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In Vitro Studies in *Enicostema Littorale* Blume

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Abstract: ***Enicostema littorale is popularly known as Indian Whitehead belongs to family gentiaceae. It is a 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 of Indian whitehead used in the treatment of diabetes mellitus, rheumatism, abdominal ulcers, hernia, swelling, itching, malaria and insect poisoning.During present piece of work efforts have been made to regenerate this important medicinal plant in vitro. Explants viz. shoot tip, leaves and stem were cultured on MS medium, with different concentration of BAP, KIN, NAA, and IBA. Induction of callus was recorded on MS fortified with BAP 1.5 mg/lit along with IAA 1.5 mg/lit. Friable callus was noticed after 15 days.***

Keywords: *In vitro*; *Enicostema littorale*, Regeneration, Callus

1. Introduction

Enicostema littorale (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 gorumadikoora, is eaten as a curry with pulses or other greens (Ahmedulla and Nayar, 1986**).** 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 (Goyalet al, 2006). Israeli legislative status *E. littorale* does not appear on the Israeli Ministry of Health medicinal plant, food or toxic substance lists. *E. littorale* is traditionally used in India as a stomachic, bitter tonic, laxative, carminative (Nadkarni 1976), 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)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). Medicinal use or other non-nutrition purposes of the ingredients - Clinical trial *E. littorale* was administered in Ayurvedic pill form (known as ghavantis) at a daily dosage of 2000 mg for three months to 84 patients with Type 2 Diabetes. *E. littorale* reduced blood glucose and serum insulin levels, and significantly improved kidney function, lipid profile, systolic and diastolic blood pressure and pulse rate (Goyalet,al, 2006). No side effects were reported in this study.During present piece of work efforts were made to establish protocol for rapid regeneration of this important medicinal plant.

2. Material and Method

Preparation of Explants:
Plantlets of *Enicostema* were collected from near hillock of Goga baba Tekdi and grown in the green house situated in Botanical garden, Department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Apical shoot, Axillary bud, node and meristematic tissue were taken as an explant. The explants were washed carefully in running tap water for 10 minute and followed by distilled water for 5 minutes. For surface sterilization, chemical such as 70% ethanol, $Hgcl_2$ (0.3 %) was used. Explants were surface sterilized for 5 minute by 0.3% mercuric chloride followed by three subsequent rinses with sterilized double distilled water in a laminar flow. All these explants were dissected into small pieces and inoculated in MS media.

Culture medium:
MS medium (Murashigeand Skoog, 1962) was used for propagationof apical shoot, Axillary bud and nodalsegments as explants. MS medium was supplemented with cytokinins viz. BA, KIN, whereas auxins viz. IAA, IBA, and NAA were used.

Culture conditions
MS medium fortified with 3% sucrose and gelled with 3 gm/lClerigel. The pH of the medium adjusted was 5.8 after adding of growth regulators. The medium wasautoclavedup to 15 PSIto get 121° C for 15 minutes. Culture tubes and culture vessels were transferredincubated at 16 h photoperiod supplied by cool white fluorescent cool tubes light and at 25 ± 2^0C temperatures. Ten cultures were mentioned for each treatment. Cultures were observed consistently and data was recorded. Five replicatesfor shoot multiplication and shoot length Mean (µ) values with the standard error (S.E.).

3. Results and Discussion

Induction of shoot formation was absent when apical shoot, axillary bud and nodal explants of *Enicostema were* grown on MS medium devoid of hormones.Shoot formation wasnoticed when MS media enriched with different concentrations of BAP i.e. 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 mg/l

along with IBA, NAA with 0.5. 1. 1.5 and 2.0 mg/lit. Observations have been mentioned in table 1.

During presence investigationit was noted that,apical shoot tip, axillary buds and nodal explants were responsible for development of callus and multiple shoots in *Enicostema*. Thecytokininstested during these studies were BAP and KIN respectively. BAP was more found effective than KIN for multiplication. MS media was fortified with 3% sucrose.Different concentrations of BAP employed were 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 mg/l alone and with IBA 0.2, 0.4, 0.6, 0.8, 1.0 mg/L. Secondly, concentration of BAP along with IAA viz. 0.2, 0.4, 0.6, 0.8, 1.0 mg/L gives average multiple shoots in *Enicostema* (Plate.1) Maximum number of multiple shoots recordedwith BAP was52 % 1.6 mg/L, and with IBA 0.2 mg/L.(Tabke.1)This is followed by 51 % with BAP 1.66 1nd IBA 0.2 0.2 mg/L. concentration .

Table 1: Effect of BAP and IBA for multiplication of different explant

Explant	Conc. of growth regulator (mg/L)		Shoot length (Mean)	% of shoot formation
	BAP	IAA		
apical shoot tip	1.0	0.2	1.88	30
	1.2	0.2	2.64	32
	1.4	0.2	2.52	37
	1.6	0.2	2.90	51
	1.8	0.2	2.16	49
	2.0	0.2	2.24	47
axillary bud	1.0	0.2	1.70	35
	1.2	0.2	2.04	37
	1.4	0.2	2.14	44
	1.6	0.2	2.62	52
	1.8	0.2	1.76	50
	2.0	0.2	1.82	49

***After 25 days mean of 5 replicate**

Effect of BAP shoots multiplication:

Apical shoot tip and Axillary bud explants were inoculated on MS medium supplement with 3% sucrose, 2.5% Clerigel and various combinations of growth hormones as shown in the table No. 1 Maximum average shoot length and multiple shoot formation percentage of *Enicostema* was recorded in 1.6 mg/L BAP combination with 0.2 mg/l IBA.This is followed by 51 % with BAP 1.66 1nd IBA 0.2 0.2 mg/L.concentration. Somatic embryos were noticed after 21 days of Inoculation. Same results were noted by Kakde and Pandhure (2015) in *Tylophora indica* and Birasdar and Pandhure (2012) *Jatropha*. Various combinations of IAA were added into the MS medium to achieve rooting. *In vitro* rhizogenesis was achieved by adding 0.5 mg/lit IAA. Similar kind of findings were recorded by Zimmarmann (1993) and Tokuhara, K.; Mii, M. (2001).Plants were hardened and introduced in soil for in vivo trails.

Figure 1: Callus and Multiple shoot formation

4. Conclusion

Ayurvedic preparations have great demand globally. Peoples are behind safer medicines now a day. Increasing demand could not supply sufficient plant material for the extraction of ayurvedic medicines. In vitro studies are suitable option for multiplication of rootstock of plant material and to fulfill the need of the peoples.

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