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# Somatic embryogenesis from *Tylophora indica* leaf and node explants using low cost medium supplemented with growth regulators

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**Keywords**

Low cost medium, *Tylophora indica*, Somatic embryogenesis,

**Abstract**

An efficient and reproducible protocol has been developed for the *in vitro* somatic embryogenesis production of an endangered medicinal climber *Tylophora indica* (Burm.f) viz., leaves and node explants. The MS medium was replaced by economically cheaper alternative nutrient were used in the study. Somatic embryogenesis induced by low cost media with different hormonal concentration of IAA, 2,4-D and NAA in combination with BAP and Kin. The results obtained in low cost medium maximum somatic embryogenesis in 2,4-D (1.0 mg/l) +BAP (1.0 mg/l) combination viz., 99.3% in leaf; node 70.57% respectively. The sub cultured embryogenic calli turned to somatic embryo in combination NAA + Kin. The high frequency embryogenic calli matured in node 72.54%; leaf 68.24% respectively.

## Introduction

*Tylophora indica* (Asclepiadace) is a perennial climbing plant native to the plains, forest and hills of southern and eastern India. *Tylophora indica* is believed as one of most important herbs and reported as an endangered species (Mulchandani, 1971). The pharmacological importance of this plant has been reported to contain 0.2-0.46% alkaloids viz. Tylophorine, tylophorinine, tylophorinidine, (+) septicine, isotylocrebrine, tylophorinicine, sterols, flavanoids, wax, resins, and tannins (Govindhari, 1975). Thus the plant is in great demand for the production of traditional and Modern medicines. Therefore, the availability of a reliable, *in vitro* callus propagation system would provide an low cost alternative methods of propagation to meet the pharmaceutical needs for effective conservation of this important plant species. This plant is propagated only through seeds which have low viability and the destruction of plant caused by harvesting the roots as a source of drug ( Faisal and Anis, 2003). *In-vitro* organogenesis leading to considerable frequency of plantlet production has already been reported through nodal segment culture (Faisal & Anis, 2006) but very few studies have been reported on induction of somatic embryogenesis in *T. indica*, which had limited success (Chaudhuri *et al*., 2004; Chandrasekhar *et al.*, 2006).

In present study a efficient low cost medium viz. macro, micro nutrients, carbon source, vitamin, gelling agent and growth regulators has been established for somatic embryogenesis production in order to reduce the production cost without compromising the quality.

## Materials and Methods

### Plant material and explants preparation:

Young and healthy plants of *Tylophora indica* were collected from the medicinal garden, Government Arts college, Ariyalur (Fig-1). The immature leaf and node 1.0-2.5 cm explant were excised and washed under running tap water for 30 min and then immersed in teepol solution (5% v/v) for 15 min. There after the explants were washed thoroughly under distilled water. Surface sterilization was carried out in laminar airflow by soaking leaves and node in 0.1% w/v solution of $HgCl_2$ for 2 min followed by 3-4 rinses in sterile distilled water to remove all the traces of $HgCl_2$.

**Figure- 1** Habit of *Tylophora indica*

### Low cost media preparation:

In present study conventional MS (Murashige and skoog, 1962) medium was replaced by low cost alternatives (Table- 1). The low-cost medium was supplemented with 30 g/L of table sugar and 8 g/L agar agar (AR grade) and different concentration of growth regulators IAA, NAA, 2,4-D and BAP. The volume of all the nutrients was made to 1 liter of culture media. The pH of media was adjusted to 5.8, using 1N NaOH and 1N HCL, and the media was dispensed into glass bottles / test tube. The test tube containing media were sterilized by pressurized steam at a temperature of 121°C and 15 pounds of pressure per square inch for 15 minutes in the pressure cooker. The sterile media were kept in the transfer room under sterile conditions until use.

### Effect of different growth regulator on Somatic embryogenesis

Leaf and node explants of *Tylophora indica* was cultured in low cost medium with different concentration of IAA (0.5-2.5 mg/l), NAA (0.5-2.5 mg/l) and 2,4-D (0.5-2.5 mg/l) with BAP (1.0 mg/l) for somatic embryo initiation (Table-2). The further differentiation of somatic embryogenic callus through leaf and node explants (Table- 2) were subcultured in somatic embryo maturation medium. The further subculture in low cost medium with different concentration of Kin (0.5 mg/l) with IBA (0.5 - 3.0 mg/l), NAA (0.5 – 3.0 mg/l) were used for somatic embryo maturation (Table- 3).

## Results

Tissue culture has become a routine method for propagation of plants. However, application of tissue culture technology is constrained by its high costs. In Present study was to develop the tissue culture protocol with low cost media options in *Tylophora indica*. The alternative low-cost medium was formulated *viz.* macro, micro, vitamin, carbon source, iron source, solidifying agent and other salts. (Table-1).

**Table- 1:** Low cost and conventional medium composition

| Conventional MS medium (Murashige & Skoog media 1962) | (mg/l) | Low cost Alternative medium | (mg/l) |
|---|---|---|---|
| ***Macro nutrients*** | | ***Macro nutrients*** | |
| Ammonium Nitrate ($NH_4NO_3$) | 1650 | Ammonium nitrate | 6.0g |
| Calcium chloride ($CaCl_2$) | 440 | fertilizer | |
| Potassium Nitrate ($KNO_3$) | 1900 | Calcium Chloride fertilizer | 0.6g |
| Magnesium Sulphate ($MgSO_4$) | 370 | Potassium Nitrate fertilizer | 10.0g |
| Potassium dihydrogen Phosphate ($KH_2PO_4$) | 170 | Magnesium Sulphate fertilizer | 0.6 g |
| ***Micro nutrients*** | | Single super Phosphate | 1.0g |
| Potassium iodide (KI) | 0.83 | ***Micro nutrients*** | |
| Boric oxide ($H_3BO_3$) | 6.2 | Potassium Iodide(LR) | 1.5 |
| Manganese Sulphate ($MnSO_4.4H_2O$) | 22.3 | Power B-boran, Boric powder | 15.0 |
| Zinc Sulphate ($ZnSO_4.7H_2O$) | 8.6 | Manganese Sulphate | 30.0 |
| Sodium Molybdate ($Na_2\ MOO_4.2H_2O$) | 0.25 | fertilizer | |
| | | Zinc Sulphate fertilizer | 10.0 |
| Copper Sulphate ($CuSO_4.5H_2O$) | 0.025 | Adbor powder | 0.50 |
| | | Chelated fertilizer | 0.1 |
| Cobalt chloride ($COCl_2$) | 0.025 | Grandular/ powder | 0.1 |
| ***Iron Nutrient*** | | | |
| Ethylene diamine tetra acetic acid (EDTA) | 1.9 | ***Iron Nutrient*** | |
| | | Ethylene diamine tetra | 0.50 |
| Ferrous Sulphate ($FeSO_4.7H_2O$) | 1.39 | acetic acid (EDTA) | |
| | | Ferrous Sulphate fertilizer | 0.4 |
| ***Vitamins*** | | | |
| Myo Inositol | 100 | ***Vitamins*** | |
| Glycine | 2 | Becosules B-complex | 10 |
| ThiamineHcl | 0.1 | Tablets Thiamine | 10 |
| Nicotinic acid | 0.5 | Riboflavin | 3 |
| Pyridoxine Hcl | 0.5 | Pyridoxine HCl | 100 |
| | | Ascorbic acid | 150 |
| ***Growth regulators*** | | Biotin | 1.5 |
| IAA | 0.1 | Folic acid | 50 |
| 2, 4-D | 0.1 | Calcium pantothenate | 100 |
| NAA | 0.1 | Niacinamide | |
| IBA | 0.1 | ***Growth regulators*** | |
| Kinetin | 0.1 | IAA | 0.1 |
| BAP | 0.1 | 2, 4-D | 0.1 |
| GA3 | 0.1 | NAA | 0.1 |
| | | IBA | 0.1 |
| ***Carbon source*** | | Kinetin | 0.1 |
| Sucrose | 30 | BAP | 0.1 |
| | | GA3 | 0.1 |
| ***Solidifying agent*** | | | |
| Agar - Agar | 8 | ***Carbon source*** | |
| | | White refined sugar (Table sugar) | 30 |
| | | ***Solidifying agent*** | |
| | | Agar Agar (AR) | 8 |

A suitable somatic embryogenesis protocol was developed in *Tylophora indica*. In these experiments the leaf and node explants were used with different concentration of auxin (IAA, NAA and 2,4-D) produced greenish embryogenic callus. In different combination of IAA, NAA, 2,4-D (0.5 to 2.5 mg/l) with the concentration of BAP (1.0 mg/l) (Table- 2). The high frequency somatic embryogenesis was in low cost medium supplemented with auxin 2,4-D, (1.0 mg/l) + BAP (1.0 mg/l) were produced 99.34% in nodal explants likewise in leaf 70.59% respectively (Table – 2, Fig- 3). In case of IAA and NAA, the embryogenic callus formation was observed in low in compare with the maximum combination (Table- 2).

**Table 2:** Effect of low cost medium supplemented for with different concentration of IAA, NAA, 2,4-D in combination of BAP 1.0 mg/l on somatic embryogenesis from leaf and node explants of *Tylophora indica.*

| Low cost medium +Growth regulator BAP (1.0 mg/l) | | | Somatic embryogenesis induction in Leaf | | Somatic embryogenesis induction in Node | |
|---|---|---|---|---|---|---|
| **IAA** | **NAA** | **2,4-D** | **% response** | **Number of embryo/explant Mean± S.E** | **% response** | **Number of embryo/explant Mean± S.E** |
| 0.5 | | | 19.34±2.22 | 2.05±0.25 | 40.34±2.44 | 3.95±0.75 |
| 1.0 | | | 24.45±3.36 | 2.01±0.30 | 46.52±3.65 | 6.40±1.00 |
| 1.5 | | | 31.58±1.78 | 2.55±0.11 | 55.38±4.51 | 6.96±0.70 |
| 2.0 | | | 48.32±4.64 | 4.25±0.10 | 76.62±1.30 | 7.95±1.30 |
| 2.5 | | | 36.24±3.72 | 2.20±0.21 | 68.46±4.28 | 2.20±0.21 |
| | 0.5 | | 30.38±5.63 | 2.60±1.05 | 35.28±2.34 | 2.90±1.05 |
| | 1.0 | | 44.26±2.30 | 3.54±0.60 | 48.46±5.46 | 4.74±1.40 |
| | 1.5 | | 62.42±3.28 | 4.75±0.50 | 56.24±4.32 | 5.65±0.60 |
| | 2.0 | | 48.34±2.48 | 4.15±0.25 | 60.39±3.54 | 5.10±0.55 |
| | 2.5 | | 34.22±6.54 | 2.01±1.02 | 54.38±2.26 | 5.00±0.72 |
| | | 0.5 | 54.42±4.36 | 5.80±0.60 | 95.48±3.58 | 13.50±0.80 |
| | | 1.0 | **70.57±2.24** | **5.60±0.90** | **99.34±2.46** | **15.60±0.50** |
| | | 1.5 | 65.46±3.62 | 4.80±1.01 | 85.65±1.60 | 13.80±0.56 |
| | | 2.0 | 45.18±2.35 | 2.88±0.10 | 74.64±4.72 | 10.04±1.30 |
| | | 2.5 | 35.26±1.24 | 2.40±0.45 | 68.25±2.18 | 8.10±0.75 |

Data presented as the mean value ± standard error after 30 days of culture from four independent experiments each with 10 replicates.

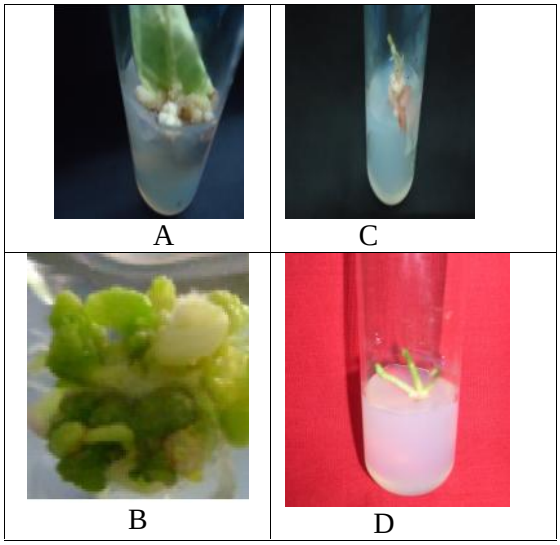
The embryogenic calli sub cultured in maturation medium containing IBA, NAA, and Kin. embryogenic calli was turned to somatic embryo in different concentration of IBA (0.5 to 3.0 mg/l), NAA (0.5 to 3.0 mg/l) with Kin (0.5 mg/l). The high frequency of maturation response was obtained in node embryogenic calli (72.54± 1.62); in leaf (68.24±1.28) respectively (Table -3) (Fig- 2).

**Table- 3.** Effect of Low cost medium supplemented with different concentration of IBA, NAA and Kinetin 0.5 mg/l on somatic embryo maturation from embryogenic calli of *Tylophora indica*.

| **Plant growth regulator** | | **Somatic embryo maturation from leaf and node (Mean ± SD)** | | | |
|---|---|---|---|---|---|
| Kin -0.5 mg/l | | **Embryogenic calli of leaf** | | **Embryogenic calli of Node** | |
| IBA | NAA | % response | Number of Somatic embryo matured | % response | Number of Somatic embryo matured |
| 0.5 | | 10.24±2.26 | 1.92±1.00 | 16.32±2.55 | 1.05±0.25 |
| 1.0 | | 16.25±3.38 | 3.90±0.20 | 25.36±3.42 | 2.10±0.30 |
| 1.5 | | 35.54±2.48 | 2.15±0.54 | 36.72±4.34 | 2.92±0.11 |
| **2.0** | | **52.12±3.24** | **4.02±1.05** | **56.26±2.18** | **3.04±0.10** |
| 2.5 | | 29.44±1.56 | 2.25±0.40 | 45.38±1.24 | 2.20±0.31 |
| 3.0 | | 20.58±4.42 | 1.60±0.50 | 28.56±3.44 | 1.58±1.05 |
| | 0.5 | 24.36±1.35 | 1.84±0.10 | 26.54±1.36 | 2.24±0.60 |
| | 1.0 | 42.52±2.26 | 2.35±1.20 | 48.32±2.24 | 1.80±0.50 |
| | **1.5** | **68.24±1.28** | **3.56±0.35** | **72.54±1.62** | **4.15±0.25** |
| | 2.0 | 44.12±4.56 | 2.04±0.22 | 55.36±3.54 | 3.51±1.02 |
| | 2.5 | 32.43±2.24 | 2.25±0.75 | 36.64±2.48 | 2.80±0.60 |
| | 3.0 | 21.27±5.62 | 1.80±0.30 | 26.42±3.24 | 1.60±0.90 |

Data presented as the mean value ± standard error after 30 days of culture from four independent experiments each with 10 replicates.

Figure - 2 : Low cost medium supplemented with different growth regulators on indirect somatic embryogenesis from *Tylophora indica* leaf and node explant




A, B- Indirect somatic embryogensis from *Tylophora indica* C,D -Somatic embryo Maturation

## Discussion

Somatic embryogenesis is one of the most intensively investigated phenomena in plant biotechnology (Zimmermann, 1993). In our results embryogenic calli were induced with the leaf and node sections in most of the media containing 2,4-D, BAP and NAA alone concentrations. Similarly the concentrations of these hormones produced somatic embryos in different plant species (Tabira 1994; Mikuła *et al*.2001). In contrast BAP was effective in inducing shoots in this species with axillary bud cultures (Rajavel and Stephan, 2014).

However, similar results that successful induction of somatic embryogenesis from *A. praecox sp. minimus* leaves also occurred through additions of 2,4-D and combinations of BAP and NAA. Besides, successful production of somatic embryos from other explants (Jamilah Syafawati, *et al*., 2012)

Pratibha Chaturvedi and Abhay Chowdhary, 2013 reported the callus of *Tylophora indica* was successfully initiate and established in the laboratory by using 0.2 ppm of BAP and 2ppm of NAA in the MS medium supplemented with 3% of sucrose and 0.8% of agar.

Similarly nodal explants of *Tylophora indica* produced maximum callusing potential in the medium supplemented with 2,4-D at 5mg/l. Chaudhuri *et al*., 2004; Thomas and Philip, 2005; Sivakumar *et al*., 2006. Reported on somatic embryogenesis various factorial combination of growth regulators elucidated different morphogenetic potential in leaf and node explants of *Tylophora indica*. The morphogenetic potential of tissue explants is also altered by genetic and physiological age of the mother plant.

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