

High Frequency and Rapid *in vitro* Plant Regeneration of *Coleus forskohlii* Briq.

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Abstract

Coleus forskohlii Briq. (Lamiaceae) is an important medicinal plant with excellent export potential in herbal drug trade. The pharmacological properties of *Coleus forskohlii* makes it useful to cure high blood pressure, spasmodic, obesity and constipation, asthma and certain cancers. The present work is aimed to develop a protocol for minimizing time duration to get complete plants through tissue culture technique. Within 35 ± 2 d using shoot tips as explants; we developed complete plants of *Coleus forskohlii* in MS medium containing 2.0 mg/l BAP through direct multiplication at the rate of 15-20 shoots per explant. The rooting occurred in all the plants and survival rate of full grown plants is 100%. This protocol is very efficient for the mass multiplication of *Coleus forskohlii* and is the fastest method as compared to all the reported ones.

Keywords: 6-Benzylaminopurine; *Coleus forskohlii*; Shoot tip; tissue culture

Introduction

Coleus forskohlii Briq. is a herbaceous plant with annual stem and perennial root stock of the mint family (Lamiaceae), with a strong, camphor-like odor that was first discovered in the lower elevations of India. It grows at 600-1800 m elevation on sun-exposed hill slopes and plateaus in arid and semiarid climatic zones. In India it occurs at high altitude up to the height of 2500 m in Himalayas in the regions from Garhwal kumamon to Nepal. It also occurs in Deccan Peninsula and Parasnath hills in Bihar [1]. Medicinal plants occupy an important place in human health, food and cosmetics industries all over the world. Herbal drugs are more preferred than allopathic drugs because of higher efficacy, affordability, easy availability and causing less or nil side effects. *Coleus forskohlii* is an important plant in Indian Ayurvedic medicine because its roots produce a labdane diterpenoid, forskolin [2]. Many members of the *Coleus* species (Lamiaceae), such as *Coleus forskohlii*, *Coleus blumei* and *Coleus amboinicus*, are important sources of secondary metabolites, such as forskolin, rosmarinic acid, thymol and carvacrol [3]. The therapeutic properties of forskolin, the main diterpene constituent of this plant contributed to the immense medicinal importance of *C. forskohlii*. Forskolin is used for cardiovascular disorders, ophthalmic problems (decrease in intraocular pressure) and also for skin disorders, e.g. psoriasis [4]. Approximately 1-500 g of root material can be obtained from a single plant. The forskolin content of the roots varies from 0.07% to 0.58% of dry matter [5]. *C. forskohlii* is traditionally propagated by means of vegetative cuttings but it is time consuming and provides a limited number of propagules. Apparently due to non-availability of quality planting materials, commercial plantation of this aromatic plant species have not been attempted [6]. Micropropagation using meristem, shoot tip, and nodal explants is the most favored method for producing clones of elite varieties of crops. Sometimes slight difference in the genetic makeup of regenerated plantlets is observed especially when regeneration is from undifferentiated tissue like callus or cell suspensions. Somatic embryos derived from such tissue may also show variations that are basically tissue culture induced. These variations are called somaclonal variations [7,8]. Much efficient work has not been done in developing the protocol for tissue culture of *Coleus forskohlii*. So an advance protocol needs to be developed for the proper and rapid mass micropropagation of *Coleus forskohlii*. Thus, in the present work an appropriate and efficient protocol

was developed from shoot tips and nodes, focusing on the identification of effective hormone combinations for rapid multiplication of shoots and their rooting within a considerably shorter period than previously reported.

Materials and Methods

Collection of plant material and explants preparation

Apical and Axillary meristem were used as explants excised from the plant growing in green house of the institute (Grow tips biotech, Bhopal, Madhya Pradesh, India). Explants were washed with soap solution followed by tap water for 25 min. It was surface sterilized with 0.1% HgCl₂ solution for 10 minutes and followed by rinsing with sterilized distilled water for 4-5 minutes.

Media and culture conditions

Sterilization of nutrient media was performed by autoclaving. The nutrient media was sterilized by autoclaving at 121°C and 15 lbs for 20 minutes. The explants were cultured in jam bottles containing 100 ml of semisolid MS medium (Murashige and Skoog) supplemented with either (IAA; 0.5-3 mg/l), (IBA; 0.5-3 mg/l), (BAP; 0.5-3 mg/l) and kinetin (0.5-2 mg/l) and different combinations of IAA, IBA and kinetin. Each treatment consisted of 3 replicates and the experiment was repeated 3 times. The pH of the medium was adjusted to 5.8 by using 0.1 N NaOH or 0.1 N HCl before the addition of agar. Incubation of culture vials was carried out in incubation room under cool white fluorescent light (200-300 flux) of 16 hours photoperiod and 8 hours dark period at a temperature of 25 ± 1°C and relative humidity of 50-60%. The cultures were observed continuously.

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Root regenerations and hardening

Root regeneration and shoot development no individual action was needed because regeneration of shoots and induction of roots were concurrent in most treatments. Individual plants were separated carefully from the bunch and transferred to small pots containing vermiculate. Rooting in *Coleus* has significance as herbal preparation extracted from the root of this plant is used to cure many diseases. Then these plantlets are placed in the controlled conditions green house for 5 days and then transferred to field in natural environmental conditions for hardening.

Data analysis

All data was subjected to analysis of variance (ANOVA) and comparisons of the mean were made with the least significant difference test at the 5% level probability.

Results and Discussion

Different explants like leaf lamina, node and shoot tip of *C. forskohlii* were cultured on different concentration of auxins (IAA and IBA) and cytokinins (BAP and Kinetin). It was observed that explants showed growth responses like enlargement, initiation of callus and shoot induction. The most significant advantage of shoot tip culture is that in a relatively short time a large number of true-to-type disease free plants could be produced. The best morphogenetic response (15-20 shoots per explants) was observed when shoot apices were cultured in medium with 2.0 mg/l BAP. Following this treatment, each bunch of shoots produced well-developed roots after 35 ± 2 d.

The explants show better response when collected and inoculated from the months of august to march. But when collected in other months, the response of the explants was poor. The explants when treated with 0.1% HgCl₂ solution for 4-5 minutes, offered 80-95% sterile culture. When the treatment of the explants is below 4 minutes and above 5 minutes the percentage of sterilization is low and dehydrated respectively. Also when the explants were treated with 70% alcohol for any duration the explants got dehydrated. Thus treatment of alcohol is avoided in the explants of *Coleus forskohlii*.

In the preliminary experiment, effects of cytokines alone or in combination were tested. Maximum (70-80%) number of bud break and initiation of shoots were reported in the medium containing BAP

(0.5-1.0 mg) alone (Figures 1 and 2). About 1-2 shoots developed in every culture (Figure 3 and Table 1). The combination of BAP and Kinetin contained media shows 40-45% of initiation of only one shoots with lots of callus formation. Maximum length of shoots (1-2 cm) was observed in the medium containing BAP alone in comparison to medium supplemented with BAP and Kinetin. Higher concentration of BAP gives short and weak shoots with the formation of non-fragile callus.

For the multiplication of shoots from a single initiated nodal region, the highest numbers of shoots were observed in the medium containing concentration (1.0-2.0 mg/l) of BAP (Figure 4 and Table 2).



Figure 1: Effect of cytokinins alone or in combination was tested.



Figure 2: Maximum (70-80%) number of bud break and initiation of shoots were reported in the medium containing BAP (0.5-1.0mg) alone.

S. No.	Growth hormones (mg/l)	Shoot induction percentage	No. of shoots per culture	Average shoot length in cm.	Callusing
1	0.5 BAP	80%	1.66 ± 1.15 ^a	2.66 ± 1.15 ^a	-
2	1.0 BAP	70%	1.66 ± 0.57 ^a	1.66 ± 0.57 ^a	-
3	1.5 BAP	68%	1.33 ± 0.57 ^a	1.33 ± 0.57 ^a	-
4	2.0 BAP	60%	1.66 ± 0.57 ^a	1.33 ± 0.57 ^a	-
5	0.5 KN	40%	1 ± 0 ^a	1.66 ± 0.57 ^a	+
6	1 KN	35%	1.33 ± 0.57 ^a	1.66 ± 0.57 ^a	+
7	KN+BAP	40%	1 ± 0 ^a	0.66 ± 0.28 ^b	++
8	KN+ BAP	45%	0.66 ± 0.57 ^a	0.83 ± 0.28 ^c	++
LSD ≤ 0.05			0.873	0.896	
F value			1.126	2.853	
F tab			0.39	0.03	

Mean ± SD.

Different small letters mean that they significantly differ from each other at P=0.05. Table 1: Effect of growth regulations on shoot induction in *Coleus forskohlii* in MS medium.

These media shows about 15-20 number of shoots per culture, when sub cultured in the same fresh medium after 15 days duration. The length of shoots elongates 4-5 cm and increases with culture duration. For further improvement in proliferation of shoots, they were sub cultured in the medium containing same BAP concentration. Cultures shows



Figure 3: Development of shoot in culture.



Figure 4: Axillary buds sprouting by the nodal explants.

S. No.	Growth hormones (mg/l)	Percent response	Average no. of shoots	Average shoot length in cm.	Callusing
1	1.0 BAP	70%	13.33 ± 1.52 ^{ae}	2.66 ± 0.57 ^{ab}	-
2	2.0 BAP	80%	17.33 ± 2.51 ^{ab}	4 ± 1 ^a	-
3	3.0 BAP	30%	4.66 ± 0.57 ^{cef}	3.66 ± 1.15 ^{ab}	-
4	1.0 BAP+1.0KN	20%	6.33 ± 1.57 ^{cdf}	1.33 ± 0.57 ^{bd}	+
5	2.0BAP+0.5KN	60%	9 ± 1 ^e	3.33 ± 1.52 ^{abd}	+
6	1.0 BAP+2.0 KN	20%	6.33 ± 2.51 ^f	1.33 ± 0.57 ^{bc}	++
LSD ≤0.05			2.566	1.414	
F value			23.08	4.29	
F tab			9.00	0.01	

*Mean ± SD.

Different small letters mean that they significantly differ from each other at $P=0.05$.

Table2: Effect of growth hormones on multiplication of shoots in MS medium.



Figure 5: Response of cultures in same medium for shoot numbers and shoot length.

better response in shoot number (20-25) and shoot length (3-6 cm) in the same medium (Figure 5). Thus the medium containing BAP (1.0-2.0 mg/l) were standardized as the best media for mass production and elongation of *Coleus forskohlii*. Following this treatment, each bunch of shoots produced well-developed roots after 35 ± 2 d. The tallest group of plantlets attained a height of 3-5 cm. Any further increase (3 mg/l BAP) decreased the number of shoots as well as the percentage of response. About 4-10 shoots with 1-5 cm were achieved in the medium containing Kinetin and BAP and produces callus. Nodal explants, when cultured in medium with a similar hormone supplement, exhibited 90% sprouting of their existing axillary buds which grew as individual shoots (Figure 4). Additional shoots were formed occasionally from the nodal junctures. Increasing the concentration of growth regulators resulted in more callus induction and callus-mediated regeneration by using (1.0 BAP + 2.0 KN).

In *Coleus forskohlii* shoot tip multiplication was obtained maximum in presence of BAP alone. The dose of cytokinin is critical in shoot organogenesis [9]. BAP alone in the medium induced bud break and multiple shoot formation in *Mentha* [10]. Geetha et al.

[11] also reported high frequency shoot multiplication from the cotyledonary nodes of pigeon pea in the presence of BAP alone. The shoot regeneration frequency increases with increase in concentration of cytokinin and 2 mg/l was found to be optimum for maximum shoot multiplication. Concentration above 2 mg/l drastically decreased shoots bud regeneration. BAP in combination with Kinetin decreases shoot multiplication. Karta [12] reported high frequency shoot multiplication and elongation from shoot apical meristem of *Lycopersicon esculentum*. He suggested meristem proper culture as an efficient plant propagation system producing fewer polyploids or chromosomally aberrant plants.

Genetic stability is conserved in the case of plants derived from shoot tip culture [13]. The active meristem located on the nodes could attribute to the formation of multiple shoot in the present investigation. Continuous presence of cytokinins in the medium resulted in shoot length suppression that has also been reported in guava [14]. Rooting of the micro propagated plants were usually achieved in auxin containing medium [15]. The multiple shoot production protocol developed in this study may be utilized for the cultivation practices of this commercially important medicinal plant.

Conclusion

Coleus forskohlii is a very important medicinal plant. The herb contains an active ingredient called forskolin. The demand of this powerful herb is increasing day by day and thus a large multiplication of this plant is necessary. Previous protocols reported about 80% plant recovery. We developed an efficient micro propagation procedure which is useful for conservation as well as mass propagation of this important medicinal plant. Pilot plant propagation system however need to be launched for the scaling up of production of superior planting material to make the cultivation of *Coleus forskohlii* in India as economically viable venture, and contribute to sustainability of economy.

Conflict of Interest

We declare that we have no conflict of interest.

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