Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI:10.26479/2019.0501.48**

EFFECT OF 6-BAP ON CALLUS CULTURE AND SHOOT MULTIPLICATION OF *COLEUS FORSKOHLII* (SYN: *PLECTRANTHUS FROSKOHLLI*; WILD) BRIQ.

Raj Kamal Vibhuti [1*], Deepak Kumar [2]

1. Department of Microbiology, Central University of Haryana, Mahendragarh, India.

2. Renaissance Biotech. Pvt. Ltd., Patna, India.

ABSTRACT: *Coleus forskohlii / Coleus barbatus* (Lamiaceae family) is an important plant in Indian Ayurvedic medicine. It is the only source of forskolin among the plant kingdom. It is an important medicinal plant with excellent potential in herbal drug trade. The root of this plant is medicinally useful for high blood pressure, spasmolysis, obesity and constipation. The present work was aimed at effect of 6-benzylaminopurine (6-BAP) on callus culture and shoot multiplication of *Coleus forskohlii.* Tissue explants from leaf, node and shoot tip were cultured on Murashige and Skoog medium supplemented with different concentrations (0.5 - 2.0 mg/l) of 1-Naphthaleneacetic acid, 2, 4-Dichlorophenoxyacetic acid 6-benzylaminopurine and Kinetin. Shoot multiplication was obtained in vitro within 20-25 days from shoot tip explants of 30 days old aseptically germinated seedlings of *Coleus forskohlii*, using 2 mg/l of 6- benzylaminopurine (6-BAP). Shoot multiplication was further enhanced with the gradual decrease in the level of BAP, and its final omission after 4 months. It was observed that shoot tip elicited maximum callusing, shooting and rooting response than that of leaf and node. Multiple shoots were obtained from shoot tip explants and callus from leaf explants.

KEYWORDS: *Coleus froskohlii*, Froskolin, *Coleus barbatus*, 6-Benzylaminopurine.

Corresponding Author: Raj Kamal Vibhuti*

Department of Microbiology, Central University of Haryana, Mahendragarh, India.

Email Address: rajkamalvibhuti@gmail.com

1.INTRODUCTION

Plants are the first medicines for mankind and thousands of plant species are harvested for their medicinal properties all over the world. *Coleus forskohlii* is an important indigenous medicinal plant

in India. It has been used in traditional Ayurvedic medicine for curing various disorders and this is the only source of the diterpenoid forskolin. Forskolin is used for the treatment of eczema, asthma, cardiovascular disorders and hypertension [1]. *C. forskohlii* is a member of the mint family, Lamiaceae. It is indigenous to India and is documented in Ayurvedic *Materia Medica* under the Sanskrit name 'Makandi' and 'Mayani" [2]. Indian sub- continjment is considered as the place of origin of *C. forskohlii* [3]. It grows wild in the subtropical temperate climates of India, Nepal, Burma, Sri Lanka and Thailand. Apparently, it has been distributed to Egypt, Arabia, Ethiopia, tropical East Africa and Brazil [4]. In India, the plant is found mostly on the dry and barren hills [5]. *C. forskohlii* is a perennial plant that grows to about 45 - 60 cm tall. It has four angled stems that are branched and nodes are often hairy. Leaves are 7.5 to 12.5 cm in length and 3 to 5 cm in width, narrowed into petioles. *C. forskohlii* is the only species of the genus to have fasciculate tuberous roots. The entire plant is aromatic [6]. The leaves and tubers have quite different odors [7, 8]. *Coleus forskohlii Briq*. (Lamiaceae) is a highly important subtropical, warm temperate medicinal species, which grows wild in the subtropical Himalayas. The species is highly valued for a labdane diterpenoid alkaloid 'forskolin', which is mainly present in the brownish red tuberous roots of this plant that is the only source of forskolin [9]. Forskolin-based drugs are widely used for treatment of congestive cardiomyopathy, glaucoma, obesity, and asthma [3]. It is characterised by a hypotensive property and inhibitory action on thrombocyte aggregation [9]. *C. forskohlii* is the only source of this compound detected so far. Indiscriminate collection of this plant from their natural habitat is leading to depletion of its resources [10]. Hence, a procedure for rapid *in vitro* propagation of this species has been described in this paper for production of high drug yielding plants [11, 12, 13]. The scope of this study includes conditions of explanting, effects of 6-BAP and other plant hormones on shoot multiplication. Recently medicinal plants occupy an important place in health care, cosmetics and food industries throughout the world [14]. Herbal drugs are more preferred than allopathic drugs because of higher efficacy, affordability, easy availability and causing less or nil side effects. Even western world begins to use herbal drugs and herbal formulations described in traditional medicines and Indian traditional medicines like Ayurveda for curing various diseases [15]. In order to ascertain quality of herbal drugs, their identity, quality, efficacy and safety are to be established standardization of herbal drugs thus include establishment of botanical identity, cultivation or collection, harvesting, processing, storage, preservation, formulation and packaging, therapeutic efficacy of herbal drugs depend upon the quality and quantity of biological active compounds. Plant tissue culture is a modern tool available to rapidly propagate plants. It can be successfully used to conserve rare and endangered medicinal plants and multiply them in a short duration [16, 17, 18]. Tissue culture technique is potentially valuable for studying the biosynthesis of secondary product and may also eventually provide an efficient means of producing commercially important plant products [19]. Tissue explants of an important Indian medicinal plant, *Coleus forskohlii* were taken

from the hardening unit of plant tissue culture laboratory, cultured *in vitro* and their growth responses like callusing, shooting and rooting were elucidated in the present investigation. Explants from leaf lamina, node and shoot tip were cultured on Murashige and Skoog medium [21] supplemented with different concentration and combination of plant hormones of auxins like 1-Naphthaleneacetic acid, 2,4-dichlorophenoxyacetic and cytokinin like 6-benzylaminopurine and kinetin.

2. MATERIALS AND METHODS

All explants were soaked 1-2 Hrs. in water, washed with 5% Teepol detergent solution for 10 min and surface sterilized with 0.1% of $HgCI_2$ for 8-10 min [22]. Explants were rinsed thrice in sterile distilled water. For shoot tip, leaves and nodes multiplication, 0.7-1 cm long shoot tips with two cotyledonary leaves from 20 and 30 days old aseptically germinated plants and nodal segments and excised leaves from one month old regenerated shoots [23], were cultured on induction medium consisting of MS basal medium [24] supplemented with different concentrations and combinations of auxins and cytokinins. The pH of the media was adjusted to 5.6, solidified with 0.8% agar-agar, and sterilised for 15 min at 1.05 kg/cm^2 pressure. Explants were transferred to fresh medium every 3-4 weeks. Each experiment was set up with three replicates and repeated thrice. The cultures were grown at 22±2^{O}C with a maximum relative humidity of 55-60% under Philips fluorescent day light tubes emitting 2000 lux for 16/8 h light/dark period [25, 26, 27, 28].

3. RESULTS AND DISCUSSION

Total 440 explants inoculated in which leaves, node and shoot tip are respectively 180, 200 and 160. Different explants like leaf, node and shoot tip of sterilized *Coleus forskohlii* were inoculated in solid MS media. Prior to treatment with plant growth regulators NAA at 1.0 mg/L in BAP at 2.0 mg/L and Kinetin at 1.0 mg/L, after 7 days, it was noticed that adventitious shoot from shoot tip (Fig 1) and initiation of callus from leaf (Fig 2 and Fig 3) started originating directly from inoculated explants [29, 30].

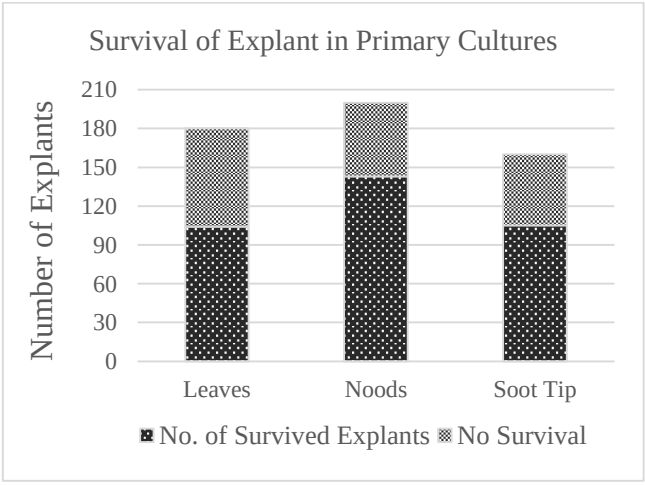

Fig 1: Survival of explant in primary culture

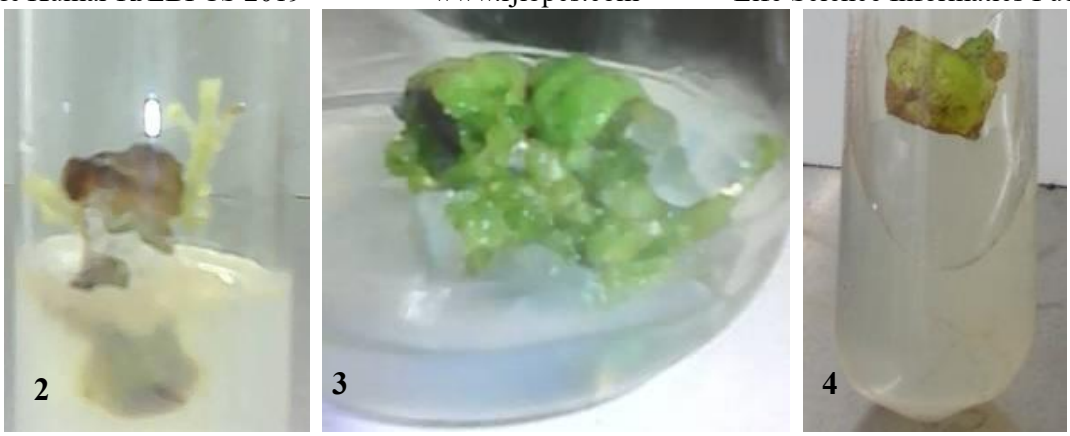

Fig 2-4: 2, Initiation of shoot from the shoot tip explants in 2.0 mg/L BAP. 3, Initiation of callus from the leaf explants in 1.0 mg/L Kinetin. 4, Initiation of callus from the leaf explants in 2.0 mg/L BAP

Only those cultures showing healthy and non-contaminated plantlets were transferred to media containing different growth hormones and cultured for 30 days. Shoots were originated directly at the axillary position of the nodal explants in presence of cytokinins in the medium. Among two cytokinins (BAP and Kinetin), BAP at 1.5 mg/L was found to be superior and generation of multiple shooting (Fig 4 and 5).

Fig 5: Generation of multiple shoots in BAP 1.5 mg/L

In leaf explants cultured on MS medium supplemented with BAP (2mg/L) + 2,4-D (1.5mg/L) produced white and friable callus (Fig 6). The shoot tip explants were cultured on different concentration of BAP induced both multiple shooting and callusing (Fig 7). The medium with BAP (1mg/L) + 2,4-D (1.5mg/L) induced green and friable callus in nodal explants.

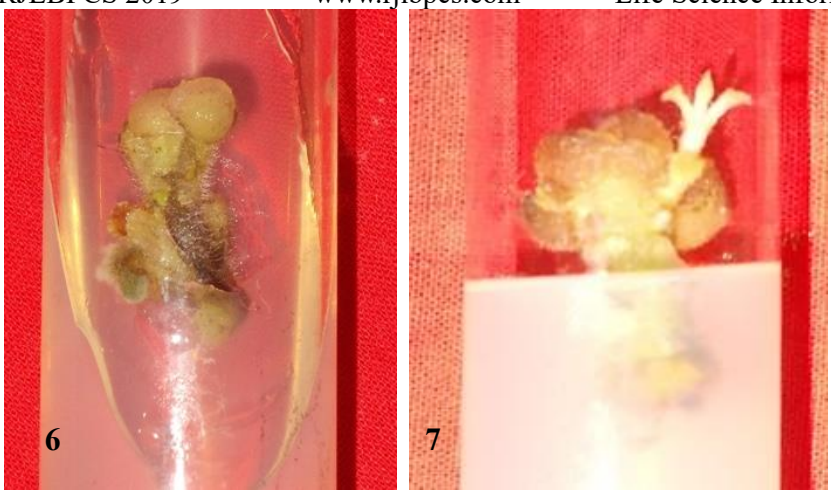

Fig. 6 & 7: 6, White and friable Callus in BAP 2mg/L+2,4-D 1.5mg/L. 7, Shoot tip explant shows both multiple shooting and callusing in BAP 2.0 mg/L

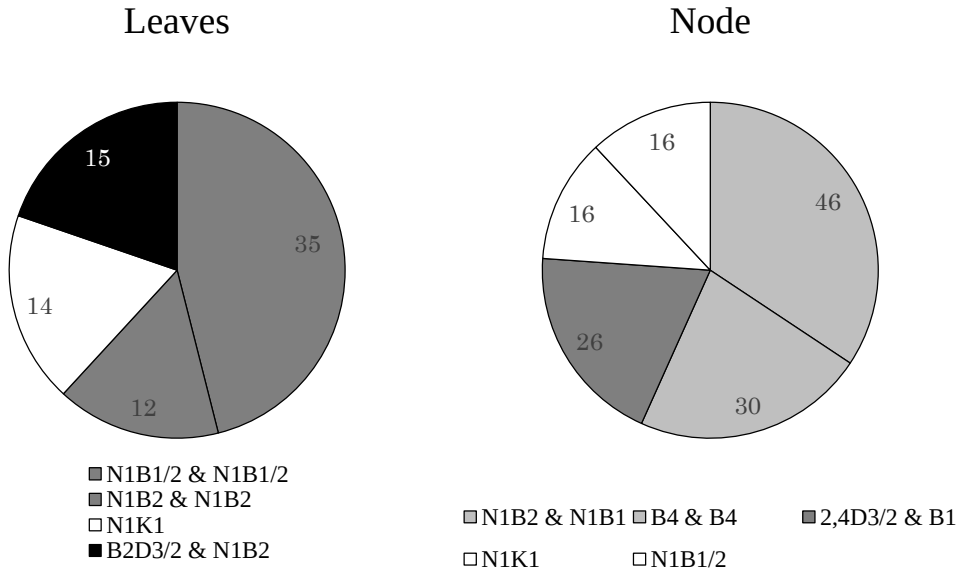

Fig. 8: Sub culturing of survived leaves and node explants after primary culture. Medium of 1st sub culturing and 2nd sub culturing showing in colourful bullets. White background shows no response in 1st culture, light grey background shows no response after 2nd sub culture grey background shows green callus and black shows white friable callus.

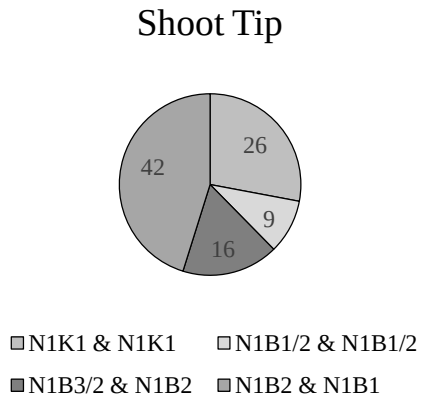

Fig. 9: All shoot tip explants are survived in primary culture. Medium of 1st sub culturing and 2nd sub culturing showing in colourful bullets. 9 explants give rise to 1-2 shoots, 26 explants give rise to 2-3 shoots, 42 explants give rise to 4-5 shoots and 16 explants give rise to 4-6 shoots.

4. CONCLUSION

From the above result and the table given below it was observed that Callus was initiated in all the explants after 1 week in culture. The different concentration of 6-benzylaminopurine induced more growth compared to Kinetin (Fig. 8 & 9). At the 2.0 mg/L concentration of BAP with 1.5 mg/L concentration of 2,4-D leaf explants showed white and friable callus. At the 2.0 mg/L concentration of BAP, nodal explants showed maximum growth in comparison to other concentration of BAP or Kinetin. Induction of multiple shoots were absent in nodal explants culture containing medium BAP (1 mg/L) + 2,4-D (1.5 mg/L). Shoot tip of *Coleus forskohlii* is suitable for induction of morphogenesis in culture as maximum multiple shoots were obtained from it. MS media Supplemented with higher concentration (2.5 mg/L) of BAP showed good callus induction of explants.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Alok Narain Singh, Head, Department of Botany, Maharaja College, Ara, and Dr. Binod Kumar Sinha, Coordinator Department of Bio-Technology, Maharaja College, Ara, for providing laboratory facilities for some experiments.

CONFLICT OF INTEREST

Both authors declare that they have no conflict of interest.

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