



Direct Organogenesis from Nodal Explant of *Cynoglossum zeylanicum* (Vahl) Thunb. ex Lehm

Duraiswamy M, Jahirhussain G*, Saravanan A and Karuniya Raja Viella G

PG and Research Department of Botany, Government Arts College (Autonomous), Thanthonimalai, Karur-639005

Affiliated to Bharathidasan University, Tiruchirappalli-620 024, Tamil Nadu, India.

E mail- jahirmaya@gmail.com

ABSTRACT

An efficient *In vitro* micropropagation protocol for boraginacean member *Cynoglossum zeylanicum* was developed under aseptic condition. The plantlets were regenerated from nodal explants of the healthy elite in the MS medium supplemented with BAP and NAA for shooting and rooting. The hormonal range of the culture medium was 2- 10 micromolar. The nodal explants gave full regeneration frequency for shoot and root. The developed plantlets showed no variation from the *in vivo* plantlets. 10 μ M BAP gave 100 % shoot regeneration frequency with 17.6 ± 0.54 number of shoots that had a shoot length of 5.48 ± 0.31 cm. The rooting was best observed at 10 μ M NAA that had 8.6 ± 0.54 roots of 4.34 ± 0.11 cm lengthy roots. The well developed plantlets were hardened and acclimatized. The study aid in multiple plantlet production of *Cynoglossum zeylanicum* that can be used for further advanced studies and research.

Keywords: Micropropagation, Nodal explants, Hormones, Plantlets

Abbreviation: BAP-6- Benzylaminopurine, NAA-Napthalene Acetic acid, μ M- micromolar, cm- centimetre

Received 06.05.2022

Revised 25.05.2022

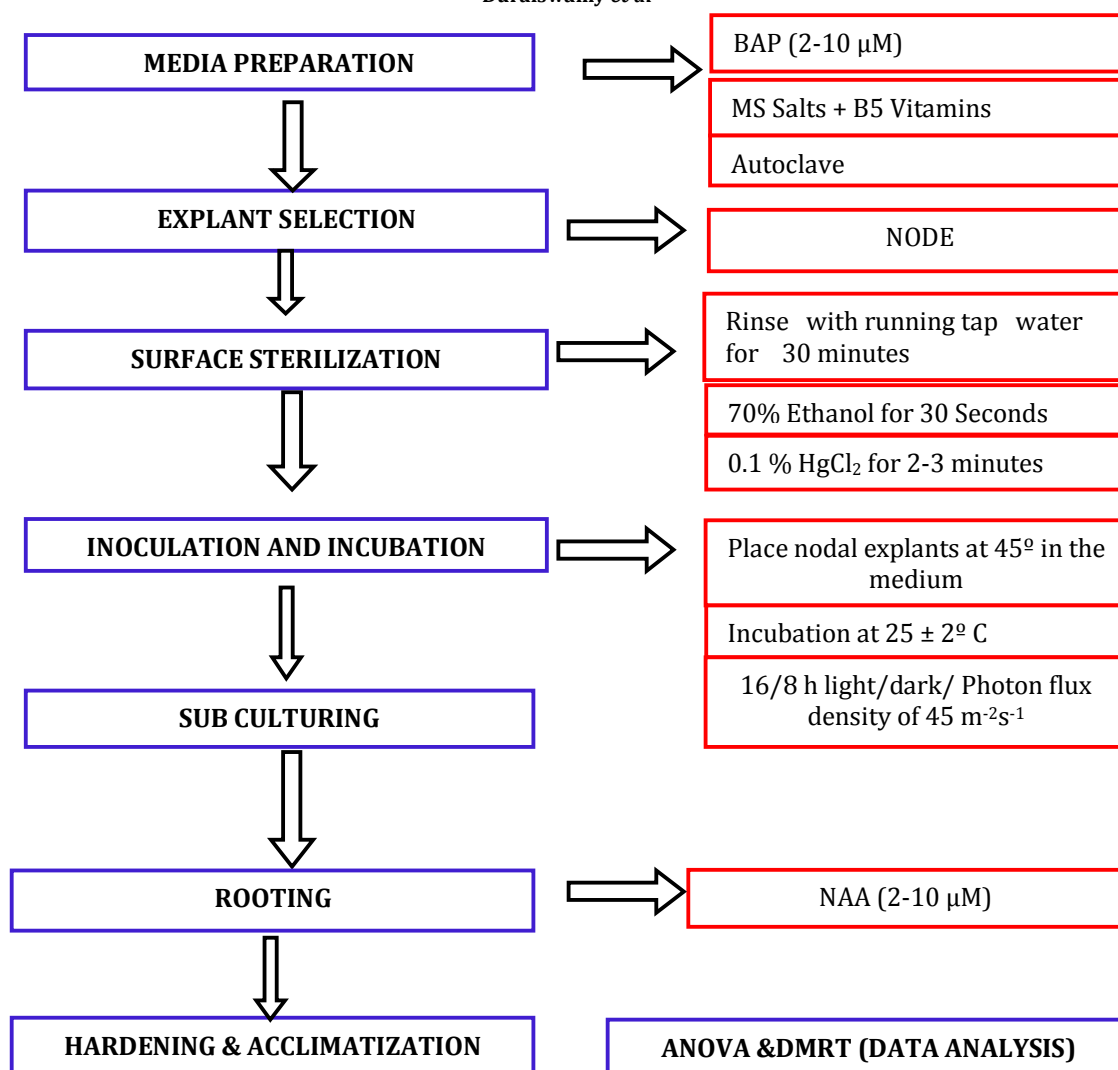
Accepted 12.06. 2022

INTRODUCTION

The earth inhabits the most vital and complex feature called biodiversity. Life interplays with the physical features and the network interactions have made earth a living planet. The enormous diversity of plant kingdom makes the mankind curious and exploring the hidden wealth. The plants with curative chemo-compounds are the medicinal plants. The plant has been destroyed to a greater extent by anthropogenic activities. So it becomes an adverse need to conserve the plants from depletion to maintain the equilibrium. One such technique is the plant tissue culture. It helps in conserving and elucidation of phytochemicals for drug discovery and other important needs of mankind. The plant *Cynoglossum zeylanicum* belongs to the family boraginaceae. The genus contains 75 species and is commonly called as hound's tongue. Common names of the plant *Cynoglossum zeylanicum* include Ceylon Forget Me Not in English, Hindi- Andhahuli, Malayalam- Kayooram, Marathi- Lichardi, Tamil- Picinottarai, Telugu- Kada Anthrinta, and Tapa in Tibet. The plant is an erect herb of 1 m tall. The plant is a soft pubescent with alternate leaves that are oblong, lanceolate with cute apex. *C. zeylanicum* is a medicinal herb that is being employed in traditional system of medicine. It is being reported to possess antiseptic, corneal conjunctivitis. It dissolves uterine tumors, takes out lymph fluids; leaf juice aids in making eye drops and used as ailment for sores, swellings, cough and fractured bone [1]. Maruthiah Anitha *et al.*, [2] reported anti-inflammatory activity of *C. zeylanicum*. It is said to be used in treatment of skin infection [3]. The plant has undergone pharmacological studies but it is not revealed for other branches of study. The study aims in investigating the other areas of science that could uplift the plant species and aid in human health.

MATERIAL AND METHODS

The nodal explant 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 $25 \pm 2^\circ$ C under $45 \text{ m}^{-2}\text{s}^{-1}$ photon density for a photoperiod of 16/8. The plantlets were transferred to rooting medium and finally hardened and acclimatized. The data's were collected and analyzed using ANOVA and DMRT.



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. The plant has been studied for ethnobotanical and pharmacological activities.

The plantlets from the nodal explants of *Cynoglossum zeylanicum* in the MS supplemented hormonal medium gave the regeneration response from 95 and above. Some of the cultures showed hyperhydricity so they did not give full response. The highest response of the explants was seen in the highest concentration 10 micromolar in both shooting and rooting. The highest concentration gave much finer result than the other tested hormones. The number of shoots and roots and their length gradually elevated with increasing concentration and we found no interruption. The highest shoot number was 17.6 with a standard deviation of 0.54 having a shoot length 5.48 cm. If we look from the highest concentration the parameters show a descending trend. The same trend was observed in rooting medium also, the highest hormonal concentration gave 8.6 ± 0.54 number of roots with a root length of 4.34 ± 0.11 cm. The lowest concentration reported 3.8 ± 0.83 roots having 2.32 ± 0.34 cm as their root length.

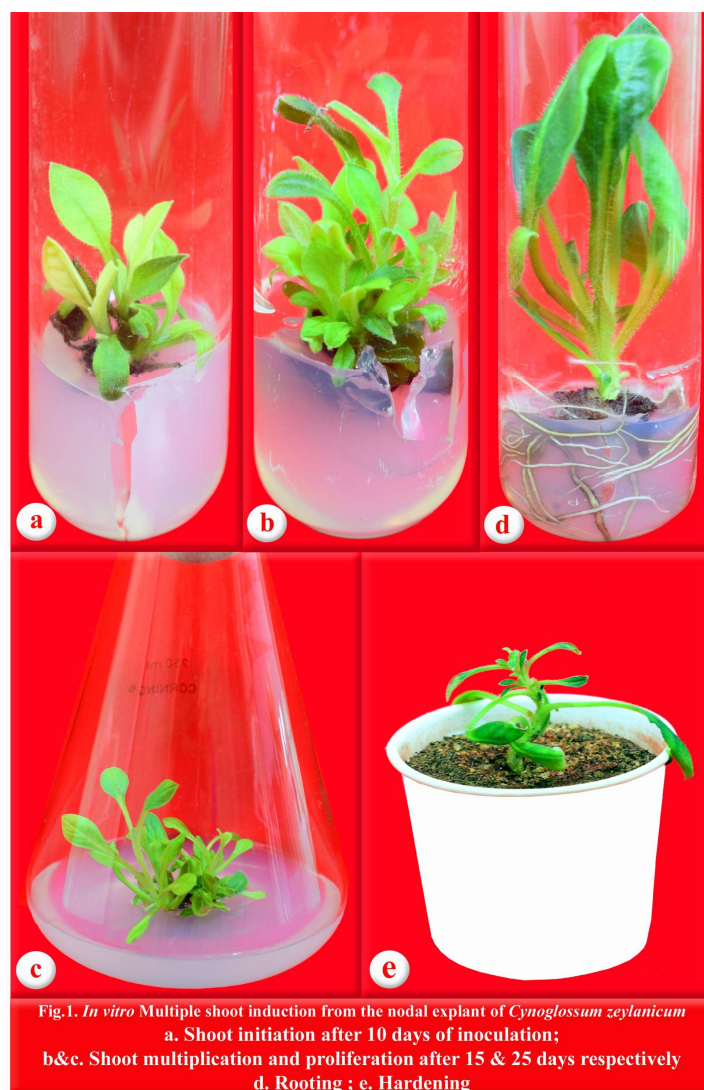
The investigation reported a lowest number of 12 shoots in the BAP supplemented medium but Zakia *et al.*, [4] recorded highest number of shoots 11.8 shoots per explants with a maximum shoot length of 12.15 cm in *Aloe vera* at 0.5 mg l^{-1} . Mahipal *et al.*, [5] reported 13.2 shoots maximum and 5.2 cm shoot length from nodal explants of *Rungia pectinata* L at the combinational concentration of 0.5 mg/L BAP and KIN + 0.1 mg/L IAA. BA was recorded to be the best cytokinin for shoot regeneration by Sahoo and Chand [6]. Several studies accorded BAP as the suitable medium for shoot induction and regeneration [7-13]. Kumari *et al.*, [14] recorded 18 shoots from nodal explants of *Bacopa monnieri* and the root induction frequency was 95% with 12 roots and of root length 5.6 cm in IBA rich medium. The result was higher

than compared with our investigation. Similarly Rashid, [15] reported 85.3 shoots in 1.0 mg/l BAP with 6.8 cm shoot length and at last summarized that NAA was effective than IBA when used alone for rooting. This investigation reports the first report of *Cynoglossum zeylanicum* for plant tissue culture.

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

BAP	NAA	PERCENTAGE OF RESPONSE (%)	NUMBER OF SHOOTS	SHOOT LENGTH (cm)	NUMBER OF ROOTS	ROOT LENGTH (cm)
2	-	95	12.8±0.83	3.08±0.42	-	-
4	-	97	14.8±0.83	3.54±0.20	-	-
6	-	98	15.6±0.54	3.82±0.31	-	-
8	-	99	16.8±0.44	4.48±0.23	-	-
10	-	100	17.6±0.54	5.48±0.31	-	-
-	2	96	-	-	3.8±0.83	2.32±0.34
-	4	98	-	-	4.4±0.54	2.56±0.11
-	6	100	-	-	5.4±0.54	3.26±0.23
-	8	99	-	-	6.2±0.44	3.72±0.49
-	10	100	-	-	8.6±0.54	4.34±0.11

Mean±Standard deviation of five replicates of three experiments



CONCLUSION

The mass production of *Cynoglossum zeylanicum* using a standard protocol was successful at the highest concentration which can be used for commercial production of the plantlet within a short span of time.

These plantlets can be used for *Ex vitro* culture of the plant and phytochemical extraction for pharmacological studies. The future studies can aid in helping humans for their benefits.

ACKNOWLEDGEMENT

We extend our sincere gratitude to Dr. N. Thajuddin, Professor & Head, Department of Microbiology, Bharathidasan University, Tiruchirappalli – 24, Dr. S. Palanivel, Head, Department of Botany, Govt. Arts college, Karur -5, Dr. R Pari, Former Principal, Govt. Arts College, Karur, and Dr. C. Jothi Venkateswaran, DCE, Chennai for their valuable support and guidance.

Conflict of Interest: The authors have no conflict of interest

Author Contributions: **Duraismamy M-** Contributed in conducting experiment, collecting and analysing data, paper preparation; **Dr. Jahirhussain G-** Research supervisor and Research designing; **Saravanan A-** Article correction and Data analysis ; **Karuniya Raja Viella G-** Research assistance and data interpretation.

REFERENCES

1. Kalpana Joshi. (2016). *Cynoglossum L.*: A review on phytochemistry and chemotherapeutic potential. *Journal of Pharmacognosy and Phytochemistry*, 5(4): 32-39.
2. Maruthiah Anitha., Edison Dalmeida Daffodil., Sabapathy Muthukumarasamy., & Veerabahu Ramasamy Mohan. (2013). Anti-Inflammatory activity of whole plant of *Cynoglossum Zeylanicum* (VAHL Ex Hornem) Thunb. Ex. Lehm, *Journal of Advanced Pharmacy Education & Research*, 3 (1): 20-23.
3. Francis Xavier, T., Kannan M., & Auxilia, A. (2015). Traditional Medicinal Plants Used in the treatment of different skin diseases. *Int.J.Curr.Microbiol.App.Sci*; 4(5): 1043-1053.
4. Zakia Shehnaz, Yousaf Zahid Najma, Yaseen Mehwish, Abbasi Nadeem, Hafiz Ishfaq, & Mohamood Nasir. (2016). Standardisation of micropropagation techniques for *Aloe vera*: A pharmaceutically important plant, *Pakistan Journal of Pharmaceutical Sciences*, 26: 1083-7.
5. Mahipal S Shekhawat, Manokari, M., & Ravindran, C. P. (2016). Micropropagation, Micromorphological Studies, and *In vitro* Flowering in *Rungia pectinata L.*, *Scientifica*, 7 pages.
6. Sahoo, Y., & Chand, P.K. (1998). Micropropagation of *Vitex negundo L.* a woody aromatic medicinal shrub, through high frequency axillary shoot proliferation, *Plant Cell Rep.*, 18: 301-307.
7. Kannan K.R., & Jasrai, Y.T. (1996). Micropropagation of *Gmelia arborea*, *Plant Cell Tiss. Org. Cult.*, 46: 269-271.
8. Sarker, R.H., Islam, M.R., & Hoque, M.I. (1997). *In vitro* propagation of Neem (*Azadirachta indica A. Juss.*) plants from seedling explants – *Plant Tiss. Cult.*; 7: 125 – 133.
9. Handique, P.J., & Bora, P. (1999). *In vitro* regeneration of medicinal plant *Houttuynia cordata* Thunb. from nodal explants. *Curr. Sci.*; 6: 1245-1247.
10. Komalavalli, N., & Rao, M.V. (2000). *In vitro* micropropagation of *Gymnema sylvestre* - A multipurpose medicinal plant. *Plant Cell Tiss. Org. Cult.*, 61:97-105.
11. Jawahar, M., Amalan Robert, G., & Jeyaseelan, M. (2004). Rapid Proliferation of Multipleshoots in *Solanum trilobatum L.* *Plant Tissue Culture*, 14: 107-112.
12. [12]. Sanatombi., & Sharma, G.J. (2007). Micropropagation of *Capsicum annuum L.*, *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 35: 57-64.
13. Pooja Bhatnagar., Madhurima Bhatnagar., Amarjit Nath, K., & Sharma, D.R. (2004). Production of Solasodine by *Solanum laciniatum* using Plant Tissue Culture Technique, *Indian Journal of Experimental Biology*, 42: 1020-1023.
14. Ritika Kumari., Meenakshi Priyadarshni., Kumari Anjali., & Shukla, L. N. (2014). *In Vitro* Mass Multiplication of *Bacopa monnieri (L.)* An Endangered and Valuable Medicinal Herb, *Indian J.Sci.Res.*, 7 (1):1248-1253.
15. Rashid, M.H., Khalekuzzaman, M., Hasan, M.F., Das R., Hossain M.S., & Mahabbat- E Khoda, S. (2009). Establishment of An Efficient Method for Micropropagation of an Important Medicinal Herb (*Scoparia dulcis L.*) From Shoot Tips and Nodal Segments, *Int. J. Sustain. Crop Prod.*, 4(1): 5-9.

CITATION OF THIS ARTICLE

Duraismamy M, Jahirhussain G, Saravanan A and Karuniya Raja Viella G. Direct Organogenesis from Nodal Explant of *Cynoglossum zeylanicum* (Vahl) Thunb. ex Lehm. Bull. Env.Pharmacol. Life Sci., Vol 11 [8] July 2022 : 97-100