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Hormonal Efficiency of Plantlet Production in *Cynoglossum zeylanicum* (Vahl) Thunb. Ex Lehm

Duraiswamy M, Jahirhussain G*, Karuniya Raja Viella G
PG and Research Department of Botany, Government Arts College (Autonomous), Thanthonimalai, Karur-639005
Affiliated to Bharathidasan University, Tiruchirrappalli-620 024, Tamil Nadu, India.
E mail- jahirmaya@gmail.com

ABSTRACT
An efficient protocol for the in vitro plantlet regeneration from nodal explants of Cynoglossum zeylanicum was developed. The nodal explant was inoculated in the MS medium supplemented with 2+1 to 10+5 of BAP+KIN for shooting and 2+1 to 10+5 of NAA+IAA for rooting. The shootlets initiated after 7 days of incubation and the highest combinational concentration (10+5) had the highest number of shoot length 9.66 cm and shoots 15.2 of an average. The lowest concentration 2+1 had 8.6 shoots with 4.84 cm of shoot length. The roots increased with increasing concentration. The highest numbers of roots 8.2 were recorded at 8+4 micromolar combination of NAA and IAA with an average root length of 4.36 cm. The well developed plantlets were hardened and had a survivality of 90% under Ex vitro condition.
Keywords: Micropropagation, Nodal explants, Hormones, Plantlets
Abbreviation: BAP-6- Benzylaminopurine, NAA-Napthalene Acetic acid, IAA-Indole 3- Acetic Acid, KIN- kinetin, µM-micromolar, cm- centimetre

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INTRODUCTION

Plants have formed the basis of a chic traditional medicine system that has been in existence for thousands of years. Herbal medicine system is still the span of about three-quarters of the world's population which rely upon traditional therapies for their health care. Plant materials remain as an important resource to conflict solemn diseases in the world. The flora of India is very outrageous in plant diversity with a predictable 50,000 species, of which about 15,000 are flowering plants, of these 5,000 species are endemic to India, while quite a lot of a hundred species are threatened. Owing to snowball use of medicinal plants, their prospect is being threatened advanced biotechnological methods of culturing plant cells and tissue provide unconventional means for rapid propagation and conservation of rare endangered and commercially important medicinal plants and provide a source for extraction of secondary metabolites.

The technique of *in vitro* regeneration of plants is based on the principle that plants can be separated into their component parts like organs, tissues or cells which can then be manipulated under aseptic culture to be grown back to complete plants. Cell and tissue culture techniques involve the growth of tissue or organ segments on a suitable nutrient medium which stimulates the development of shoot formation and organogenesis used for large scale plant multiplication [1]. *In vitro* regeneration or tissue culture is a method of vegetative propagation conducted in the laboratory condition and it has a significant impact on plant breeding, horticulture and medicine. It is the ever-ready tool for specialization in hybridization either by sexual or asexual methods. It is a clean and rapid way of genetic engineering by which the materials can be grown for identification and manipulation of genes or transfer of characters from one plant to another. The current investigation focuses on the aseptic culture of *Cynoglossum zeylanicum* a Boraginacean member. *Cynoglossum zeylanicum* is also called as the Ceylon hound's tongue, Ceylon forget-me-not, and Indian hound's tongue. The plant has been used in the traditional medicines of tribes and has numerous uses in ethnobotany. The plant has no literature concerned to plant tissue culture and there are a few pharmacological investigations in this plant. The research aims in developing a standard protocol using combination of hormones for improving the plant from being overused.

MATERIAL AND METHODS

The nodal explants from the field grown *Cynoglossum zeylanicum* was surface sterilized with tween, running tap water and distilled water for about 30 minutes. The sterilized explants were inoculated in the autoclaved MS medium augmented with MS salts, B5 vitamins and shooting hormone. The inoculated tubes were incubated at 25 ± 2ºC under 45 $m^{-2}s^{-1}$ photon density for a photoperiod of 16/8Hrs. The plantlets were transferred to rooting medium and finally hardened and acclimatized. The plantlets were analyzed for the physical parameters like shoot length, number and root length by ANOVA and DMRT statistical tools.

RESULT AND DISCUSSION

The nodal explants of *Cynoglossum zeylanicum* initiated after 7 days from the date of inoculation and it showed a high significance in the regeneration frequency. The initiated shoots developed into plantlets that resembled the *in vivo* plant with no somoclonal variations. The MS medium fortified with combinations of cytokinin and auxin for shootlet and rootlet production in the concentration range of 2 to 10 micromolar gave finer results beyond our expectations. This is the first report on the tissue culture of this plant Table-1.

The nodal explants were inoculated vertically into the shooting medium of concentration range 2+1 to 10+5 BAP+KIN. The shootlets initiated after 7 days of incubation at the standard photoperiod and temperature. The shoot number increased with increasing concentration and attained the maximum number of 15.2 at 10+5 µM BAP+KIN. The highest combinational concentration had the highest number of shoot length 9.66 cm. The prime concentration 2+1 had 8.6 shoots with 4.84 cm of shoot length. The rooting medium was supplemented with NAA and IAA in combination of 2- 10 micromolar NAA and 1-5 micromolar of IAA. We observed that the root number increased gradually with increasing concentration and dropped after a particular combination of concentration. The response of root induction from the shootlets of nodal explants ranged between 95-100%. The highest numbers of roots 8.2 were recorded at 8+4 micromolar combination of NAA and IAA with an average root length of 4.36 cm. After this concentration the root numbers decreased to 7.4 and length dropped to 4.04 cm indicating the prior concentration is much suitable for multiple root regeneration in *Cynogossum zeylanicum.*

Nagarajan *et al.,* [2] recorded highest frequency (100%) of shoot induction in 2.0 µM BAP and 1.5 µM KIN with maximum number of shoots 6.4±1.94, 5.4±1.51 respectively from nodal explants of *Bacopa*. The isolated shoots were transferred to rooting medium and then followed by hardening. The shoot tip explants of *Enicostema axillare* in combination at 1.0 mg/l BAP and 0.2 mg/l KIN gave 98.51 % shoot induction with 8.41 shoots/explants. The plants were rooted in half strength IBA medium. The rooted plantlets were successfully hardened with 90 % of survival rate [3]. The result of our investigation is higher when compared to the results in *E. axillare.* The maximum shoot number of our study was 15.2 while Nagarathnamma *et al*., [4] observed 86.8 ± 3.9 shoots on MS medium supplemented with BAP (3mg/l) in combination with NAA (1mg/l). The result is five times greater than our result. BAP in combination with KIN and NAA in combination with IAA were more effective in inducing plantlet production. The result showed harmony with *Swertia chirata* [5-7]. Ashwini *et al.,* [8] reported BAP (10 µM) in combination with KIN (2 µM) had maximum number of shoot 19.33 ± 1.09 per explant from nodal explants of *Exacum bicolor*.

Table 1: Hormonal efficiency of plantlet production in *Cynoglossum zeylanicum* (Vahl) Thunb. ex Lehm

BAP+KIN	NAA+ IAA	PERCENTAGE OF RESPONSE (%)	NUMBER OF SHOOTS	SHOOT LENGTH (cm)	NUMBER OF ROOTS	ROOT LENGTH (cm)
2+1	-	97	8.6±0.54	4.84±0.26	-	-
4+2	-	98	12.6±0.89	5.72±0.25	-	-
6+3	-	100	12.8±0.44	6.64±0.32	-	-
8+4	-	100	13.4±0.54	7.76±0.27	-	-
10+5	-	100	15.2±0.83	9.66±0.30	-	-
-	2+1	95	-	-	4.6±0.54	2.44±0.08
-	4+2	99	-	-	5.8±0.83	2.74±0.11
-	6+3	100	-	-	5.6±0.54	3.42±0.19
-	8+4	99	-	-	8.2±0.44	4.36±0.33
-	10+5	100	-	-	7.4±0.54	4.04±0.55

Mean±Standard deviation of five replicates of three experiments

Fig.1. *In vitro* Multiple shoot induction from the nodal explant of *Cynoglossum zeylanicum*
a. Shoot initiation after 10 days of inoculation;
b&c. Shoot multiplication and proliferation after 15 & 25 days respectively
d. Rooting ; e. Hardening

CONCLUSION

The standard protocol form combination of hormones (cytokinin and auxin) for *Cynoglossum zeylanicum* showed 10+5 micromolar of cytokinin and 8+4 micromolar auxin suited for healthy plantlet production for conservation. This hormonal combination can be used to develop millions of plantlets to meet the commercial need in various fields and study.

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Conflict of Interest: The authors have no conflict of interest

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