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MINI-REVIEW

Coleus forskohlii: advancements and prospects of in vitro biotechnology

Monisha Mitra[1] · Saikat Gantait[2] · Nirmal Mandal[1]

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Abstract

Coleus forskohlii syn. *Plectranthus barbatus* is a popular medicinal plant belonging to the family *Lamiaceae* and order *Lamiales*. The leaf and root extracts can be utilized for the treatment of various ailments like bronchitis, asthma, hay fever, angina and abdominal disorders. The major metabolite that is found exclusively in the cork cells of the root in *C. forskohlii* is forskolin, which is used commercially for the treatment of glaucoma, asthma and several heart ailments. The essential oil extracted from the tubers of the plant also exhibits anti-microbial properties. The present review recounts the existing reports on biotechnological approaches like direct, indirect organogenesis and somatic embryogenesis for mass propagation of plantlets; the amelioration of forskolin production through cell suspension and genetic transformation as well as slow growth storage for germplasm conservation. Additionally, the unexplored arenas and the prospective novel approaches are also addressed in this review that can be utilized in designing new experiments in near future on this plant.

Keywords Cell suspension · Forskolin · Genetic transformation · Micropropagation · Somatic organogenesis

Introduction

Coleus forskohlii syn. *Plectranthus barbatus* is a popular medicinal plant belonging to the family *Lamiaceae* (popularly known as mint family). The genus '*Coleus*' was coined by De Loureiro in 1970 which means 'sheath enveloped around style', whereas, the species name '*forskohlii*' was designated to honour eminent Swedish botanist Forsskåhl (Kavitha et al. 2010). In the Ayurvedic scriptures, the Sanskrit names were recorded as '*Mayani*' and '*Makandi*' (Shah 1996). The cytological reports reveal that the plant is diploid and the basic chromosome number (x) is 14. However, there are contradictory reports regarding the variation in basic chromosome number since the South African cultivars possess $n = 16$, whereas the North Indian and South Indian cultivars possess $n = 17$ and $n = 15$, respectively (Kavitha et al. 2010). The major metabolite found exclusively in the cork cells of the root in *C. forskohlii* is forskolin. It is used in the treatment of glaucoma, asthma and cardiac and gastrointestinal ailments (Alasbahi and Melzig 2010). Conventional propagation of *C. forskohlii* is carried out chiefly through seeds and stem cuttings. The major shortcomings faced in traditional propagation of this plant are slow growth and lower levels of forskolin, since the accumulation of the alkaloid is majorly influenced by environmental factors (Chandel et al. 1991). Hence, there is an urgent need of interference via in vitro strategies for enhancement of large-scale propagation of *C. forskohlii*, retaining its genetic uniformity, and amelioration of forskolin production simultaneously. This review encompasses the in vitro strategies that have been conducted so far in *C. forskohlii*.

Monisha Mitra and Saikat Gantait contributed equally to this work.

✉ Saikat Gantait
saikatgantait@yahoo.com

[1] Department of Agricultural Biotechnology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal 741252, India

[2] Crop Research Unit (Genetics and Plant Breeding), Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal 741252, India

Geographical distribution

The distribution of *C. forskohlii* is abundant in the tropical and sub-tropical zones of the world. The origin of this plant is found in India (Valdes et al. 1987). The countries in which this medicinal plant is evenly distributed in the Indian

subcontinent are, namely, India, Nepal, China, Myanmar and Sri Lanka and other countries, namely, Thailand, Brazil, Ethiopia and Egypt (Khan et al. 2012). In India, its cultivation is localized in Kumaon and Garhwal region of Himachal Pradesh, Parasnath hills in Bihar and the Western Ghats (Chandel and Sharma 1997).

Botanical description

C. forskohlii is an herbaceous, medicinal and aromatic plant belonging to the family *Lamiaceae* and order *Lamiales* (Lokesh et al. 2018). The height of the plant ranges from 30 to 60 cm, possessing an erect stem with four branches and with the presence of pubescence in nodal regions (Fig. 1a, b). The leaf length and width are 7.5 cm and 5 cm, respectively. The leaves are greenish in colour with a characteristic teardrop shape. The centre of leaf may display a purplish shade and pubescence is also present (Khan et al. 2012) (Fig. 1c). The inflorescence is raceme, the dimensions are 2 to 2.5 cm, the flowers are cross-pollinated and colour is pale purple or lilac with a hairy calyx (Fig. 1d). The stigma is modified into two lobes and the ovary is parted into four locules (Fig. 1d) (Bhowal and Mehta 2017). The main economic part of the plant is root, which is conical or fusiform in shape, found in fasciculated form with a dimension of 0.5 to 2.5 cm in diameter and is golden brown in colour (Kavitha et al. 2010).

Economic importance and phytochemistry

The plant has multifaceted uses in many arenas; it has its uses in the pharmaceutical industry to manufacture various drugs and also utilized by the food industry mainly as a condiment. The tubers of this plant are edible and can be consumed in the form of pickles (Chowdhary and Sharma 1998). In Ayurvedic scriptures, it is mentioned that the leaf and root extracts can be utilized for the treatment of various ailments like bronchitis, asthma, hay fever, angina and abdominal disorders (Foster and Johnson 2006). The essential oil extracted from the tubers of the plant also exhibits anti-microbial properties (Chowdhary and Sharma 1998). A wide array of various secondary metabolites is present in the entire plant, viz. methyl quinine, barbatusin and coleon in leaves and coleosol, coleonol and other biomolecules like glycosides, phenols and terpenoids in the roots (Chandel et al. 1991).

The major metabolite found exclusively in the cork cells of the root in *C. forskohlii* is forskolin (Fig. 1f). It is a labdane diterpenoid produced through a non-mevalonate pathway that

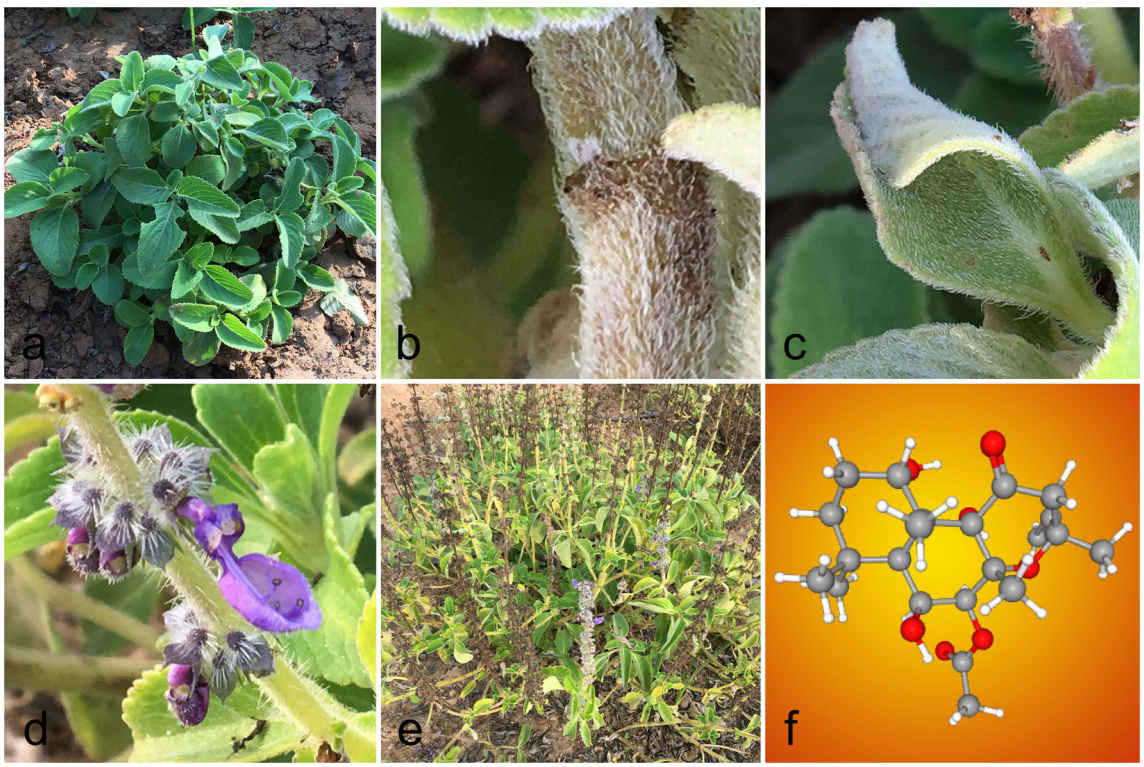

Fig. 1 Growth and propagation of *Coleus forskohlii* syn. *Plectranthus barbatus*, in its natural environment. **a** Young and actively growing plant of *Coleus forskohlii* in its natural habitation. **b** Stems with hairs. **c** Leaves with hairs. **d** Flowers at different stages of development. **e** Matured plants. **f** Forskolin ($C_{22}H_{34}O_7$) (molecular weight: 410.5 g/mol), the key secondary metabolite (structure source: PubChem) (photographs are not in scale) (source: unpublished photographs of Saikat Gantait)

consecutively regulates the cyclic adenosine monophosphate levels, which is a major compound in cell regulatory pathway by increasing its concentration (Dubey et al. 1981).

The action of forskolin results in increase in the basal metabolic rate; this property is harnessed in manufacturing supplements for weight loss (Alasbahi and Melzig 2010). It is used in the treatment of glaucoma since forskolin resulted in reduction of intraocular pressure (Matsumoto et al. 1990); forskolin coupled with sodium cromoglycate is utilized for asthma treatment since it produces bronchodilator effects (González-Sánchez et al. 2006); it also exhibits cardiotonic properties, since it causes reduction in systolic and diastolic pressure and the cardiac output is enhanced (Alasbahi and Melzig 2010; Baumann et al. 1990); and gastrointestinal ailments can also be cured since forskolin causes an upsurge in the marker enzyme concentration thus preventing the increase in size of the liver. Lately, some metabolites found exclusively in *C. forskohlii* like coleolic acid, demethylcryptojaponol, lupeol and oleanolic acid exhibit the property of apoptosis and tumour suppression (Sun et al. 2011).

Conventional propagation and its shortcomings

Conventional propagation of *C. forskohlii* is done via seeds that are sown in nursery beds with proper irrigation. After 15 to 20 days, it is transplanted in the main field. The crop is vegetatively propagated through stem cuttings measuring 10 to 12 cm with four pairs of leaves. It is planted in nursery and after a month root is formed, consequently it is transplanted to the main field (Lokesh et al. 2018). The major shortcomings faced in traditional cultivation of the plant are slow growth and lower levels of forskolin, since the accumulation of the alkaloid is majorly influenced by environmental factors (Chandel et al. 1991). Hence, there is an urgent need of in vitro strategies to enhance large-scale propagation of *C. forskohlii* and the enhancement of forskolin.

In vitro regeneration

Micropropagation is the most apt method for production of plantlets on a large scale and it assures continuous supply of plantlets (Gantait et al. 2018). Tissue culture–mediated technologies require minimum amount of materials for initiating cultures that are disease-free and resemble the mother plant (Gantait and Kundu 2017a). Furthermore, for overcoming the problems of low metabolite concentration in the plants, other approaches like genetic transformation and cell suspension culture were also adopted. This review encompasses the present scenario of the in vitro approaches followed in *C. forskohlii* along with the major factors affecting the success of such approaches.

Selection of explant

The proper selection of explant that is absolutely free from any sort of contaminant is the obligatory aspect of any micropropagation experiment. In *C. forskohlii*, a number of explants, namely shoot tip, nodal segment, root, hypocotyl and leaf, have been utilized in both direct and indirect organogenesis experiments (summarized in Tables 1 and 2). In *C. forskohlii*, shoot tip (Sen and Sharma 1991; Sen et al. 1992; Bhattacharyya and Bhattacharya 2001; Rajasekharan et al. 2010; Vibhuti and Kumar 2019) and nodal segments (Sharma et al. 1991; Rajasekharan et al. 2010; Dube et al. 2011; Sahai and Shahzad 2013; Sreedevi and Pullaiah 2014; Thangavel et al. 2014) are prevalently used in almost all micropropagation experiments. Shoot tip is the explant of choice since it is easily obtainable and also produces genetically stable propagules (Rout et al. 2006). In addition, the presence of high concentration of meristematic cells in shoot tip ensures accumulation of desirable growth regulators and exhibits totipotency (Akin-Idowu et al. 2009). Similarly, nodal segment is also preferred as explant since the axillary buds that are present here aid in initiating new propagules in much lesser time (Gantait and Kundu 2017b). Leaf is also used for both direct and indirect organogenesis experiments (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). The other explants that are utilized in indirect organogenesis experiments are hypocotyl and root (Sen et al. 1992; Tripathi et al. 1995).

Surface disinfection

The sterilization procedure is regarded as the crucial phase for any in vitro experiment. The sterilization methodology is dependent upon the type, dosage and period of exposure of the surface sterilants used, which further rely on the type of tissue used that can be either juvenile or mature (Panigrahi et al. 2017). In *C. forskohlii*, the basic sterilization procedure that is recurrent in most of the experiments include treatment with minuscule amounts of Teepol for 5–10 min, followed by washing with sterile water subsequent to treatment with 0.1% (w/v) $HgCl_2$ for 10 min (Table 1) (Sen and Sharma 1991; Sharma et al. 1991; Sreedevi and Pullaiah 2014; Vibhuti and Kumar 2019). The usage of 70% (v/v) ethanol (Rajasekharan et al. 2010; Sreedevi and Pullaiah 2014; Thangavel et al. 2014) and antibiotics like streptocycline (Bhattacharyya and Bhattacharya 2001; Dube et al. 2011)

Table 1 Factors involved and their influence on in vitro direct organogenesis of *Coleus forskohlii* syn. *Plectranthus barbatus* (arranged in chronological order)

Explant	Surface sterilization	Basal media	Carbon source	PGR (mg/l or μM*)	Additive (mg/l)	Gelling agent	Physical factors (temp/LI/PP/RH)	Regeneration response	Acclimatization	Reference
Shoot tip	2–3 drops 5% Teepol for 10 min → 0.1% $HgCl_2$ (w/v) for 7–10 min	MS	Not mentioned	2 BAP	Not mentioned	0.6% agar	23–25 °C; 2000 lx; 16 h; 55–60%	Multiple shoot	Not carried out	Sen and Sharma (1991)
Nodal segment	2–3 drops 5% Teepol for 15 min → 0.1% $HgCl_2$ for 10 min	MS	3% sucrose	2 kinetin + 1 IAA 1 IAA	100 myo-inositol	1% agar	25 ± 3 °C; 3000 lx; 16 h; NM	Multiple shoot Root induction	Soil and sand (1:1)	Sharma et al. (1991)
Shoot tip	Not mentioned	MS ¼ MS	1% sucrose	2 BAP 0.5 IBA	Not mentioned	0.6% agar	Not mentioned	Multiple shoot Root induction	Not carried out	Sen et al. (1992)
Shoot tip	2–3 drops 5% Teepol for 5 min → 0.1% Bavistin (w/v) + 0.02% streptocycline (w/v) for 30 min → 0.1% $HgCl_2$ for 2 min	MS	3% sucrose	0.46* kinetin + 0.57* IAA	Not mentioned	Not mentioned	25 ± 2 °C; 41.76 μmol/-m^2/s; 18 h; NM	Multiple shoot	Soilrite and loamy soil (1:1)	Bhattacharyya and Bhattacharya (2001)
Nodal segment	70% (v/v) ethanol for 30 s → 0.1% $HgCl_2$ for 8 min	MS	Not mentioned	2 BAP ½ MS	Not mentioned	Not mentioned	Not mentioned	Multiple shoot Root induction	Soilrite and garden soil (1:1)	Ashwinkumar (2006)
Nodal segment	1% Bavistin + 0.5% streptocycline for 5 min → 0.1% $HgCl_2$ for 3 min	MS	3% sucrose	1.5 BAP PGR free	Not mentioned	Not mentioned	22 ± 2 °C; 40–80 μmol/-m^2/s; 16 h; NM	Multiple shoot Root induction	Not carried out	Dube et al. (2011)
Leaf	0.1% $HgCl_2$ for 4 min	MS ½ MS	Not mentioned	5 BAP	Not mentioned	Not mentioned	25 ± 2 °C; NM; 16 h; NM	Shoot induction Root induction	Not carried out	Krishna et al. (2010)
Shoot tips; nodal segment	4% Cleansol for 15 min → 70% ethanol for 90 s → 0.01% $HgCl_2$ for 5 min	½ MS ½ MS	3% sucrose	8.87* BAP + 0.54* NAA	Not mentioned	0.7% agar	25 ± 2 °C; 31.55 μmol/-m^2/s; 16 h; NM	Multiple shoot Root induction	Soilrite	Rajasekharan et al. (2010)
Leaf	1% Bavistin for 30 min → 5% Labolene (v/v) for 15 min → 0.1% $HgCl_2$ for 3 min	MS	3% sucrose	2 BAP 2 BAP + 0.1 NAA 5* NAA	Not mentioned	0.8% agar	25 ± 2 °C; 50 μmol/m^2/s; 16 h; NM	Shoot induction Multiple shoot Root induction	Garden manure + garden soil + soilrite (1:2:1)	Sahai and Shahzad (2010)
Nodal segment	1% Bavistin for 30 min → 5% Labolene for 15 min → 0.1% $HgCl_2$ for 3 min	MS	3% sucrose	5 μM BAP 1 μM NAA	Not mentioned	0.8% agar	24 ± 2 °C; 50 μmol/m^2/s; 16 h; NM	Shoot induction Root induction	Garden manure + garden soil + sand (1:2:1)	Sahai and Shahzad (2013)
Nodal segment	2–3 drops 5% Teepol for 5 min → 70% ethanol for 15 min → 0.1% $HgCl_2$ for 7 min → 0.05% $HgCl_2$ for 4 min	MS	Not mentioned	0.25 BAP + 0.25 kinetin 1 BAP + 1 NAA ½ MS + 0.5 IAA	Not mentioned	Not mentioned	25 ± 2 °C; 2000 lx; 12 h; 90%	Shoot induction Shoot proliferation Root induction	Soil + vermiculite + sand (2:1:1)	Sreedevi and Pullaiah (2014)
		MS	3% sucrose		Not mentioned	0.8% agar		Multiple shoot		

Table 1 (continued)

Explant	Surface sterilization	Basal media	Carbon source	PGR (mg/l or μM*)	Additive (mg/l)	Gelling agent	Physical factors (temp/LI/PP/RH)	Regeneration response	Acclimatization	Reference
Nodal segment	2–3 drops 5% Teepol for 3 min → 2% Bavistin → 70% ethanol for 30 s → 0.1% $HgCl_2$ for 2 min			4.65* kinetin + 1.73* GA_3 7.38* IBA			25 ± 2 °C; 80 μmol/m^2/s; 16 h; NM	Root induction	River sand + garden soil + sand dust (1:1:1); 90%	Thangavel et al. (2014)
Apical and axillary meristem	0.1% $HgCl_2$ for 10 min	MS	Not mentioned	0.5 BAP 2 BAP	Not mentioned	Not mentioned	25 ± 2 °C; 200–300 lx; 16 h; 50–60%	Shoot induction Multiple shoot	Not carried out	Kaul et al. (2015)
Shoot tip	2–3 drops 5% Teepol for 10 min → 0.1% $HgCl_2$ for 8 min	MS	Not mentioned	2 BAP	Not mentioned	0.8% agar	22 ± 2 °C; 2000 lx; 16 h; 60%	Shoot induction	Not carried out	Vibhuti and Kumar (2019)

BAP, 6-benzylaminopurine; *GA_3*, gibberellic acid; *IAA*, indole acetic acid; *IBA*, indole-3-butyric acid; *LI*, light intensity; *MS*, Murashige and Skoog (1962); *NAA*, α-naphthalene acetic acid; *NM*, not mentioned; *PGR*, plant growth regulator; *PP*, photoperiod; *RH*, relative humidity

was found in some literature, in order to minimize the bacterial contamination.

Basal medium

Basal media consist of various proportions of organic and inorganic nutrients that include vitamins and minerals, which are essential for proper development of the in vitro plantlets (Gantait and Kundu 2017a). In *C. forskohlii*, Murashige and Skoog (MS) (Murashige and Skoog 1962) basal medium was utilized for all direct and indirect organogenesis experiments (summarized in Tables 1 and 2).

Carbon source

Carbohydrates are the prime repository of carbon. It helps in the morphological development of plants and also acts as an osmotic agent (Gantait and Kundu 2017b). The carbon concentration and type used in almost all experiments was 3% (w/v) sucrose (Tables 1 and 2) (Rajasekharan et al. 2010; Sahai and Shahzad 2010), since it promotes translocation and absorption of energy easily and results in active growth (Nayeem et al. 2014). Sreedevi et al. (2013b) suggested the usage of cane sugar instead of sucrose displayed positive response and it can be regarded as a cheaper alternative.

Physical conditions (temperature, light intensity and relative humidity)

The physical conditions, namely light, temperature and relative humidity, play an indispensable role for proper success of any in vitro experiment (Gantait and Kundu 2017a). Photosynthesis and photomorphogenesis are the key spectacles that plants acquire from light. Further, any in vitro culture is dependent on photoperiod and light intensity (Nayeem et al. 2014). In *C. forskohlii*, the available literature upon scrutinization showed that the desirable photoperiod for better in vitro propagation was 40 to 200 μmol/m^2/s (Tables 1 and 2) (Bhattacharyya and Bhattacharya 2001; Rajasekharan et al. 2010; Sahai and Shahzad 2010, 2013; Dube et al. 2011; Thangavel et al. 2014). Further, the photoperiod duration was similar in all of the in vitro experiments, i.e. 16 h. Temperature plays a direct role in governing the rate of photosynthesis along with respiration in the in vitro cultures, since temperature is directly proportional to the photosynthesis rate (Gantait and Kundu 2017b). In *C. forskohlii*, the temperature regimes for all the in vitro experiments were maintained in the range of 22 to 25 °C (Kaul et al. 2015; Vibhuti and Kumar 2019). Relative humidity also plays a vital role and its ambient range needs to be fixed because at high concentrations it

Table 2 Factors involved and their influence on in vitro indirect organogenesis of *Coleus forskohlii* syn. *Plectranthus barbatus* (arranged in chronological order)

Explant	Surface sterilization	Basal media	Carbon source	PGR (mg/l* or μM)	Additive (mg/l)	Gelling agent	Physical factors (temp/LI/PP/RH)	Regeneration response	Acclimatization	Reference
Hypocotyl	Not mentioned	MS	1% sucrose	2 NAA + 1 BAP	Not mentioned	0.6% agar	Not mentioned	Hard; green	Not carried out	Sen et al. (1992)
				1 NAA + 1 BAP				Friable; white		
Root	Not mentioned	MS	7% sucrose	1 2,4-D + 4 NAA	Not mentioned	Not mentioned	Not mentioned	Callus induction	Not carried out	Tripathi et al. (1995)
Leaf	0.1% (V/V) Triton-X for 15 min → 0.05% $HgCl_2$ + 2 drops Tween-20 for 8 min	MS	3% sucrose	2.3* kinetin	Not mentioned	0.8% agar	25 ± 2 °C; 50 μmol/m^2/s; 16 h; NM	Callus induction (nodular, compact green)	Vermiculite	Reddy et al. (2001)
				4.6* kinetin + 0.54* NAA				Shoot regeneration		
				0.4 μM IBA				Root induction		
Leaf	Not mentioned	MS	Not mentioned	2 2,4-D	Not mentioned	Not mentioned	Not mentioned	Callus induction	Not carried out	Ashwinkumar (2006)
				0.5 kinetin				Shoot regeneration		
Leaf	2–3 drops of 3% Tween 20 for 10 min → 0.1% $HgCl_2$ for 5 min	MS	3% sucrose	2 monuron + 0.5 diuron	Myo-inositol (100 mg/L)	0.8% agar	25 °C; 50 μmol/m^2/s; 16 h; NM	Callus induction	Not carried out	Srinivasan et al. (2006)
				0.5 TDZ				Shoot regeneration		
Leaf	Bavistin for 1 h → 0.1% $HgCl_2$ for 1 min	MS	3% sucrose	1 2,4-D + 0.5 BAP	Not mentioned	0.8% agar	22 ± 2 °C; 40–80 μmol/-m^2/s; 16 h; NM	Callus induction, light brown,	Not carried out	Atulkar et al. (2006)
				0.5 BAP + 0.1 NAA				Shoot regeneration		
Leaf	2–3 drops of Tween 20 → 0.1% Bavistin for 45 min → 0.05% $HgCl_2$ for 10 min → 0.1% $HgCl_2$ for 3 min	B5	Not mentioned	3 picloram 3 2,4-D	Not mentioned	0.7% agar	22 ± 2 °C; NM; 16 h; NM	Friable, Amorphous callus	Not carried out	Balasubramanya et al. (2012)
				3 BAP				Hard nodular green callus		
				5 picloram + 0.5 BAP				Friable, white, callus		
Leaf	0.05% $HgCl_2$ for 7 min → 0.01% $HgCl_2$ for 4 min	B5 and MS (shoot, root)	3% sucrose	2 2,4-D	Not mentioned	0.8% agar	26 ± 2 °C; NM; 16 h; NM	Compact, green callus	Soil rite + ½ MS salt (without sucrose and vitamin)	Sreedevi et al. (2013a)
				2 BAP + 1 NAA				Shoot regeneration		
								Root induction		

Table 2 (continued)

Explant	Surface sterilization	Basal media	Carbon source	PGR (mg/l* or μM)	Additive (mg/l)	Gelling agent	Physical factors (temp/LI/PP/RH)	Regeneration response	Acclimatization	Reference
				¼ MS + 0.5 IBA						
Leaf	2–3 drops of Tween 20 → 0.1% $HgCl_2$ for 5 min	MS	3% sucrose	2 2,4-D	Not mentioned	Not mentioned	26 ± 2 °C; NM; 16 h; 75%	Callus induction, white coloured	Not carried out	Gopi and Mary (2014)
				1 BAP + 1 kinetin + 1 NAA				Somatic embryo induction		
Nodal segment	2–3 drops 5% Teepol for 5 min → 70% ethanol for 15 min → 0.1% $HgCl_2$ for 7 min → 0.05% $HgCl_2$ for 4 min	½ MS	Not mentioned	2 NAA	Not mentioned	Not mentioned	25 ± 2 °C; 2000 lx; 12 h; 90%	Callus induction	Not carried out	Sreedevi and Pullaiah (2014)
Leaf	Not mentioned	MS	3% sucrose	2 2,4-D	Not mentioned	Not mentioned	22 ± 2 °C; 40 μmol/m^2/s; 16 h; NM	Callus induction	Not carried out	Gangopadhyay et al. (2016)
Leaf	Not mentioned	MS	3% sucrose	3 NAA + 1 BAP	Not mentioned	Not mentioned	Not mentioned	Callus induction	Not carried out	Swaroopa et al. (2016)
Leaf	2–3 drops 5% Teepol for 10 min → 0.1% $HgCl_2$ for 8 min	MS	Not mentioned	1.5 2,4-D + 2 BAP	Not mentioned	0.8% agar	22 ± 2 °C; 2000 lx; 16 h; 60%	Callus induction	Not carried out	Vibhuti and Kumar (2019)

2,4-D, 2,4-dichlorophenoxyacetic acid; *BAP*, 6-benzylaminopurine; *GA_3*, gibberellic acid; *IAA*, indole acetic acid; *IBA*, indole-3-butyric acid; *LI*, light intensity; *MS*, Murashige and Skoog (1962); *NAA*, α-naphthalene acetic acid; *NM*, not mentioned; *PGR*, plant growth regulator; *PP*, photoperiod; *RH*, relative humidity, *TDZ*, thidiazuron

results in contamination and hyperhydricity in the cultures (Gantait and Kundu 2017b).

Plant growth regulators

The morphogenesis and development of plantlets under in vitro condition, irrespective of direct organogenesis or indirect organogenesis experiments, are majorly influenced by the kind of growth regulators used and their respective dosages. Plant growth regulators (PGRs) help in maintaining a harmony among the growth rates of different parts of a plant that would eventually result in formation of a complete plantlet (Gantait and Kundu 2017a). In *C. forskohlii*, there is an array of reports available wherein the effects of PGRs have been well documented both in the cases of direct (Table 1) and indirect organogenesis (Table 2) as well as in somatic embryogenesis.

Direct organogenesis

The shoot organogenesis includes multiple shoot induction and multiple shoot proliferation, which can be achieved by cytokinin application solely or in combination with any auxin (Fig. 2a) (Reddy et al. 2001). In *C. forskohlii*, there are substantial reports available on direct organogenesis experiments, wherein sole application of cytokinin, mainly N^6-benzylaminopurine (BAP), exhibited adequate results (Sen and Sharma 1991; Sen et al. 1992; Krishna et al. 2010; Sahai and Shahzad 2010, 2013; Dube et al. 2011; Kaul et al. 2015; Vibhuti and Kumar 2019). Another type of cytokinin that was utilized was kinetin along with auxins (viz. α-naphthalene acetic acid (NAA), indole-3-acetic acid (IAA)) for multiple shoot proliferation (Fig. 2b) (Sharma et al. 1991; Bhattacharyya and Bhattacharya 2001). Furthermore, BAP along with auxins like NAA (Sreedevi and Pullaiah 2014; Sahai and Shahzad 2010; Rajasekharan et al. 2010) exhibited similar results as well. There is also a sole reference available wherein combination of two cytokinins, viz. BAP and kinetin, yielded promising results (Sreedevi and Pullaiah 2014). Interestingly, Thangavel et al. (2014) utilized gibberellic acid (gibberellin A_3 or GA_3) along with BAP for multiple shoot formation of *C. forskohlii*, and attained favourable results. The utilization of 'topolins' for multiple shoot induction and proliferation which is regarded as a novel group of aromatic cytokinins is seldom reported. It has been regarded as a potent replacement to BAP due to its ability for delaying senescence and preventing necrosis and hyperhydricity (Aremu et al. 2012).

Callogenesis

Indirect organogenesis is defined as the mechanism of plantlet regeneration under in vitro condition wherein the primary step is callus induction and its proliferation. Callus signifies a mass of unorganized cells (Fig. 2d). It is further categorized into friable calli and embryogenic calli that can be further utilized in cell suspension culture and regeneration of multiple shoots in a specific PGR-supplemented media, respectively (Gantait and Kundu 2017a). In *C. forskohlii*, there are only a handful of reports that are available on callus induction, proliferation and complete plantlet regeneration (summarized in Table 2). It has been observed that sole application of picloram (Balasubramanya et al. 2012) or picloram in combination with BAP (Balasubramanya et al. 2012) gave satisfactory results regarding induction of friable calli. Other PGRs like 2,4-dichlorophenoxy acetic acid (2,4-D) and NAA at higher doses were successful in the induction of friable calli (Sen et al. 1992; Gopi and Mary 2014). Swaroopa et al. (2016) utilized a combination of BAP and NAA that resulted in callus induction exhibiting higher biomass. The number of reported experiments corresponding to both callus induction and simultaneous indirect regeneration is quite limited until date. The induction of embryogenic callus using cytokinin (Reddy et al. 2001; Balasubramanya et al. 2012) resulted in the development of compact calli and their subsequent regeneration. Interestingly, a combination of synthetic analogues, viz. monuron and diuron, which are urea derivatives, resulted in callus induction, and plantlet regeneration was carried out with the help of thidiazuron (TDZ) (Srinivasan et al. 2006). There are no such reports on the usage of abscisic acid or brassinosteroids for callus induction and it can serve as an alternative to auxin or cytokinins.

Rooting

In any in vitro experiment, rooting of regenerated plantlets is important and the success of the entire micropropagation experiment is entitled to the rooting percentage of the regenerants (Fig. 2c) (Gantait and Kundu 2017b). In *C. forskohlii*, the rooting of the in vitro plantlets in both direct and indirect organogenesis experiments is summarized in Tables 1 and 2, respectively. The rooting of explants was achieved in PGR-free media, viz. full ¼ MS (Sen et al. 1992) and ½ MS (Krishna et al. 2010; Rajasekharan et al. 2010). The efficiency of the rooting can be increased by the inclusion of PGRs in the basal medium. The PGRs that were chiefly used for rooting were NAA (Rajasekharan et al. 2010; Sahai and Shahzad 2010, 2013; Sreedevi and Pullaiah 2014), IAA (Sharma et al. 1991; Sahai and Shahzad 2013; Sreedevi and Pullaiah 2014) and indole-3-butyric acid (IBA) (Sen et al. 1992; Thangavel et al. 2014). There are no such clear reports available on the influence of activated charcoal on in vitro rooting. Activated charcoal in combination with a plant growth regulator helps in root initiation since it creates a less luminous environment that favours a rhizospheric state, and is quite effective in absorption of polyphenolic compounds that

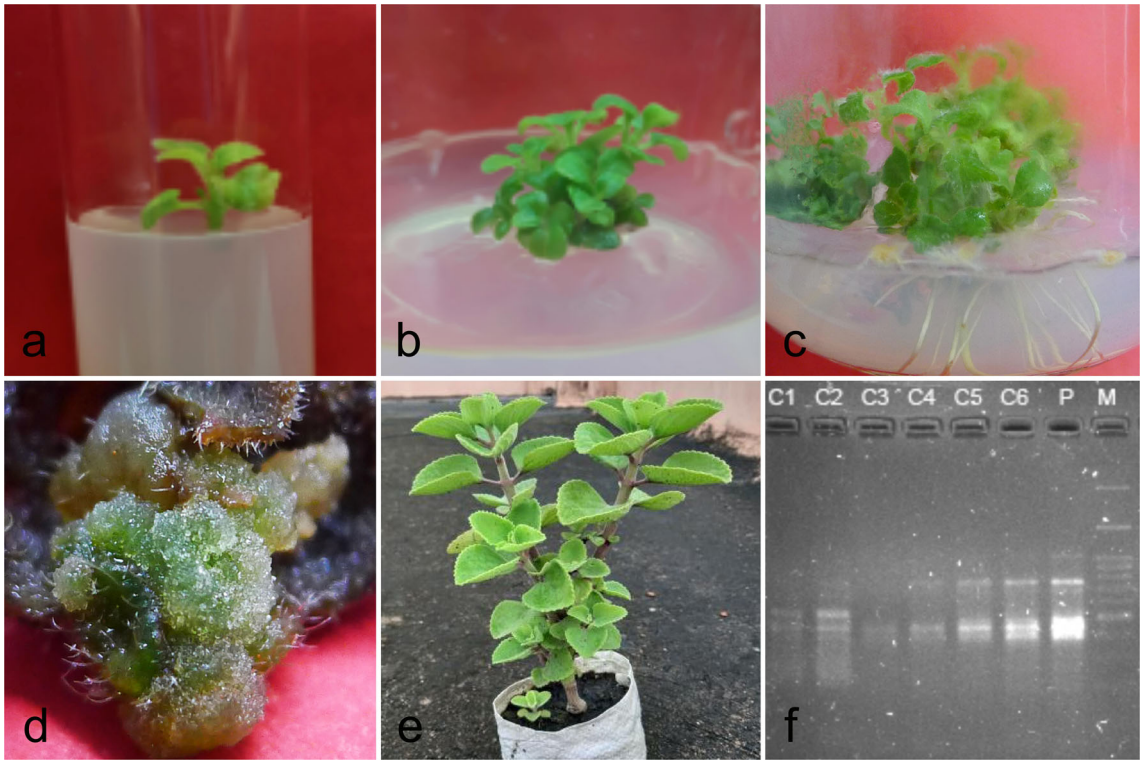

Fig. 2 In vitro propagation of *Coleus forskohlii* syn. *Plectranthus barbatus*. **a** Direct shoot bud initiation. **b** Multiple shoot induction. **c** Full-grown in vitro plantlet with multiple shoots and roots. **d** Development of organogenic calli. **e** Acclimatized plantlet. **f** Assessment of clonal fidelity of in vitro regenerated plantlets using ISSR marker ($(GT)_8T$) (photographs are not in scale) (source: unpublished photographs of Monisha Mitra)

are responsible for detrimental effects like browning (Gantait et al. 2009).

Somatic embryogenesis

Somatic embryos usually develop from the embryogenic calli that are induced by application of specific PGRs. Thus, the process where somatic embryos are formed from amorphous mass of somatic cells is defined as somatic embryogenesis (Gantait and Kundu 2017b). In *C. forskohlii*, there is only a single instance of somatic embryogenesis reported (Gopi and Mary 2014) using BAP (1 mg/l) and 2,4-D (mg/l). There are no such elaborate experiments conducted on somatic embryogenesis via direct or indirect means. The utilization of cytokinins and auxins, individually, can be adopted for development of bipolar propagules and further its regeneration. Histological assays and scanning electron microscopic studies can be conducted for identification of different stages of embryo, i.e. globular, heart and torpedo.

Acclimatization

Acclimatization is an eventual accomplishment of any in vitro propagation experiment, coupled with low-cost incentives and higher survival rates (Fig. 2e). However, proper care should be taken at this stage since the explants tend to be highly susceptible to dehydration and microbial attack (Mukherjee et al. 2019). In *C. forskohlii*, limited experiments on acclimatization had been carried out where the usage of soilrite and sand in a fixed ratio is recurrent (Sharma et al. 1991; Sahai and Shahzad 2013; Sreedevi and Pullaiah 2014; Thangavel et al. 2014). Soilrite has also been used solely in some cases as well (Rajasekharan et al. 2010; Sahai and Shahzad 2010). The details of the acclimatization procedure adopted and the survivability percentage are not mentioned clearly in all the experiments reported until date. Some innovative approaches that can be adopted are foliar spray of fertilizers or micronutrients and the utilization of gas permeable vessels where the aeration and carbon dioxide levels can be elevated for providing better durability to the regenerants (Teixeira da Silva et al. 2005).

Clonal fidelity assessment

Clonal fidelity assessment is defined as a tool that ensures whether the tissue-cultured plantlets are true-to-type and similar to the mother plant so that the genetic integrity is conserved (Fig. 2f) (Chaturvedi and Mitra 1975). This step is

the most important one to ensure that there is no somaclonal variation and that all the in vitro regenerants are true-to-type. However, until date, there are no such reports available on clonal fidelity assessment of in vitro regenerants of *C. forskohlii*.

Synthetic seed development and their storage

In synthetic seed technology, bipolar explants like somatic embryo, protocorm-like body and unipolar propagules like shoot tip, nodal segment are encapsulated with the help of an encapsulation matrix that aids in mimicking the endosperm available in true seed (Gantait et al. 2015). Synthetic seed technology has multifaceted uses in major arenas for instance handling, exchange and storage of germplasms etc. (Mukherjee et al. 2019). In *C. forskohlii*, there is a lone report on synthetic seed production, wherein shoot tips and nodal segments were encapsulated using 3.5% (w/v) sodium alginate and 90 mM calcium chloride and were inoculated in full-strength MS semisolid medium. The regeneration percentage achieved was 92%. The synthetic seeds were stored at 4 °C for 6 months. Post-storage results show that 61% regeneration efficiency was achieved with stable forskolin content (Swaroopa et al. 2007). Apart from this single report, there is no information on the use of encapsulation or cryopreservation technology for mid- to long-term storage of *C. forskohlii*. However, a unique way of storage of in vitro regenerants was conducted by Dube et al. (2011), wherein growth retardants, viz. 3 M mannitol and 3 M sorbitol, were applied in medium and the resultant cultures were stored at room temperature for 30 days. Post-storage results displayed that 90% recovery was observed among the regenerants.

In vitro secondary metabolite production

Biotechnological-based tools offer a reliable way of production of secondary metabolites on a large-scale throughout the year. The major metabolite present in *C. forskohlii* is forskolin, which has a major contribution in the pharmaceutical sector. However, only a limited number of reports on secondary metabolite production in *C. forskohlii*, wherein desirable level of forskolin was found in the roots of the in vitro regenerants, are available (Sen et al. 1992). Tripathi et al. (1995) reported that callus cells exhibited 0.075% (w/v) forskolin levels. Interestingly, Malathy and Pai (1999) reported the presence of forskolin 0.002 to 0.01% in the callus derived from roots, stems and leaves when compared with naturally grown plants, wherein forskolin was absent in the aerial part of the plant. Balasubramanya et al. (2012) conducted an experiment where callus was induced in B5 medium supplemented with 0.5 mg/l NAA; the resultant callus that formed was rhizogenic and the forskolin content was 1178 mg/kg of dry weight, suggesting that rhizogenic masses are crucial for forskolin production since in ex vitro plant forskolin is localized in the root. Gangopadhyay et al. (2016) suggested an innovative approach to elevate the forskolin levels in friable callus (induced from leaves) via exposing these calli to ultraviolet C (UVC) (254 nm) for 10 min before being inoculated in regenerative media supplemented with 1.5 mg/l BAP. Then, these regenerants were subjected to HPTLC analysis, which showed enhanced levels of forskolin in the UVC-treated regenerants, since it provided protection to the root and acted as an elicitor by upregulating the key genes involved in forskolin synthesis (Schmidlin et al. 2008).

Cell suspension culture

Cell suspension culture has paved a way for an incessant production of secondary metabolites. This approach is beneficial in many ways, for instance it is not season dependent; the cells that are harvested are free from biotic contaminants; any type of metabolite can be extracted with ease; there is reduction in manpower costs and direct extraction from cells is also feasible (Vijaya Sree et al. 2010). In *C. forskohlii*, a few reports are available on cell suspension culture. Mersinger et al. (1988) initiated calli in media supplemented with 0.5 mg/l 2,4-D and 0.2 mg/l kinetin, which were suspended in liquid B5 medium supplemented with 1 mg/l IBA and 0.01 mg/l kinetin. Swaroopa et al. (2013a) initiated the cell suspension culture by inoculating friable calli in MS liquid medium supplemented with 3 mg/l NAA and 1 mg/l BAP, which were maintained at temperature 25 ± 2 °C under dark conditions, on a rotatory shaker at 120 rpm. Further, elicitors of biological origin, viz. *Aspergillus niger*, *Penicillium notatum*, *Rhizopus oryzae* and *Fusarium oxysporum*, were employed to enhance the forskolin accumulation. It was observed that *Aspergillus niger* outweighed the other elicitors since it proved to be 6 times better than convention cell suspension because the secretion of oligosaccharides from the cell wall of *Aspergillus niger* along with chitin served as elicitors. Similarly, Swaroopa et al. (2013b) utilized an array of elicitors of bacterial origin, namely *Bacillus subtilis*, *Escherichia coli*, *Proteus aureus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, for increasing the forskolin accumulation in the cultures maintained in MS media supplemented with 3 mg/l NAA and 1 mg/l BAP under dark conditions at temperature 25 ± 2 °C. *Bacillus subtilis* was found promising among the other elicitors since its application resulted in elevation of forskolin concentration. Further, Swaroopa et al. (2015) reported the use of some abiotic elicitors in order to enhance the forskolin level in the suspension cultures.

In vitro induction of polyploidy

Induction of polyploidy via artificial means has been employed recently for simultaneous improvement of plant biomass along with secondary metabolite profile. In medicinal plants, there are instances of accumulation of secondary metabolite in vegetative parts of the plant exhibiting higher biomass (Mukherjee et al. 2019). In *C. forskohlii*, there is only a single report on artificial polyploidy available, wherein shoot tips were treated in 0.25% (w/v) colchicine for 3 h per day for a period of 3 days, under ex vitro conditions. A change in morphological features was observed in the form of increased stomata and pollen size. Further, the forskolin concentration in the autotetraploid plant showed 17% increase over its diploid counterpart (Hegde and Krishnan 1991).

Genetic transformation

Genetic transformation is defined as the method in which genetic amendment and modification is directed by the transfer of specific gene from other species in order to achieve desirable expression (Liu et al. 2008). In *C. forskohlii*, *Agrobacterium*-mediated transformation had been attempted. Mukherjee et al. (1996) attempted genetic transformation via *A. tumefaciens*, where shoot tips were transformed using *A. tumefaciens* strain C58 (wherein the strain was maintained in solidified media supplemented with tryptone and yeast extract) (Depicker et al. 1980). The transformed callus formed was subjected to forskolin estimation via HPLC and there was 0.014% improvement as compared with that of the untransformed line.

There are few reports available until date in *C. forskohlii*, where *A. rhizogenes*–mediated hairy root culture had been attempted. Sasaki et al. (1998) utilized strain MAFF 03-01724 for hairy root induction. After co-cultivation, the emerging hairy roots were subcultured in woody plant (WP) medium supplemented with Claforan for getting rid of bacteria and finally maintained in liquid WP media at 25 °C under dark conditions. The forskolin concentration was 1.6 mg/100-ml flask which was significantly higher than the non-transformed ones. Similarly, strain A4 was used to induce hairy root by using leaf as explant. The hairy roots obtained after infection were subcultured in MS medium supplemented with 500 mg/l cefotaxime. The transformed roots were confirmed by the presence of mannopine and finally maintained in liquid MS medium supplemented with 1 mg/l IBA and 600 mg/l casein hydrolysate, and the resultant forskolin concentration was 1.449 ± 0.023 mg/g fresh weight. Additionally, at the 14th day, elicitor methyl jasmonate resulted in an increase in forskolin concentration by 2.7-fold (Reddy et al. 2012). Until date, Pandey et al. (2014) employed MTCC2364 strain to induce infection in nodal parts for 15 min and co-cultivation was done for 72 h before being inoculated in MS medium supplemented with 500 mg/l cefotaxime. The transformed roots were confirmed through molecular analysis using *rolA* sequence and finally maintained in PGR-free liquid MS medium at 25 °C under dark conditions. The forskolin level analysed through HPLC showed 2.36 mg/g in dry weight which was significantly higher than the untransformed plants (1.16 mg/g).

Conclusion

The biotechnological advancements achieved until date on *C. forskohlii* have been described intricately in this review. There are sufficient reports available on direct and indirect organogenesis, but there still prevails an insufficiency in terms of the availability of reports on of different types of basal media, carbon source, additives, synthetic growth hormones and in vitro physical conditions. The use of somatic embryogenesis for regeneration of plantlets also needs to be addressed extensively and in depth. There are no reports on assessment of clonal fidelity by using molecular markers; therefore, the use of markers like RAPD and ISSR, wherein no prior sequence information is not necessary, can be fruitful. The major arena of encapsulation and germplasm storage for short and longer duration needs to be addressed, since the reports available are very few and are not reproducible. The enhancement of secondary metabolite through cell suspension culture can be enriched by utilizing more elicitors of abiotic origin. The reports available on genetic transformation are still inadequate and it could be explored and presented in a more elaborate way. It is essential to mention that there is no report available on in vitro–based polyploidy induction that could have been attempted by using colchicine or other anti-mitotic agents to enhance the quantity of forskolin production. Since the plant is a rich hub of secondary metabolites and has high pharmaceutical values, cutting-edge nanotechnology can also serve as a unique way for enhancing its medicinal value. Henceforth, this review provides ample information regarding the present scenario of the biotechnological interventions achieved so far in *C. forskohlii* and also multiple shortcomings that can be explored to create a new podium for upcoming innovative approaches.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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