Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.3, No.5, 2013

In Vitro Mass Propagation of Enicostemma Littorale Blume from Shoot Tip Explants

Nalini, P. and *Velayutham, P.
Government Arts College (Autonomous), Karur 639 005, Tamil Nadu - INDIA
*Corresponding author : vela_utham@yahoo.com

Abstract:
In vitro rapid regeneration and mass propagation was deliberated from shoot tip explants of *Enicostemma littorale* Blume. The explants were cultured on MS (Murashige and Skoog, 1962) medium containing various concentration of cytokinins ranging from 5 µM to 25 µM. When compared to KIN, BAP was found to respond well in shoot multiplication and number of shoots. Large number of shoots was produced from all concentration of BAP and KIN. Maximum number of shoots and highest frequency of 100% shoot induction was observed on MS medium containing 15 µM KIN and BAP. The excised shoots were then transferred to MS medium augmented with IBA and NAA in various concentration for root induction. The roots were initiated and well developed in 2µM of both the auxins. The *in vitro* raised plantlets were successfully transferred to soil through hardening and acclimatization.
Key words : *Enicostemma littorale*, cytokinins, auxins
Abbreviations : BAP - 6-benzylaminopurine; KIN - kinetin (6-furfuralaminopurine; IBA-indole butyric acid; NAA - Naphthalene acetic acid

1. Introduction:

Enicostemma littorale Blume, one of the medicinal plants of Gentianaceae, is a perennial herb. Traditionally it is used as a stomachic and bitter tonic due to the presence of glycosides and ophelic acid, used as a substitute for *Swertia Chirata* (the famous Indian bitter) and hence commonly referred as Chota Chirayata. Mainly it is used along with other herbs for the treatment of Diabetes Type 2. The whole plant is useful as ayurvedic herbal medicine. The medicinal uses include antidiabetic, antitumours, antimalarial, antimicrobial, anti-inflammatory, antioxidant and antipyreric activities (Nadkarni, 1908; Sadique *et al.,* 1987; Kavimani and Manisenthilkumar, 2000; Murali *et al.,* 2002; Maroo *et al.,* 2003; Babu *et al.,* 2004; Jaishree *et al.*, 2008).
The medicinal value of this plant is due the presence of bitter glycosides (Hostettemann-Kaldas et al., 1981) and alkaloids. The major chemical constituents are swertiamarin, a glycoside (Viswakarma et al., 2004) and gentianine, a bitter alkaloid (Delaude,1984). Epigenin, genkwanin, swertisin, saponarin and gentiocrucine are also reported to present in minor amounts (Gosal et al., 1974)
There are only a few reports on this plant for rapid multiplication (Velayutham *et al.,* 2005; Nagarathnamma, *et al.,* 2010) prompting the authors for attempting to propagate plants from shoot tip explants under *in vitro* conditions. Therefore, there is a need to develop a means for rapid regeneration of plantlets.
Plant tissue culture is an essential component of plant biotechnology. It offers new techniques in the production, multiplication, alternation and preservation of plants. The tremendous advancement in the field of science and technology has made it into a powerful industrial technology. With tissue culture techniques, a variety of organs, tissues, or cells can serve as source material for the propagation and regeneration of plants on a chemically defined culture medium (Haissig et al., 1987). The success of plant biotechnology relies on the fundamental techniques of plant tissue culture. Understanding basic biology of plants is a prerequisite for proper utilization of the plant system or parts thereof. Plant tissue culture helps in providing a basic understanding of physical and chemical requirements of cell, tissue, organ culture, their growth and development. Establishment of cell, tissue and organ culture and regeneration of plantlets under in vitro conditions has opened up new avenues in the area of plant biotechnology (Dagla, 2012).
In recent years there has been an increased interest in *in vitro* culture techniques which offer a viable tool for mass multiplication and germplasm conservation of rare, endangered and threatened medicinal plants (Tiwari *et al*., 2000). This technique is an alternative method of propagation as there is an increase in the propagation rate of plants,

availability of plants throughout the year, protection of plants against pests and pathogens under controlled conditions and the availability of uniform clones and uniform production of secondary metabolites. (Bajaj *et al*., 1988). Thus cloning of medicinal plants to increase biomass production and production of bioactive compounds through cell cultures has assumed significance (Rao *et al*., 1996). Hence, an alternative method of propagation with increased bioactive compounds was needed.

Shoot tip culture is extensively applied in horticulture, agriculture and forestry. Morel (1960) pioneered the work of shoot apex culture of orchid, Cymbidium, *for the clonal multiplication. Yadav* et al. *(1990) successfully cultured shoot tips and nodal explants of two tropical trees,* Morus nigra *and* Syzygium cuminii. *From these explants multiple shoots and roots were proliferated by providing different nutrient conditions. Finally the regenerated plants were transferred into the soil. The two common cytokinins, BAP (6-benzylaminopurine) and Kinetin (6-furfualaminopurine), are widely used for micropropagation of plants from shoot tip and nodal explants with axillary buds (Philip et al., 1992; Velayutham and Ranjithakumari, 2003; Velayutham et al., 2005a,b; Ranjan et al., 2010; Padmapriya et al., 2011; Abo El-soud, et al., 2011; Gnanaraj et al., 2011; Kantamaht et al., 2012).*

2. Materials and Methods

2.1. Source of explants

Enicostemma littorale Blume from natural habitats were collected from the agricultural field at Sukkaliyur in Karur. The shoot tips were used for the regeneration in the investigation. The explants were excised with sterile blade and were collected in a beaker.

2.2. Sterilization of explants

The excised explants were thoroughly washed with running tap water for 10 to 15 min with few drops of liquid soap (Teepol 5%). The explants were then washed with distilled water for 3 to 4 times and further sterilization was carried out in the Laminar Air Flow Chamber under aseptic condition prior to inoculation.

The explants were sterilized with 70% alcohol for 30-45 sec and finally disinfected with 0.1% (W/V) $HgCl_2$ for 3-5 min, in the chamber. The explants were then washed 4-5 times with sterile distilled water to remove the traces of mercuric chloride.

2.3. Sterilization of Glasswares

All the glasswares were washed thoroughly with chromic acid (Potassium dichromate and sulphuric acid 2:1 w/v), rinsed in tap water and then with distilled water. Sterilization of glasswares, forceps and scalpels for micropropagation was done in an autoclave at 121ºC for 20 minutes at 1.06 kg cm^{-2}.

2.4. Culture media

Murashige and Skoog (Murashige and Skoog 1962) solid medium containing 3% (w/v) sucrose and 0.8% (w/v) agar were used in the experiments. Different concentrations of plant growth regulators were added to the medium for shoot multiplication (BAP and KIN) and rooting (IBA and NAA). Media were adjusted to pH 5.8±0.1 with 0.1N NaoH and 1N Hcl before gelling with 8 gl^{-1} agar, prior to autoclaving (121ºC at 1.06 kg cm^{-2} for 20 min).

2.5. Inoculation procedure

The explants were inoculated into the culture tubes on the medium containing different concentrations of plant growth regulators. By means of a long stainless steel forceps, one explant per tube was placed. It is a routine process to flame the mouth of the test tube after uncapping and before recapping the tubes to reduce contamination.

2.6. Shoot induction

For multiple shoot induction, the shoot tip explants were placed on MS medium supplemented with different concentrations of benzylaminopurine (BAP: 5-25 µM) or Kinetin (KIN: 5-25 µM). The shoot number and length were measured in each tube.

2.7. Root induction

In vitro raised shoots of 2 cm and above were excised from the culture tube and subcultured into MS medium supplemented with various concentrations of indole butyric acid (IBA: 2-10 µM) or naphthalene acetic acid (NAA: 2-10 µM). The root number and length were measured in each culture medium.

2.8. Culture maintenance and conditions

All cultures were maintained at 25± 2ºC under a 16/8 h light/dark regime, under a photon flux density of 45-50 mol $m^{-2}s^{-1}$ provided by cool white fluorescent tubes (40W, Phillips, India). The relative humidity (RH) within culture room was maintained at 55±5%. The media was refreshed at 3wk intervals.

2.9. Hardening and acclimatization

Plantlets with well-developed roots were dislodged from the culture medium and roots were washed gently under running tap water to remove the adhering medium. Plantlets were transferred to plastic cups (10 cm diameter) containing autoclaved garden soil, farmyard manure and sand (2:1:1).Each plantlet was irrigated with distilled water every 2 days for 2 weeks followed by tap water for one week. The potted plantlets were initially maintained under culture room conditions in 3 weeks and later transferred to normal laboratory condition in 2 weeks. The potted plantlets were initially covered with porous polyethylene sheets to maintain high humidity and were maintained inside the culture room.The relative humidity was reduced gradually. After 30 days the plantlets were transplanted to the field under shade for 3 weeks and then transplanted to the field under shade for 3 weeks and then transplanted to the soil for further growth and development.

2.10.Experimental design, data collection and statistical analysis

All the experiments were preformed using a randomized completely block design and each experiment consisted of five tubes with one explant each and five replicates. The parameters recorded were shoot multiplication frequency (number of culture responding in terms of multiple shoot proliferation and root development), number of shoots per explant, shoot length, number of roots per shoot, root length and survival rate(%). The analysis of variance (ANOVA) appropriate for the design was carried out to detect the significance of differences among the treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% level of significance (Gomez and Gomez, 1976).

3. Results and Discussion

3.1. Shoot multiplication

The shoot tips were initially grown on MS medium supplemented with BAP or KIN alone in different concentrations ranging from 5-25µM. Of the two cytokinins, BAP was found to induce more number of shoots when compared to KIN. Shoot multiplication frequency was observed on both the hormones. The shoot induction frequency ranged from 75-100% and 80-100% on MS medium supplemented with BAP and KIN respectively. Large number of shoots was produced in 30 days of culture. The highest frequency of 100% shoot induction was observed on MS medium supplemented with 15 µM BAP and KIN. However, variations among the two hormones were observed in number of shoots and shoot length.

Among the different concentrations of BAP, the basal medium supplemented with 15 µM BAP showed the highest number of 29.2 shoots per explant followed by 10 µM BAP with 24.4 shoots. Among the different concentrations of KIN, the highest number of 28.3 shoots produced on the basal medium supplemented with 15 µM KIN followed by 20 µM KIN with 25.8 shoots were showed after 45 days of inoculation. Of the two cytokinins tested, BAP was found to respond well in shoot multiplication and number of shoots from shoot tip explants when compared to KIN (Fig.1a-d; Table 1).

The basal medium supplemented with 15 µM BAP showed the highest mean shoot length of 8.4 cm per explant followed by 10 µM BAP with 7.53 cm. Shoots growing on MS medium with 15 µM KIN reached the highest shoot length of 8.33 cm followed by 20 µM KIN with 7.71 cm in the same period of culture. These results showed both the cytokinins tested were found to initiate and proliferate shoots from shoot tip explants of *E. littorale* Blume. From this survey 15 µM BAP was found to be the best and favourable concentration for promoting shoot multiplication from the shoot tip when compared to KIN.

In many plants, multiple shoots were obtained from the shoot tips or axillary buds by administering BAP or KIN(Kackar *et al*., 1991; Varghese *et al*., 1993; Bennet *et al*., 1994; Kumar *et al*., 1998; Sahoo and Chand 1998; Baskaran and jayabalan, 2005). In the present study, BAP was found to be more effective for shoot multiplication. Similar results were observed in *Tridax procumbens* (Sahoo and Chand, 1998), *Solanum trilobatum* (Emmanuel *et al*., 2000), and *Cichorium intybus* (Velayutham and Ranjithakumari, 2003).

In several studies BAP was more effective in inducing bud break resulting in the sprouting of a large number of shoots (Sahoo and Chand, 1998; Kadota and Niimi, 2003; Velayutham and Ranjithakumari, 2003). Karthikeyan et al. (2009) obtained 15.23 shoots per nodal cutting in *Centella asiatica* by administering 2 mg BAP alone. The number of shoots were increased to 18.12 when 0.5 mg KIN was added together with 2 mg BAP. Similarly, Padmapriya *et al.* (2011) also produced large number of shoots (more that 40) from the nodal explants of Solanum nigrum on MS medium supplemented with 15 µM BAP or KIN alone. Ranjan *et al.* (2010), Gnanaraj *et al*. (2011), Abo El-soud *et al.* (2012) and Kantamaht *et al.* (2012) obtained more number of shoots from the shoot tip explants of *Capsicum annuum, Alternanthera sessilis, Rumex vesicarius* and *Anubias barteri* respectively on MS medium supplemented with BAP than KIN. Gnanaraj *et al.* (2011) obtained a mean value of 23.4 shoots from shoot tip and nodal explants

of *Alternanthera sessilis* on a medium with 2 mg l^{-1} BA alone. But the number of shoots was 3 to 4 folds increased (81.6 shots) when 1.5 mg^{-1} KIN was fortified with 3 mg l^{-1} BAP. The earlier studies on *Enicostemma littoral* also, BAP favoured more number (Velayutham *et al*., 2005a; Nagarathnamma *et al.,* 2010). In the present investigation also BAP was found to be more efficient in shoot multiplication.

3.2. Rooting of regenerated shoots

The isolated shoots were cultured on half strength MS medium supplemented with different concentrations of IBA and NAA ranging from 2-10 µM. Roots were initiated and well established in all the concentrations of the two auxins studied. Of the different concentrations of IBA, maximum number of 7.1 roots were induced on 2 µM IBA with mean root length of 2.65 cm. However, higher frequency of root induction(100%) was observed in 2 µM IBA. The MS basal medium (half strength) with 2 µM NAA showed 100% root induction frequency with 8.1 roots per shoot. The root length was 3.01 cm in 2 µM NAA (Fig.2e-f; Table 2). The observation showed, the basal medium supplemented with NAA was found to induce more number of roots than IBA. In the present study, minimum concentration of hormone was needed for root induction. Root induction frequency was gradually reduced in higher concentration of hormone.

In many studies, IBA and NAA were used to induce rooting. Higher frequency of rooting was achieved by IBA in *Aristolochia indica* (Manjula *et al*., 1997), *Gymnema sylvestris* (Komalavalli and Rao, 2000), *Avicennia marina* (Al-Bahrany and Al-Khayri, 2003) and *Eclipta alba* (Baskaran and Jayabalan, 2005), *Centella asiatica* (Karthikeyan *et al.,* 2009), *Enicostemma littoral* (Nagarathnamma *et al.,* 2010), *Alternanthera sessilis* (Gnanaraj *et al.,* 2011), *Rumex vesicarius* (Abo El-soud *et al.,* 2012). Higher frequency of roots were observed in *Cichorium intybus* at 5 µM NAA (Velayutham and Ranjithakumari, 2003; Velayutham *et al*., 2006), *Rubus chamoemorus* (Martinussen *et al*., 2004), *Viburnum odoratissimum* (Schoene and Yeager,2005) and *Plumbago zeylanica* at 3 µM NAA (Velayutham *et al*., 2005b), *Solanum nigrum* (Jabeen *et al*.,2005) also showed that NAA was found to induce more number of roots when compared to IBA .In the present study also NAA was found to induce more number of roots than IBA.

3.3. Hardening and acclimatization

The well rooted plantlets were transplanted to the paper cup for hardening. The survival rate of these plants was 80-90%. The established plants were transferred to the field for acclimatization.

References

Abo El-soud I.H., AL-azab, A.A., Koriesh, E.M. and Atta-Alla, H.K. 2011. Micropropagation of *Rumex vesicarius* L. Through Shoot Tip Culture. *Catrina Journal : Archive* 6(1),Doaa.

Al-Bahrany, A.M. and Al-Khayri, J.M. (2003). Micropropagation of grey mangrove *Avicennia marina*. *Plant Cell, Tissue and Organ Culture* 72,87-93.

Babu, P.S. and Stanely Mainzen Prince, P. (2004). Antihyperglycaemic and antioxidant effect of hyponidd, an ayurvedic herbomineral formulation in streptozotocin-induced diabetic rats. *Jour. Pharm. Pharmacol*. 56(11),1435-1442.

Bajaj,Y.P.S., Furmanowa, M. and Olszowsks, O., (1988). Biotechnology of the micropropagation of medicinal and aromatic plants. In: Biotechnology in Agriculture and Forestry, Medicinal and aromatic Plants I. (ed.) Bajaj, Y.P.S.). Springer – Berlin, Heidelberg, New York, Tokyo, vol.4, pp 60-103.

Baskaran, P. and Jayabalan, N. (2005). An efficient micropropagation system for *Eclipta alba* – a valuable medicinal herb. *In vitro Cell. Dev. Biol. Plant*. 41, 532 - 539

Bennet, I.J., McComb,J.A., Tonkin, C.M. and McDavid, D.A.J. (1994). Alternating Cytokinins in Multiplication Media Stimulates In vitro Shoot Growth and Rooting of Eucalyptus globulus Labill. *Ann. Bot.,* 74,53-58.

Dagla, H.R. 2012. Plant Tissue Culture. Resonance, 17(8),759-767.

Delaude, C. 1984. Gentianine, alkaloid from *Exacum quinquenervium* Griseb. (Gentianaceae). *Bull. Soc. R. Sci. Liege* 53,54–56.

Emmanuel,S., Lgnacimuthu,S. and Kathiravan.(2000). The effect of thiodizuron and BAP on shoot multiplication in the medicinal plant *Solanum trilobatum*. *Jour. Med. Arom. Pl. Sci*. 22(1),71.

Gnanaraj, W.E., Marimuthu, J., Kavitha, M.S. and Selvan, N. 2011. Micropropagation of *Alternanthera sessilis* (L.) using Shoot tip and Nodal segments. *Iranian Jour. Biotech.* 9(3),206-212.

Gomez, K. A. and Gomez, K. A. (1976). Statistical procedures for agricultural research with emphasis on Rice. Philippines International Rice Research Institute, Los Bans.

Gosal, S., Singh, A.K. and SP, RK C. 1974. Chemical constituents of gentianaceae. Xanthone-o-glucosides of Swertia

purpurscens Wall. Jour. Pharma Sci. 36,944
Haissig,B.E., Nelson,N.D. and Kidd, G.H. (1987). Trends in the use of tissue culture in forest improvement. *Bio. Technology*. 5,52-59.
Hossain,M., Rahman, S.M., Zaman, A., Joarder, O.I. and Islam, R. (1992). Micropropagation of *Morus laevigata* Wall.from mature trees. *Plant Cell Rep.*11,522-524.
Hostettmann-Kaldas, M., K. Hostettmann and O. Sticher, 1981. Xanthones, flavones and secoiridoids of american *Gentiana* species. Phytochem., 20,443-446.
Jabeen,F.T.Z., Venugopal,R.B., Kiran,G., Kaviraj,C.P. and Rao,S. (2005). Plant regeneration and i*n vitro* flowering from leaf and nodal explants of *Solanum nigrum* (L). – An important medicinal plant. *Plant Cell Biotech Mol. .Bio*. 6(1&2),17-22.
Jaishree, V., Shrishailappa, B. and Suresh, B. (2008). *In vitro* Antioxidant Activity of *Enicostemma axdillare. Jour. Health Sci.,* 54(5),524-528.
Kackar, N.L., Solanki, K.R., Singh,M. and Vyas,S.C. (1991). Micropropagation of *Prosopis cineraria*. *Ind. J. Exp. Biol.* 29,65-67.
Kadota,M. and Niimi,Y. 2003. Effects of cytokinin types and their concentrations on shoot proliferation and hyperhydricity in *in vitro* pear cultivar shoots. *Plant Cell Tiss. Org. Cult*. 72,261-265.
Kantamaht, K., Panyaros, C. and Kamnoon, K. 2012. Micropropagation of *Anubias barteri* var. *Nana* from Shoot Tip Culture and the Analysis of Ploidy Stability. *Not Bot Horti Agrobo*, 40(2),148-151
Kavimani, S. and Mani Senthilkumar, K.T. (2000). Effect of methanolic extract of *Enicostemma littorale* on Dalton's ascetic lymphoma. *Jour. Ethnopharmacol*. 71(1-2),349-352.
Komalavalli, N. and Rao, M.V. (2000). *In vitro* micropropagation of *Gymnema sylvestre* – A multipurpose medicinal plant. *Plant Cell Tiss. Org. Cult.* 61,97-105.
Kumar,S., Chander,S., Gupta, H. and Sharma,D.R. (1998). Micropropagation of *Actinidia deliciosa* from axillary buds. *Phytomorphology* 48(3),303-307.
Manjula,S., Anita,T., Benny,D. and Nair,C.M. (1997). *In vitro* plant regeneration of *Aristolachia indica* through axillary shoot multiplication and organogenesis. *Plant Cell Tiss. Org. Cult,* 51,145-148.
Maroo, J, Vasu, V.T. and Gupta, S. (2003). Dose dependent hypoglycemic effect of aqueous extract of *Enicostemma littorale* blume in alloxan induced diabetic rats. *Phytomedicine,* 10(2-3),196-199.
Martinussen,I., Nilsen,G., Svenson,L., Junttila,O. and Rapp, K. (2004). *In vitro* propagation of cloudberry (Rubus chamaemorus). *Plant Cell Tiss. Org. Cult.* 78,43-49.
Morel G. 1960. Producing virus-free cymbidiums. American Orchid Society 29,495–497.
Murali, B., Upadhyaya, U.M. and Goyai, R.K. (2002). Effect of chronic treatment with *Enicostemma littorale* in non-insulin-dependent diabetic (NIDDM) rats. *Jour. Ethnopharmacol.* 81(2), 199-204.
Murashige, T. and Skoog,F.,1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol Plant,* 15,473-497.
Nadkarni,K.M. (1908). Indian Materia Medica. Popular Prakashan Private Limited, Mumbai. V-1: 990-993.
Nagarathnamma, M., Sudarshana, M.S., Nirajan, M.H. and Pandurangamurthy 2010. Rapid regeneration of Enicostemma littorale Blume from leaf and stem cultures. Journal of Plant Interactions, 5(1),69-73.
Padmapriya, H., Karthikeyan, A.V.P., Jahirhussain, G., Karthi, C. and Velayutham, P. 2011. An efficient protocol for *in vitro* propagation of *Solanum nigrum* L. from nodal explants. *Journal of Agricultural Technology* 7(4),1063-1073
*Philip, V.J., Joseph, D., Triggs, G.S. and Dickinson, N.M. 1992. Micropropagation of black pepper (*Piper nigrum *Linn.) through shoot tip cultures. Plant Cell Rep. 12(1),41-44.*
Ranjan, K., Chakrabarthi, A.K., Singh, S.K. and Pragya.2010. Micropropagation of Chilli (*Capsicum annuum* L.) Plants through shoot tip culture. *Veg. Sci.* 37(1),Doaa
Rao, P.S., Supresanna, P. and Ganapathi, T.(1996). Plant biotechnology and Agriculture: Prospects for crop improvement and increasing productivity. *Science and culture* 62(7-8), 185-191.
Sadique, J., Chandra, T., Thenmozhi, V. and Elango, V. (1987). The anti-inflammatory activity of *Enicostemma littorale and mollugo cerviana.* Biochem. *Med. Metab. Biol.* 37(2), 167-176.
Sahoo,Y. and Chand, P.K. (1998). *In vitro* multiplication of a medicinal herb, *Tridax procumbens* L. (Mexican daisy, coat buttons) : Influence of explanting season, growth regulator synergy, culture passage and planting substrate. *Phytomorphology,* 48(2), 195-205.
Schoene,G. and Yeager,T. (2005). Micropropagation of sweet viburnum (*Viburnum odoratissimum*). Plant Cell Tiss.

Org. Cult. 83,271-277.
Tiwari KN, Sharma NC, Tiwari V, Singh BD (2000). Micropropagation of *Centella asiatica* (L.), a valuable medicinal herb. *Plant Cell Tiss. Org. Cult.,* 63,179-185.
Varghese,S.K., Inamdar,J.A., Kalia, K., Subramanina,R.B. and Nataraj, M. (1993). Micropropagation of *Aegle marmelos* (L.) Corr. *Phytomorphology* 43(1&2),87-92.
Velayutham, P. and Ranjitha Kumari, B.D. (2003). Direct shoot regeneration from leaf explants of chicory(*Cichorium intybus* L.*). Plant Cell Biotech Mol Bio.* 4(3&4),125-130.
Velayutham, P., Malaimuthu, C., Baskaran, P. and Ranjithakumari, B.D. 2005a. *In vitro* plant regeneration system for Enicostemma axillare (Lam.) A. Raynal – A versatile Medicinal Plant. *Jour. Swamy Bot. Club*. 22,105-108.
Velayutham,P., Jahir Hussain, G. and Baskaran, P. (2005b). *In vitro* plantlet formation from nodal explants of *Plumbago zeylanica* L. – an important medicinal plant. *J. Swamy Bot. Cl.* 22, 117-120.
Velayutham,P., Ranjithakumari,B.D., and Baskaran,P. (2006). An effeicient in vitro plant regeneration system for *Cichorium intybus* L. an important medicinal plant. *Jour. Agri. Tech.* 2(2), 287-298.
Vishwakarma, S.L., Rajani, M., Bagul, M.S. and Goyal, R.K. 2004. A Rapid Method for the Isolation of Swertiamarin from Enicostemma littorale. Pharmaceutical Biology 42,400-403.
Yadav, V., Madan, L. and Jaiswal, Y.S. 1990/ Micropropagation of *Morus nigra* L. from shoot tip and nodal explants of mature trees. *Scientia Hort*. 44,61-67.

Table 1: Effect of different concentrations of cytokinins on Micropropagation from shoot tip of *Enicostemma littorale* Blume.

Concentration of growth regulators KIN	BAP	**Shoot induction** frequency (%)	**Number of** shoots	**Shoot** length (cm)
5	—	80	15.2±1.38 de	4.55±0.49 e
10	—	90	22.8±1.53 bc	7.53±0.30 bc
15	—	100	28.3±1.15 a	8.33±0.28 a
20	—	95	25.8±1.98 ab	7.71±0.31 ab
25	—	85	18.1±1.44 d	6.06±0.31 d
—	5	80	16.3±1.05 de	5.78±0.43 de
—	10	95	24.4±1.09 b	7.53±0.35 b
—	15	100	29.2±1.19 a	8.4±0.21 a
—	20	90	17.4±1.54 cd	6.01±0.39 cd
—	25	85	18.3±0.83 c	6.19±0.38 c

Values are Mean of 5 replicates recorded after 30 days of culture.
Values in the last two columns are Mean ± SE of Mean followed by the letters within the column indicating the level of significance at $P<0.05$ by Duncan's Multiple Range Test (same letter within the column of the respective growth regulator indicates the absence of difference; different letters indicate the significant difference; and combination of letters indicate no significant difference)

Table 2: Effect of different concentrations of auxins on rooting of isolated shoots of *Enicostemma littorale* Blume.

Concentration of growthregulators (µM) IBA	NAA	Root induction frequency (%)	Number of roots	Root length (cm)
2	—	100	7.1±0.39 a	2.65±0.14 a
4	—	95	6.3±0.54 b	2.5±0.16 ab
6	—	90	5.8±0.40 bc	2.49±0.11 bc
8	—	80	4.7±0.49 d	1.8±0.10 d
10	—	75	4.6±0.47 de	1.69±0.13 de
—	2	100	8.1±0.33 a	3.01±0.09 a
—	4	95	7.2±0.43 ab	2.75±0.08 ab
—	6	90	6.2±0.43 cd	2.44±0.08 cd
—	8	85	6.4±0.42 c	2.55±0.10 bc
—	10	80	4.4±0.39 e	1.63±0.12 e

Values are Mean of 5 replicates recorded after 30 days of culture.
Values in the last two columns are Mean ± SE of Mean followed by the letters within the column indicating the level of significance at $P<0.05$ by Duncan's Multiple Range Test (same letter within the column of the respective growth regulator indicates the absence of difference; different letters indicate the significant difference; and combination of letters indicate no significant difference)

Fig.1. *In vitro* propagation of *Enicostemma litorale* Blume from shoot tip explant. a. shoot tip on MS medium supplemented with cytokinins; **b.** shoot initiation 10 days after subculture; **c**. shoot proliferation after 30 days; **d.** proliferation and elongation of leafy shoots after 45 days; **e**. root initiation from the isolated shoot; **f**. well established roots.

This academic article was published by The International Institute for Science, Technology and Education (IISTE). The IISTE is a pioneer in the Open Access Publishing service based in the U.S. and Europe. The aim of the institute is Accelerating Global Knowledge Sharing.

More information about the publisher can be found in the IISTE's homepage: http://www.iiste.org

CALL FOR PAPERS

The IISTE is currently hosting more than 30 peer-reviewed academic journals and collaborating with academic institutions around the world. There's no deadline for submission. **Prospective authors of IISTE journals can find the submission instruction on the following page:** http://www.iiste.org/Journals/

The IISTE editorial team promises to the review and publish all the qualified submissions in a **fast** manner. All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Printed version of the journals is also available upon request of readers and authors.

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar