

## STANDARDIZATION FOR CLEAN MOTHER CULTURE AND MICROPROPAGATION FROM FIELD GROWN PLANT OF *COLEUS FORSKOHLII*

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### ABSTRACT

A protocol was developed for *Coleus forskohlii* for high frequency shoot organogenesis from apical buds and shoot tips on MS (Murashige & Skoog, 1962) medium supplemented with different concentrations of 6-benzyl amino purine (BAP) or Kinetin with added charcoal. The highest rate of shoot growth and survival was achieved at the hormone BAP 0.5 mg/lit. Where highest multiplication of shoots on MS medium supplemented with BAP 2.0 mg/l and high rooting frequencies were obtained in basal media. Regenerated plants were easily acclimatized in greenhouse conditions and later transferred to soil with 90% survival. This protocol yielded a higher number of shoots within a short period. Consequently, the protocol developed in this study offers a simple and improved in vitro method to regenerate *C. forskohlii*.

**Keywords:** *Coleus Forskohlii*, Clean Culture, Micropropagation, 6-Benzyle Amino Purine, Kinetin

### INTRODUCTION

*Coleus forskohlii* is a perennial herb of Labiatae (Lamiaceae) that includes the favorable herbs and mints and lavenders. *C. forskohlii* is also known as Makandi, Mayini and Puravai. This plant grows wild in warm, subtropical and temperate areas in India, Burma, Thailand, Bhutan and Nepal (Saha *et al.*, 1980). It grows well at 600-1800 meter elevation in loamy and sandy loams soil with pH of 6.4-7.9. The plant is valued for the production of forskolin (diterpene) drug which is used in glaucoma, congestive cardiomyopathy and asthma (Valdes *et al.*, 1987) hypertension, psoriasis, eczema, anti obesity (Henderson *et al.*, 2005). Forskolin is found in almost all part of the plant, roots are the main source of compound (Shah *et al.*, 1980). The principle mechanism by which forskolin exerts its hypotensive activity is by stimulation of adenylate cyclase and thereby increasing cellular concentrations of the second messenger cyclic AMP (cAMP) (Seamon *et al.*, 1981). Forskolin content from natural habitats of dry weight of root varies from 0.04 – 0.44% (Viswakarma *et al.*, 1988). *C. forskohlii* plant is also categorized in endangered species and need to be conserve. Natural calamities such as typhoons, floods, droughts cause devastating losses in *C. forskohlii* plantation. The consequent need for fresh seedlings at regular intervals has led to very large increase in the demand for clean planting material. Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants under aseptic conditions using modern plant tissue culture methods with the advantage of rapid multiplication, requirement of fewer mother plant, product uniformity, seasons' independent production, disease free, and high return. The present study aims at developing protocol for clean mother culture from shoot tip explants of *C. forskohlii* so as to give rise to true - to - type clones for potential application in large - scale propagation.

### MATERIALS AND METHODS

Young branches of *Coleus forskohlii* were collected from CIMAP Bangalore and were identified by Taxonomist. Explants were grown in pots with suitable soil conditions. Shoot tip explants was collected from potted plants and processed for aseptic culture. Explants were surface sterilized by cleaning thoroughly under running tap water for 10 min. The explants were then surface sterilized with detergent wash for 15 minutes and are rinsed 4-5 times tap water for 30 minutes. Then, after wash with 70% alcohol for 3-5 second only and wash with double distilled water for 3-4 times. Further sterilization procedure

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was carried out inside Laminar air flow chamber. The explants were then dipped into 0.1% bavistin for 45 minutes, add the cleaned explants were finally treated with HgCl<sub>2</sub> (0.05%) for 10 min then add HgCl<sub>2</sub> (0.1%) to this solution and wait for 5 minutes. Finally wash under aseptic condition with sterile distilled water for 4-5 times. After surface sterilization the explants were trimmed into small uniform pieces and inoculated in seven different concentration and combinations of MS supplemented media with hormones BAP (0.0, 0.5, 1.0, 2.0 mg/l), Kinetin (0.5, 1.0, 2.0 mg/l) with added charcoal, prepared before 48 hrs. of inoculation. All cultures were maintained 20-25°C in fluorescent tube light (40-80  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 7-10 days. The grown shoot tips were subculture in same media. Further, explants of basal media and 0.5 mg/lit BAP were subculture in higher concentration (1.0 and 2.0 mg/lit) of BAP supplemented MS medium for multiple shoots growth then observe for viability, shoot length and number of leaves during incubation. After incubation of 20-25 days the individual shoots with two to three nodes were inoculated on MS medium supplemented with (0.0, 0.2, 0.5 and 1.0 mg/lit) of IAA for 30-40 days and observed for best rooting. Each set of experiment were repeated twice. The well developed rooted plants were removed from cultured bottles, and then washed to make them free of agar. These rooted plantlets were transplanted in a sterilized mixture of garden soil and sand (1:1) in small plastic pots and leave in shaded area for 3-4 weeks for hardening process. These plants were transferred in field for further growth. The statistical analysis was done using MS Excel software.

## RESULTS AND DISCUSSION

The present study of *in-vitro* propagation of *C. forskohlii* includes multiple shoot formation from shoot tip explants, elongation of regenerated shoots, rooting and hardening of the elongated shoots. In the regeneration experiments, the shoot tips explants were inoculated on MS medium supplemented with BAP (0.5, 1.0 and 2.0 mg/L) or kinetin (0.5, 1.0 and 2.0 mg/L) with added charcoal. Survivability and shoot bud formation was observed (Table 1). The frequencies of explants survivability and forming shoot buds was maximum with 0.5 mg/lit BAP concentration but the growth on other concentration were also significant (Figure a). The maximum  $2.8 \pm 0.23$  shoots per explants was observed at 2.0 mg/lit BAP in the initial culture (Table 1). After four weeks every explants were subcultured and  $4.5 \pm 0.43$  shoots could be recovered up to the first subculture. The number of shoots could be recovered up to the second subculture, resulting in  $6.9 \pm 0.43$  shoots from a single explant (Figure b). No significant shoot generation observed in kinetin hormone combination media. These explants were transferred to rooting media and after 20 days best root growth was found in basal MS media in compared with IAA supplemented media (Table 2). Afterward, individual cups with single plant were partially covered with polythene bag to maintain high humidity. When the plants had shown signs of new leaf growth, the polythene covers were removed. The rooted plants were acclimatized in garden and about 90% of plants survived in the hardening process, and then the plants were established successfully in the experimental field. The present protocol of *in vitro* propagation technique holds a huge probable for a large scale propagation and conservation of *C. forskohlii* which is medicinally important.



**Figure a–c: Direct Regeneration of Shoots from Shoot Tip Explants of *C. Forskohlii* on MS Medium; (a) Shoot Bud Initiation on BAP (0.5, 1.0 control and 2.0 mg/L); (b) Development of Multiple Shoots in Second Subculture on BAP (0.5, 1.0 and 2.0 mg/L); (c) Explants with Rhizogenesis**

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**Table 1: Effect of Different Concentrations of BAP and Kinetin on Multiple Shoot Formation from 50-60 Days Old Shoot Tip Explants of *C. Forskohlii* on MS Medium**

| Plan Regulator | Growth | Number Explants Inoculated | of | Percentage Responded Explant | of | Initial Culture       |     |                                  | First Subculture      |     |                           | Second Suculture      |     |                           |
|----------------|--------|----------------------------|----|------------------------------|----|-----------------------|-----|----------------------------------|-----------------------|-----|---------------------------|-----------------------|-----|---------------------------|
|                |        |                            |    |                              |    | No. Shoots of Explant | per | Average Height of Explants (cms) | No. Shoots of Explant | per | Average height of explant | No. shoots of explant | per | Average height of explant |
| Control        |        | 25                         |    | 60%                          |    | 2.0±0.25              |     | 0.9±0.09                         | 2.1±0.25              |     | 1.1±0.08                  | 2.5±0.3               |     | 1.2±0.09                  |
| BAP 0.5 mg/lit |        | 25                         |    | 68%                          |    | 2.3±0.31              |     | 1.2±0.12                         | 3.3±0.52              |     | 1.4±0.15                  | 3.3±0.35              |     | 1.5±0.12                  |
| BAP 1.0 mg/lit |        | 25                         |    | 58%                          |    | 2.5±0.15              |     | 1.4±0.15                         | 3.8±0.55              |     | 1.7±0.25                  | 4.9±0.32              |     | 1.8±0.15                  |
| BAP 2.0 mg/lit |        | 25                         |    | 60%                          |    | 2.8±0.23              |     | 1.6±0.17                         | 4.5±0.43              |     | 1.9±0.37                  | 6.9±0.43              |     | 1.9±0.27                  |
| Kinetin mg/lit | 0.5    | 20                         |    | 32%                          |    | 1.2±0.22              |     | 0.7±0.05                         | 2.2±0.27              |     | 0.8±0.06                  | 3.2±0.25              |     | 0.9±0.07                  |
| Kinetin mg/lit | 1.0    | 20                         |    | 24%                          |    | 1.1±0.12              |     | 0.5±0.06                         | 1.5±0.15              |     | 0.7±0.07                  | 2.1±0.22              |     | 0.7±0.06                  |
| Kinetin mg/lit | 2.0    | 20                         |    | 20%                          |    | 1.2±0.21              |     | 0.6±0.08                         | 2.0±0.11              |     | 0.6±0.08                  | 3.2±0.31              |     | 0.8±0.05                  |

**Table 2: Effect of IAA Root Induction from Regenerated Shoots of *C. Forskohlii***

| Plant Growth Regulator - IAA | Percentage of Culture Producing Roots | Mean Number of Roots / Shoots | Mean Length of Roots (cms) |
|------------------------------|---------------------------------------|-------------------------------|----------------------------|
| Control                      | 93%                                   | 6.6±0.54                      | 3.8±0.36                   |
| IAA 0.2 mg/lit               | 90%                                   | 5.6±0.43                      | 3.2±0.26                   |
| IAA 0.5 mg/lit               | 90%                                   | 5.2±0.33                      | 2.8±0.33                   |
| IAA 1.0 mg/lit               | 88%                                   | 5.0±0.36                      | 2.9±0.41                   |

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