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In Vitro Regeneration of *Ennicostemma littrolae* L. (Blume)

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ABSTRACTS

Einnicostemma littrolae L. highly medicinal plant and belongs to the family Gentianaceae. The plant is pungent and very bitter, antihelmintic, cures fever and vata diseases. It is also used as stomachic, laxative, antidiabetic, and crushed plant material is applied to snake-bites. In vitro rapid propagation was deliberated from shoot tip node and leaf explants of E. littrollae. The explants were cultured on (MS Murashige and Skoog 1962) medium supplemented with B5 vitamins. In various concentrations of cytokines and auxins ranging from 0.1 mg to 3.0 mg combinations of BAP and KN was good response from shoot tip, nodal explants. Highest number of callus induced in the concentrations of 2.5 mg BAP+2 mg NAA. Multiple shoot was noticed in 2.5 mg BAP and 1.5 mg NAA and 2 mg IBA. The present study enables the large scale production of E. littrollae using in vitro conditions and disease free plants.

Keywords: *Einncostemma littrolae Plant regeneration, Shoot regeneration, Sterilization, Growth regulators, (BAP, KN, NAA, IAA, and IBA), Medicinal uses.*

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INTRODUCTION

Ennicostemma littorale commonly, Blume and belongs to the family Gentianaceae is one of the important potential medicinal plants widely distributed in India. It is an erect or procumbent perennial herb of 5-30 cm tall, simple, branched at the base. This plant is characterized by the presence of many flowered auxiliary clusters around the stem. The plant is pungent and very bitter, antihelmintic, cures fever and vata diseases. It is also used as stomachic, laxative, antidiabetic, and crushed plant material is applied to snake-bites [1]. Traditionally 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. It is mainly used along with other herbs for the treatment of diabetes type. The medicinal uses include antidiabetic, antitumours, antimalarial, antimicrobial, and antipyreric activities.[2,26].The medicinal value of this plant is due the presence of bitter glycosides, alkaloids. The major chemical constuients bitter alkaloids. epigenin, genkwanin, swertisin, saponarin and gentiocrucine are also reported to present in minor amounts [3,25]. The explants were cultured on MS medium containing various concentrations 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 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 initiated and well developed in 2µM of both raised plantlets were successfully transferred to soil through hardening and acclimatization. [15,22].The medicinal value of this plant is due the presence of bitter glycosides are swertiamarin, a glycoside gentianine, a bitter alkaloid epigenin,

genkwanin, swertisin, saponarin and gentio crucine are also reported to prompting the authors for attempting to propagate plants from shoot tip explants under in vitro conditions. [19,20]. The whole plant are highly medicinal important used in several preparations of ayurvedic and folk medicine. It has been reported that the phytochemical studies revealed the presence of secondary metabolites most highly present in orientin, isoorientin, D-pinitol, norepinepherin mucilage, tannins, non protein amino acid, tannins, flavonoids, C-glycosides, steroids, terpenoids, fattyacids, saponins and coumarin, major and minor chemical constituents are also present[13].The present results showed potential plant has been applied for therapy to possess pharmacological actions human and treatment of anti-cancer, [23,24]

MATERIAL AND METHODS

Plant collection and Sterilization of explants

E. littrolae was collected from **A.V.V.M SRI PUSHPAM COLLEGE** (Autonomous) Poondi Thanjavur, Tamil nadu India. The explants were then prewashed 10% percent W/V of bavistain methyl 1-3 benzimidizole carbonate solution and washed thoroughly in running tap water. The explants were subsequently and disinfection surface sterilized with 0.12% $HgCl_2$ mercuric chloride solution for 3-5 minutes and washed 2-3 times in sterile distilled water. $HgCl_2$ was very penetrating that it destroyed the microorganism present in most tissues of the explants. The surface sterilized explants were trimmed gently with help of sterile surgical blade [1, 7]

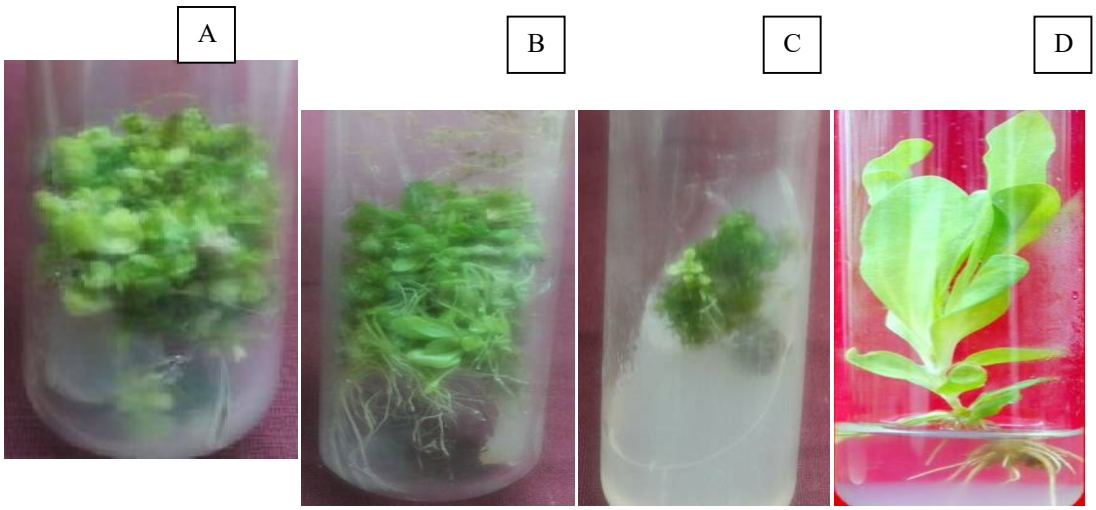
Culture medium

Nutritional support must be essential for optimal growth of tissue by in vitro. The nutrient media basically consists of inorganic nutrients. Carbon source, vitamins, iron source, amino acids and natural supplements. All the stock solution were prepared and stored in Amber – sterilized coloured bottles and preserved in a refrigerator at 4^0C. In present study MS medium supplemented of different concentrations of growth regulators were added, like Benzyl amino purine (BAP) Kinetin (KIN) 2, 4-Dichlorophenl acidic acid (2,4-D) Naphtali acetic acid (NAA), Indole-3 butyric acid (IBA) were 0.5 to 3.5mg concentration was used. Multiple shoot was noticed BAP, NAA, IBA 0.1 to 3.5mg were used. The required sucrose and other organic supplements nutrients were added. The final volume was made up of with sterile distilled water. To the above said media 0.8% W/V sugar and Hi-media agar was added. P^H was adjusted to 5.8 – 5.9 with either 0.1 N Na OH or 0.1 N Hcl using a P^H meter [23, 21].

RESULTS

Plant tissues normally grow in an organized fusion in which specific cell types differentiate from non specialised meristematic cells. Plant developmental processes can be modified by culture in vitro for suitable nutrients medium with the application of growth regulators. The interactions of the main growth regulators can be used but at the simplest level auxins can cause cell enlargement and division. Cytokines cause cell division and shoot development.

Fig 1**[A]Callus induction [B&C] Root initiation [D] Shoot initiation and shoot development**



IBA -2.0 mg/ 40 DAYS NAA -3.0 mg/ 20 DAYS BAP -2.0 mg/ 10 DAYS IBA -3.0 mg/ 40 DAYS

The explants shoot and nodal explants for micro propagation were selected from the same. The result showed that shoot formation was generated from direct organogenesis for explants, were detected in all

treatments. After 15 days growth of callus was observed [Fig-1]. The MS medium was supplemented with different concentration of hormones NAA, BAP, KN, IBA combination of it initiates basal callus and shoot and root proliferation.[Fig -1]

Callus induction

The requirement of various plant growth regulators for inducing callus and differentiation from shoot tip and nodal explants. The explants began to after 15 days of culturing and callus proliferation occurred [Fig-1]. The concentration of hormone BAP, KN, NAA, large amount of callus induction from MS media at 4 weeks [Table-1]

Table -1: Effect of various concentrations of BAP, KN, and NAA in Callus induction on MS media from shoot tip explants after 4 weeks of culture.

Growth regulators (mg/L)			Culture showing response (%)	Basal callus
BAP	KN	NAA		
0.5	-	0.5	55	+
1.0	-	1.0	65	+
1.5	-	1.5	72	++
2.0	-	2.0	85	+++
2.5	-	2.5	95	++++
-	0.5	0.5	70	+
-	1.0	1.0	72	+++
-	1.5	1.5	80	+++
-	2.0	2.0	90	+++
-	2.5	2.5	92	+++
0.5	0.5	0.5	80	++
1.0	1.0	1.0	82	++
1.5	1.5	1.5	95	+++
2.0	2.0	2.0	81	+++
2.5	2.5	2.5	90	+++

Callus induction: + poor ++ moderate +++ high response.

Table-2: Effect of various concentrations of BAP, IBA, and KN in shoot regeneration on MS medium from shoot tip and nodal explants after 4 weeks of culture

MS Medium Growth regulators (mg/L)			Culture Showing Response %)	Mean Shoot/ explants	Mean Shoot length (cm)	Basal Callus
BAP	IBA	KN				
0.5	0.5	-	60	3	4.5	+++
1.0	1.0	-	70	4	4.8	+++
1.5	1.5	-	80	5	4.2	++
2.0	2.0	-	75	4	5.1	+++
2.5	2.5	-	95	6	5.0	+++
-	0.5	0.5	55	2	2.5	++
-	1.0	1.0	65	4	4.0	+++
-	1.5	1.5	80	3	4.2	+++
-	2.0	2.0	90	5	5.0	+++
-	2.5	2.5	85	4	5.1	+++
0.5	0.5	0.5	65	3	5.1	+++
1.0	1.0	1.0	75	2	4.9	+++
1.5	1.5	1.5	90	5	4.2	+++
2.0	2.0	2.0	82	4	5.2	++
2.5	2.5	2.5	90	5	5.0	++

Shoot induction have been indifferent grades viz.,

- No response + poor ++ moderate +++ high

Table -3 Effects of various concentrations of BAP, NAA, and IBA in root formation on MS medium from shoot tip and nodal explants after 4 weeks of culture.

MS Medium Growth regulators (mg/L)			Culture Showing Response (%)	Mean root/ explants	Mean root length	Basal callus
BAP	NAA	IBA				
0.5	0.5	-	60	10	09.95	-
1.0	1.0	-	65	12	11.05	-
1.5	1.5	-	72	12	11.05	-
2.0	2.0	-	85	14	13.45	-
2.5	2.5	-	92	14	13.45	-
0.5	-	0.5	70	13	12.75	-
1.0	-	1.0	75	12	11.05	-
1.5	-	1.5	82	10	09.95	-
2.0	-	2.0	90	15	13.75	-
2.5	-	2.5	95	14	13.80	-
0.5	0.5	0.5	80	13	12.75	-
1.0	1.0	1.0	85	12	13.45	-
1.5	1.5	1.5	75	10	09.95	-
2.0	2.0	2.0	90	15	13.75	-
2.5	2.5	2.5	95	14	13.80	-

Shoot induction has been indifferent grades viz.

- No response + poor ++ moderate +++ high

Shoot regeneration

The highest frequency of shoots from shoot tip explants were observed in MS media containing 0.5 mg to 2.5 mg BAP, NAA, IBA, and KN was used. [Table -2]. The maximum shoot regeneration frequency i.e. 95% on MS basal medium supplemented with 2.5mg/L BAP, NAA 2.5mg/L and IBA 2.0mg/L respectively. The shoot regenerated shoots obtained from both the explants. Shoot multiplication and elongation took place on the same medium

Shoot multiplication

A large number of lateral shoots were recorded on the explants cultivated at media many authors emphasize the influence of proper cytokines and auxins combination on the formation of shoots under the in vitro conditions. Among the different concentration of BAP, NAA, IBA 0.5 to 2.5mg/L used, IBA at 1.5mg/L has given 85% growth of shoot with a mean shoot length of 4.2cm.The maximum amount of multiple shoot was observed at 2.5mg/L with 85% of response with mean shoot length 4.0cm [Fig-3&4]. The highest numbers of shoots from flower bud explants were observed by on 45 days it has given 15 shoots per explants [Table-3].

Root formation

Among the different concentration of BAP, NAA, IBA 0.5 to 2.5mg/L were used, IBA at 1.5mg/L has given 95% growth response of roots. A large number of lateral roots were recorded on the explants cultivated at MS media to proper cytokines and auxins combination on the formation of roots.[Table -3] The maximum amount of roots was observed at IBA 2.5mg/L [Fig-2 & 3]. The highest number of roots from flower bud explants was observed by on 45 days it has given 15 roots per explants [Table- 3].

DISCUSSION

Nutrition since possess the nutrients namely vitamins, minerals, secondary metabolites and fibre proclaiming its exceptional health benefits. The breakdown products of the sulphur containing principles in exhibiting the anticancer property at stage [14,24]. For the present study, the results revealed direct organogenesis of leaf explants there was callus formation and the duration of shoot initiation on nodal explants were 15-20 days this result is agreement with finding in number of plants initiation. [7,18]. For the present study, the results when sub cultured on 35th day 15 shoots per explants were achieved [Table-3]. The shoots were aggregated. The mean shoot lengths were 13.80 measured. [7,9]. KN, BAP, played a role on inducing shoot multiplication. It was suggested that the use of BAP as a cytokines and NAA as an auxins in an appropriate ratio. They investigated that BAP induced more shoot produced. Many investigators examined that uses of auxins and Cytokines KN, IBA, BAP was the optimum concentration for shoot and root regeneration. [3].This study has presented 95% regeneration at BAP 0.5 to 2.5mg KN 0.5mg to 2.5mg for initiation of shoot BAP, IBA, KN= 0.5 to 2.5 mg multiple shoot formation in explants. [12, 10] The effect of different media and cytokines on shoot regeneration of E. littrolae shoot tip and nodal explants were cultured on three types of media supplemented with various concentrations of BAP and KIN. The degree of

growth and differentiation varied considerably with the medium constituents [8, 7]. Comparing the effect of cytokines type BAP and KIN on shoot production, the best response was achieved by BAP [19]. In general and also found in the present study, higher concentrations of cytokines above 2.5 mg/l reduced the shoot number as well as shoot length. This finding is also in line with the finding of who reported that higher concentrations of cytokinin reduced the number of micro propagated shoots. A similar response was also observed. [16, 17].The regeneration of explants was cultured on the medium containing strong auxins and cytokines for the production moss of callus. These calli were transferred to medium supplemented with cytokinin and a weak auxin for shoot regeneration [9, 13]. In the present study, the semi friable calli obtained from shoot tip and nodal explants were transferred in to MS-B5 medium augmented with constant concentration of BAP 1.5 mg/l with different concentrations of KIN 0.5-3.0 mg/l in combinations for the purpose of organogenesis [16, 24]The previous and earliest observation best rooting was achieved in the medium with reduced basal nutrients and IBA. This step is very important for the plant survival and critical step in the production of complete entire plantlets. [6].In the present experiment from full to half strength in the basal medium was sufficient for the rooting of shoots. In many plant species IBA is considered as an important and effective growth regulator for the induction of roots. In the present experiment 2 mg/l IBA produced maximum number of healthy roots similar observation. [11,14]. In present study the highest frequency of shoots from leaf explants was observed in MS media containing 2.5 mg BAP +2.5 mg KIN this combination showed 82 % response. Root explants E. axillare were cultured on MS medium supplemented with KIN, BAP used alone in combinations (2.22 m in combination with 4.64 m of KIN induced the maximum number of adventitious shoot buds 24.60 + 0.54 shoots per explants with the shoot length of 1.54 + 0.36. Explants from in vitro shoots in BAP supplemented medium were found with more response than those of wild plants. [25] The type of callus is determined by the explants used and organ of the plant, the hormones and their concentrations, the chemical constituents of the culture medium. The combinations of external growth regulators cytokines with auxins are essential requirement to stimulate shoots formation from callus. The difference in response are depends on regeneration media could be due to the kind of endogenous hormones in cells which control many circumstances expressed by cells [10] MS medium supplemented with different concentrations of BAP/KN resulted in initiation of callus and shoots from shoot tip and nodal explants (Table-1). Maximum number of multiple shoots were induced in MS medium supplemented with 1.5 mg/l BAP (Fig-1.a & b) when compared to other and higher concentrations used. Hence it is suggested that this optimum concentration of BAP promotes multiple shoot induction. Similar reports were also obtained with the cultures of Phyllanthus amarus, Celastrus paniculatus [3, 4].

CONCLUSION

E. littotrae is a highly potential and medicinal plant. The in vitro regeneration protocol is an efficient means of ex-situ conservation of plant diversity. We have demonstrated in this study the effects of growth regulators on morphogenic response of cultivated in vitro. Appropriate combinations and concentration of plant hormones result in higher yield of plant biomass and mass propagation by tissue culture technique.

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