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In vitro CALLOGENESIS OF MEDICINALLY IMPORTANT AYURVEDIC HERB *Enicostema littorale* BLUME

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AUTHOR'S CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author MDT designed the study and wrote the first draft of manuscript. Author MAM managed the literature searches. Author TI edited the manuscript and analyzed the data. Author JMW wrote the protocol. Author TAW edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Purpose: The practice of in vitro culturing of medicinally important plants has gained much attention in enhancing the secondary metabolite production. In this perspective, the current study was carried out to promote a rapid and standard method for in vitro callogenesis of *Enicostema littorale* Blume using different explants.

Research Methods: In vitro callogenesis of *Enicostema littorale* was done on Murashige and Skoog's media. Explants were cautiously sterilised and later put on MS medium added with variable combinations and combinations of growth regulators and were maintained in culture room at temperature of 25 ± 2°C with photoperiods of 16 h. The cultures were observed at regular intervals for callus initiation and results were recorded regularly.

Findings: Maximum callus was yielded from nodal explants when Murashige and Skoog medium was added with various growth promoters (6-Benzylaminopurine and Kinetin -3.0 and 2,4-dichlorophenoxyacetic acid -1.5 mg each followed by Kinetin-2.0 and Naphthyl Acetic Acid -0.5 mg) per liter amount of media. Similarly, it was also revealed from the present investigation that leaf explants proved better for callogenesis on MS media added with 6-Benzylaminopurine-3.0 and Naphthyl Acetic Acid -1.0 mg/l followed by Kinetin-1.5 and NAA-0.5 mg/l. However, shoot tip explants weakly responded for callogenic induction during the present study. The present study while using combinations of growth regulators at different concentrations and combinations, all the selected explants responded distinctly.

Value: The developed tissue culture protocol can be proved as rapid and reliable method for enhancing and extracting the secondary metabolite production, and as a landmark to meet the industrial need in the near future.

Keywords: Callogenesis; *Enicostema littorale*; growth regulators; ms medium; explants.

1. INTRODUCTION

The proclivity towards herbal medicines and medicinally important secondary metabolites is gaining much attention these days. The technique of *in vitro* culturing of novel medicinal and aromatic plants (MAPs) has enhanced their production both quantitatively and qualitatively [1]. *In vitro* generation

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of cell, tissue organ culture and regeneration of plantlets under has opened up new prospects in the field of biotechnology [2].

Enicostemma littorale is a perennial herb 10–15 cm high. It is branched from the base with erect stems. Leaves are sessile and can be linear or linear-oblong [3]. It is commonly known as "chota chirayta", used as a substitute for chiretta (*Swertia chirata*), a well-known anti-diabetic drug [4, 5]. It is widely distributed throughout the greater part of India and common in coastal areas. *E. liittorale* is hub of secondary metabolites. These secondary metabolites possess various therapeutic properties and due to this, they are considered as a medicine or drug [6]. It is used as stomachic, laxative, antidiabetic, and crushed plant material is applied to snake-bites [7]. Traditionally it is used as antipyretic, to cure stomach ache, laxative and as a tonic for anorexia [8, 9]. The medicinal herb is said to possess antitumor, hypoglycaemic, antimalarial, anticancer, antidiabetic, antimicrobial, antifungal, anti-inflammatory, antioxidant, antipyreric, antihelminthic, antinociceptive, aepatoprotective and antihyperlipidaemic properties [10, 11, 12].

The medicinal value of *E. Littorale* is owing to the occurrence of bitter glycosides and alkaloids such as swertiamarin and gentianine [13, 14].

The plant produces high seed set, however under natural conditions the seed germination rate is too poor. The drug quantity accumulated by the plant species is very low *ca. 0*.3%. So, in to extract the ample drug, a huge quantity of plant biomass is required. Because of low seed germination rate, propagation via explants is an alternative method to increase its population. Micropropagation is the most operative methods to preserve such germplasms. Since there is less report on *in vitro* callogenesis of *E. littorale* [15]. Therefore, current study was carried out with an aim to induce a rapid and reliable method for *in vitro* callogenesis of medicinally important ayurvedic herb *E. littorale* which could be used for the extraction of valuable secondary metabolites of immense therapeutic importance.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

For present study, different explants of *E. littorale* such as nodal, shoot tip and leaf explants were collected from the main campus of Sant Gadge Baba Amravati University (SGBAU), Amravati (MS-444602).

2.2 Media Preparation and Sterilization

During the present investigation, a standard protocol was followed in which MS [16] media augmented with sucrose-3% and agar-0.8% were used. Variable combinations/concentrations of growth regulators were mixed carefully in the growing media and pH was maintained to 5.6-5.8.

2.3 Excision and Surface Sterilization of Explants

Disease free explants were cautiously selected and rinse with tap water and further with de-ionised water to avoid contamination. Furthermore, explants were treated with standard concentration of 0.1% surface steriliser (Mercuric Chloride) for about 2-3 min followed by washing with distilled water for 4-5 times. Moreover, the explants were strelised with 70% ethyl alcohol for 2-3 min, and further followed by rinsing with de-ionised water to eliminate the remaining traces of surface sterilizers.

2.4 Inoculation Procedure

The sterilized explants were then sliced into appropriate pieces, inoculated in MS medium added with variable combinations and concentrations of various growth regulators and were further maintained in culture room at the optimum temperature around 25 ± 2ºC with photoperiod of 16 hrs. The cultures were monitored carefully at regular intervals for callus initiation and results were listed regularly.

3. RESULTS AND DISCUSSION

During the present study explants such as nodal, shoot tip and leaf were inoculated on MS media added with different combinations and concentrations of auxin (Naphthyl Acetic Acid and 2,4-dichlorophenoxyacetic acid), cytokinin (6-Benzylaminopurine) and Kinetin. Growth regulators are consistently used to promote callogenic responses in various medicinally important plant species [17].

It was revealed from the results that the callus obtained from the nodal explants took nearly 20-25 days after inoculation (Fig. 1A). For callus induction from nodal explants two combinations of 6-Benzylaminopurineand Kin-2.0-3.0 + Naphthyl Acetic Acid-0.5-1.0 and Kinetin-2.0-2.5 + 2, 4-D-0.5-1.5 mg/l was used. Callus obtained from these combinations was healthy, ranges in colour from yellowish white to brownish green and fragile in texture (Table 1).

Table 1. Percentage (%) of callogenic response of nodal, leaf and shoot tip explants of *Enicostema littorale* Blume at different concentrations and combinations of various growth regulators after 30–35 days of subculture

Explants Used	No of cultured explants	Growth regulators (mg/l)				Callogenic response (%)	Callus colour and texture
		Kin	NAA	BAP	2, 4-D		
Nodal	30	2.0	0.5	0.0	0.0	35	Brownish/ green
	30	0.0	0.0	2.0	0.5	50	Yellowish/ Fragile
	30	2.5	1.0	0.0	0.0	49	Brownish green/ Fragile
	30	2.0	1.0	0.0	0.0	53	Yellowish white/ Fragile
	30	0.0	1.0	2.5	0.0	65	Green/ Fragile
	30	0.0	0.0	2.5	1.0	61	Brownish green/ Fragile
	30	0.0	0.0	3.0	1.5	70	Yellowish white/ Fragile
Leaf	30	1.5	0.5	0.0	0.0	30	Green/ soft
	30	2.0	0.0	0.0	0.5	55	Yellowish/ soft
	30	0.0	1.0	3.0	0.0	60	Yellowish white/ soft
	30	0.0	0.5	2.5	0.0	51	Green/ soft
	30	2.5	1.0	0.0	0.0	45	Green/ soft
Shoot tips	30	2.0	1.0	0.0	0.0	05	Green/ Fragile
	30	0.0	1.0	2.5	0.0	07	Green/ Fragile
	30	0.0	0.0	2.5	1.0	09	Green/ Fragile
	30	0.0	0.0	3.0	1.5	12	Green/ Fragile

The maximum callogenic response (70%) was observed from nodal explants (Fig. 1B) when MS media was augmented with BAP-3.0 and 2, 4-D-1.5 mg/l while as minimum callogenic response (35%) was observed during the combination of Kin-2.0 mg and NAA-0.5 mg/l after 30-35 days of subculture. Similar findings were reported by John et al., [2018], that nodal explants proved better for callogenesis on similar medium.

Similarly, leaf explants responded well to callus formation. For callus induction BAP-2.5-3.0 mg + NAA-0.5-1.0, and Kin-1.5-2.5 + NAA-0.5-1.0 or 2, 4-D-0.0-0.5 mg/l were used. The callus obtained from leaf explants in these combinations was healthy, green and soft. During current study the callogenic effectiveness of the callus were induced with the combination of higher concentration level of BAP or Kin with lower one of NAA and the same response has been recorded in few medicinal plant species [18, 19]. It was pragmatic from the results as shown in Table 1, that maximum callogenic response (60%) was observed from leaf explants (Fig. 1D) when MS media was added with BAP-3.0 and NAA-1.0 mg/l while as minimum callogenic response (30%) was observed during the combination of Kin-1.5 and NAA-0.5 mg/l after the period of 30-35 days of subculture.

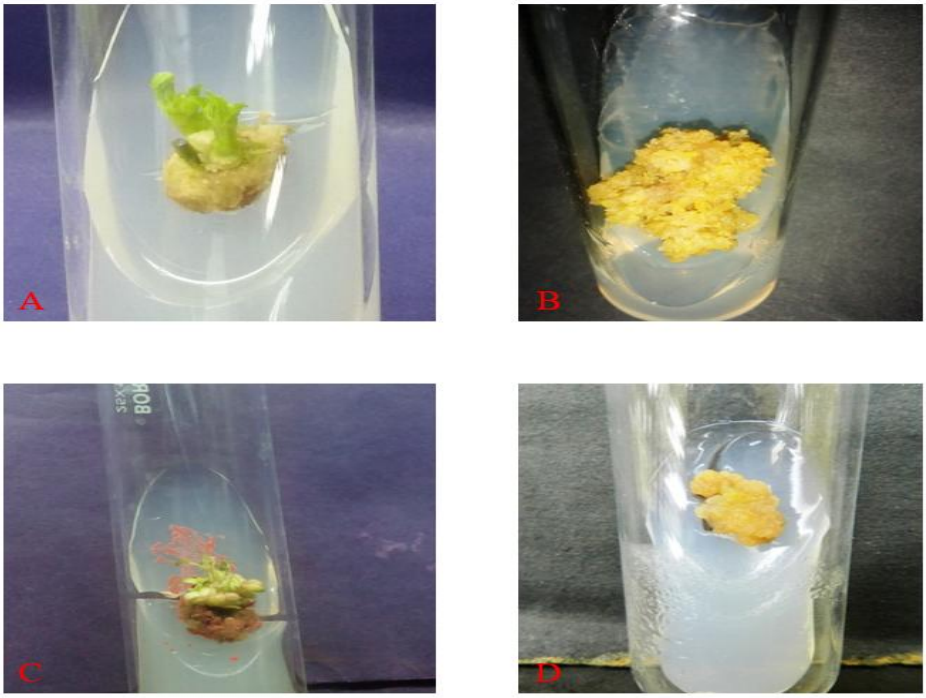

Fig. 1. (A-D). *In vitro* callogenic response from nodal and leaf explants of *Enicostema littorale* Blume. (A) Initiation of callus (nodal explants) (B) Regeneration of callus (nodal explants) (C) Initiation of callus (leaf explants) (D) Regenerative callus (leaf explants)

Furthermore, callus initiation in nodal and leaf explants was started earlier in medium, added with BAP-2.0 and NAA-0.5 mg/l. It was also observed from the results (Fig. 1C) that during the 1st week of *in vitro* callogenesis, leaf explants start initiation but no callus induction until the third week. After that, inoculated tissues showed the sign of callus induction in MS medium containing BAP-1.0 and NAA-0.5 mg/l. However, current investigation proved that nodal and leaf explants responded better at a elevated levels of NAA-1.0 mg and BAP-3.0 mg/l. These findings support the work of Nagarathnamma et al., [15] that developed a higher occurrence and rapid regeneration process for callus initiation from both leaf and nodal explants of *E. littorale* on MS medium added with BAP-3.0 mg and NAA-1.0 mg/l. Likewise, the shoot tip explants does not show satisfactory results of callogenesis when added with growth regulators.

4. CONCLUSION

It was concluded from the present study while using combinations of growth regulators at different concentrations and combinations, all the selected explants responded distinctly. Callusing was observed in nodes and leaf explants when supplemented with 6-Benzylaminopurineand Kinetin-3.0 mg and 2,4-dichlorophenoxyacetic acid-1.5 mg followed by Kinetin-2.0 mg and Naphthyl Acetic Acid-0.5 mg per liter amount of media respectively. The callus induced was yellowish white and friable in nodal, green and soft in leaf explants. However, shoot tip explants showed less callogenic response when MS media was augmented with diverse combinations/concentrations of growth regulators. Furthermore, developed tissue culture protocol can be proved as rapid and reliable method for enhancing and extracting the secondary metabolite concentrations, and as a landmark to meet the industrial need in the near future.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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