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# *In vitro* Plant Regeneration from Nodal Explants of *Coleus forskohlii* Briq. - An Important Medicinal Plant

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*Key words:* *In vitro* regeneration, *Coleus forskohlii*, Medicinal plant, Micropropagation

## Abstract

An efficient *in vitro* mass propagation and promising protocol has been successfully standardized and developed for *Coleus forskohlii* through direct organogenesis from nodal explants. Nodal explants cultured onto MS basal medium supplemented with 4.44 μM BAP recorded the highest response and produced 24.3 ± 0.2 shoots per explant with an average shoot length 5.6 ± 0.4 cm after 30 days of culture. The *in vitro* shoots recorded higher response for development of rooting on half strength MS fortified with 2.46 μM IBA which produced the best response 7.8 ± 0.6 roots per *in vitro* shoot with an average root length of 4.3 ± 0.1 cm after 25 days. The *in vitro* rooted plantlets were transferred for hardening and 90% of the plantlets survived were successfully acclimatized and established in small plastic pots. This protocol recorded to be a highly repeatable, successful and rapid technique that could be utilized for the commercial mass propagation and *ex situ* conservation of *Coleus forskohlii*. It is important to note that the morphology of the *in vitro* plantlets of *Coleus forskohlii* showed a true-to-type growth habit, both *in vitro* and when transferred to *ex vitro* growth conditions.

## Introduction

Medicinal plants play an important role in the development of potent therapeutic agents. Plant based drugs provide outstanding contribution to modern therapeutics. *Coleus forskohlii* Briq. a valuable medicinal plant belongs to Lamiaceae with well-known efficacy in the Ayurveda and Indian indigenous system of medicine for the treatment of asthma, heart ailments, insomnia, bronchitis, epilepsy, anaemia and inflammation (Sunita et al. 2013). It is a perennial herb, widely growing throughout India and is also found in

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Thailand, Australia and other parts of South East Asia. It grows up to a height of 30 - 62 cm tall and aromatic in nature. It has four angled stems that are branched and nodes are often hairy. Leaves are 7.5 - 12.5 cm in length, 3 - 5 cm in width, usually pubescent, narrowed into petioles. Inflorescence is raceme, 15 - 30 cm in length, flowers are stout, 2 - 2.5 cm in size, usually perfect and calyx hairy inside. The crop is being commercially grown in large areas in Madhya Pradesh, Maharashtra, Kerala, Karnataka and Tamil Nadu (Poornima et al. 2015). A natural compound, forskolin isolated from tuberous roots of *Coleus forskohlii* is used as a potential drug for hypertension, obesity, asthma, respiratory disorder, cancer, inflammatory painful urination (Vivek et al. 2015).

*Coleus forskohlii* is traditionally propagated through vegetative cuttings or seeds. But, in vegetative propagation the success rate is always very low and time consuming. In addition, the seeds of *C. forskohlii* show a very low germination percentage and propagation by seed does not produce homogenous populations, resulting in great variability in potent metabolites. Also due to non-availability of sufficient quality planting materials, commercial plantations of this important aromatic and medicinal plant species have not been widely attempted. At present, production of forskolin is completely dependent on the commercial collection of wild and a few cultivated *C. forskohlii* plants in India. Hence, there is an urgent need for an efficient plant regeneration protocol to be developed.

Moreover, due to the large scale and indiscriminate collection of wild plants from forests and insufficient attempts either to allow its replenishment or its cultivation, *C. forskohlii* is rapidly disappearing and now it has been listed as one of the vulnerable to extinction plant species in India. Hence, it is necessary to develop methods for the conservation of this threatened species. *In vitro* propagation methods offer powerful tools for plant germplasm conservation and mass multiplication. The present study therefore aims at developing a simple, rapid, economical and high frequency mass propagation protocol from nodal explants of *Coleus forskohlii* for the potential application in large scale propagation.

## Material and Methods

The plant material *Coleus forskohlii* (Fig. 1A) was collected from Namakkal district, Tamil Nadu, India and were raised in pots containing soil and farm yard manure (1 : 1) under greenhouse conditions at Department of Biotechnology, University of Madras, Guindy Campus, Chennai-600 025.

Nodal explants were washed thoroughly in running tap water and washed with a 0.1% (v/v) aqueous solution of Tween 20 (Qualigens, Mumbai, India) for 5 min, and again washed with sterile distilled water. The cleaned explants were subjected to surface sterilization with 0.1% (w/v) $HgCl_2$ (disinfectant, Hi-Media, India) for 5 min under aseptic conditions in laminar air flow cabinet (Bio-Clean Air, Chennai). These were finally rinsed 5 - 6 times with sterilized distilled water to remove traces of $HgCl_2$.

After surface sterilization, disinfected nodal explants were trimmed to 0.8 - 1.0 cm and cultured on MS supplemented with different concentrations of BAP (1.11, 2.22, 4.44 and 8.88 µM), TDZ (1.14, 2.27, 4.54 and 9.08 µM) and Kn (1.16 , 2.32, 4.65 and 9.20 µM) for shoot multiplication. The number of shoots and their length were measured after 30 days of culture. The proliferated shootlets produced from *in vitro* multiplication stage were individually separated and cultured on half strength MS supplemented with IBA (0.49, 0.98, 2.46 and 4.92 µM) for *in vitro* rooting. Root number and root length were recorded after 25 days in culture.

After 25 days, the *in vitro* grown plantlets were thoroughly washed with tap water to remove residual agar from roots, then immersed in 0.2 % aqueous solution of bavistin (a fungicide) for 15 - 20 min and washed with tap water. Thereafter, the treated plantlets were transplanted in small plastic pots filled with a mixture of red soil, vermiculite and farm yard manure (1 : 1 : 1), covered with moistened polythene bags for hardening. After 15 days, the fully acclimatized plantlets were transferred to pots of 6 cm diameter for their growth and development.

MS media were solidified using 0.9% (w/v) agar containing 3% (w/v) sucrose. The pH of the media was adjusted to 5.6 ± 0.2 before autoclaving at 121°C for 15 min under 1.16 kg/cm$^2$ pressure and transferred to culture room. All the plant cultures in the studies were maintained in 16 hrs light/8 hrs dark photoperiod with a photosynthetic photon flux density (PPFD) of 50 µmol/m$^2$s$^2$ provided by cool white fluorescent lamps (Phillips, India) with 60 - 65 % relative humidity. All cultures were incubated at 25 ± 1°C.

## Results and Discussion

Shoot proliferation developed from nodal explants cultured on MS supplemented with BAP (1.11, 2.22, 4.44 and 8.88 µM), TDZ (1.14, 2.27, 4.54 and 9.08 µM) and Kn (1.16, 2.32, 4.65 and 9.20 µM). The shoot initiation response varied depending on plant growth regulators. Initiation of multiple shoots in most of the treatment was observed within three weeks of culture. The highest shoot number was recorded on MS medium supplemented with 4.44 µM BAP with better growth response (80.00 ± 5.00) and recorded 24.30 ± 0.2 shoots of per explant with an average length of 5.60 ± 0.40 cm after 30 days of culture (Table 1; Fig. 1B, C). At lower and higher concentrations of BAP (1.11 and 8.88 µM), TDZ (1.14 and 9.08 µM) and Kn (1.16 and 9.20 µM) did not significantly induce the number of shoots per explant. BAP has been considered to be one of the most active cytokinins for organogenic differentiation in plant tissue culture (Janarthanam et al. 2009 and 2011, Biswas et al. 2009, Sudharson et al. 2014). Nodal explants on MS without growth regulator did not initiate shoot differentiation.

Half strength MS was used throughout the experiment for rooting. Individual *in vitro* proliferated microshoots were placed in half strength MS supplemented with various concentrations of IBA (0.49, 0.98, 2.46 and 4.92 µM). Rooting occurred in all concentrations but with different rooting percentages, and the optimal response was

observed on half strength MS supplemented with IBA (2.46 µM) in terms of average number of roots (7.80 ± 0.60) with mean root length of 4.30 ± 0.10 cm per shoot with 85.00 ± 5.00 % of rooting response recorded after 25 days of culture (Table 2; Fig. 1D, E). In the present study root induction was obtained with lower concentration of IBA. The reports are in accordance with the findings of Janarthanam and Sumathi (2012) in which high rooting percentage in *Ocimum citriodorum* was achieved with IBA (0.5 mg/l ), wherein increasing the concentration of IBA (4.92 µM) resulted in a decrease in the percentage of root induction response with decreased number of roots and root length. The excised shoots did not show rooting on culture medium without plant growth regulators.

**Table 1. Effect of different concentrations of cytokinin (BAP, Kn, TDZ) on *in vitro* shoot multiplication from nodal explants of *Coleus forskohlii.***

| Plant growth regulator (µM) | | | Shoot induction (%) | Number of shoots per explant | Shoot length (cm) |
|---|---|---|---|---|---|
| BAP | TDZ | Kn | | | |
| 1.11 | | | 31.67 ± 2.89 | 4.33 ± 1.15 | 2.13 ± 0.15 |
| 2.22 | | | 58.33 ± 2.89 | 4.60 ± 0.40 | 2.90 ± 0.10 |
| **4.44** | | | **80.00 ± 5.00** | **24.30 ± 0.2** | **5.60 ± 0.40** |
| 8.88 | | | 40.00 ± 5.00 | 3.67 ± 1.53 | 2.10 ± 0.20 |
| | 1.14 | | 20.00 ± 0.00 | 3.33 ± 1.15 | 1.93 ± 0.15 |
| | 2.27 | | 55.00 ± 5.00 | 6.10 ± 1.60 | 2.93 ± 0.06 |
| | 4.54 | | 35.00 ± 5.00 | 3.00 ± 0.00 | 1.57 ± 0.06 |
| | 9.08 | | 33.33 ± 5.77 | 2.57 ± 1.20 | 1.40 ± 0.10 |
| | | 1.16 | 33.33 ± 2.89 | 3.00 ± 1.73 | 1.83 ± 0.06 |
| | | 2.32 | 35.00 ± 5.00 | 3.40 ± 0.50 | 2.03 ± 0.15 |
| | | 4.65 | 45.00 ± 0.00 | 4.00 ± 1.00 | 2.17 ± 0.15 |
| | | 9.20 | 18.33 ± 5.77 | 2.00 ± 0.00 | 1.27 ± 0.06 |

*In vitro* grown plantlets with 2 - 3 leaves and well-developed roots were taken from the culture tubes and washed in running tap water to remove traces of agar. Gradual acclimatization of *in vitro* grown plants to external environment was essential for *Coleus forskohlii* plantlets. Thus, the plantlets were subsequently immersed in 1% fungicide (bavistin) solution and were hardened in small plastic pots containing a mixture of red soil, vermiculite and farmyard manure (1 : 1 : 1) for 3 weeks. More than 90 % trans-planted plants survived in this way and resumed growth (Fig. 1F). There was no detectable phenotypic variation among the acclimatized plants. In conclusion, the results showed the ability of the nodal explants to produce higher number of shootlets without any intervening callus phase, where all the plantlets were uniform in height and growth.

Hence, we propose this protocol as a simple, economical, rapid and highly reproducible to obtain more plantlets within a short period of time.

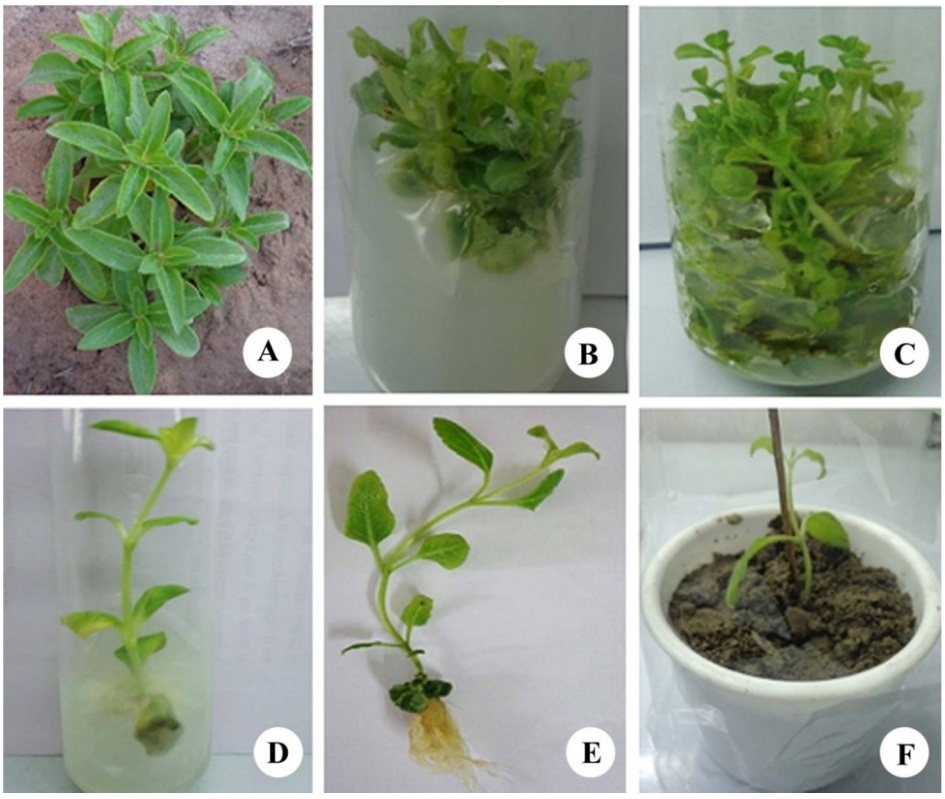


Fig. 1A. Mother plant of *Coleus forskohlii* (B-F). Stages of *in vitro* plant regeneration from nodal explants of *Coleus forskohlii***.** B. Initiation of multiple shoots from nodal explant inoculated on MS medium supplemented with BAP 4.44 µM after two weeks of culture. C. Proliferation of multiple shoots from nodal explants on MS containing BAP 4.44 µM after 30 days of culture. D. Rooting of *in vitro* regenerated shoots inoculated on half strength MS containing 2.46 µM IBA. E. Healthy *in vitro* developed plantlet. F. Well established and hardened *in vitro* plants transferred to small paper pots.

**Table 2. Effect of different concentrations of IBA in half strength MS medium on rooting response of *Coleus forskohlii***

| IBA (µM) | Rooting response (%) | Number of roots per shoot | Root length (cm) |
|---|---|---|---|
| 0.49 | 30.00 ± 5.00 | 2.67 ±0.58 | 2.43 ± 0.12 |
| 0.98 | 46.67 ± 2.80 | 3.67 ±0.58 | 3.43 ± 0.06 |
| **2.46** | **85.00 ± 5.00** | **7.80 ±0.60** | **4.30 ± 0.10** |
| 4.92 | 30.00 ± 0.00 | 2.00 ±1.00 | 2.30 ± 0.30 |

Explants were cultured on MS supplemented with BAP, TDZ and Kn. Data were recorded after 30 days of culture. Results represent mean ± Sd of six replicated experiments

*In vitro* shoots were cultured on half-strength MS supplemented with IBA. Data were recorded after 25 days of culture. Results represent mean ± Sd of six replicated experiments.

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