

Review Article

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Medicinal Coleus (*Coleus forskohlii* Briq): Different Methods of Propagation – A Review

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Abstract *Coleus forskohlii* (Briq.), a prominent member of the Lamiaceae family, is a highly valued medicinal herb in Indian Ayurvedic medicine and the global pharmaceutical industry. Its primary therapeutic significance is attributed to forskolin, a unique labdane diterpene concentrated in the root tubers. Forskolin serves as a potent adenylate cyclase activator, making it indispensable for treating ailments such as hypertension, glaucoma, and congestive heart failure. However, the commercial cultivation of *C. forskohlii* faces significant challenges; conventional propagation via vegetative cuttings is often hindered by slow multiplication rates, seasonal limitations, and susceptibility to soil-borne pathogens. This review provides a comprehensive analysis of various propagation methodologies and biotechnological advancements aimed at enhancing biomass and forskolin yield. It evaluates the impact of plant density, cutting types, and planting methods on root productivity. A significant focus is placed on in vitro techniques, including shoot induction and callus formation using Murashige and Skoog (MS) media supplemented with specific growth regulators like BAP, NAA, and 2,4-D. Furthermore, the review explores the efficacy of brassinosteroids in promoting root development and the application of somatic embryogenesis and direct organogenesis for rapid, large-scale clonal propagation. These biotechnological approaches not only ensure the production of genetically uniform, disease-free planting material but also offer sustainable strategies for the conservation of this endangered medicinal species. By integrating tissue culture with elicitation strategies, the industry can better meet the rising global demand for forskolin while preserving natural germplasm.

Keywords Medicinal coleus (*Coleus forskohlii* Briq); Lamiaceae family; Direct organogenesis; Somatic embryogenesis; Brassinosteroid; Leaf explants; Stem cuttings; Forskolin

1 Introduction

Coleus forskohlii belongs to the family Lamiaceae is an important plant in Indian Ayurvedic medicine and herbaceous plant that usually grows at a height of 600–1,500 ft and is spread over the subtropical warm temperate climatic zones of India. It is an ancient and important root drug claimed to improve appetite, increase vitality and useful by curing the ailments like inflammation, flatulence, dropsy etc. (Rupp et al., 1986). It occurs in sub-tropical Himalayan regions from Kumaon to Nepal, Bihar and Deccan plateau of southern India. It is widely cultivated in Rajasthan, Maharashtra, Gujarat, Karnataka, Tamil Nadu and Andhra Pradesh and its present annual production is about 100 tons from the area of 700 ha in India, cultivation of *C. forskohlii* is picking up in recent years. It is a fast-growing herb and hence more amenable to get exposed to variety of environmental stress.

It is Commercially propagated by the means of vegetative cuttings (Reddy et al., 2001). Conventional means of propagation through vegetative cuttings is not suitable to meet the increasing demand of this species due to limited number of propagules produced in each cycle. In vitro propagation is the most efficient and reliable technique for production of ample planting stock of genetic uniformity. Its rapid mode of vegetative propagation makes it a suitable candidate for micro propagation (Biondi and Thorpe, 1982).

It is highly valuable as a medicinal plant due to a biologically active compound labdane diterpene present in the root tubers called 'forskolin' (Bhat et al., 1977). An active diterpenoid in roots, it poses multiple biological activities such as positive inotropic, anti-hypertensive and antiglaucoma (Seamon and Daly, 1981), which is

widely used by pharmaceutical industry due to its wide range of therapeutic effects. The main feature of forskolin is in its roots having unique mechanism of generating cyclic adenosine monophosphate in the cells through direct activation of the catalytic unit of adenylate cyclase enzyme (Yanagihara et al., 1996). For commercial cultivation of this crop optimization of plant population per unit area, method of planting and type of cutting are the prime factors in terms of root yield of medicinal coleus.

The most important problem to the commercial growers of coleus is optimum plant density, appropriate type of cutting, method of planting and selecting suitable cultivar. The species is known well for its pharmacological importance.

2 Botanical and Phytochemical Background

2.1 Taxonomical classification

The plant belongs to the family Lamiaceae and the order Lamiales. Commonly known as the mint family, it includes a number of potent medicinal plants. It consists of 236 genera and 7,000 species, it is the largest family of the order Lamia. The genus *Coleus* was first described in 1790, with *C. forskohlii* classified under the family Lamiaceae, highlighting its botanical significance. This classification underscores the plant's historical use in traditional medicine and its potential for further research in phytochemistry.

2.2 Phytochemistry and medicinal relevance

A Biologically active compound labdane diterpene present in the root tubers called 'forskolin' a key compound extracted from the roots, activates adenylate cyclase, leading to increased levels of cyclic adenosine monophosphate (cAMP) in the cells (Seamon and Daly, 1981). These cAMP plays a key role in various body functions, including heart muscle contraction, smooth muscle relaxation, insulin secretion, and thyroid function and has various physiological effects (Kavitha et al., 2010). The study identifies several minor diterpenoids and other phytochemicals present in *C. forskohlii*, emphasizing the complexity and potential of its chemical profile.

3 Conventional Propagation Methods

3.1 Rooted cuttings with different planting methods and varieties

The yield of medicinal coleus is influenced by plant density, cutting type, and planting methods (Chandrasekhar et al., 2016). Chintapalli local and K-8 varieties were planted using ridge and furrow and flatbed methods, with ridge and furrow methods yielding the highest dry root yield. Genetic factors also significantly impact root yield performance.

3.2 Role of leaves and cutting type in rooting

The physiological status of the cutting is vital for successful rooting. Semi-hardwood stem cuttings that retain apical leaves show significantly higher rooting percentages and quality (Belniaki et al., 2018). Studies found that cuttings with leaves produced an average of 16 roots, compared to only 5.7 roots in leafless cuttings, suggesting that endogenous auxins or carbohydrates from the leaves facilitate better establishment.

3.3 Ex situ propagation through stem cuttings

The methodology involved selecting disease-free, mature stem cuttings from the mother plant are grown in prepared beds and poly bags in a combination of sand, soil, and manure was used to create optimal growing conditions for the stem cuttings (Patel, 2016). A well-structured water and drainage system is crucial for the successful growth of *Coleus forskohlii*, preventing water logging and plant damage. Results indicated that the mixed media in poly bags facilitated rapid root and shoot development, supporting the plant's propagation and contributing to its ex-situ conservation efforts.

4 In vitro Regeneration and Micropropagation

4.1 In vitro shoot induction using MS medium

Leaf explants were collected and subjected to thorough surface sterilization using running water, antifungal agents, and detergents to ensure aseptic conditions (Murashige and Skoog, 1962). After sterilization, explants were cut into smaller pieces and inoculated in MS media with varying concentrations of hormones 2,4-D (0, 0.01, 0.02,

0.03, 0.04, 0.1, 0.5, 0.1, 0.2, 2.5 mg/L), BAP (0.0, 0.01, 0.02, 0.03, 0.04, 0.1, 0.5, 0.1, 1.5 mg/L) in different concentration and combination to promote callus formation. The pH of the culture media was maintained between 5.4-5.7, adjusted with NaOH or HCl, and solidified with 0.8% agar.

Cultures were kept at a controlled temperature of (22±2) °C with a 16-hour photoperiod, with sub culturing performed every two weeks. Callus induction involved sub culturing leaf explants in different concentrations of BAP and NAA to enhance the shoot formation. In regular intervals of two weeks were maintained for monitoring and promoting the number of shoots generated from the callus (Dodds and Roberts, 1982). The study found that MS medium supplemented with BAP and 2,4-D was more effective for callus induction compared to NAA and BAP. Callus exhibited a range of colours (Lisowska and Wysokinska, 2000) and showed significant genetic variability, leading to successful multiple shoot formation from the leaf explants.

4.2 Effective propagation method for salt tolerance

Using apical tip as meristem as explant it resulted in quicker plant growth than Nodal Segment. This Medicinal coleus explant grown in the NaCl Medium of different Concentration from 0.1% to 0.4%. Later they found that the explant of medicinal coleus was able to withstand and grow at the low concentration of 0.2% of NaCl with the support of BAP and NAA (Sharan et al., 2014).

Generally, the use of BAP is considered to be most suitable for promoting large-scale multiplication and micropropagation of various plant species (Shrivastava and Banerjee, 2008). In *C. forskohlii*, BAP alone has shown best result in promoting multiple shoots formation from the nodal segments and shoot tips (Sen and Sharma, 1991).

4.3 Effect of brassinosteroid in rooting

Swamy et al. (2021) found that the application of brassinosteroid significantly increased root numbers in medicinal coleus plants. The application of 28-homobrassinolide at 100 µM resulted in an 85% increase in root numbers by day 15, indicating its effectiveness in promoting rooting. The findings suggest that brassinosteroid could be pivotal in commercial cultivation practices for medicinal plants, enhancing both rooting and vegetative growth (Rao et al., 2010). Terminal cuttings from healthy coleus plants were treated with BRs, monitored for 160 days, and found to increase carbohydrate levels and forskolin content in roots, suggesting a link between growth regulators and secondary metabolite production.

4.4 Effect of cytokinin combined elicitors in coleus

In vitro culture techniques using cytokinins combined with elicitors can enhance both the propagation efficiency and secondary metabolite production of *Coleus* spp., a plant group with notable medicinal value. Cytokinins promote cell division, shoot differentiation, and tissue proliferation, while elicitors stimulate defense-related physiological responses and activate secondary metabolic pathways. Their combined application may therefore improve the accumulation of bioactive compounds associated with antioxidant, anti-inflammatory, and pharmacological activities.

Compared with conventional cultivation, tissue culture provides controlled growth conditions, stable nutrient availability, and a shorter production cycle. These factors create a favorable environment for secondary metabolite biosynthesis in *Coleus* spp. Previous studies have shown that controlled culture conditions and nutrient supply can increase secondary metabolite levels in tissue-cultured plants (Govindaraju et al., 2018). Therefore, optimizing cytokinin types and elicitor combinations is an effective strategy for improving both in vitro propagation and medicinal compound production in coleus.

4.5 Shoot regeneration from proximal, middle and distal segment of *Coleus forskohlii* leaf explants

Rapid shoot regeneration was examined in Three different segments of coleus leaf explant (Proximal, Middle and Distal) where cultured on Murashige and Skoog (MS) Medium with different concentrations of BAP, KIN, NAA, and IAA were tested for shoot elongation. Although callus proliferation was observed, KIN was found to be ineffective in the present study. However, Sharma et al. (1991) previously reported that KIN-enriched media

increased the frequency of callus formation from leaf explants. BAP with different concentrations (0.5, 1.0, 2.5, 5.0) in which BAP at 5.0 mg/L yielded the highest shoot regeneration rate is found to be effective for micropropagation of *C. forskohlii* then Distal leaf segments showed superior regeneration compared to proximal and middle segments, likely due to the presence of more meristematic tissues in the proximal area (Krishna et al., 2010).

4.6 Shoot organogenesis

Focuses on the development of callus from *C. forskohlii* leaf segments in a sterile environment under controlled conditions. The results show that cytokinin and auxin plays a crucial role in shoot differentiation. Kinetin, when combined with NAA or IAA, leads to the highest shoot regeneration frequency. The study also found that rooting efficiency is highest in half-strength MS medium without growth regulators. The study confirms that in vitro-produced plants maintain clonal purity and forskolin content, making it viable for commercial propagation (Sairam Reddy et al., 2001).

4.7 Large scale clonal propagation

Large scale clonal propagation leaf lamina is used as an explant and it is achieved in the media containing 2 µM BA + 0.1 µM NAA Where a highest number of 35 shoots/explant were produced. Later they transferred to root induction medium comprising of IBA, NAA and IAA (1-5 µM) in half-strength MS medium to determine the Most suitable shoot length for proper root induction. The rooted plantlets were acclimatized in field conditions after proper hardening (Sahai and Shahzad, 2010).

4.8 Roots cultivated in shake flask

Forskolin production from *C. forskohlii* roots can be achieved through various cultivation methods, including shake flasks and bioreactors. The transformed hairy root cultures, initiated via *Agrobacterium rhizogenes*, can produce forskolin in significant quantities, with optimal conditions yielding up to 14 mg/L after 21 days. Hormone-free media and sucrose additions enhance growth and forskolin production, with specific elicitors like methyl jasmonic acid boosting yields. Cutting roots without negatively impacting growth or productivity is a notable advantage in scaling up production (Krombholz et al., 1992).

4.9 Somatic embryogenesis

In vitro plant regeneration through somatic embryogenesis in *Coleus forskohlii* Briq. has been successfully established using leaf explants (Gopi and Mary, 2014). Research indicates that the combination of plant growth regulators (PGRs) significantly influences the regeneration process (Yasmin et al., 2001).

Specifically, a medium containing 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP) yielded the highest frequency of direct somatic embryogenesis, achieving up to 80% success in embryo maturation and conversion to plantlets. Additionally, alternative methods utilizing direct organogenesis from leaf explants have shown promise, with protocols achieving up to 35 shoots per explant using optimized concentrations of BAP and auxins (Dode et al., 2003).

These findings highlight the potential for both somatic embryogenesis and direct organogenesis as effective strategies for the mass propagation and conservation of this medicinally important species, although the reliance on specific PGR combinations may limit broader applicability.

5 Conclusion

The comprehensive evaluation of propagation strategies for *Coleus forskohlii* (Briq.) underscores its standing as a premier medicinal resource, primarily due to the unique pharmacological profile of forskolin. As the global demand for natural adenylate cyclase activators grows, the limitations of traditional vegetative propagation such as low multiplication indices, high susceptibility to soil-borne pathogens, and seasonal dependency have become significant bottlenecks for the pharmaceutical supply chain.

This review identifies a critical shift toward biotechnological interventions as the most viable path forward. The synthesis of current research demonstrates that:

- (1) Micropropagation Efficiency: In vitro protocols, specifically direct organogenesis from leaf lamina and apical meristem culture, offer a robust mechanism for the rapid turnover of disease-free, genetically uniform planting material. The optimization of BAP and NAA concentrations remains the most effective hormone regime for maximizing shoot induction.
- (2) Regenerative Innovation: Somatic embryogenesis has emerged as a high-efficiency alternative, with maturation rates reaching 80%. This method provides a sophisticated platform for germplasm conservation and large-scale clonal propagation that exceeds the capacity of conventional stem cuttings.
- (3) Enhanced Phytochemistry: The integration of brassinosteroids and elicitors (such as methyl jasmonic acid) during the culture process not only facilitates superior rooting but also significantly enhances the biosynthetic pathways of secondary metabolites, leading to higher forskolin concentrations.
- (4) Sustainability and Scaling: Advanced cultivation techniques, including hairy root cultures and shake-flask bioreactors, present a sustainable model for metabolite extraction that bypasses the need for destructive harvesting of wild or field-grown plants.

In conclusion, while field-level optimizations like the "ridge and furrow" method provide incremental gains in yield, the future of *C. forskohlii* lies in the synergy between tissue culture technology and molecular elicitation. Adopting these biotechnological frameworks is essential for ensuring a consistent, high-quality supply of forskolin while simultaneously preserving the genetic diversity and sustainability of this endangered medicinal species. Future research should prioritize the refinement of bioreactor parameters and the exploration of genetic markers to further stabilize forskolin yields in commercial-scale production.

Authors' contributions

Sanjaai Kumar and Santhosh Raj conducted the literature review, collected the data regarding various propagation methods, and drafted the manuscript. Jones Ponuraj conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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