

## **A Review on Plant Tissue Culture of *Coleus Forskohlii* and Its Gene Expression**

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### **Abstract**

*Coleus forskohlii* Briq. is a perennial herb, belonging to the family Lamiaceae. The tuberous roots of *Coleus forskohlii* are a rich source of forskolin (coleonol). It is an endangered plant so it is necessary for the Plant Tissue Culture of *Coleus forskohlii*. It is a most important medicinal as well as aromatic values. *Coleus forskohlii* has been successfully micro propagated under *in vitro* conditions. Aromatic *Coleus* acts as a natural source of drug for hypertension, cardiovascular health, obesity, glaucoma and many other diseases without any side effects. Worldwide medicinal plants are being investigated because product safe, cheaper, and efficacious in overall.

The pharmacological and biochemical investigations established that forskolin possess multifaceted biological activities. It is a valuable addition in medicinal plants biodiversity. The demand of this plant is increasing day by day multiplication of this plant is necessary. The herb contains an active ingredient called Forskolin. Forskolin being the major chemical constituent of

the tuber, herbal preparations of it act on various multiple pharmacologic mechanisms. From gene expression we identified several reference genes that are suitable for qRT-PCR data normalization.

Keywords: *Coleus forskohlii*, ornamental plant, forskolin, plant tissue culture and gene expression.

### **Introduction**

Plant tissue culture is collection of techniques used to maintain or grow plant cells tissue or organs under sterile conditions on a nutrient culture medium of known composition. Medicinal plants are of great significance to the health of individuals and communities. *Coleus* is an aromatic perennial, with an erect stem and tuber like roots, reaching 60 cm. *Coleus* is a member of the mint family and native to India, and grows in the subtropical temperate climates of India, Nepal, Thailand and Sri Lanka. The name *Coleus* is derived from the Greek word *Koleos*, which means sheath around the style. There are more than 500 varieties of *Coleus* in cultivation all over the world. *Coleus* is commonly known as Pashanbhedi (Sanskrit), Patharchur (Hindi), Makandiberuor Manganiberu (Kannada) and is grown throughout the country. Medicinal plants like *Coleus* have curative properties due to the presence of various complex chemical substances of different chemical nature, which are found as secondary plant metabolites in one or more parts of these plants. Medicinal plants are important for pharmacological research and drug development, not only when constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds (Mukherjee, 200). Complete plants of *Coleus forskohlii* was developed in MS medium containing 2.0 mg/l BAP through direct multiplication at the rate of 15-20 shoots per explant. The survival rate of full grown plants is 100% and rooting occurred in all plants (Kaul et al., 2015). Best shoot formation was reached from the callus using 0.5 mg/l BAP and 0.1 mg/l NAA after 2 weeks of incubation. Green, light green, cremish, brown colour callus was stimulated with (2,4-D) auxin alone and in combination with (BAP) cytokinines (Atulkar et al., 2012).

Transcriptome is the full range of m-RNA, molecules expressed by an organism. Forskolin, a complex labdanediterpendoid found in the root of *Coleus forskohlii* (Lamiaceae), has received attention for its broad range of pharmacological activities. Irini Pateraki (2014) detected Forskolin in the root cork of *Coleus forskohlii* in a specialized cell type containing characteristic structure

with histochemical properties consistent with oil bodies. Organelle purification and chemical analysis confirmed the localization of Forskololn and of its simplest diterpene precursor backbone (13R) manoyl oxide, to the oil bodies. The labdanediterpen backbone is typically synthesized by two successive reactions catalyzed by two distinct classes of diterpenesynthases. IriniPateraki et al.(2014) recently described the identification of small gene family of diterpenesynthase candidates (CfTPSS) in *Coleus forskohlii*.

#### Geographical Distribution

The *Coleus* group of plant grows in tropical situations and in warm temperature climate zone on mountains of India, Nepal, Burma, Srilanka, Thailand and Africa. It comes up well on the sun exposed dry hill slopes from 300 m to 1800 m altitude. A well-drained medium fertile soil is suitable for its cultivation.

#### Botanical Description

*Coleus forskohlii* is 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 3-5 cm in width, usually pubescent, narrowed in to petioles. Inflorescence is raceme, 15-30 cm in length; flowers are stout, 2 to 2.5 cm in size, usually perfect and calyx hairy inside. Upper lip of calyx is broadly ovate. The ovary is four parted and stigma is two lobed and flower is cross pollinated by wind and insect. The root is typically golden brown, thick, fibrous and radially spreading. Roots are tuberous fasciculate 20 cm long 0.5 to 2.5 cm in diameter, conical fusiform, straight, orange red within and strongly aromatic. *Coleus forskohlii* is only species of genus to have fasciculate tuberous roots.

#### Taxonomical Classification of *Coleus Forskohlii*

*Coleus forskohlii* Briq. is a member of mint family, Lamiaceae. It is an indigenous to India and is recorded in Ayurvedic material medica under the Sanskrit name Makandi and Mayani (Shah, 1996). The taxonomic position of *C. forskohlii* is as follows:

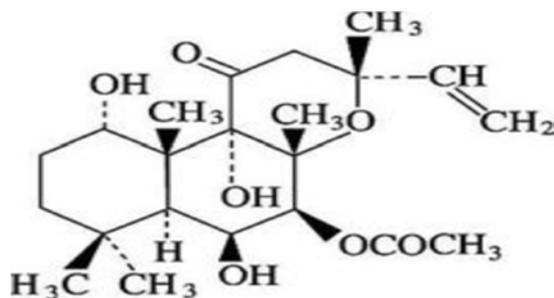
Kingdom - Plantae  
 Divisio - Magnolophyta  
 Class - Magnoliopsida  
 Order - Lamiales  
 Family - Lamiaceae  
 Genus - *Coleus*  
 Species - *forskohlii*

#### Genetic Variation in *Coleus*

Reddy (1952) reported that *Coleus forskohlii* is diploid with  $n = 14$ . However, Riley and Hoff (1961) from their studies on chromosome numbers in South African dicotyledons reported that *C. forskohlii* is diploid with basic chromosome number  $n = 16$ . Birand Saggoo (1982 and 1985) reported that Central Indian collections have basic number of  $n = 17$ , while South Indian collections have  $n = 15$  and concluded that variability in base number of various members of the family could be due to aneuploidy at generic level which ultimately leads to morphological variations. Shah (1989) reported that populations from different ecogeographic areas vary greatly in their morphology.

#### Phytochemistry

The tuberous root extracts of *C. forskohlii* contain minor diterpenoids viz., deacylforskolin, 9-deoxyforskolin, 1,9-deoxyforskolin, 1,9-dideoxy-7-deacylforskolin in addition to forskolin (7 $\beta$ -acetoxy-8, 13-epoxy-1 $\alpha$ , 6 $\beta$ , 9 $\alpha$ -trihydroxy-14-en-11-one) (Ammon and Kemper, 1982 and De Souza and Shah, 1988).



Chemical structure of forskolin Adapted from Khan et al., (2012).

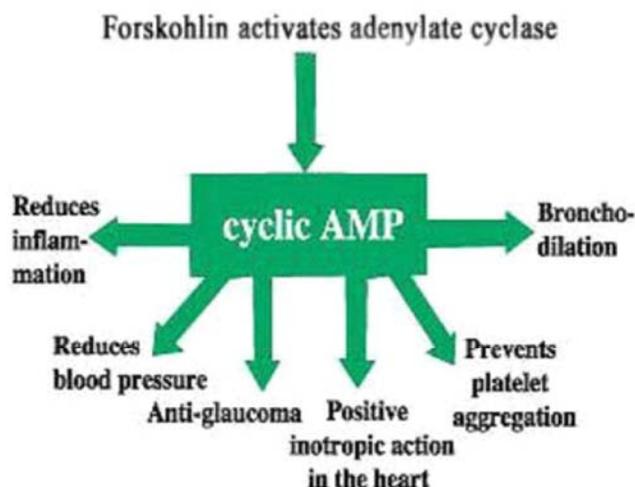
### Importance of *Coleus Forskohlii*

In traditional Ayurvedic system of medicine, *Coleus forskohlii* has been used for treating heart diseases, abdominal colic, respiratory disorder, insomnia, and convulsions bronchitis, intentional disorder, burning sensation, constipation, epilepsy and angina. When roots mixed with mustard oil, the extract is applied to treat eczema and skin infection. Forskolin is also used in the preparation of medicines preventing hair greying and restoring essential oil has potential color. The plant is used for veterinary purpose (De Souza 1993). Essential oil has potential uses in food flavoring industry and can be used as an antimicrobial agent (Chowdhary et al., 1998).

### Action of *Forskolin*

Forskolin being the major chemical constituent of the tuber, herbal preparations of it act on various multiple pharmacologic mechanisms. De Souza (1977) establish that the methanol extracted from the root tuber is helpful in lowering blood pressure and positive inotropic activities in animal models. The principle mechanism by which forskolin exerts its hypertensive activity is by stimulation of adenylate cyclase and thereby increasing cellular concentrations of the second messenger cyclic AMP (cAMP) (Seamon et al., 1981). Forskolin directly stimulates almost all

hormone sensitive adenylate cyclases in intact cells, tissues and even solubilized preparation of adenylate cyclase (Metzger and Lindner, 1981). The unique feature of this activation is that the site of action for forskolin is the catalytic subunit of the enzyme or a closely associated protein (Seamon and Daly, 1981). Of the 9 types of adenylate cyclase in humans, forskolin can activate all except type IX, which is found in spermatozoa (Iwatsubo et al., 2003). Stimulation of adenylate cyclase is thought to be the mechanism by which forskolin relaxes a variety of smooth muscles. This action of forskolin improved the potential use of the molecule, not only as an invaluable research tool for understanding cyclic – AMP dependent physiological processes, but also as a potential therapeutic agent for diseases like cardiac insufficiency, hypertension, glaucoma, thrombosis, asthma and metastatic condition (Seamon, 1984).



Adapted from Soni et al., 2012

#### Use of forskolin

*Coleus forskohlii* is a herb that has been used before this ancient times in Ayurvedic and Hindu traditional medicine. The roots has been used to treat colic, heart failure, congestive, convulsions, eczema, hypertension, painful urination, insomnia and respiratory disorders (Khan et al., 2012). Uses of *Coleus* also produce therapeutic benefit in angina, psoriasis, asthma and prevention of cancermetastases.

#### Cosmetic use

Forskolin is extracted from *Coleus forskohlii* plant. It is increase isoform selectivity via acting on adenylyl cyclase to enhance the intracellular levels of AMP (cyclic adenosine monophosphate) (Khan et al., 2012). Forskolin in combining with hydroxycitric acid (HCA) can be apply for body shape and fat management (Gupta, 2004). The mechanism of rising and maintaining skimp body mass is link to the availability of cyclic AMP. Aside facilitating hormonal action, cyclic AMP may manipulate thermo genic response of the body to food, rising the utilization of body and enhance the metabolic rate of the body (Khan et al., 2012).

#### For skin problems

*Coleus* has antimicrobial properties. Its oil is useful in topical preparations. *Coleus* oil is an

especially effective against propionic bacterium acnes, the microorganism responsible for acne, and it has been found effective against other microorganism known to be responsible for skin infection and eruptions (Khan et al., 2012). This oil suppress the growth of skin pathogens. It has also been found active against yeast culture (Majeed&Prakash, 2007).

#### Antioxidant activity

Plant of *Coleus forskohlii* extract express higher antioxidant activity and high amount of polyphenols in comparison to other *Coleus* species (Khan et al., 2012). Leaf extract of this plant showed importantly high amount of total polyphenols, flavones and flavonols and high antioxidant activity. Rasineni et al. (2008) intimate that *Coleus* can be used as an important source of phenolic compound.

#### Perfumery uses

*Coleus* is a rich source of diterpenoids with different oxygen patterns. Over forty compounds consisting to four different classes of aroma compounds have been isolated from oils found from different indigenous genotypes of *Coleus forskohlii*. These include monoterpenoids, diterpenoids, sesquiterpenes alcohol and sesquiterpenes. The presence of 3-decanone, bornyl acetate and g-eudesmol compounds were describe in their experimental studies (Majeed&Prakash, 2007).

#### Miscellaneous uses

*Coleus* also well work in treating cancer, asthma, cardiopathy, congestive heart failure, cramp, convulsions, depression, dermatosis, dyspepsia, dysuria, glaucoma, eczema, high blood pressure, hypothyroidism, infertility, insomnia, ischemia, myocardosis, obesity psoriasis, respirosis, water retention and thrombosis (Duke et al., 2002).

#### Heart disorder

Forskolin has a positive inotropic action on cardiac tissue via increased cAMP levels. Forskolin lowered normal or elevated blood pressure in different animal species through a vasodilatory effect and it had a positive inotropic action on the heart muscle (De Souza et al., 1983; Dubey et al., 1981).

#### Glaucoma

The topical utilization of forskolin lowered the intraocular pressure in rabbits, monkeys and healthy

human volunteers and it was associated with a reduction in aqueous inflow and no change in outflow facility indicating the potential of forskolin as a therapeutic agent in the treatment of glaucoma. It also showed no influence on humans in reducing aqueous flow when applied topically to the eye (Brubaker et al., 1987).

#### Asthma

Forskolin was studied as bronchodilator for its potential use in the treatment of asthma (Bruka, 1986). In human basophils and mast cells, forskolin blocked the release of histamine and leukotriene C-4 (Marone et al., 1987). A study involving human revealed that inhaled forskolin powder formulations were capable of causing bronchodilation in asthma patients (Bauer et al., 1993).

#### Antithrombotic effect

Forskolin inhibits platelet aggregation through adenylatecyclase stimulation, augmenting the effects of prostaglandins (Siegl et al., 1982; Adnot et al., 1982).

#### Anti-obesity

The antiobesity effects of *C. forskohlii* were investigated in ovariectomized rats (Han et al., 2005) and the administration of *C. forskohlii* extracts reduced body weight, food intake and fat accumulation in those rats suggesting that *C. forskohlii* may be useful in the treatment of obesity.

#### Other uses

In addition to its cAMP stimulating activity, forskolin inhibits the binding of platelet-activating factor (PAF), independently of cAMP formation (Wong, 1993). Forskolin also appears to have an effect on several membrane transport proteins and inhibits glucose transport in erythrocytes, adipocytes, platelets and other cells (Mills et al., 1984). The safety of *C. forskohlii* and forskolin has not been fully evaluated. It should be avoided in people with ulcers, because it may increase stomach acid levels (Seamon et al., 1981).

#### Plant Tissue Culture of *Coleus Forskohlii*

Plant tissue culture refers to *in vitro* techniques used to cultivate the plants, seeds and various

parts of the plant like embryo, tissues, protoplast etc. Plant cell has the ability of totipotency that can differentiate and regenerate from meristematic cell to whole plant. *Coleus forskohlii* is an endangered plant so it is necessary for growth and development of its by plant tissue culture. Develop complete plant free from microbes by the plant tissue culture technique.

#### High frequentation Shoot generation of *Coleus forskohlii*

In plant tissue culture developed complete plants of *Coleus forskohlii* in MS medium containing 2.0 mg/l BAP through multiplication at the rate of 15-20 shoots per explants (Kaul et al., 2015).

#### Shoot regeneration from callus

As callus culture can be obtained as rapid plant regeneration system which could be used for the somaclonal variation for producing transgenic plants. Developed plant of *Coleus forskohlii* from the leaf explants of callus. For callus formation using the growth hormones 2,4-D (1.0 mg/l) alone and in combination with BAP (0.5 mg/l) induced green colour, brown colour, green fragile, and light green colour are formed. Best shoot induction are achieved from the callus using BAP 0.5 mg/l and NAA 0.1 mg/l after two week of inoculation. Where highest number of shoot induced and maximum frequency was regenerated. Pratibhadube (2011) was developed the plant of *Coleus forskohlii* from the adventitious shoots in MS medium supplemented with 1.5 mg/l BAP.

#### Callus formation of *Coleus forskohlii*

Malathy and J.pai (1999) have obtained the callus using the hormone of cytokinin and auxin. E sreedevi (2013) was developed plant from the callus on MS medium containing in BAP (2mg/l) and NAA (1mg/l). The highest rate of shoot multiplication was achieved and more than 200 shoots were produced from the callus clump. Callus induction and proliferation system are known to be very useful for the study of bio-synthesis of natural product of *Coleus forskohlii* (Sen et. al., 1992; Mukherjee et. al., 1996; Reddy et.al., 2001).

#### Gene expression

Stable reference genes are necessary to normalize the gene expression data (Shin-young hong et al (2008). Reference genes are particularly useful when stress responsive gene are analyzed in order to produce transgenic plants that exhibit enhanced stress resistance. Genes encoding tubulins, actin or elongation factors are widely used in gene expression studies. We will use three genes

Actin7 (*ACT7*), Tubulin alpha 6 (*TUA6*), and Ubiquitin18 (*UBC18*) for gene expression studies. *UBC18* and *ACT7* genes are the most stable genes that might be widely used for multiple purposes. The function of *ACT7* genes encode one of the six distinct and ancient subclasses of actin protein in the complex Arabidopsis actin gene family. *ACT7* actin genes and investigated its tissue specific expression and regulation. The highest level of *ACT7* mRNA are found in rapidly expanding vegetative organs. The *ACT7* mRNA levels varied by 128- fold among different tissue and organs. The *ACT7* promoter may contain a high density of hormone response elements (J. McDowell et al, 1996). *TUB6* genes the expression patterns of the affected tubulin genes and the phenotypes of the lefty double mutant to address increasing effects of microtubule defects on cell morphogenesis. Tubulin genes were expressed ubiquitously in examined tissue and cell types in Arabidopsis (Tatsuya Abe, et al 2004). The *UBC18* gene encoding an ubiquitin-conjugating enzyme exhibits the most stable expression in different plant tissue or in plants treated with various growth hormones. The ubiquitin-conjugating enzyme *UBC18* function redundantly to control an early step of pharyngeal morphogenesis. The regulation of protein stability through ubiquitin-mediated proteolysis has been show to play a critical role in a wide range of cellular and developmental processes. In *Brachypodium distachyon* the expression of the polyubiquitingenes are most stable in different plant tissue and growth hormone treated plant sample, the expression of the S- adenosymethionine decarboxylase gene (*SAM DC*) ranked are most stable in plant grown under various environmental stresses (Shin-Young Hong et al. 2008).

### Conclusion

The present evidence shows that *Coleus forskohlii* is the only known natural source of the forskolin (diterpenoid). Moreover still, there is paucity for the mechanism of other bioactive principles present in the herb except forskolin. Further researches in view of applicability of forskolin for treating human ailments without any side effects. It is an ornamental plant with many herbal component and herbal properties. It is a valuable addition in medicinal plants biodiversity. The expression of growth regulatory genes were seen to be upregulated in *Coleus* treated explants and hence it can be observed that *Coleus* can be propagated by plant tissue culture method in a short duration and conserved environment.

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