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Conservation of *Vitex trifolia* L. through *in vitro* Micropropagation

R. Mary Sujin and A. John De Britto *

Plant Molecular Biology Research Unit, St. Xavier's College (Autonomous), Palayamkottai, Tamilnadu, India.
*e.mail: bjohndesxc@gmail.com

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Abstract

An efficient micropropagation system for *Vitex trifolia*, an important medicinal plant has been developed. Shoot organogenesis occurred from *V. trifolia* nodal explants inoculated on medium with appropriate supplements of plant growth regulators. 65 % of shoot regeneration frequency and 4 shoots per nodal explants were achieved when cultured on a medium containing 2 % of 6-benzylaminopurine (BAP) and 1 % of 1- naphthaleneacetic acid (NAA). Shoot organogenesis was improved and 80 % of shoot regeneration frequency was recorded within 24 days, and regenerated plantlets survived and grew vigorously in greenhouse condition. The regeneration protocol developed in this study provides a basis for germplasm conservation and for further investigation of medicinally active constituents of this medicinal plant.

Keywords: *Vitex trifolia., in vitro* micropropagation, plant tissue culture, conservation

Introduction

Plants are the main source of medicines and play a key role in world health and global economy (Constable 1990; Srivastava *et al.,* 1995). Most of the pharmaceutical industry is highly dependent on wild populations for the supply of raw materials for extraction of medicinally important compounds. The genetic diversity of medicinal plants in the world is getting endangered at an alarming rate because of ruinous harvesting practices and over-harvesting for production of medicines, with little or no regard to the future. Hence there is a strong need for proactive understanding in the conservation, cultivation, and sustainable usage of important medicinal plant species for future use (Nalwade and Tsay, 2004).

Plant tissue culture offers a potential to deliver large quantities of disease-free, true-to-type healthy stock within a short span of time (Hussain *et al.,* 2001). Micropropagation has become a reliable and routine approach for large-scale rapid plant multiplication, which is based on plant cell, tissue and organ culture on well defined tissue culture media under aseptic conditions. Any micropropagation system must produce large number of genotypically uniform plants similar to the original plant from which they were propagated (Bajaj *et al.,* 1988). Also, plant tissue culture is useful for conservation and rapid propagation of medicinal plants.

Vitex trifolia L. (Verbenaceae) is a stout, aromatic shrub or a small tree found wild in several parts of India, which is traditionally used by the tribes and native medical practitioners for the treatment of various ailments including liver disorders, tumors, rheumatic pains, inflammation, sprains, fever and used in the treatment of tuberculosis (Anonymous, 2003). *Vitex trifolia* possess larvicidal, wound healing, anti HIV, anticancer, trypanocidal, antibacterial and antipyretic activities (Kannathasan *et al*., 2007).

Therefore, the aim of the present study was to develop a procedure for shoot multiplication from nodal and intermodal explants derived from mature trees of *V. trifolia.*

Materials and Methods

This research was conducted at the Centre for Biodiversity and Biotechnology (CBB), St. Xavier's College (Autonomous), Palayamkottai, Tamiladu, India. The samples of *Vitex trifolia* collected from Agasthiamalai Biosphere Reserve of Western Ghats, Tamil Nadu, India (Plate 1).

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a). Selection of explants

Young shoots were collected from healthy plant and thoroughly washed under running tap water for 30 min. After rinsing 3-4 times with sterile distilled water, nodes and internodes were cut into smaller segments (1 cm) and used as explants.

b). Surface sterilization procedure

Explants treated with 5% tween-20 for 5 minutes with constant stirring followed by 3-4 rinses in sterile distilled water and further treated with an antifungal agent (Bavistin) for 2 hours and were further with detergent for 10 min. and rinsed 4-5 times tap water. Further sterilization procedures were carried out inside laminar air flow chamber, where explants were surface sterilization through single dip in 70% (v/v) for half minute followed by three times rinses in sterile distilled water.

There after mercuricloride (0.1%) treatment was given to explants for 8 minutes followed by four times rinsed in sterile distilled water. Thereafter explants were carefully transferred to be placed over sterile petridish & were then inoculated into the culture establishment medium inoculated into the culture establishment medium (MS Medium; Murashige & Skoog 1962) using sterile forceps under aseptic conditions.

c). Chemicals

Nodes and internodes were cultured on MS basal medium supplemented with 2,3,4,5% sucrose, 0.7% (w/v) agar (Hi-Media, Mumbai. The pH of the medium was adjusted to 5.8 and autoclaved at 121°C for 15 min. The surface sterilized explants were placed vertically on the culture medium. The cultures were incubated at 25 ± 2°C under cool fluorescent light (2250 lux 12 hr/d photoperiod). All the cultures were incubated at 25±2°C under 16h light/8h dark photoperiod MS medium supplemented with different concentrations of Benzyl amino purine (BAP), α-naphthaleneacetic acid (NAA) and thidiazuron (TDZ) for in vitro regeneration of shootlets.

d.) Statistical analysis

95 explants were assigned randomly and the date record was subjected to statistical analysis in MS Excel.

Results and Discussion

In the present study, the type and percentage of BAP and NAA influenced the average number of shoots produced per explants as well as mean length of the shoots. There was no result shown in internodal explants. There was no sign of growth when explants were cultured in TDZ in the media. The percentage (0.5, 1.0, 1.5, 2.0 and 2.5 mgl^{-1}) of BAP showed shoot production (Table 1). The percentage (0.5, 1.0, 1.5 and 2.0 mgl^{-1}) of BAP showed shoot production (Table 2).

Among various concentrations best response in terms of multiple shoot formation was observed on MS supplemented with 2.0 mgl-1 BAP (Table 1). 2.0 mgl^{-1} BAP, proved to be optimal, producing an average shoot length of 5.73±0.2 cm per explants Table 1& Plate1.

The best result for shoot proliferation was obtained from MS medium supplemented with 2.0 mgl-1 BAP(4.00±1.0). At a low level of BAP concentration (0.5 mgl^{-1}), fewer shoots were obtained (1.33±0.5). At a high level of BAP concentration (2.5 mgl^{-1}), fewer shoots were recorded (2.66±2.06).

Among various concentrations of NAA best response in terms of multiple shoot formation was observed on MS supplemented with 1.0 mgl-1 BAP (Table 2). 1.0 mgl^{-1} NAA, proved to be optimal, producing an average shoot length of 4.50±0.3cm per explants Table 2& Plate 1.

Shoot proliferation was obtained from MS medium supplemented with 1.0 mgl-1 NAA (4.00±1.0). At a low level of NAA concentration (0.5 mgl^{-1}), fewer shoots were obtained (2.33±0.5). At a high level of NAA concentration (2.0 mgl^{-1}), fewer shoots were recorded 1.33±0.5).

TDZ alone or in combination of NAA did not show any positive response on shoot formation and development. The combination of BAP with NAA and TDZ with NAA treatments also did not promote new shoot formation.

In 2003, Bhau and Wakhlu reported that higher concentration of BAP decreases shoot multiplication in mulberry plant. In present study , the maximum shoot length (5.73±0.2) cm was obtained from explants on a MS medium with

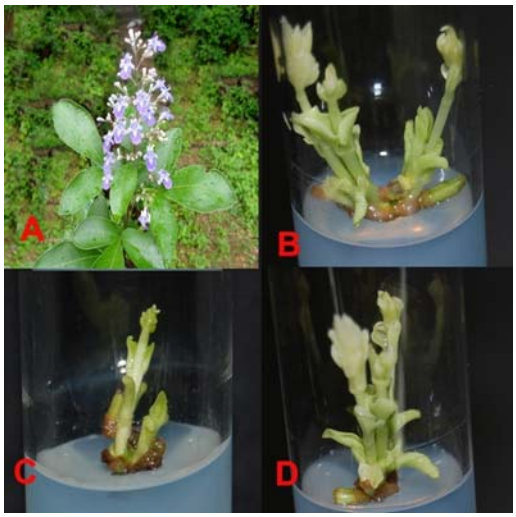
2.0 mgl-1 BAP. Similar finding reported by Faheem *et al.,* (2008), that is maximum rate of shoot induction (80%) at 0.1 mgl-^{1} BAP in *Gladiolus hybridus*.

Table -1: Effect of concentration BAP on *in vitro* shoot multiplication of *V. trifolia*

Concentration of BAP (mg/l^{-1})	Average no shoots per explant (Mean±SE)	Average length of shoots(cm) Mean± SE)
0.0	0.0±0.0	0.00±0.0
0.5	1.33±0.5	1.64±0.2
0.1	2.00±1.0	2.16±0.3
1.5	3.33±1.5	4.36±0.4
2.0	4.00±1.0	5.73±0.2
2.5	2.66±2.06	3.20±0.4

Table -2: Effect of concentration NAA on *in vitro* shoot multiplication of *V. trifolia*

Concentration of NAA (mg/l^{-1})	Average no shoots per explant (Mean±SE)	Average length of shoots(cm) Mean± SE)
0.0	0.0±0.0	0.00±0.0
0.5	2.33±0.5	2.76±0.4
0.1	4.00±1.0	4.50±0.3
1.5	2.66±1.1	3.70±0.1
2.0	1.33±0.5	2.83±0.3
2.5	0.0±0.0	0.00±0.0



A. Habit; B. Shoot proliferation on 2.0 mgl-1 BAP; C. Shoot proliferation on 2.5 mg/l^{-1} BAP; D. Shoot proliferation on 1.0 mgl^{-1} NAA;
Plate -1: Habit and Shoot proliferation of *V. trifolia*

Maximum number of multiple shoot bud (3.6±0.51) per explants was induced on MS medium supplemented with 2.0 mg/l-^{1} BAP combination with 0.5 mgl-^{1} NAA in *Vitex negundo* reported by Rahman and Bhadra (2011), But in our result even a single hormone 1.0 mg/l-^{1} NAA shows maximum number of multiple shoots (4.00±1.0) and shoot length (4.50±0.3).

Conclusion

A protocol was developed for *in vitro* shoot multiplication of *V. trifolia* using nodal explants from healthy plants. BAP was found to be essential for shoot multiplication from nodal explant on MS medium. BAP at 2.0 mgl-^{1} gave the best results for the proliferation of cultures from explant among the experiment. The procedure described here provides a rapid and prolific micropropagation system. The shoot induction protocol developed in this study provides a basis for germplasm conservation and for further investigation of medicinally active constituents of the elite medicinal plant. Further work for standardization of efficient in-vitro protocol for best shoot multiplication & in-vitro rooting is under progress in our laboratory.

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