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In Vitro Multiple Shoot Induction of *Eupatorium triplinerve* Vahl through direct regeneration from nodal segments

***P. Samydurai[1], R. Sarvesan[1] and P. Eganathan[2]**

[1]Department of Plant Biology & Plant Biotechnology, Presidency college, (Autonomous), Chennai-600 005, India.
[2]Biotechnology & Bioprospecting laboratory, M.S. Swaminathan Research Foundation, Taramani, Chennai – 600 113, India

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The objective of this study was to develop a rapid growth for regeneration of the highly valuable medicinal plant *Eupatorium triplinerve* Vahl from nodal explants. Single node explants were inoculated on MS basal medium containing 3% sucrose, supplemented with different concentration and combinations of 6-benzyl amino purine with GA_3 and thidiazuron with GA_3 for direct plant regeneration. Maximum number of shoots were (5.0) observed on MS medium fortified with 0.2mg/l BAP + 0.02mg/l GA_3 after 30 days of culture. Maximum shoot length of 2.85cm was achieved. Optimum root induction was achieved with half strength MS liquid medium fortified with 0.2mg/l of IBA and 0.2mg/l IAA. Plantlets were transferred to sterile vermiculite soil under controlled conditions. Well established plantlets were later transferred to plastic pots containing garden soil and sand (3:1). After this the plants were transferred to field conditions for acclimatization.

Eupatorium triplinerve /tissue culture

***E**upatorium triplinerve* Vahl is commonly known as ayapana, a highly valuable medicinal plant which belongs to the family Asteraceae. It is an ornamental erect perennial herb with aromatic leaves that grows up to 20 to 30cm in height. Ayapana is rich in naturally occurring coumarin (7-methoxy coumarin) chemicals, which may helps to explain why the plant is used in herbal medicine as an anti-tumour remedy. Recent research reported that this chemical was toxic to cancer cells including multi drug resistant cancer cells and leukemia cells. In Aregentina an infusion of the entire plant is also used to stimulate menstruation.

More than 90% of the medicinal plants for herbal industries in India and for export are drawn from the natural habitats thus challenging their existence (Gupta et al. 1998). Considering the adverse effects of synthetic drugs the western population is looking for natural remedies, which are safe and effective (Gijtenbeek et al. 1999, Johnson and William, 2002). India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the Third World Countries (Rajshekharan, 2002). The present investigation was carried out to develop a standard procedure for *in vitro* production of multiple shoot induction by using nodal segments of *Eupatorium triplinerve*.

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**To whom correspondence may be addressed. Email: samydurai.bio@gmail.com

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Material and methods

Plant material and explant source

Explants were collected from one year old field grown mature plants. This was cut into 1-2 cm of nodal segments and used for induction of multiple shoots. Explants were washed thoroughly under running tap water for 30 minutes and treated with surfactant Tween 20 (2-3 drops per 100mL of sterilized distilled water). Then these explants were surface sterilized with 0.1% mercuric chloride (w/v) for 5 minutes and washed with sterilized distilled water 5-7 times for avoiding or minimizing contamination under aseptic conditions.

Culture media and conditions

The explants were inoculated on MS basal (Murashige and Skoog, 1962) medium either individually or in various combinations with growth regulators (BAP + GA_3, TDZ + GA_3) containing 3% (w/v) sucrose. The pH was adjusted to 5.8 prior to the addition of 2.5 g/l phytagel and autoclaved at 121^0C for 20 minutes. Cultures were then incubated at 20 ± 2^0C with 16 h photoperiod by cool white fluorescent tubes (Das et al.1996) and 75-80% related humidity (Mukherjee et al. 1991).

Induction of multiple shoots formation

The aseptic nodal explants were cultured on MS medium supplemented with different concentrations and combination of 6- benzyl aminopurine with GA_3 (0.2+0.02, 0.4+0.04, 0.6+0.06, 0.8+0.08, 1.0+0.1mg/l). These same concentrations are used for Thidiazuron. Thirty explants were used for each treatment and the experiment was repeated three times. The cultures were incubated at 20 ± 2^0C under above mentioned culture conditions. Responses of the cultures were observed and recorded over a period of five weeks. The shoot length and number of shoots were recorded. Explants that produced more than two shoots were considered as multiple shoots.

Root induction

For root induction, shoots were transferred to half strength MS basal medium supplemented with different concentrations of IBA and IAA (0.2-1.0mg/l) and 2% (w/v) sucrose. Rooted plantlets were thoroughly washed to remove the adhering gel and planted in specially made plastic pots containing vermiculite, soil (3:1) and kept in the greenhouse for acclimatization.

Statistical analysis

Analysis of Variance (ANOVA) was performed on all data to compare concentration effects of growth regulators and influence of days. Means were separated using Duncan's Multiple Range Test (DMRT).

Result and Discussion

Shoot induction

In a significant development it was observed that multiple shoot induction derived from nodal explants, when MS was supplemented with different concentration and combination of cytokinins with gibberellic acid. Shoot buds initiated on nodal segments after 7days of inoculation. The higher frequency (85%) formation of maximum number of shoots (5.0) was observed in 0.2 mg/l BAP combination with 0.02 mg/l GA_3. After 4 weeks an average shoot length of culture was obtained as 2.85cm (Table -1). Second batch of experiment was conducted in TDZ with GA_3. This Thidiazuron also induced maximum number of shoots (3.70cm) in lower concentration like BAP. After 4 weeks the shoot length was observed to be 2.20cm.

Table-1: Effects of different concentrations of BAP+GA_3, TDZ+GA_3 in MS basal medium for multiple shoot induction from nodal explants of *Eupatorium triplinerve*

Plant growth regulators (mg/l) Concentrations	BAP+GA_3		TDZ + GA_3	
	No. of Shoots/ Explant	Shoot length (cm)	No. of Shoots/ Explant	Shoot length (cm)
0.2+0.02	5.00a	2.85a	3.70a	2.20a
0.4+0.04	4.40ab	2.31ab	2.40bc	1.33b
0.6+0.06	3.30ab	2.29ab	3.20ab	1.36b
0.8+0.08	2.70b	2.05ab	2.50bc	1.08b
1.0+0.1	2.70b	1.26b	2.00c	0.96b

Mean in a column followed by a same letter(s) are not significantly ($P<0.05$) different according to Duncan's Multiple Range Test.

Table-2: Effect of different concentrations of IBA and IAA in half-strength MS medium on root induction from regenerated shoots

Plant growth regulators (mg/l)	% of rooting response	Number of roots	Average root length (cm)
IBA			
0.0	0.0	0.00	0.00
0.2	75	2.4a	2.750a
0.4	56	1.8ab	2.070b
0.6	56	1.8ab	1.621bc
0.8	53	1.50b	1.401c
1.0	48	1.10b	0.774d
IAA			
0.0	0.0	0.00	0.00
0.2	67	2.600a	1.930a
0.4	55	1.501b	1.161b
0.6	54	1.800b	1.260b
0.8	51	1.700b	1.110b
1.0	49	1.301b	0.921b

Mean in a column followed by a same letter(s) are not significantly (P<0.05) different according to Duncan's Multiple Range Test.

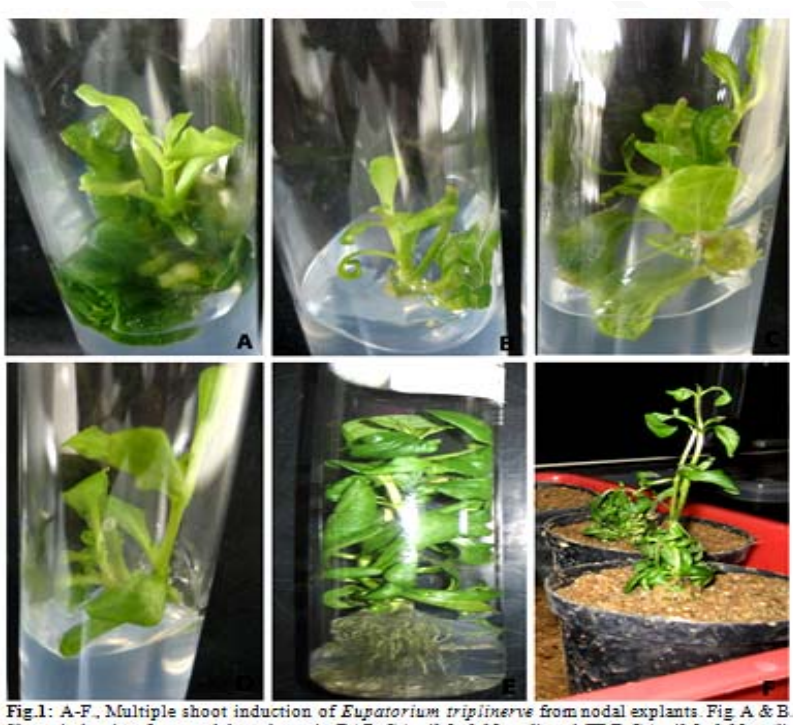

Fig.1: A-F., Multiple shoot induction of *Eupatorium triplinerve* from nodal explants. Fig. A & B. Shoot induction from nodal explants in BAP+GA_3 (0.2+0.02mg/l) and TDZ+GA_3 (0.2+0.02mg/l). Fig. C. Formation of multiple shoots from nodal explants on MS medium fortified with 0.2mg/l BAP+0.02mg/l GA_3. Fig. D. Elongation of multiple shoot on 0.2mg/l BAP+0.02mg/l GA_3. Fig. E. Well developed rooted plantlet. Fig. F. Plant acclimatized to the green house.

Martin, (2003) reported that the shoot initiation was rapid from the nodal segments of *Eupatorium triplinerve* cultured on MS medium fortified with 8.867 µM BAP and 2.46 µM IBA. The best response was noticed in BAP+GA_3 better than TDZ+GA_3 and also combination of cytokinins was most suitable for high frequency of shoot induction. Maximum of shoot induction probably due to the synergistic activity of BAP and KN as observed in *Pisonia alba* (Jagadish chandra et al.1999). However, increased levels of these growth regulators in combination of BAP + GA_3 and TDZ +GA_3 inhibited the number of shoot initiation.

Root induction

Well developed *in vitro* raised shoots were transferred to MS media having various concentrations of IBA and IAA for root induction. The maximum numbers of rootlets were observed (2.60) in half strength of MS medium fortified with 0.2 mg/l IAA (Fig.1E, Table 2) followed by (2.4) 0.2mg/l IBA. Thus, 0.2 mg/l IBA and 0.2mg/l IAA was found to be best for root induction. Various studies had also showed that higher concentration of cytokinin generally inhibited root formation of plants (Scharudolf and Reinert, 1959). Similar results were also reported in several medicinal and fruit plants, such as *Carica papaya* (Islam et al. 2000), C*entella asiatica* (Mohapatra et al. 2008), *Ocimum basilicum* (Sahoo et al.1997), *Gymnema sylvestre* (Komalavalli and Rao, 2000), *Holostemma adakodien* (Martin, 2002), *Swainsona salsula* (Yang et al. 2001).

Acclimatization

Plantlets were removed from rooting medium after three weeks of incubation and transferred to sterile vermiculite under controlled conditions. Well developed plantlets were transferred to plastic pots containing garden soil and sand (3:1)**,** after two week of the plants was transferred to field.

Conclusion

In the present study the *in vitro* multiplication shoot induction of *Eupatorium triplinerve* has been optimized through nodal explant. This developed protocol is successful and could be used for large scale production and clonal propagation of this important medicinal plant.

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References

Das S., Jha TB., Jha S. 1996. *In vitro* propagation of Cashew nut. *Plant Cell Rep*., 15: 615-619.

Gijtenbeek JMM, Vanden Bent MJ., Vecht CJ. 1999. Cyclosporine neurotoxicity. *Journal of Neurolo*., 246: 339-346.

Gupta Akshey K., Vats SK., Brij L. 1998. How can cheap a medicinal plant species. *Current Science*, 74: 565-566.

Islam M., Islam R., Hossain M., Alam MF., Banu NA. 2000. Callus induction and plant regeneration from immature zygotic embryos of *Carica papaya* L. Bangl. *Journal of Genetics and Biotechn*., 1(1): 123-128.

Jagadish Chandra K., Rachappaji SS., Gowda R.D., Tharasaraswathi KJ.1999. *In vitro* propagation *Pisonia alba* (C) Spanoge (Letuce tree)–A threatened Species. *Phytomorpho*., 49: 43-47.

Johnson WC., William WO. 2002. Benefits morbidity and mortality associated with longterm administration of oral anticoagulant therapy to patients with peripheral arterial bypass procedures: A prospective randomized study. *Journal of vascular surg*., 35(3): 413-421.

Komalavalli N., Rao MV. 2000. *In vitro* micro propagation of *Gymnema sylvestre* – a multipurpose medicinal plant. *Plant Cell Tissue and Organ Cultu*., 61(2): 97-105.

Lakshmana Rao, P.U. 1994. *In vitro* plant regeneration of scented leaved geranium *Pelargonium graveolens*. *Plant Sci*., 98: 193-198.

Martin KP. 2003. Rapid auxiliary bud proliferation and *in vitro* rooting of *Eupatorium triplinerve. Biologia Planta*., 47: 589-591.

Mohapatra H., Barik DP., Rath SP. 2008. *In vitro* regeneration of medicinal plant *Centella asiatica*. *Biologia Planta*, 52(2): 339-342.

Mukherjiee A., Unnikrishnan M., Nair NG. 1991. Growth and Morphogenesis of immature embryos of Sweet potato (*Ipomea batata* L.). *In vitro Plant Cell Tissue and Org. Cultu*., 26: 97-99.

Murashige T., Skoog, 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiologia planta*, 15: 473-497.

Rajshekharen PE. 2002. Herbal medicine. In World of Science. *Employment news*, 21:.3.

Sahoo Y., Pattnaik SK., Chand PK. 1997. *In vitro* clonal propagation of an aromatic medicinal herb *Ocimum basilicum* L. (Sweet basil) by auxiliary shoots proliferation. *In vitro Cellular and Developmental Biol. Plan*., 33(4): 293-296.

Schraudolf H., Reinert J. 1959. Nature In: Plant propagation by Tissue Culture. (George, E.F. & Sherrington, P.D. eds.) 1984. Eastern Press, England. 184: 465-466.

Yang J., Hu Z., Guo GQ., Zheng GC. 2001. *In vitro* plant regeneration from cotyledon explants of *Swainsona salsula* Taubert. *Plant Cell Tissue and Org. Cultu*., 66(1): 35-39.