

Plant Regeneration from Nodal explants Culture through Organogenesis in *Eupatorium triplinerve vahl*

*S.Usha¹ and Karpagam, S²

1. Department of Biotechnology, New Prince Shri Bhavani Arts and Science College, Medawakkam, Chennai - 600100

2. Department of Botany, Queen Mary's college, Chennai - 600 004, Tamil Nadu, India

Corresponding Author: S.Usha

Abstract: An efficient plant regeneration protocol was developed for *Eupatorium triplinerve* Vahl, an important medicinal herb. Nodal explants inoculated on Murashige and Skoog medium supplemented with 4.44 μ M 6-Benzyladenine(BA), showed better growth response and produced 18.3 \pm 1.15 number of shoots per explant with an average length of 6.4 \pm 0.06 cm after 30 days. Rooting of shoots was achieved in half strength MS medium supplemented with 2.46 μ M Indol-3-butyric acid, this produced 6.02 \pm 0.2 number of roots per shoot with an average height of 3.5 \pm 0.1 cm after 30 days. The rooted plantlets were transferred in the pots hardening, 80 percent of plantlets survived and resumed growth in the mixture of soil, vermiculite and farm yard manure (1 : 1 : 1).

Keywords: *Eupatorium triplinerve*, Nodal explants, Invitro tissue culture, Plant regeneration.

Date of Submission: 10-07-2017

Date of acceptance: 22-07-2017

I. Introduction

Medicinal plants are the most important source of life saving drugs for the majority of the world's population. Plant secondary metabolites form the economically important drugs, fragrances, pigments, food additives and pesticides. *Eupatorium triplinerve* Vahl is a valuable medicinal herb belonging to the family Asteraceae commonly known as Ayapana found in the Western and Eastern Ghats of India. The leaves are used to prepare infusions, decoctions, baths and plasters. It is a liver protectant, used to reduce the inflammation of the urinary tract, and cure tetanus. An infusion of the leaf and stem is used as a digestive stimulant. Ayapana is an antineoplastic and used for cancerous tumours in Peru and Argentina[1]. *E. triplinerve* leaf extract of the plant is used as antiseptic in the treatment of various ulcers and haemorrhages[2]. Antimicrobial activity of leaf extracts of *E. triplinerve* against some human pathogenic and bacteria and phytopathogenic fungi was reported by[3]. *Invitro* culture of axillary bud of the plant *E. triplinerve* has been attempted through organogenesis by [1] with 8.87 μ M benzylaminopurine (BAP) and 2.46 μ M Indol-3-butyric acid (IBA) and developed a mean of 8.1 shoot per node. The present study aims to developed simple, rapid, economical, and high frequency regeneration protocol from nodal explants of *E. triplinerve* for large scale propagation, as the plant found rare in the locality and has high medical value.

II. Material methods

2.1 Selection of explant

Healthy plants of *E. triplinerve* collected from Azhiyar (Coimbatore), Tamil Nadu, India were raised in pots containing soil and farm yard manure (1:1) under green house conditions. The twig of the mature plant is collected cleaned thoroughly under running tap water for 20 minutes. The nodal region of the twig is cut and trimmed to 0.8 -1.0 cm and used as nodal explant. The nodal explant is surface sterilized using Tween 20 (2 drops in 100 ml of water) for 1 min, and again washed with sterile distilled water. The cleaned explants were finally treated with 0.1 % HgCl₂ (w/v) for 4-5 minutes under aseptic conditions and washed 5 times with sterile distilled water to remove traces of HgCl₂.

2.2 Culture Media and Condition

The MS basal[4] medium supplemented with 3% (w/v) sucrose was used for all *in vitro* culture studies. The pH of the medium was adjusted to 5.6 \pm 0.2 prior to adding 0.9% (w/v) agar and autoclaved at 121°C for 15 minutes. The plant growth regulators were filter sterilized using 0.2 μ m filter (Minisart®, Sartorius) prior to addition to culture media.

2.2.1 Shoot Induction

The surface sterilized explants were inoculated on the MS medium supplemented with different concentrations of BA (1.11, 2.22, 4.44 and 8.88 μM) and KN (0.46, 2.32, 4.65 and 9.20 μM) for shoot initiation. The cultures were maintained at $25 \pm 1^\circ\text{C}$ under 16/8 hr photoperiod by cool white fluorescent tubes ($50\mu\text{mol m}^{-2}\text{s}^{-2}$) with 55 - 60% relative humidity. The proliferated shootlets of 4.5-5.0 cm length were produced after 30 days in culture.

2.2.2 Root Induction

The shoots were transferred to MS medium supplemented with different concentrations of IBA (0.49, 1.23, 2.46, 4.92 μM) for root development the same culture condition was maintained as for the shoot induction, the roots recorded after 30 days. For *ex vitro* establishment, well rooted plantlets were rinsed thoroughly with sterile water to remove residual nutrient from the plant body and transplanted to plastic pots containing a mixture of red soil, vermiculite and farm yard manure ratio (1 : 1 : 1), covered with moistened polythene bags for hardening. After 15 days, the fully acclimatized plantlets were transplanted to pots (6 cm dia).

2.3 Statistical Analysis

The study was analysed using (ANOVA) Analysis of Variance to compare all the data for various concentration of growth regulators for shoot induction and root induction depending on the days. The means obtained in the data were separated using Duncan's Multiple Range Test (DMRT).

III. Results and Discussion

The important biotechnological tool in vitro plant tissue culture is used for rapid production of disease free, herbicide, drought, pesticide and insect resistant plants which is of economic importance and also to conserve the germplasm of valuable rare plants

Eupatorium triplinerve Vahl. is a rare plant with high medicinal value and used for various treatments. Due to the increasing demand the plant is propagated in the present study by *invitro* propagation. The plant part is used in small pieces and cultured in vitro irrespective of season it superior to the conventional method as it results in cent percent plants which is disease free.

3.1 Nodal Explant Establishment

The nodal explant is considered superior to other explants reported by *Aristolochia indica* [5], *Jatropha curcas* [6], *Spilanthes acmella* [7], *Withania somnifera* [8], *Mentha piperta* [9], *Eupatorium triplinerve* [10] hence nodal explants were taken from potted plants from green house.

3.2 Shoot Induction

Multiple shoots developed from nodal explants cultured on MS medium supplemented with BA (1.11- 8.88 μM) and KN (0.46 – 9.20 μM) showed different response according to the hormonal concentration used. Initiation of multiple shoots in most of the treatments was observed within 3 weeks of culture in application of BA. Higher number of multiple shoot proliferation from nodal explants was observed in MS containing 4.44 μM BA showed better growth response (80%) and produced 18.3 ± 1.15 shoots per explant with an average length of 6.4 ± 0.06 cm after 30 days of culture (Table 1, Fig. 1A,B,C,D). Similar study was observed by [10] in *invitro* multiple shoot induction of *E. triplinerve* through direct regeneration from nodal segments were maximum shoots number of 5 observed on the MS medium supplemented with 0.2mg/l BAP and 0.02mg/l GA_3 after 30 days of culture maximum shoot of 2.85 cm was observed. This decrease in the length of shoot length may be because of the hormonal combination BAP and GA_3 . Hence it clearly proves the concentration of BA and KN supplement in the MS medium used in present study has better shoot length. Maximum shoot induction due to the synergistic activity of BAP and KN as observed in *Pisonia alba* [11]. The stimulating effect of BAP on formation of multiple shoots has been reported earlier in several medicinal plants [12], [13]. The hormonal concentrations of BA and KN are not much used in the plant propagation. Whereas the higher concentration of BA (8.88 μM) produced dark green callus and compact structures which ultimately turned brown and failed to develop into normal shoots. BA has been considered to be one of the most active cytokinins for organogenic differentiation in plant tissue culture [14], [15], [16] but it should be specific concentration.

3.3 Root induction

The regenerated shoots were inoculated on half strength MS medium. The first roots appeared after 2 weeks of culture and after 30 days, the root system was well developed (Figure 1E, F). The percentage of rooting was 80% and developed 6.0 ± 0.2 roots per *invitro* shoot with an average root length 3.5 ± 0.3 cm induced after 30 days old culture on root induction medium (Table 2). The high number of roots per shoot

produced on half strength growth regulator medium in *E. triplinerve* with subsequent high survival rate. Various studies had also showed that higher concentration of cytokinin generally inhibited root formation of plants[17]. The roots were directly induced from the base of shoot in MS medium supplemented with(5 mg/L) IBA[18] without an intervening callus.

3.4 Acclimatization

Hundred percent plantlet survival was recorded after hardening of the regenerated *E. triplinerve* in red soil, vermiculite and farmyard manure (1:1:1) for 3 weeks of culture. However, the rate decreased as some plants died over the next 4-10 weeks after transfer to soil. It was observed that very gradual acclimatization of *in vitro* grown plants to the external environment is most essential for *E. triplinerve*. Eighty percent of the plants transferred to pots survived and resumed growth (Figure 1 G, H, I). compared to the regeneration protocol of *Spilanthes acmella* by[7] where adventitious roots produced after 10 week in the present work the shoots grown *in vitro* possess the competence and developed adventitious roots within 30 days.

IV. Conclusion

The present protocol for *in vitro* regeneration of *Eupatorium triplinerve* reported here can be used to make this plant for biotechnology applications. Pharmaceutical usages, germplasm conservation, commercial cultivation in addition to production of secondary metabolites.

Table 1. Effect of BA and NAA on Shoot regeneration from nodal explants of *Eupatorium triplinerve*

PGR concentration (BM)		Shoot Induction (%)	Number of shoots / Explant	Shoot length (cm)
BA	NAA			
1.11	-	50.0 ± 0.0	6.3 ± 1.1	2.6 ± 0.1
2.22	-	55.0 ± 5.0	7.0 ± 1.0	3.0 ± 0.2
4.44	-	80.0 ± 5.0	18.3 ± 1.15	6.4 ± 0.06
8.88	-	45.0 ± 8.7	5.3 ± 1.1	3.6 ± 0.1
-	0.46	30.0 ± 5.0	4.1 ± 0.2	2.7 ± 0.26
-	2.32	40.0 ± 10.0	4.3 ± 0.5	3.1 ± 0.1
-	4.65	60.0 ± 0.0	5.5 ± 1.8	3.2 ± 0.15
-	9.20	35.0 ± 5.0	3.4 ± 0.15	2.8 ± 0.29

Results represent mean ± SD of three replicated experiments. Data were recorded after 30 days of culture.

Table 2. Effect of auxins on rooting from *in vitro* shoots of *Eupatorium triplinerve*

PGR Concentration – IBA		Root Induction (%)	No of roots	Root length(cm)
	0.49	40.00±5.00 ^b	3.63±0.37 ^b	2.28±0.48 ^a
	1.23	45.00±5.00 ^b	3.63±0.37 ^b	3.06±0.61 ^a
	2.46	80.00±5.00 ^c	6.02±0.29 ^c	3.51±0.31 ^b
	4.92	30.00±5.00 ^a	2.63±0.29 ^a	2.47±0.39 ^a
F Value		56.750	57.745	4.484
P Value		<0.001**	<0.001**	<0.040*

Data were recorded after 30 days of culture

Note: 1. **denotes significant at 1% level

2. Different alphabets among solvents denote signification's at 5% level using Duncan Multiple Range Test (DMRT).



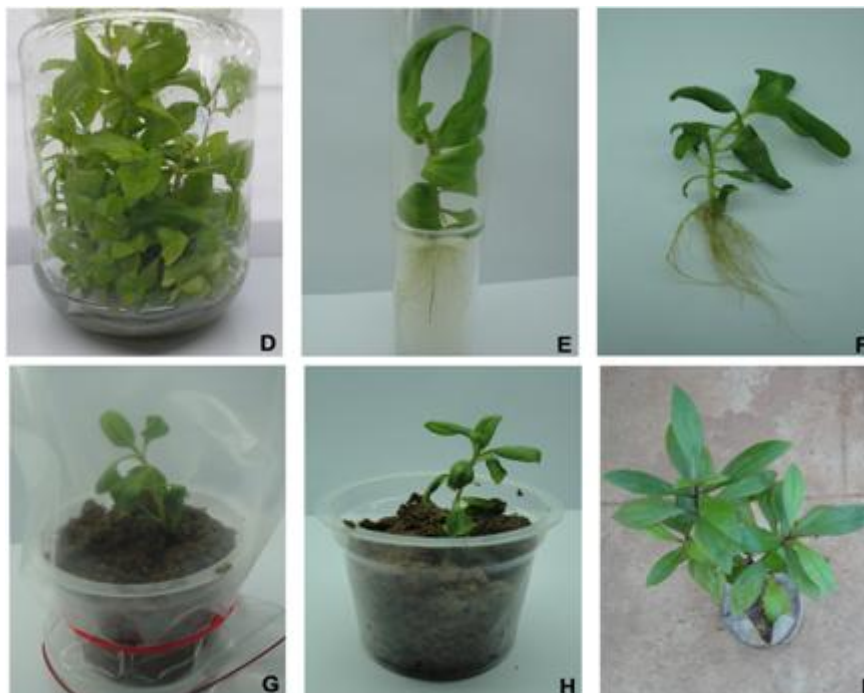


Fig. 1. plant regeneration from nodal explants of *Eupatorium triplinerve*
Stages in the micropropagation of *Eupatorium triplinerve*

- A. Mother plant of *Eupatorium triplinerve*
 B. Initiation of shoot from nodal explants after 2- 3 weeks of culture.
 C. Proliferation of multiple shoots from nodal explants at 30 days of cultured on MS medium containing 4.44 μ M BA.
 D.& E. .Rooted plantlet after 30 days of culture on growth regulator with $\frac{1}{2}$ strength MS medium
 F. A well established plant.
 G. & H. Hardened *in vitro* plant successfully transplanted to the plastic cup
 I. Acclimatized plantlets successfully transplanted to the pots.

Reference

- [1]. K.P. Martin. Rapid axillary bud proliferation and *ex vitro* rooting of *Eupatorium triplinerve*. 47, *Biologia Plantarum*, 2003, 589-591,
- [2]. A. Ghani., *Medicinal plants of Bangladesh : Chemical constituents and uses.* (1st edn. Asiatic Society of Bangladesh. 1998) (174).
- [3]. S. Rahman, and M.Junaid, Antimicrobial activity of leaf extracts of *Eupatorium triplinerve* vahl. against some human pathogenic bacteria and phytopathogenic fungi. 37(1), *Bangladesh J. Bot.* 2008, 89-92.
- [4]. T. Murashige, F. Skoog. A revised medium for rapid growth and bioassay with tobacco tiss..cult. 15, *Physiol.Plant.* 1962, 473-479.
- [5]. P.V Pattar, and M Jayara. In vitro plant regeneration of Plantlets from Leaf and Nodal explants of *Aristolochia indica* L.- An Important Threatened Medicinal Plant.1691(12), *Asian Paci. J Trop. Biomed.* 2012, 60257-60259.
- [6]. L. Laxmidhar maharana, M. Durga Kar, S. Snigdha Patthhaik, K. Sahu, C.S Sudam, In Vitro antioxidant activity of aqueous leaf extract of *Solanum nigrumlinn.* 3, *Pharmacology on line.*2010,333-345.
- [7]. K. Kuldeep yadav, N. Narender sing. Rapid regeneration from nodal explants of *spilanthes acmella* (L)MURR-an endangered medicinal plant.1, *Fascicula biologie* , 2012, 35-39
- [8]. S. Syed saema ,I. Iffat Z. Zareen, A. Ahmad,Pratibha Misra. Rapid in vitro plant Reneration from nodal explants of *withania somnifera* . *somnifera*(L).Dunal: a valuable medicinal plants. 4(6), *J. Sci &Rec.*, 2015, 1649-1652.
- [9]. P. Sujana, and C.V. Naidu,High Frequency Rapid plant regeneration from shoot tip and Nodal Explants of *Mentha piperita*(L)..3(5), *J. Phyt.* 2011,09-13.
- [10]. P. Samyuraj, R. Sarvanan and P. Eganathan in vitro multiple shoot Induction of *Eupatorium triplinervei*. 6 *Inter. J App. Bio .Sci.* 2012, 19-22.
- [11]. k . Jagadish chandra, SS Rachappaji , RD. Gowda, KJ. Tharasaraswathi..In vitro propagation *pisonia alba*(C) Spanoge (Lettuce tree)-A threatened Species. 49, *Phytomorpha.*1999, 43-47.
- [12]. Pandey, R. Metha, R. Upadhyay. In-Vitro Propagation of an endangered medicinal plant.6, *Psoralea corylifolia* linn. *Asian J. Pharm. and Clinic Rec.* 2013,
- [13]. H.B. Gururaj, P. Giridhar. and G.A. Ravishankar. Micropropagation of *Tinospora cordifolia* (Willd.) Miers ex Hook.F & Thoms – A multipurpose medicinal plant. 9, *Curr. Sci.*, 2007, 23-26.

- [14]. B. Janarthanam, and S. Seshadri. . In vitro manipulations of *Rosa bourboniana*. 769, *Acta Horticulturae.*, 2008, 357-370.
- [15]. B. Janarthanam, and E. Sumathi, *In vitro* Regeneration of *Justicia gendarussa* Burm. f. *Libyan Agricul. Rese. 5 Cen. J. Internat.*, 2010, 284-287.
- [16]. H. Schraudolf, J. Reinert. *Nature in: plant propagation by Tissue1959* (George,E.F.& Sherrington, P.D. eds.) 1984. *Eastern prees*, England. 184:465-466.
- [17]. J. Valizadeh, and M.Valizadeh, *In vitro* callus induction and plant regeneration from *Withania coagulans*. 2(21), *Pakistan J. Biol.Sci.*2009, 1415-1419.

IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) is UGC approved Journal with SI. No. 5012, Journal no. 49063.

S.Usha. "Plant Regeneration from Nodal explants Culture through Organogenesis in *Eupatorium triplinerve vahl*." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)* 12.4 (2017): 28-32.