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RESEARCH ARTICLE

# Low Cost *In Vitro* Propagation of *Tylophora indica* (Burm f.) Merrill. using different Carbon Sources

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## Abstract

*Tylophora indica* (Burm f.) Merrill. is an annual perennial medicinal plant in certain regions of India used as folk remedy for the treatment of bronchial asthma, bronchitis, allergies and dermatitis. An efficient protocol is described for the rapid *in vitro* multiplication of *Tylophora indica* using various carbon sources, viz. AR grade sucrose, white refined sugar (table sugar), unrefined brown sugar, jiggery and sugarcane juice was investigated. In this node, explants were initially cultured on Murashige and Skoog's (MS) medium supplemented with AR grade sucrose in addition with BAP (6-Benzylamino purine) and NAA (α-Naphthalene acetic acid), particularly BAP (1.5 mg/L) and (0.5 mg/L) NAA was very effective in inducing shoot development. Selected concentrations were used to supplement different carbon sources among the different sugars (2% and 3% w/v) as an alternative ingredient for the MS medium composition. The study revealed that the percentage of response was high in AR grade sucrose (95.2%) followed by white refined sugar (94.8%), sugarcane juice (76.8%), unrefined brown sugar (73.8%) and jaggery (67.6%) respectively. Instead of laboratory grade sucrose, the alternative carbon source may be used as a cheaper substitute for the MS media to propagate *T. indica.*

**Keywords:** *Tylophora indica*, *in vitro* multiplication, carbon source, 6-benzylamino purine, α-naphthalene acetic acid.

## Introduction

*In vitro* clonal propagation of medicinal plants enables large scale production of therapeutically high value taxa for commercialization and sustainable utilization in the industrial sector (Chandrasekhar *et al.,* 2006). *Tylophora indica* (Bum. f.) Merrill. (Asclepiadaceae) commonly known as "Antmool" is an important medicinal plant, traditionally used as a folk remedy in treatment of bronchial asthma, bronchitis, rheumatism, allergies and inflammation (Kaur *et al.,* 2011). The roots have a sweetish taste turning acrid, aromatic odor and a brittle fracture. They possess stimulant, emetic, cathartic, expectorant, stomachic and diaphoretic properties and are used for the treatment of asthma (Shivpuri *et al.,* 1968), bronchitis, whooping cough, dysentery, diarrhoea and in rheumatic gouty pains (Anonymous, 1976). The powdered leaves, stem and root contain several alkaloids (Rao *et al.,* 1971) including tylophorine ($C_{24}H_{27}O_4N$), tylophorinine ($C_{23}H_{25}O_4N$) which are pharmacologically active and anticancer tylophorinidine ($C_{22}H_{22}O_4N$) has also been isolated from the roots of three-year old plant (Mulchandani *et al.,* 1971). Apparently due to non-availability of sufficient quality planting materials, commercial plantations of this important aromatic and medicinal species have not been widely attempted and presently the wild population is exploited for extraction purposes. Due to over exploitation and lack of organized cultivation, the wild populations have declined fast.

There are a number of constraints for the propagation and conservation through conventional methods like vegetative and seed propagation. The major one is variations in edaphic and climatic factors, low percentage of seed set and seasonal dormancy. The propagation in its natural habitat is a rare phenomenon evidenced by close field observation. The above mentioned causes prompted us to find an alternate method of rapid micropropagation of this species. This necessitates the need to source for alternative low cost facilities, equipments and chemicals. In many developing countries, production cost of micropropagated plant is high (Savangikar, 2002; Dhanalakshmi and Stephan, 2014). *In vitro* multiplication and subsequent growth of plant are affected by several growth medium supplements. The AR grade sucrose is one of the factors added to the cost of MS media ingredients. So in view of medicinal importance, there is an urgent need to conserve this species *ex situ* through *in vitro* methods. Reports on *in vitro* propagation are limited (Sharma and Chandel, 1992; Chaudhuri *et al.,* 2004). So, in this study we are giving efficient and reproducible protocol for low cost micropropagation in *T. indica*. Therefore, the present study evaluated low cost *in vitro* propagation of *Tylophora indica* (Burm f.) Merrill. using different carbon sources.

## Materials and methods

*Plant material:* The plant were collected from Poovalur, Trichy Dt. TN, India (Geographic location Latitude $10^0$ 54'1"N; Longitude $78^0$ 49'51"E) (Fig. 1). The node explants collected from mature plant were washed with distilled water for two times and then rinsed with 1% (v/v) detergent (Teepol) for 5 min, later surface sterilized with 0.1% (w/v) aqueous solution of $HgCl_2$ for 5 min followed by 4-5 rinses in sterilized $ddH_2O$. Node explants were cut into small bits and used in further studies.

Fig. 1. Habit of *Tylophora indica* (Burm f.) Merrill.

*Media and culture conditions:* The glassware was subjected to chromic and sulphuric acid mixture (1:3) for 24 h and washed thoroughly with teepol (10%) detergent solution. It was then cleaned under running tap water, further rinsed with distilled water and oven dried. The vessels were decontaminated by autoclaving them for 20 min, then washed with detergent, tap water, distilled water and finally oven dried. The conventional MS salts Murashige and Skoog (1962) supplemented with 30 g/L of sucrose and 8 g/L agar agar and different concentration of BAP (0.5, 1.0, 1.5, 2.0 mg/L) and NAA (0.5 mg/L) was used as the control for shoot development (Table 1). Among these different concentrations of BAP and NAA, the highest shoot induction combination was selected for further studies. Selected hormonal concentrations were used as control and treated with different carbon sources (2% and 3%) viz. white refined sugar, unrefined brown sugar, jaggery and sugar cane juice (3% w/v) (Table 2). The pH of the medium was adjusted to 5.8 by 1N NaOH or 1N HCl before being autoclaved for 121°C for 20 min and all the cultures were incubated under light provided by cool white fluorescent lamp for the photoperiod of 16 h at 25±2°C. Data were taken in triplicate on the following parameters; number of shoots and shoots length (cm).

## Results

Various concentrations of plant growth regulators were tested on regeneration of *Tylophora indica.* Number of shoot regeneration was observed in the MS media supplemented with 1.5 mg/L BAP and 0.5 mg/L NAA (Table 1).

Table 1. Effect of MS medium supplemented with different concentration of BAP and NAA (mg/L).

| Hormone concentration (mg/L) | | Percentage of shoot induction (Mean±SE) |
|---|---|---|
| BAP | NAA | |
| 0.5 | 0.5 | 12.3±1.2 |
| 1.0 | 0.5 | 18.2±2.1 |
| 1.5 | 0.5 | 20.1±1.6 |
| 2.0 | 0.5 | 15.2±0.5 |

Results are mean ± S.E of 20 replicates.

Fig. 2. Shoots induction from MS medium supplemented with BAP (1.5 mg/L) + NAA (0.5 mg/L).

The best combination was used as a control and supplemented with different concentrations (2% and 3%) of carbohydrates. Among different carbohydrates used, sucrose and white refined sugar performed well followed by sugar cane juice, unrefined brown sugar and Jaggery in terms of inducing multiple shoot number. The results were depicted in Table 2 and Fig. 2 respectively. The maximum shoot number (16.5±1.90) was recorded at 3% sucrose, supplemented with MS medium (1.5 mg/L BAP and 0.5 mg/L NAA). The next best concentration for obtaining maximum number of shoots was at 3% white refined sugar (15.5±1.83), least number of shoots (9.30±0.90) was obtained in MS medium supplemented with 3% jaggery. High frequency of shoot regeneration was observed at 3% sucrose and white refined sugar respectively. But maximum number of shoots was obtained at 3% sucrose only. Shoots induced on MS medium supplemented with 3% sucrose resulted in maximum (4.45±0.22) when compared to other carbon sources used (Table 2, Fig. 2). The second best carbon source was white refined sugar **(**4.15±0.12) followed by unrefined brown sugar, Jaggery and sugarcane juice.

## Discussion

Different types and concentrations of carbon sources were used to study their effect of shoot induction from nodal explants of *T. indica*. The growth and multiplication of shoots *in vitro* are affected by various factors, one of which is the concentration and type of exogenous carbon source added to the medium (Hossain *et al*., 2005). The carbon source serves as energy and osmotic agents to support the growth of plant tissue (Lipavska and Konradova, 2004).

Table 2. Effect of different carbon sources on regeneration of *Tylophora indica* (Burm f.) Merrill.

| Carbon source | Conc. (g/L) | Percentage of response (Mean±SE) | Number of shoots (Mean±SE) | Shoot length (cm) (Mean±SE) |
|---|---|---|---|---|
| AR grade sucrose | 2% | 95.2±0.29 | 14.0±1.70 | 3.57±0.20 |
| | 3% | 97.4±0.58 | 16.5±1.90 | 4.45±0.22 |
| White refined sugar | 2% | 94.1±0.36 | 14.58±1.30 | 3.10±0.30 |
| | 3% | 96.5±0.66 | 15.5±1.83 | 4.15±0.12 |
| Unrefined brown sugar | 2% | 73.8±0.84 | 10.30±1.20 | 1.73±0.25 |
| | 3% | 82.0±0.90 | 11.5±1.69 | 2.95±0.60 |
| Jaggery | 2% | 67.6±0.42 | 8.10±0.40 | 1.50±0.54 |
| | 3% | 71.2±0.74 | 9.30±0.90 | 2.55±0.60 |
| Sugar cane juice | 2% | 76.5±0.56 | 10.60±0.70 | 1.20±0.45 |
| | 3% | 86.7±0.22 | 12.40±0.27 | 2.10±0.80 |

Results are mean±S.E of 20 replicates.

Even though carbohydrates are of prime importance for cell growth, maintenance and differentiation *in vitro*, the fundamental aspects of carbon utilization and metabolism in cell and tissue cultures have yet to be fully understood (Romano *et al.,* 1995). In the present study also, growth of *T. indica* is greatly influenced by different carbon sources supplemented in the media. In plant tissue culture, AR grade sucrose serves as a carbohydrate supply to provide energy for cell. In order to reduce the cost of the culture medium, commercially available white refined sugar (table sugar), unrefined brown sugar, sugarcane juice and jaggery at different levels were studied. But in the present study, high frequency, maximum number of shoots was induced on white refined sugar supplemented medium. The results obtained are in line with the earlier observations in *Mulbury* (Vijaya Chitra and Padmaja, 2001), where addition of white refined sugar instead of sucrose in the multiplication medium increased the shoot number and also growth of the shoots. Many authors have reported sucrose as a better source for shoot proliferation than other carbon sources in micropropagation of several plant species such as Patchouli *Pogostemon cablin* Berth (Kumaraswamy *et al.,* 2010), *Centellea asiatica* (Anwar *et al*., 2005), Peach root (Tauquer *et al*., 2007). Sucrose has been reported to be the best source of carbon and energy (Bridgen, 1994). However in the present study, the use of white refined sugar has shown best results than the use of sucrose. The results of commercial white refined sugar and AR grade sucrose in the media have shown comparable results. This suggests that sucrose can be replaced by white refined sugar for *T. indica* tissue culture. Many laboratories have reported the use of table sugar in plant propagation medium (Ganapathi *et al*., 1995; Kaur *et al*., 2005). Zapata (2001) has successfully reduced the cost of banana tissue culture by 90% by replacing the tissue culture AR grade sucrose with a commercial sugar. Beside, utilization of locally available table sugar can reduce the cost of potato tissue culture by 34-51% without any quality problems of tissue cultured plants (Demo *et al*., 2008). It is therefore recommended that white refined sugar can be considered as low cost substitute for *T. indica* micropropagation.

The plants cultured on jaggery and unrefined brown sugar had poor growth compared to other carbon sources. Similar results are reported by Ill-Wan and Korban (1998). Bouza *et al.* (1992) reported that the addition of table sugar to the medium results in hyperhydricity which leads to low cellulose and chlorophyll contents, less ethylene production and abnormal nitrogen and sugar metabolism. The decrease in shoot multiplication at higher concentration of carbon may be due to the inhibition of organogenesis and induction of callus proliferation. Locally available white refined sugar (table sugar) at concentration of 3% (w/v) enhanced shoot proliferations and vigorous growth of plantlets similar to AR grade sucrose (3%). This may be mainly due to easy translocation and assimilation of these energy sources available in medium by the explants resulting in cell division and then leading vigorous growth. In similar way, good performances of *in vitro* plantlets of banana, chrysanthemum, peanut and chickpea in table sugar supplemented medium are reported (Kodym and Zapata-Arias, 2001).

## Conclusion

It can be concluded from the study that among the different carbon sources used, white refined sugar performed well followed by AR grade sucrose in term of shoot induction. Since, the white refined sugar is the better carbohydrate choices for *in vitro* shoot induction of *Tylophora indica*. However, further research is highly required to explore the effect of different variety of carbon sources on *in vitro* plant regeneration of *T. indica*.

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