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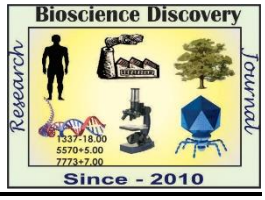

Micropropagation of *Euphorbia fusiformis* Buch-Ham. A rare medicinal plant

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Abstract

Micropropagation has in the recent years proved to be a very effective technique for rapid and large-scale propagation of many plant species. The cost of production of a plant plays a major role in commercial viability of micro propagation protocol. Especially in plant systems where alternative in vitro propagation technique are available. Shoot tip explants were used for micropropagation of *Euphorbia fusiformis* Buch-Ham. For culturing of explants MS medium was used with (0.5-3.0 mg/l) BAP, Kn and TDZ. High percentage response of single shoot induction (85% and 77%) was observed on MS mediumwith Kn (2.0 mg/l) and BAP (2.5 mg/l) respectively. MS medium with TDZ(2.5mg/l) shows high response (92%) of multiple shoot induction with averageshoot length (6.00 ± 0.32). IBA is the most effective auxin for induction of rootsfrom *in-vitro* shoots than IAA on half-strength MS medium. It is first attempt of the regeneration studies in *E. fusiformis* through micropropagation from shoot tip explants.

INTRODUCTION

Euphorbia fusiformis Buch-Ham. (Synonym: *Euphorbia aqualis*, Euphorbiaceae) is a rare medicinal plant found in Bengal, Uttar Pradesh, Konkan and Central Eastern Ghats of Tamil Nadu, Andrapradesh, and Telangana State in Pakhala forest regions. The ethno botanical value of this plant is due to its action as a remedy for several diseases like rheumatism, gout, paralysis and arthritis, liver disorders and diarrhea. The tuberous roots of this plant were used by *Bhagats* (tribal physicians) of Dangs forest for the treatment of various abdominal disorders, especially for tumors of abdomen, and urinary stones. However, after extensive literature survey, it came to conclude that only few pharmacological studies have been carried out on this plant, namely, its anti-inflammatory and antibacterial activities (Natarajan *et al.*, 2005). Further there is no work reported on *in vitro* micro propagation. It is said to be of medicinal value, its latex being used as an antidote for snake and scorpion bites. The tuber pulp is used as a cure for arthritic pains in some regions of the Himalaya.

MATERIALS AND METHODS

Collection of plant materials and surface sterilization: The plant material was collected from the Forest of Pakhala, Warangal, in Telangana State. Freshly grown shoot tips, were selected as an explant source. Shoot tips were washed in running tap water for 10 minutes to remove the dust or sand particles. The shoot tips were surface sterilized by using 0.5% of Sodium hypochlorite for 20 minutes. Few drops of Tween-20 were also added as a surfactant.

After 20 minutes the plant material was washed three times with sterile distilled water to remove the traces of bleach with gentle shaking under sterile conditions. To avoid the latex the explants of shoot tip were pretreated with ascorbic acid before inoculation for 15 min following the sterilization with mercuric chloride (0.05%) for 3 to 5 min and washed several times with sterile, distilled water and then were inoculated on culture tubes containing culture medium.

Culture media and culture conditions: MS media containing 3.0% sucrose and supplemented with various concentrations of cytokines such as BAP (0.5 – 3.0 mg/l), Kn (0.5 – 3.0 mg/l) and TDZ (0.5 – 3.0 mg/l) were used. The initial pH of the culture media was adjusted to 5.8 before addition of 0.8% (w/v) agar- agar. The medium was dispensed into culture tubes (25 + 150 mm) each containing 15ml of the culture medium capable with non-absorbent cotton and was autoclaved at 121^{o}C for15 minutes. In each cultures tube one shoot tip explant was implanted. The cultures were maintained under 16 h light provided with white fluorescent tubes (40 µ mol m-2s-2) at 25 ± 2^{o}C.

RESULTS AND DISCUSSION

Effect of BAP on shoot proliferation: Direct shoot proliferation was observed in shoot tip explants. After 6 weeks of shoot tip explant culture developed Single shoot (Plate-I). The various treatment tested on MS medium with BAP at (2.5 mg/l) resulted in maximum elongation (5.8 ± 0.44). But at high concentration of BAP (3.0 mg/l) considerably the elongation of shoot induction was found to be reduced. As the concentration of BAP was increased up to 2.5mg/l. The shoot elongation was increased but as the concentration of BAP above (2.5mg/l) resulted the shoot elongation were reduced.

Effect of Kn on shoot proliferation: Shoot tip explants were capable of directly developing of single shoot on MS basal medium containing different concentrations of Kn (0.5 – 3.0 mg/l). Single shoot initiation from shoot tip explants was observed within 20 -25 days after inoculation. The maximum length of shoots (5.9±0.35) was observer on MS medium containing (2.5 mg/l) Kn. Thehighest percentage (70 %) of shoot induction was also observed in the same combination (Table –1) (Plate-I)

Effect of TDZ on shoot proliferation: Direct shoot proliferation was observed in shoot tip explants. After 6 weeks of shoot tip explant culture, 2-3 shoots were developed (Plate-I-). The maximum of 2- 3 shoots was emerged on the medium containing TDZ (2.5mg/l) with 92% responded. When the concentration of TDZ was increased up to 3.0mg/l induction of multiple shoots was not increased but length of the shoots was increased (Table – 1).

Induction of Rooting: *In vitro* regenerated shoot cultured on MS medium supplemented with (0.5- 2.0 mg/l) IBA or IAA were used for root formation IBA was proved to be most effective for production of roots on the excised margins. Among different concentrations, 2.0 mg/l IBA was found to be the best for proper rooting in which 100% of shoots were rooted within three weeks of culture (Plate I). Supplemented with 2.0 mg/l IBA compared to other two growth regulators. The results obtained are in coincidence with the earlier workers. (Bhatt *et al.*, 2006) and *Lins culinaris* Medik (Omran *et al*., 2008).

Acclimatization: The *in vitro* derived fully elongated healthy shoots and rooted plantlets were removed from the culture medium and the roots were washed under running tap water to remove agar. Then the plantlets were transferred to polypots containing pre-soaked vermiculite and maintained inside a growth chamber at 28^{o}C and 70 – 80 % relative humidity. After Root formation was induced from three weeks they were transplanted to poly bags containing mixture of soil + sand + manure in 1: 1:1 ratio and kept under shade house for a period of three weeks. The potted plantlets were irrigated with Hogland's solution every 3 days for a period of 3 weeks.

DISCUSSION

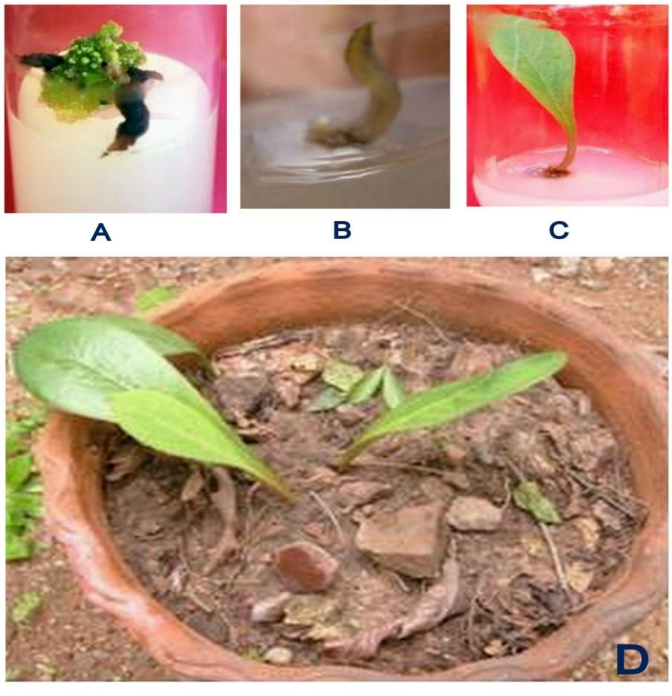
These are the first attempts to establish shoot tip cultures of this species. The results obtained show the unusual primitive effects of TDZ on shoot tip. There was an increase in the number of axillary buds and proliferation of the shoots was observed which served as source material for further multiplication. Similar results were obtained for *Leptadenia reticulata* shoot cultures, where IBA, NAA and IAA stimulated significantly the number of nodes per plantlet in comparison to cytokines (Kalidass *et al.*, 2009). However, for other Euphorbia species, such as *E. tenellus, E. niruri, E. caroliniensis,* and *E. fraternus* (Catapan, 2000; Saradhi and Islamia, 1997), *Excoecaria agallocha* L. (Rao *et al.*, 1998) cytokinins stimulated shoot proliferation.

Table-1: Effect of BAP, Kn and TDZ on shoot initiation of *Euphorbia fusiformis* from shoot tip explants on MS medium.

Plant Growth regulator (mg/L)	Shoot elongation *Mean S.D	Response	Culture establishment (%)
BAP			
0.5	1.83 ± 0.32	Single shoot	65
1.0	2.83 ± 0.40	Single shoot	73
1.5	4.00 ± 0.38	Single shoot	77
2.0	4.12 ± 0.32	Single shoot	70
2.5	5.88 ± 0.44	Single shoot	65
3.0	3.93 ± 0.32	Single shoot	60
Kn			
0.5	1.86 ± 0.32	Single shoot	70
1.0	2.90 ± 0.32	Single shoot	78
1.5	4.23 ± 0.23	Single shoot	80
2.0	4.32 ± 0.34	Single shoot	85
2.5	5.90 ± 0.35	Single shoot	70
3.0	4.00 ± 0.35	Single shoot	60
TDZ			
0.5	2.96 ± 0.32	Multiple shoot	75
1.0	3.00 ± 0.32	Multiple shoot	78
1.5	4.50 ± 0.42	Multiple shoot	80
2.0	4.60 ± 0.34	Multiple shoot	86
2.5	6.00 ± 0.32	Multiple shoot	92
3.0	4.03 ± 0.32	Multiple shoot	73

*** Mean ± Standard Error**

PLATE-1



A. Shoot bud initiation on MS medium with BAP

B. Single shoot initiation on MS medium with Kn

C. Direct shoot prolifiration on MS medium with TDZ

D. Plantlets acclimitized in the green house

The result of present investigation shows that the shoot tip explants from mature plants of *Euphorbia fusiformis* Buch. Ham could be induced to produce shoots *in vitro*. Maximum numbers of shoots length were induced on MS medium fortified with various concentrations of BAP, Kn and TDZ. These results are also in agreement with those on *Tectona grandis* (Gupta *et al*., 1980), *Abizzia lebbeck* (Gharyl and Maheshwari, 1982) multiple shoot induction was also observed in *Ziziphus manritiana* (Sudharshan *et al*., 2000) and *Vanilla plantifolia* (Geetha *et al*., 2000) shoot tips cultured on MS + cytokinin alone as it was observed in the present studies. Nasir *et al*. (1997) have studied the shoot meristem culture in 16 cultures of cotton using several media formation. They observed the best shoot development on MS media containing Kn alone compared to other media with NAA / IAA in combination with Kn. These results are similar to the present observation in *Euphorbia fusiformis* Buch. Hamwhich contain cytokinins showed the increased number of shoots/ explants. Sharma and Dhiman (1998) have also observed the similar results when they have cultured the shoot tips of F1 hybrids of *Paulownia*. The capacity of shoot bud differentiation and shoot proliferation from shoot tip explants of *Euphorbia fusiformis* Buch-Ham depended on hormonal variation. There was good shoot bud induction and proliferation response only in the presence of cytokinin and no response in the basal medium. Similar results are well documented in several medicinal plants (Pattnaik and Chand, 1996), *Emblila officinale* (Verma and Kant, 1996) and *Withania somnifera* (Deka *et al*., 1999). From our study it was clear that 2.0 mg/l BAP and Kn were significantly more effective for inducing shoot organogenesis. Well-developed shoot lets when transferred to rooting medium containing 1.5mg/l IBA/IAA induced higher frequency (14.32 and 12.68) of roots / explant. Similar effect of IBA was reported in *Ocimum americanum*, *O. canum* and *O. sanctum* (Pattnaik and Chand, 1996) and also in *Heracleum candicans.* However, IBA (1.5mg/l) was found to be the bestrooting hormone than other auxins. Auxin support for *in vitro* multiplication of shoots, similar results were observed in Sunflower (Patil *et al.*, 1993), in Mulberry (Naik and Lata, 1996) and in Coriander (Stephan and Jayabalan, 1998). Among different concentrations, 2.0 mg/l IBA was found to be the best for proper rooting in which 100% of shoots were rooted within three weeks of cultures maintained (plate I). These findings are in coincidence with those observed in other plant species *Phyla nodfolia*, *Leptadenia reticulata* (Bhatt *et al.,* 2006) and *Lins culinaris* Medik (Omran *et al.*, 2008). About 80 - 90% of the regenerated plantlets could tolerate and survive under field conditions. A number of plantlets were lost due to damping off and necrosis during acclimatization in *ex vitro* condition. From our experimental data, it is evident that BAP and KN are the best suited for inducing multiple shoots and IBA for rooting and *in vitro* flowering. In conclusion, this communication describes an efficient rapid propagation system of *Euphorbiafusiformis* Buch. Ham.

The protocol here in described is very much efficient for the *In vitro* multiplication of*Euphorbia fusiformis* Buch. Ham. In the light of our results, it can be suggested that enhanced shoot bud's formation can be achieved by using the MS media with 0.5 mg/l of BAP. The high concentrations of exponentially increase the axillary shoots formation. The *In vitro* roots were successfully induced by 2.0mg/l of IBA. The rooted plants were then effectively acclimatized with the potting mix of 80% sand and 20% farm yard manure (v/v).

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