of cells for extraction of biologically active component is an attractive option for the future.

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Table 4 Effect of combinations of Auxins and cytokinins in MS medium along with 10%(v/v) Coconut Water on Callus induction in nodal explants

MS media + Plant Growth Regulators + 10% (v/v) Coconut Water	Concentration in mg/L	No. of Tubes inoculated	Callus Response
2,4-D + BAP	1.0+2.0	60	53.33±1.33
	2.0+2.0	60	40±0.00
	3.0+2.0	60	60±1.53
	4.0+2.0	60	53.33±1.76
	5.0+2.0	60	70±1.53
	6.0+2.0	60	83.33±0.58
	7.0+2.0	60	76.66±0.67
NAA+BAP	1.0+2.0	60	70±0.58
	2.0+2.0	60	50±1.53
	3.0+2.0	60	63.33±2.19
	4.0+2.0	60	93.33±0.33
	5.0+2.0	60	80±0.58
	6.0+2.0	60	73.33±0.88
	7.0+2.0	60	93.33±0.00
IAA+BAP	1.0+2.0	60	76.66±0.67
	2.0+2.0	60	83.33±1.45
	3.0+2.0	60	73.33±1.20
	4.0+2.0	60	66.66±1.67
	5.0+2.0	60	90±0.00
	6.0+2.0	60	76.66±1.33
	7.0+2.0	60	86.66±0.33
2,4-D + Kinetin	1.0+2.0	60	70±0.00
	2.0+2.0	60	63.33±1.86
	3.0+2.0	60	56.66±1.20
	4.0+2.0	60	50±0.58
	5.0+2.0	60	36.66±2.08
	6.0+2.0	60	96.66±0.33
	7.0+2.0	60	93.33±0.33
NAA+Kinetin	1.0+2.0	60	76.66±1.33
	2.0+2.0	60	90±0.58
	3.0+2.0	60	80±1.00
	4.0+2.0	60	76.66±1.86
	5.0+2.0	60	70±1.53
	6.0+2.0	60	86.66±0.88
	7.0+2.0	60	83.33±0.33
IAA+Kinetin	1.0+2.0	60	83.33±0.88
	2.0+2.0	60	76.66±1.33
	3.0+2.0	60	86.66±0.33
	4.0+2.0	60	60±1.53
	5.0+2.0	60	53.33±1.53
	6.0+2.0	60	83.33±2.33
	7.0+2.0	60	96.66±0.33

The values represent the means (Mean±SE). Mean values within column followed by the significantly different by Duncan's multiple range test (P>0.05)

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