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Micropropagational studies in *Enicostema littorale*

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Abstract
Present investigation aim to develop standard protocol for micropropagation of herbal drudge *Enicostema littiralae*. This perennial herb distributed in West Indies, tropical Africa, India and Sri Lanka. It is found almost throughout India up to an altitude of about 450 meter. The whole plant is used in curing from various diseases including endemic disease diabetes mellitus, very frequently throughout the India. It is also utilised in rheumatism, abdominal ulcers, hernia, swelling, itching, malaria and insect poisoning. Present investigation revealed that induction of callus and multiple shoots on MS medium supplemented with various concentration of growth regulator alone or in combination. Maximum callus induction frequency was achieved on MS media along with 2.0 mg/L of 2, 4 D alone with Somatic embryo and 2.0 mg/L of BAP in combination of 0.5 mg/L of IAA. Induction of multiple shoots was achieved on IAA in combination of BAP 0.5 mg/L and 1.5 mg/L. Standard protocol was established for micro propagation of herbal drugs using IAA, 2, 4 D, BAP and KIN.

Keywords: *In vitro*; *Enicostema littoralae*, micro propagation; callus

Introduction
Enicostema littoralae (Indian Whitehead) belongs to family gentiaceae is a perennial herb growing up to 40 cm tall, with 4-angled stems. Leaves are narrow- oblong, lance shaped. Stalk less white flowers are borne in dense clusters in leaf axils. This species is globally distributed in West Indies, tropical Africa, India and Sri Lanka. It is found almost throughout India up to an altitude of about 450 m., from Punjab and the Gangetic plains southwards, most commonly in coastal areas. This is important medicinal plant in ayurveda and Indian herbal medicines. Entire plant of Indian Whitehead is used to treat diabetes mellitus, rheumatism, abdominal ulcers, hernia, swelling, itching, malaria and insect poisoning.
Enicostema littorale is an uncultivated leafy green eaten in southern India as a source of iron and calcium. Greens (quelites) are important supplemental sources of nutrients such as iron, calcium, magnesium, vitamin C, B vitamins, betacaroten, in traditional societies. *E. littorale*, locally known as gorumadi, or gorumadi koora, is eaten as a curry with pulses or other greens (Ahmedulla and Nayar, 1986**) [1].** In a clinical trial with 84 diabetic patients who ingested 2g of *E. littorale* per day for 3 months, no adverse side effects were reported (Goyal *et al*, 2006) [2]. *E. littorale* is traditionally used in India as a stomachic, bitter tonic, laxative, carminative (Nadkarni 1976) [5], to reduce fever and as a "tonic" for appetite loss. Many other genera in the gentian family have similar traditional uses worldwide (Kirtikar and Basu 1935) [3] lists species with similar uses worldwide; Weiss 1988:40-42 describes uses of European Gentian spp.). In Ayurvedic (India) medicine, *E. littorale* is taken in combination with other herbs, especially for diabetes (Nadkarni 1976) [5]. During present investigation efforts were made to establish micropropagation protocol for rapid regeneration of this important medicinal plant.

Material and Method
Preparation of Explants
Different explants viz. leaf, shoot tip, nodal region of *Enicostema* were collected from Botanical garden, Department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. These explants washed twice with running tap water for 3 minutes. Then after surface sterilization was carried out by washing twice with distilled water followed by mercuric chloride, leaf explants sterilized with 0.1 % whereas shoot tips and nodal segments were sterilized by 0.3% of $Hgcl_2$ and finally appropriate size of explants were cuts and inoculated on MS medium.

Culture medium and culture conditions
All experiment of present investigation were carried out on MS media supplemented with various concentration of 2, 4 D, IAA, BAP and KIN alone or in combination. MS medium fortified with 3% sucrose and gelled with 3 gm/l Clerigel. The pH of the medium was adjusted to 5.8 before sterilization. After inoculation all culture tubes were transfer into culture rooms where all conditions were control and aseptic. Cultures were maintained at 25±1ºC with a 16-h photoperiod with 40 mol m^2/ s provided by cool white fluorescent tubes. Observations were recorded after every week interval and tabulated in to table.

Results and discussion
Standard protocol for surface sterilization was developed by trial and errors methods. For leaf explant 0.1% and for nodal segment 0.3% of mercuric chloride concentration was found to be best for sterilization of explants. Induction of callus was achieved on both 2, 4D alone and IAA in combination of BAP. Maximum induction of callus recorded on 2.0 mg/L of 2, 4D alone and 0.5 mg/L of IAA along with 2.0 mg/L of BAP

supplemented in MS medium. Lower concentration of 2, 4D induced poor callus however higher concentration resulted in induction of callus along with somatic embryo for both explant leaf and nodal segment. Nodal segment was found best explant for induction of callus and somatic embryogenesis in *Enicostema* using 2, 4D growth hormone incorporated in to MS media.

Table 1: Response of Explant to various growth regulator combinations.

Growth regulators (mg/L)				Response of explants	
2,4 D	**BAP**	**KIN**	**IAA**	**Axillary leaves**	**Nodal segments**
0.5				--	20.00 PC
1.0				20.00 PC	26.66 PC
1.5				20.00 PC	33.33 PC
2.0				40.00 C/SE	53.33 C/SE
2.5				33.33 PC	53.33 C/SE
	0.5			26.66 PC	26.66 PC
	1.0			40.00 C/S	53.33 C/S
	1.5		0.5	53.33 C/S	73.33 C/S
	2.0			33.33 PC	60.00 MC
	2.5			33.33 PC	53.33 AC
		0.5		20.00 PC	26.66 PC
		1.0		26.66 PC	33.33 C/S
		1.5	0.5	26.66 PC	40.00 C/S
		2.0		33.33 C/S	40.00 C/S
		2.5		33.33 PC	33.33 PC

Percentage response on three separate experiments, each based on a minimum of five replicates.
PC=Poor Callus. C/SE= Callus along with somatic embryo. C/S=Callus along shoot. MC= Massive Callus.

Response of BAP/KIN on induction of multiple shoots

Present investigation revealed that, induction of multiple shoot on MS media supplemented with 0.5 mg/L of IAA in combination with different concentration of BAP and KIN. Both the combinations BAP and KIN along with IAA revealed the induction of callus after three weeks and later on shoot induction were achieved. High frequency of multiple shooting was noticed on 0.5 mg/L of IAA in combination with 1.5 mg/L of BAP with 73.33 % regeneration. KIN was found less frequent in induction of multiple shooting from both the explant.

Fig 1: Somatic embryos

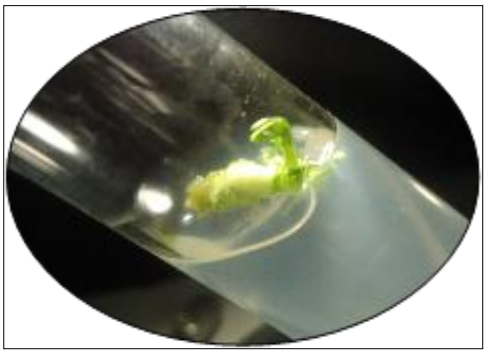

Fig 2: Callus along with Shoot

Fig 2: Multiple shooting on BAP

Fig 4: Shooting on KIN

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