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Application of *in vitro* Biotechnological Tools in *Coleus forskohlii*: An Important Source of Therapeutic Products

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ABSTRACT: The various plant species of *Coleus genus* are commercially important in the field of medicines, components of food and as ornamental plants. Among them, *Coleus forskohlii* is known for sole source of forskolin production naturally. Since the initiation of plant tissue culture, it has boosted the improvement of crops through various ways. The present review summarized various systems of *in vitro* regeneration for commercial production of plantlets as well as establishment of suspension culture and transformation protocol for improving forskolin production *in vitro*. Traditional regeneration system for this plant are not that much efficient and content of forskolin in each plant differed hence, the application of *in vitro* regeneration system and suspension culture can be a useful tool to overcome such limitations. Several studies have been carried out to develop a rapid and efficient protocol for regeneration of plantlets as well as suspension culture for *Coleus forskohlii*. It has been observed that various factors like choice of explants, sterilizing agents, selection of basal medium with appropriate plant growth regulators are important for desirable results. It is also concluded by this review that there is still requirement of more studies for improvement of regeneration efficacy as well application of somaclonal variation and determination of genetic stability in tissue cultured plantlets. More studies should be conducted to establish genetic engineering protocol using direct as well as indirect methods of gene transfers to achieve ample yield of forskolin.

Keywords: *Coleus forskohlii*, forskolin, Organogenesis, Suspension culture, Callus.

INTRODUCTION

Medicinal plant products were initially collected for the treatment of numerous ailments in people and animals all over the world. Although new technology has been developed in pharmaceutical science, medicinal plants are still an important part of the medicines available in the market. Some conventional formulations prepared from locally available medicinal plants are cheap sources of treatment for multiple ailments in various regions (Kavitha *et al.,* 2010). Forskolin, an important labdane diterpene molecule with significant therapeutic qualities, is derived from the curative plant *Coleus forskohlii* (Loftus *et al.,* 2015). Forskolin is an important component of various drugs. The Subcontinent region of India is believed to be the centre of origin for *Coleus forskohlii* (Valdes *et al.,* 1987). It is found in wild areas of various countries like Nepal, India and Sri Lanka, but its appearance is not limited to this region and is dispersed throughout the African and North American regions.

It grows wild in the subtropical temperate climates of India, Nepal, Burma, Sri Lanka and Thailand (Willemse, 1985). This plant is usually located in dry and unproductive hills. In the tubers of this plant, cork cells possess cytoplasmic vesicles that are distinctively yellowish to radish brown in colour. These vesicles are storage sites for various secondary metabolites, along with forskolin (Abraham *et al.,* 1988). Several ethnomedical uses of the tuberous roots and leaves of *C. forskohlii* for human as well as veterinary ailments are noted in the ancient literature. Visually, the roots of this plant are broad, hairy, radially extended and golden brown in colour (Kamini *et al.,* 2013). The root extract of this species has a wide variety of biochemical constituents (Ammon and Kemper 1982; De Souza and Shah 1988) among which forskolin is the major secondary metabolite, which has a huge medicinal value (Kavitha *et al.,* 2010). *C. forskohlii* is the only known plant to produce forskolin naturally (Velmurugan *et al.,* 2010). Forskolin is used for the treatment of a wide range of disorders, such as glaucoma, cardiac diseases and some types of cancer (Mitra *et al.,* 2020). It is also helpful in curing respiratory diseases, skin diseases such as psoriasis and eczema and hypertension (Rupp *et al.,* 1986).

C. forskohlii is traditionally propagated by sowing seeds in nursery beds with appropriate irrigation. It is replanted in the main field after 15 to 20 days. Stem cuttings with four pairs of leaves and a diameter of 10 to 12 cm are used for vegetative propagation of the crop. It is planted in the nursery, where it takes a month to establish roots before being moved to the main field (Lokesh *et al.,* 2018). But conventional propagation

methods encounter some limitations, like the slow growth rate of plantlets as well as the lower accumulation of forskolin content (Chandel *et al.*, 1991). It is found mainly in the wild, so excessive collection of the roots from these sources makes it count as an endangered species. The annual production of the roots of this plant in India is around 100 tonnes from an area of 700ha. This market demand also promoted the cultivation of this plant (Kavitha *et al.*, 2010). Limitations faced by conventional methods have forced the application of *in vitro* strategies for commercial production of plantlets of *C. forskohlii* which have a higher yield of forskolin and a uniform genetic constituent (Mitra *et al.*, 2020).

In vitro regeneration methods are useful tools to achieve large scale plantlet production and the conservation of germplasm. Plant tissue culture offers rapid production of genetically stable plants. It can be applied to the conservation of rare medicinal plants and their subsequent multiplication in a short period of time (Ananthu *et al.,* 2021). Tissue culture is also necessary for germplasm dissemination, preservation, and secure circulation of internal planting material (Rodge *et al.*, 2023). The market demand for the roots of the plant can be fulfilled by this technology. The best benefit that tissue culture offers is the quality of the raw herbal material and its homogeneity. The technique is also significantly important for studies related to the development of active components within plant cells and their subsequent enhancement of production, as well as modifications in the production technology (Praveena *et al.,* 2012). *In vitro* regeneration protocols for various plants are reported, but continuous optimisation is still in progress to obtain a more efficient and rapid system to multiply plants, especially in medicinal plants. This review covers various systems involved in the regeneration of *C. forskohlii*.

Plant tissue culture for *Coleus forskohlii* regeneration. Plant tissue culture comprises a set of *in vitro* culture techniques and methods like organogenesis, somatic embryogenesis, suspension culture etc. It can also be used to develop genetically variable plants using somaclonal variation. To increase the number of suitable germplasms and improve the health of the planted material, tissue culture has been used to create genetic diversity in crop plants (Brown and Thorpe 1995). The best approach for producing plantlets on a commercial level is micropropagation, which also ensures a steady supply of planting material (Gantait *et al.,* 2018). Tissue culture techniques have been improved in the last few decades as a result of progress and the need to produce on a large scale. Low secondary metabolite contents in entire plants have been a concern that has prompted researchers to seek out a big technological advancement. A number of specific procedures have been created for the commercial production of a diverse range of plant secondary metabolites as a result of advancements in current technology (Twaij *et al.*, 2020).

Selection explant and surface sterilization of explant. Every micropropagation experiment must begin with the careful selection of an explant that should be completely free from any type of contamination. In many regeneration studies of *C. forskohlii*, explants including shoot tips, nodal segments, roots, hypocotyls and leaves have been used (Table 1). According to several reports, the preferred explants are the shoot tip and nodal segment. These explants have an abundance of meristematic cells that demonstrate totipotency and an accumulation of desired growth regulators (Akin-Idowu *et al.,* 2009). Leaf explants were also applied in both direct and indirect regeneration investigations (Reddy *et al.,* 2001; Ashwinkumar, 2006; Krishna *et al.,* 2010; Sahai and Shahzad 2010; Gopi and Mary 2014; Gangopadhyay *et al.*, 2016; Vibhuti and Kumar 2019).

Surface sterilization using various groups of chemicals is a critical procedure for the establishment of any regeneration system in any plant. The success of sterilization is dependent on both i.e. type of explant and the combination of surface disinfectants used. Maturity status and size of the explant were major factors when determining the optimum dosage and period of sterilization. Most studies on *C. forskohlii* regeneration showed that the sterilization process involves treatment with any detergent for 5 to 30 minutes, such as Tween-20, labolene, Teepol, Cleansol, and Triton X-100 in varying concentrations after washing with distilled water (Table 1). Some studies also reported the utilization of antifungal components like Bavistin (Bhattacharyya and Bhattacharyya, 2001; Balasubramanya *et al.,* 2012; Sahai and Shahzad 2013; Chandra *et al.,* 2019) for 30–60 min and antibiotics like streptocyclin (Bhattacharyya and Bhattacharyya 2001) and streptomycin (Chandra *et al.,* 2019) for efficient surface sterilization to control initial contamination during the establishment of cultures. Mercuric chloride ($HgCl_2$) in various concentrations for various periods is a prominent sterilizing agent in most of the reports (Table 1). Ethanol (70%) was also used in some literature (Rajasekharan *et al.,* 2010).

Plant tissue culture medium is prepared using various concentrations of different nutrients like minerals, vitamins, carbon sources, and plant growth regulators, which are necessary for the proper development of the *in vitro* plantlets. Basal medium described by Murashige and Skoog (1962) was the most preferred medium for *in vitro* regeneration studies in *C. forskohlii* (Table 1), whereas B5 medium described by Gamborg *et al*. (1968) was also used by Balasubramanya *et al.* (2012). The morphogenesis and regeneration of plantlets in tissue culture in all regeneration systems are greatly influenced by the type and dose of plant growth regulators utilized.

A culture medium, which provide the nutrients required for growth of plant tissues and organs *in vitro*. An ideal mix of nutrients and growth regulators is called a culture medium. The fundamental nutritional needs of cultivated plant cells are quite similar to those of entire plants. The nutritional needs of plants growing in soil appear to have provided the first inspiration for the creation of a basic culture medium, with nutrient

solutions used for whole plant culture providing the final inspiration (Chimdessa, 2020). Regardless of direct organogenesis or indirect organogenesis experiments, the type of growth regulators utilized and their corresponding doses have a significant impact on the morphogenesis and development of plants during in vitro environments. Plant growth regulators (PGRs) contribute in retaining balance between the rates of growth of various plant components, which finally leads to the production of a whole plantlet (Gantait and Kundu 2017). There are several papers that demonstrate the impact of plant growth regulators for *in vitro* regeneration in *C. forskohlii*. An enormous variety of plant growth regulators like 6-benzylaminopurine (BAP), kinetin, 1-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and 2, 4-dichlorophenoxyacetic acid (2,4-D) were used in several studies (Table 1).

Direct organogenesis in *Coleus forskohlii.* Plant regeneration is the primary result of plant tissue culture, which is founded on the principle of totipotency. Organogenesis refers to the development of organs from cultured explants. Shoot buds or monopolar structures formed in cultures by modulating the cytokinin-to-auxin ratio (Bhatia and Bera 2015). The administration of cytokinin alone or in conjunction with any auxin results in multiple shoot induction and proliferation, which are components of direct organogenesis (Reddy *et al.,* 2001). Numerous investigations have been conducted to achieve direct organogenesis in *C. forskohlii*, where the use of just one cytokinin, primarily BAP, produced satisfactory results (Krishna *et al.,* 2010; Sahai and Shahzad 2010, 2013; Kaul *et al.,* 2015; Janarthanam and Sumathi 2020). BAP together with auxins like NAA (Rajasekharan *et al.*,2010) also displayed a parallel outcome. Another cytokinin namely kinetin that was utilized along with IAA (Bhattacharyya and Bhattacharyya 2001) and NAA (Senthilkumar *et al.,* 2019) for direct organogenesis. Additionally, there is only one report where combining BAP and kinetin, two cytokinins, produced positive outcomes (Sreedevi and Pullaiah 2014).

Table 1: Details of regeneration system applied in *C. forskohlii.*

Regeneration through direct organogenesis				
Explant(s)	**Sterilization**	**Medium**	**Plant growth regulators**	**Reference**
Shoot tip and single node	5% Teepol (5 min); 0.1% Bavistin and 0.02% Streptocycline (30-40 min); 0.01% $HgCl_2$ (2 min)	MS medium	0.57 µM indole-3-acetic acid and 0.46 µM kinetin	Bhattacharyya and Bhattacharyya (2001)
Leaves	Tween 20; 0.1% Bavistin (45 min); 0.05% mercuric chloride (10 min); 0.1% mercuric chloride (3 min)	B_5 medium	0.5mg/l BA for shoot bud induction	Balasubramanya *et al.,* (2012)
Nodal explant	1% Bavistin (30 min); 5% labolene (15 min); 0.1% $HgCl_2$ (3 min)	MS medium	5 µM BA for shoot formation; 5 µM BA and 0.1 µM NAA for shoot multiplication	Sahai and Shahzad (2013)
Shoot tips and nodal segments	4% Cleansol (15 min); 70% ethanol (90 s); 0.01% $HgCl_2$ (5 min)	MS medium	8.87 µM BAP and 0.54 µM NAA for multiple shoot induction	Rajasekharan *et al.* (2010)
Leaf explant	0.1% $HgCl_2$ (4 min)	MS medium	5.0 mg/l BAP for multiple shoot initiation; 0.1 mg/l BAP and 0.1 mg/l IAA for elongation	Krishna *et al.* (2010)
Leaf segment	1% Bavistin (30 min); 5% Labolene (15 min); 1% $HgCl_2$ (3 min)	MS medium	2 mg/l BAP for shoot induction 2 BAP +0.1 mg/l NAA for multiplication	Sahai and Shahzad (2010)
Nodal segment	5% Teepol (3 min); 2% Bavistin; 70% ethanol (30 s); 1% $HgCl_2$ (2 min)	MS medium	4.65 µM kinetin + 1.73 µM GA_3 for shoot induction	Thangavel *et al.* (2014)
Shoot tip	5% Teepol (10 min); 0.1% $HgCl_2$ (8 min)	MS medium	2 mg/l BAP for shoot induction	Vibhuti and Kumar (2019)
Nodal Explants	0.1% Tween 20 (5 min); 0.1% $HgCl_2$ (5 min)	MS medium	4.44 µM BAP for shoot regeneration; 2.46 µM IBA for root induction	Janarthanam and Sumathi (2020)
Apical and Axillary meristem	Soap solution (25 Min); 0.1% $HgCl_2$ (10 min)	MS medium	2.0 mg/l BAP for shoot regeneration	Kaul *et al.,* (2015)
Leaf	0.1% $HgCl_2$ (10 min)	MS medium	2.0 mg/l Kinetin and 0.1mg/l NAA for shoot regeneration	Senthilkumar *et al.* (2019)
Nodal segment	1% Bavistin + 0.5%	MS	1.5 mg/l BAP for shoot	Dube *et al.*

	streptocycline (5 min); 0.1% $HgCl_2$ (3 min)		induction	(2011)
Nodal segment	5% Teepol (5 min); 70% Ethanol (15 min); 0.1% $HgCl_2$ (4 min)	MS medium	0.25 mg/l BAP +0.25 mg/l kinetin for shoot induction 1 mg/l BAP +1 mg/l NAA for multiplication ½ MS + 0.5 mg/l IAA for rooting	Sreedevi and Pullaiah (2014)
Regeneration through indirect organogenesis				
Leaves	Tween 20; 0.1% Bavistin (45 min); 0.05% mercuric chloride (10 min)	B_5 medium	3mg/l 2,4-D and 3mg/l picloram for callus induction; 2mg/l BA for callus with shoots	Balasubramanya *et al.* (2012)
Leaf	0.05% $HgCl_2$ (7 min)	B_5 and MS medium	2 mg/l 2,4-D for callus induction 2 mg/l BAP + 1 mg/l NAA for shoot induction	Sreedevi *et al.* (2013)
Shoot apex, internodal segments with a single axillary and leaf lamina with mid-vein	0.1% Tween 20 (5 min); 1% Bavistin (60 min); 0.1% $HgCl_2$ (2-3 min); 0.5% streptomycin (5 min)	MS medium	0.5mg/l IAA and 0.5 mg/l BAP for callus induction; 1mg/l IAA and 5 mg/l BAP for shoot regeneration; 3 mg/l IAA for root induction	Chandra *et al.* (2019)
Leaf	0.1% Triton X-100 (15 min);0.05% $HgCl_2$ containing 2 drops of Tween-20 (8 min)	MS medium	2.4 µM kinetin for callus induction; 4.6 µM kinetin and 0.54 µM NAA for shoot induction	Reddy *et al.* (2001)
Nodal segment	5% Teepol (5 min); 70% ethanol (15 min); 0.1% $HgCl_2$ (7 min)	MS medium	2 mg/l NAA for callus induction	Sreedevi and Pullaiah (2014)
Leaf	-	MS medium	2 mg/l 2,4-D for callus induction	Gangopadhyay *et al.* (2016)
Leaf	-	MS medium	3 mg/l NAA + 1mg/l BAP for callus induction	Swaroopa *et al.* (2016)
Leaf, node and shoot tip	5% Teepol (10 min); 0.1% $HgCI_2$ (8-10 min)	MS medium	2.0 mg/l BAP with 1.5 mg/l 2,4-D for callus induction	Vibhuti and Kumar (2019)
Leaf	0.1% $HgCl_2$ (10 min)	MS medium	1.0 mg/l kinetin for organogenic callus induction; 1.0 mg/l kinetin and 0.1 mg/l NAA for shoot induction	Senthilkumar *et al.* (2019)
Regeneration using somatic embryogenesis				
Leaf	Tween 20; 0.1% $HgCI_2$ (5 min)	MS medium	1.0 mgl/l 2,4-D for embryogenic callus induction; 1.0 mg/l BAP and 0.5 mg/l 2,4-D for embryogenesis	Gopi and Mary (2014)

Indirect regeneration of *C. forskohlii.* The main stage in indirect organogenesis, a method of plantlet regeneration under in vitro conditions, is the induction and proliferation of calluses. A callus is a collection of dispersed cells. Callus can be further classified into friable and embryogenic calli. These types of callus are useful for establishment of cell suspension and induction of numerous shoots using a medium comprising precise combination of plant growth regulators (Gantait and Kundu 2017). Callus mediated regeneration is important for selection of variant cell lines for genetic improvement of crop plants and also considered as a pre requisite step for *in vitro* mutation works (Velmurugan *et al.,* 2010). Only a few reports on callus induction, proliferation, and full plantlet regeneration in *C. forskohlii* are currently available (Table 1).

Application of picloram along with 2, 4-D provided optimum induction of friable calli (Balasubramanya *et al.,* 2012) whereas Chandra *et al.* (2019) obtained callus induction using combination of IAA and BAP. When talking about influence of sole cytokinin i.e. kinetin in *C. forskohlii*, Reddy *et al.* (2001); Senthilkumar (2019) found success in induction of callus using leaf samples. Vibhuti and Kumar (2019) induced callus using mixture of 2,4-D and BAP.

Regeneration using somatic embryogenesis . Typically, somatic embryos arise from embryogenic calli that have been stimulated by the administration of particular plant growth regulators. Therefore, the formation of somatic embryos from an amorphous mass of somatic cells is known as somatic embryogenesis (Gantait and Kundu 2017). Only one somatic embryogenesis experiment employing a leaf explant has

been documented in *C. forskohlii* (Gopi and Mary, 2014). In this experiment, they used BAP and 2, 4-D. There aren't any detailed studies on somatic embryogenesis that are done either directly or indirectly. It is possible to use cytokinins and auxins separately to create bipolar propagules and promote their regeneration.

Cell suspension culture. Cell suspension culture*i.e.in vitro* cultivation of cells in liquid medium under continuous shaking is a system for aceaseless yield of secondary metabolites. This method has numerous advantages, including the fact that it does not require a specific season that the collected cells are free of biotic pollutants, that any type of metabolite may be extracted with ease, that there is a reduction in the cost of labor, and that direct extraction from cells is also possible (Vijaya Sree*et al.,* 2010). There are a few studies on cell suspension culture in *C. forskohlii*. Initiating calli in media treated with 2, 4-D and kinetin that were suspended in liquid B5 medium supplemented with IBA and kinetin was done by Mersinger*et al.* (1988). Swaroopa *et al.* (2013) established the cell suspension culture by inoculating friable calli in MS broth fortified with NAA and BAP.

Genetic transformation in *C. forskohlii.* Genetic transformation is a technology that allows for the creation of genetic alteration through the transfer and integration of a particular gene or DNA segment from any species in order to get desired traits in the host. In *C. forskohlii*, a few studies for transformation using either *Agrobacterium* or biolistic gun method. Mukherjee *et al.* (1996) transformed shoot tips using *A. tumefaciens* strain C58 and the transformed callus were compared for forskolin production with untransformed samples. Guleria and Gowda (2015) transformed leaf derived callus using biolistic gun method. In this study, pABC plasmid DNA containing the β -glucuronidase (GUS) reporter gene and the AtWBC19 gene from Arabidopsis thaliana as a selectable marker gene was bombarded onto the callus of *C. forskohlii* using a biolistic gun.

There are few studies in *C. forskohlii*, in which hairy root cultures were initiated through the *A. rhizogenes*–mediated hairy root culture had been attempted. Sasaki *et al.* (1998) utilized strain MAFF 03- 01724 for hairy root induction. The forskolin concentration was considerablymore than non-transformed ones. Likewise, strain A4 was used to induce hairy roots by using leaf as explant. Mannopine was used as evidence that the roots had undergone transformation and the resulting forskolin concentration was 1.449 mg/g fresh weight. Additionally, at day 14, forskolin levels increased by 2.7 times as a result of the elicitor methyl jasmonate (Reddy *et al.*, 2012). The most recent study, Pandey *et al.* (2014), used the MTCC2364 strain to cause infection in nodal regions. The altered roots were later validated using molecular analysis utilizing the *rolA* sequence and kept in PGR-free liquid MS media. Compared to untransformed plants, the forskolin level estimate was substantially greater.

CONCLUSIONS

This review elaborates on the biotechnological advances made on *C. forskohlii* upto date. There are several findings on direct and indirect organogenesis. This article addressed the impact of various explant sources, sterilization agents, and plant growth regulators on the micropropagation using direct and indirect regeneration system. Coleus nodal segments were the best source of explants for culture initiation because they tolerated sterilization better than apical shoots, demonstrating the highest survival frequency. There are some reports on somatic embryogenesis and genetic transformation but needs further work to establish robust and rapid protocols.

FUTURE SCOPE

The current study summarized a sufficient number of the findings on regeneration of plant lets and production of forskolin using suspension culture however, new methodologies, such as testing the genetic integrity of in vitro regenerated plantlets, are still needed. This can ensure production of true type plantlets and can avoid any deviation from mother plants. Opposite to genetic fidelity, studies on induction of somaclonal variation in cultures can be induced to develop lines which have potential to produce higher amount of therapeutic components. The numbers of studies available on genetic transformation are still insufficient and more studies should be conducted to explore effect of various factors involve in optimization of genetic transformation. Hairy root induction via genetic transformation can also be optimized to enhance secondary metabolite production. From this point onward, the current study offers a wealth of details regarding the state of the biotechnological interventions made in *C. forskohlii* so far and also identifies numerous techniques that may be helpful to investigate this plant for the generation of additional secondary metabolites.

Conflict of Interest. None.

REFERENCES

Abraham, Z., Srivastava, S. K. and Bagchi, C. A. (1988). Cytoplasmic vesicles containing secondary metabolites in the roots of *Coleus forskohlii*. *Current Science, 57*, 1337-1339.

Akin-Idowu, P. E., Ibitoye, D. O. and Ademoyegun, O. T. (2009). Tissue culture as a plant production technique for horticultural crops. *African Journal of Biotechnology, 8,* 3782–3788.

Ammon, H. P. and Kemper, F. H. (1982). Ayurveda: 3000 years of Indian traditional medicine. *Medizinische Welt, 33*, 148-153.

Ananthu, J. S., Pawar, S. V., Najan, B. R., Raut, Y. M. and Gulwane, V. P. (2021). Development of Sterilization and Initiation Protocol for *in vitro* Regeneration in Bamboo (*Bambusa balcooa* Roxb.). *Biological Forum – An International Journal, 13*(3a), 221-227.

Ashwinkumar, S. K. (2006). In: International Conference on Globalization of Traditional, Complementary and Alternative systems of medicine. *Tamil Nadu Agricultural University, Coimbatore,* pp 29–30

Balasubramanya, S., Rajanna, L. and Anuradha, M. (2012). Effect of plant growth regulators on morphogenesis and forskolin production in *Plectranthus barbatus* Andrews. *In Vitro Cellular Developmental Biology-Plant 48*, 208-215.

Bhatia, S. and Bera, T. (2015). Somatic Embryogenesis and Organogenesis. In *Modern Applications of Plant Biotechnology in Pharmaceutical Sciences*; *Elsevier: London, UK*. pp. 209–230.

Bhattacharyya, R. and Bhattacharya, S. (2001). *In vitro* multiplication of *Coleus forskohlii* Briq.: An approach towards shortening the protocol. *In Vitro Cellular Developmental Biology- Plant 37*, 572-575.

Brown, D. C. and Thorpe, T. A. (1995). Crop improvement through tissue culture. *World Journal of Microbiology and Biotechnology 11(4)*, 409-415.

Chandel, V. K. S., Sharma, N. and Srivastav, V. K. (1991). *In vitro* propagation of *Coleus forskohlii* Briq. A threatened medicinal plant. *Plant Cell Reports, 10*, 67–70.

Chandra, A. K., Rajak, K. K., Gururani, K., Kumar, H. and Kumar, M. (2019). Influence of explants type and phytohormones on *In vitro* callogenesis and plantlet regeneration of patharchur (*Coleus barbatus* L.), an endangered ethnomedicinal plant. *Journal of Pharmacognosy and Phytochemistry*, *8*(3), 943-953.

Chimdessa, E. (2020). Composition and Preparation of Plant Tissue Culture Medium. *Journal of Tissue Culture and Bioengineering, 3*, 120.

De Souza, N. and Shah, V. (1988). Forskolin –An adenylate cyclase activating drug from an Indian herb. *Economic and Medicinal Plant Research, 2*, 1-16.

Dube, P., Gangopadhyay, M., Dewanjee, S. and Ali M. N. (2011). Establishment of a rapid multiplication protocol of *Coleus forskohlii* Briq and *in vitro* conservation by reduced growth. *Indian Journal of Biotechnology, 10*, 228–231.

Gamborg, O. L., Miller, R. A. and Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research, 50*, 151–158.

Gangopadhyay, M., Gantait, S., Palchoudhury, S., Ali, M. N., Mondal, C. and Pal, A. K. (2016). UVC-priming mediated modulation of for skolin biosynthesis key genes against Macrophomina root rot of *Coleus forskohlii* - a tissue culture based sustainable approach. *Phytochemistry Letters, 17*, 36–44.

Gantait, S. and Kundu, S. (2017). *In vitro* biotechnological approaches on *Vanilla planifolia* Andrews: advancements and opportunities. *Acta Physiologiae Plantarum, 39*, 196.

Gantait, S., El-Dawayati, M. M., Panigrahi, J., Labrooy, C. and Verma, S. K. (2018). The retrospect and prospect of the applications of biotechnology in *Phoenix dactylifera* L. *Applied Microbiology and Biotechnology 102*, 8229-8259.

Gopi, C. and Mary, M. D. R. (2014). *In vitro* plant regeneration through somatic embryogenesis in medicinally important leaf explants of *Coleus forskohlii* Briq. *IOSR Journal of Agriculture and Veterinary Science, 7*(9), 20-23.

Guleria, N. and Gowda, P. H. R. (2015). An efficient regeneration and genetic transformation protocol of *Coleus forskohlii* using biolistic gun. *International Journal of Agriculture, Environment and Biotechnology, 8*(2), 227-235.

Janarthanam, B. and Sumathi, E. (2020). *In vitro* Plant regeneration from nodal explants of *Coleus forskohlii* Briq.- An important medicinal plant. *Plant Tissue Culture and Biotechnology, 30*(1), 143-148.

Kamini, K., Ashashri. S., Pankaj, G. and Lalit, N. (2013). Comprehensive review: *Coleus forskohlii*. *International Journal of Ayurvedic and Herbal Medicine, 3*, 1106-1113.

Kaul, T., Malik, M. A., Yaqoob, U. and Mehta, J. (2015). High frequency and rapid in vitro plant regeneration of *Coleus forskohlii*Briq. *Medicinal and Aromatic Plants, 4*, 193.

Kavitha, C., Rajamani, K. and Vadivel, E. (2010). *Coleus forskohlii*: A comprehensive review on morphology, phytochemistry and pharmacological aspects. *Journal of Medicinal Plants Research, 4*(4), 278-285.

Krishna, G., Reddy, P. S., Nair, N. A., Ramteke, P. W. and Bhattacharya, P. S. (2010). *In vitro* direct shoot regeneration from proximal, middle and distal segment of *Coleus forskohlii*leaf explants. *Physiology and Molecular Biology of Plants, 16*(2), 195-200.

Loftus, H. L., Astell, K.J., Mathai, M. L. and Su, X. Q. (2015). *Coleus forskohlii* extract supplementation in conjunction with a hypocaloric diet reduces the risk factors of metabolic syndrome in overweight and obese subjects: a randomized controlled trial. *Nutrients*, 9508–9522.

Lokesh, B., Deepa, R. and Divya, K. (2018). Medicinal Coleus (*Coleus forskohlii* Briq): a phytochemical crop of commercial significance. *Journal of Pharmacognosy and Phytochemistry, 7*, 2856–2864.

Mersinger, R., Dornauer, H. and Reinhard, E. (1988). Formation of forskolin by suspension cultures of *Coleus forskohlii*. *Planta Medica, 54*, 200–204.

Mitra, M., Gantait, S. and Mandal, N. (2020). *Coleus forskohlii*: advancements and prospects of in vitro biotechnology. *Applied Microbiology and Biotechnology, 104*, 2359–2371

Mukherjee, S., Ghosh, B. and Jha, S. (1996). Forskolin synthesis in *in vitro* cultures of *Coleus forskohlii* Briq transformed with *Agrobacterium tumefaciens*. *Plant Cell Reports 15*, 691–694.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiology Plantarum, 15*, 473–495.

Pandey, R., Krishnasamy, V., Kumaravadivel, N. and Rajamani K. (2014). Establishment of hairy root culture and production of secondary metabolites in Coleus (*Coleus forskohlii*). *Journal of Medicinal Plant Research8*, 58–62.

Praveena, R., Pandian, S.A. and Jegadeesan, M. (2012). *In vitro* culture studies on medicinal Herb - *Coleus forskohlii* Briq. *Libyan Agriculture Research Center Journal International, 3*(1), 30-35.

Rajasekharan, P. E., Ganeshan, S. and Bhaskaran, S. (2010). *In vitro* regeneration and conservation of three *Coleus* species. *Medicinal and Aromatic Plant Science and Biotechnology, 4*(1), 24-27.

Reddy, C.S., Praveena, C. and Veeresham, C. (2012). Strategies to improve the production of forskolin from hairy root cultures of *Coleus forskohlii* Briq. *International Journal of Pharmaceutical Sciences and Nanotechnology, 5*, 1720–1726.

Reddy, P. S., Rodrigues, R. and Rajasekharan, R. (2001). Shoot organogenesis and mass propagation of *Coleus forskohlii* from leaf derived callus. *Plant Cell, Tissue and Organ Culture, 66***,** 183–188.

Rodge, R. R., Mirza, A., Kaur, H., Girase, L. and Khan J. J. (2023). *In vitro* regeneration of *Musa*spp. plantlet CV. Grand Naine by plant tissue culture technique. *Biological Forum – An International Journal, 15*(4), 533-538.

Rupp, R. H., De Souza, N. J. and Dohadwalla, A. N. (1986). Proceedings of the International Symposium on Forskolin: Its chemical, biological and medical potential. *Hoechst India Limited, Bombay*, pp. 19-30.

Sahai, A and Shahzad, A. (2010). *In vitro* clonal propagation of *Coleus forskohlii* via direct shoot organogenesis from selected leaf explants. *Journal of Plant Biochemistry and Biotechnology, 19*, 223–228.

Sahai, A. and Shahzad, A. (2013). High frequency *in vitro* regeneration system for conservation of *Coleus forskohlii*: a threatened medicinal herb. *Acta Physiologiae Plantarum, 35*, 473–481.

Sasaki, K., Udagawa, A., Ishimaru, H., Hayashi, T., Alfermann, A. W., Nakanishi, F. and Shimomura, K. (1998). High forskolin production in hairy roots of *Coleus forskohlii*. *Plant Cell Reports, 17*, 457–459.

Senthilkumar, P., Vasuki, A. and Lakshmiprabha, A. (2019). Mass propagation of important medicinal plant *Coleus forskohlii* (Briq) through direct and indirect regeneration using leaf explant. *Journal of Emerging Technologies and Innovative Research, 6*(3), 178-186.

Sreedevi, E. and Pullaiah, T. (2014). Effect of growth regulators on in vitro organogenesis and long term storage of *Plectranthus barbatus* Andr. (Syn.: *Coleus forskohlii* (Wild.) Briq.). *Current Trends in Biotechnology and Pharmacy, 8*, 143–151.

Sreedevi, E., Anuradha, M and Pullaiah, T. (2013). Plant regeneration from leaf-derived callus in *Plectranthus barbatus*Andr [Syn.: *Coleus forskohlii* (Wild.) Briq.]. *African Journal of Biotechnology 12*, 2441–2448.

Swaroopa, G., Anuradha, M. and Pullaiah, T. (2013). Elicitation of forskolin in suspension cultures of *Coleus forskohlii* (wild) Briq using elicitors of fungal origin. *Current Trends in Biotechnology and Pharmacy, 7*, 755–762.

Swaroopa, G., Anuradha, M. and Pullaiah, T. (2016). Influence of sucrose and plant growth regulators on growth and forskolin production in callus cultures of *Coleus forskohlii*. *Current Trend in Biotechnology and Pharmacy, 10*, 45–54.

Thangavel, P., Prabhu, S. and Britto, S. J. (2014). High frequency shoots regeneration from nodal explants of *Plectranthus barbatus* Andrews belong to the Lamiaceae. *Journal of Andaman Science Association, 19*, 126–135.

Twaij, B. M., Jazar, Z. H. and Hasan, M. N. (2020). Trends in the Use of Tissue Culture, Applications and Future Aspects. *International Journal of Plant Biology, 11*(1), 8385.

Valdes, L. J., Mislankar, S. G. and Paul, A. G. (1987). *Coleus barbatus* (*C. forskohlii*) (Lamiaceae) and the potential new drug forskolin (Coleonol). *Economical Botany, 44*, 474-483.

Velmurugan, M., Rajamani, K., Paramaguru, P., Gnanam, R., Kannan Bapu, J. R., Davamani, V. and Selvakumar, T. (2010). Direct and indirect organogensis in medicinal Coleus (*Coleus forskohlii* BRIQ.)- A review. *Agriculture Review, 31*(1), 73-76.

Vibhuti, R. K. and Kumar, D. (2019). Effect of 6-BAP on callus culture and shoot multiplication of *Coleus forskohlii*(Syn: *Plectranthus froskohlli*; wild) Briq. *Research Journal of Life Sciences, Bioinformatics, Pharmaceuticals and Chemical Sciences, 5*(1), 574-581.

Vijaya Sree, N., Udayasri, P., Aswanikumar, V. V. Y., Ravi, B.B., Phani, K. Y. and Vijay, V. M. (2010). Advancements in the production of secondary metabolites. *Journal of Natural Products, 3*, 112–123.

Willemse, R. H. (1985). Notes on East African *Plectranthus* species (Labiatae). *Kew Bulletin, 40*, 93-96.

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