

Mass Propagation of Important Medicinal Plant *Coleus forskohlii* (Briq) Through Direct and Indirect Regeneration Using Leaf Explant

¹ Senthil kumar P ² Vasuki A and ³ Lakshmi prabha A

^{1,2} Research Scholars, ² Professor, Department of Botany, Bharathidasan University, Tiruchirappalli-620024, Tamil nadu, India

Abstract; *Coleus forskohlii* (Briq) is a threatened multipurpose medicinal plant that has prevalent applications. *C.forskohlii* is the only known natural source of forskolin. The present communication an *in vitro* rapid regeneration method using leaf explants of *C.forskohlii* through indirect and direct organogenesis. In direct regeneration methods the maximum direct shoot proliferation was observed on leaf explant in Kinetin (2.0 mg/l) with NAA (0.1mg/l). In *in direct* regeneration method the growth regulator combination which produced highest percentage of organogenic callus induction from leaf explant was Kinetin (1.0 mg/l). Highest shoot buds and multiple shoots were produced from callus clump with KIN (1.0 mg/l) + NAA (0.1mg/l). Regenerated and elongated shoots, when transferred to ½ strength MS basal medium resulted in abundant rooting plants that were transferred to acclimatization (*invitro* and *invivo* condition) and maintained in a greenhouse and field. This *in vitro* propagation protocol should be useful for conservation as well as mass propagation and production of forskolin.

Key words: *Coleus forskohlii*, Leaf callus, Medicinal plants, Organogenesis

Abbreviations: BAP: 6-benzylaminopurine, IAA: indole-3-acetic acid, KIN: kinetin, NAA: α - naphthalene acetic acid, 2,4-D: 2,4- dichloro phenoxy acetic acid

Corresponding author. Dr.A Lakshmi Prabha - E-mail: dralprabha@yahoo.com.

I. Introduction

Coleus forskohlii Briq. (Lamiaceae) is an important plant in Ayurvedic medicine. It produces the labdane diterpenoid forskolin in the tuberous roots (Bhat *et al.*, 1977). The fasciculate tubers of *C. forskohlii* on drying yield a unique labdane diterpenoid called forskolin (Dubey *et al.*,1981). Forskolin directly stimulates the catalytic subunit of adenylate cyclase and brings positive inotropic effect, lowers blood and intraocular pressure. In addition, it has been shown to have anti-inflammatory property (Rupp *et al.*, 1986.).Secondary metabolites are stored mainly in the cytoplasmic vesicles of cork cells in both fibrous and tuberous root (Abraham *et al.*, 1988). *Coleus forskohlii* plant is valued as forskolin drug and is used for the treatment of glaucoma, congestive cardiomyopathy and asthma (De souza et al., 1986; Valdes et al., 1987; Hussain et al., 1992). Using the adenylate cyclase stimulant activity, it is also valued for antiallergic activity and suppressing hair graying (Keikichi et al., 1988).*C. forskohlii* is traditionally propagated by means of vegetative cuttings but it is time for consuming and provides a limited number of propagules. Apparently due to non-availability of quality planting materials, commercial plantations of this aromatic plant species. Hence, a procedure for rapid *in vitro* propagation of this species has been described in this paper. At present, production of forskolin is commercial collection of wild and a few cultivated *C. forskohlii* plants in India. Due to large scale production of forskolin, collection of wild plants from forests and in sufficient attempts either to its cultivation, *C. forskohlii* is rapidly disappearing and now it is listed as one of the plant species in India vulnerable to extinction. It is an important to develop methods for the conservation of this threatened species. *In vitro* propagation methods help for plant germplasm conservation and multiplication. Micropropagation of *C. forskohlii* was reported using axillary buds, which yielded low number of shoots (Sharma *et al.*, 1991). This method may not be useful for the large-scale production and cultivation of the elite clones. It has been shown that the shoot organogenesis from callus cultures, and it can be used as an effective method for multiplication of medicinal plants. In the present study, achieve a higher rate of shoot multiplication by callus mediated multiple shoot organogenesis as an alternative method. This report on multiple shoot organogenesis states, the development and establishment of complete plantlets from leaf derived callus cultures of *C. forskohlii*. Callus mediated regeneration is significant for selection of different cell lines for genetic development of crop plants and also considered as a pre necessary stage for *in vitro* mutation works.

II. Materials and methods

Plant material and explant source

Plants of *C.forskohlii* were collected from Aathur, Salem in Tamilnadu, India and cultured, maintained in green house condition in departmental garden of Botany, Bharathidasan University, Tiruchirapalli, Tamil nadu, Young leaves were used as explant source for direct and indirect organogenesis.

Media and culture conditions

Murashige and Skoog's (1962) medium supplemented with 3% (w/v) sucrose was used for regeneration of plants. The media was solidified with 0.8% (w/v) agar and pH was adjusted to 5.7 prior to autoclaving at 121°C and 15 lbs for 20 minutes. Cultures were maintained at 25 ± 2 °C with 16-h light/8-h dark photoperiods. Explants were washed with soap solution followed by tap water for 25 min. It was surface sterilized with 0.1% HgCl₂ solution for 10 minutes and followed by rinsing with sterilized distilled water for 4-5 minutes. Explants are culture in MS media supplemented with cytokines (BAP, Kinetin) and auxins (IAA, NAA and 2,4-D) in different combinations and concentration. Each treatment consisted of 3 replicates and the experiment was repeated 3 times. Incubation of culture vials was carried out in incubation room under cool white fluorescent light (200-300 flux) of 16 hours photoperiod and 8 hours dark period at a temperature of 25 ± 1 °C

Direct organogenesis

Direct regeneration of shoots without intermediate callus stage is a faster way of reproducing clones preventing somaclonal variation. Hence, the present one of the study is aimed at optimizing a procedure for faster and large-scale direct shoot regeneration of *C. forskohlii* through leaf explants. Direct shoot regeneration allows large-scale multiplication of plantlets *in vitro* by preventing clonal variation as opposed to regenerated from calli alone, which frequently leads to somaclonal variation (Reddy et al., 2001). In the present study, the leaf explants at the cytokinin (BAP & Kinetin) combined with Auxins (IAA, NAA) responded with direct shoot proliferation. After incubation over a period of 25 days, best regeneration of shoots was achieved on MS medium supplemented with cytokinin and auxins combination were compared with other plant growth regulators.

In-direct organogenesis

Callus formation

MS medium with different concentrations of cytokines (BAP and Kinetin) either alone or combination with Auxins (IAA and NAA) were tested for effects on callus formation from leaf explants. Small leaf segment was inoculated on MS medium with and without hormone. Callus formation frequency was recorded and the frequency of callus formation and nature of response was determined four weeks after culture initiation. After screening of suitable medium for organogenic callus induction.

Shoot differentiation and Rooting Callus was cultured in 10 ml solid medium. In the first set, light-green with nodular compact callus was transferred to the MS medium supplemented with different concentrations of cytokinins (BAP and Kinetin) for shoot differentiation and the efficiency of the callus was evaluated. Second set of experiments were carried out with the addition of auxins (IAA, NAA) for the optimal response on shoot differentiation. Green callus was transferred onto MS medium supplemented with different concentration of Kinetin and combination with NAA & IAA for shoot buds induction. Percentage of calli forming shoots; number and length of differentiated shoots data's were recorded after four weeks of inoculation. *In vitro* derived shootlets measuring in cm were excised from leaf-derived callus and cultured on MS medium supplemented with IAA, IBA or without growth regulator and also shoot lets were cultured on half strength MS medium. Data was noted on percentage of rooting, mean number and length of the roots after four weeks removing from the rooting media.

III. Results and discussion

1.1 Direct shoot organogenesis

Micropropagation through direct shoot regeneration allows large-scale multiplication of plantlets *in vitro* by avoiding clonal variation as dissimilar to regenerate from calli alone, which often leads to somaclonal variation (Reddy et al., 2001). In the present study, leaf explants were used for their response to plant growth regulators at cytokinin (Kinetin, BAP) and auxin (IAA, NAA) at different concentrations. After initiation over a period of 25 days, an optimal regeneration of shoots (89.6 %) was achieved on MS medium supplemented with combination of KIN 2mg/l and NAA 0.5mg/l, (Fig-1a) in comparison with other plant growth regulators (Table-1) and after subculture high shoot multiplication was achieved in KIN 1mg/l and NAA 0.1 mg/l (fig-1b,c). Generally the usage of BAP is considered as most suitable for promoting large-scale multiplication and micropropagation of various plant species (Shrivastava and Banerjee, 2008) but our results shows Kinetin (2mg/l) with NAA (0.1mg/l) combination very suitable for direct regeneration from leaf explants (fig-1a), After subculture at low concentration of NAA (0.1mg/l) with Kinetin (1mg/l) combination stimulate multiple shoots from the leaf explants. These observations are reliable with previous studies where addition of NAA stimulating the proliferation and elongation of shoots in *Petasites hybridus* (Wldi et al., 1998), *Eucalyptus grandis* (Luis et al., 1999) was shown.

Table -1 Effect of plant growth regulators on direct shoot regeneration from leaf explants of *Coleus forskholii*

Concentrations Hormones	Leaf			
	Treatment (mg/l)	Percentage of response	Average No. of shoots / explant	Average length of shoot (cm)
BAP+IAA				
0.5+0.5	46.8 ⁿ	2.8 ^g	1.0 ^l	
1.0+0.5	63.2 ^h	3.8 ^f	2.2 ^h	
1.5+0.5	65.6 ^g	4.8 ^e	2.5 ^e	
2.0+0.5	67.6 ^f	5.8 ^d	2.9 ^d	
BAP+NAA				
0.5+0.5	51.6 ^m	2.6 ^g	1.0 ^l	
1.0+0.5	59.6 ^j	4.8 ^e	2.0 ^j	
1.5+0.5	61.6 ⁱ	5.8 ^d	2.1 ⁱ	
2.0+0.5	64.4 ^{gh}	6.8 ^c	2.2 ^h	
KN + IAA				
0.5+0.5	55.6 ^l	1.8 ^h	1.0 ^l	
1.0+0.5	77.6 ^e	3.8 ^f	2.1 ⁱ	
1.5+0.5	76.8 ^e	4.8 ^e	2.3 ^g	
2.0+0.5	79.6 ^d	5.8 ^d	2.4 ^f	
KN + NAA				
0.5+0.5	57.6 ^k	2.8 ^g	1.1 ^k	
1.0+0.5	84.4 ^c	16.8 ^a	3.0 ^c	
1.5+0.5	87.6 ^b	14.6 ^a	3.2 ^b	
2.0+0.5	89.6 ^a	12.6 ^b	3.3 ^a	

Means within a column followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.



Fig-1: Direct regeneration of shoots from leaf explants of *Coleus forskohlii*:

a. Shoot buds regenerated on MS medium with Kinetin 2.0mg/l+NAA 0.5mg/l after 25 days; b,c. Multiple Shoots development on KIN 1mg/l + NAA 0.1mg/l; d,e. Elongated shoots; f. Rooting of regenerated shoot on ½ Strength MS media; g,h. *In vitro* Accamalization and hardening; i. Plants transferred in green house condition

1.2 *In-direct* organogenesis-

Callus induction

Indirect organogenesis was completed in two stages. First stage was the usage of MS medium with supplemented with auxin and cytokinins alone for callus induction from the leaf explant and the second was the development of shoot buds and multiplication in MS Medium supplemented with auxin and cytokines combination from leaf derived green organogenic callus. Among the different explants tested, leaf explants favored good callogenic response and further experiments were carried out with calli derived from leaf explants only. The leaf explant revealed green organogenic callus, white callus, green nodular callus, white friable callus, green compact callus, pale greenish friable, brown callus (Table-2) and (fig 2). Callus induction and proliferation systems are known to be very suitable for the study of bio-synthesis of natural products. In *C.forskohlii*, this method of synthesis of forskolin from callus has been used (Sen *et al.*, 1992; Mukherjee *et al.*, 1996; Reddy *et al.*, 2001). Here after the optimization of cellular proliferation is the first essential step to establish cultures from plant tissues for the production of natural products. Leaf explant not only developed a good source for callus induction but also exhibited maximum percent frequency of shoot bud differentiation. Previous reports also confirmed the advantage of leaf being the source of the dedifferentiation and callus development in other plants such as *Tylophora indica* (Rao *et al.*, 1970), *Cimicifuga racemosa* (Lata *et al.*, 2002). Leaf explants when implanted on MS medium supplemented with different growth regulators viz., 2,4-D, IAA, NAA at different concentrations (0.5 to 2mg/l) (Table-1) showed various response both in expressions of morphology and biomass. The best callus response was

on 2,4-D 2.0 mg/l for pale green friable callus(Fig 2-g,h) and white friable callus(fig 2-,d) .At lower concentrations, 2,4-D 0.5mg/l induced greenish white callus(fig 1-a) .The best greenish white and nodular (organogenic) calli was produced in Kinetin 1mg/l (TABLE-2 & fig.1b).NAA produced white friable and brown callus (fig1-e,f) and IAA induced white compact callus(Fig-2c). Only greenish white and green nodular callus was able to regenerate adventitious shoots after being transferred to shooting medium.. At high concentration of auxins such as (3.0 mg/l), 2,4-D & NAA was initiate to be toxic to explants which turned brown after two weeks of incubation. Later, it turned to black at the base after four weeks of culture. This was also evident from the previous reports the maximum effective nature of 2,4-D to induce callus was observed in *Citrus* (Ling and Iwamasa, 1997) and Pommello (Goh *et al.*, 1995). Leaf derived callus growth was determined as the fresh weight after four weeks of inoculation.

Table- 2 Effect of different growth regulators on callus induction of the leaf explants in *Coleus forskholii*

Hormones	Treatment (mg/l)	Leaf	
		Percentage of Callus formation	Nature of response
BAP	0.5	26.2 ⁿ	GCC
	1.0	29.8 ^m	GCC
	1.5	37.2 ^k	GCC
	2.0	41.2 ^j	GCC
KN	0.5	69.6 ^e	GW
	1.0	95.4 ^a	GW
	1.5	92.6 ^b	GW
	2.0	90.2 ^b	GW
2,4-D	0.5	71.2 ^e	PGC
	1.0	82.2 ^d	PGC
	1.5	86.2 ^c	PGC
	2.0	92.4 ^b	WFC
IAA	0.5	35.2 ^l	WC
	1.0	41.2 ^j	WC
	1.5	51.2 ^h	BC
	2.0	53.2 ^g	BC
NAA	0.5	44.4 ⁱ	WFC
	1.0	52.2 ^g	WFC
	1.5	56.9 ^f	BC
	2.0	57.6 ^f	BC

Means within a column followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.*GCC-Green compact callus; GW-Greenish white callus(organogenic callus); WC-White compact callus ;WFC-White Friable callus; BC- brown callus; PGC-Pale green callus



Fig-2 Regeneration of different type of callus from leaf explants of *C.forskohlii* after 30 days

a.Pale green callus in; b.green callus(organogenic callus) ; c. white compact callus; d.e. white friable callus; f. brown callus; g. pale green friable callus; h-callus mass culture

Multiple shoot Formation

Green organogenic callus was transferred to MS medium supplemented with Kinetin and BAP (1mg/l) with combination IAA and NAA (0.1-0.4mg/l) (Table -3). The best shoot bud induction response was achieved on the medium containing Kinetin 1mg/l with NAA 0.1mg/l in higher percent (95.8%) and number of multiple shoots per callus (72.6) (fig-3 c,d). In contrast, the frequency of shoot bud differentiation and ten to twenty numbers of shoots were lesser in BAP concentration. The result of NAA, IAA in combination with Kinetin was also calculated for shoot regeneration from the callus. Leaves and shoot elongation (length) was noticed within four weeks of culture. Increasing the concentration of kinetin decreased the rate of callusing as well as shoots regeneration ability. It is in contrast with the earlier report (Sharma *et al.*, 1991) on *C. forskohlii* where the higher concentration of kinetin enhanced shoot multiplication. Addition of NAA (0.1mg/l) developed not only the rate of shoot regeneration but also the number and the length of the shoots. It has been shown previous that the addition of NAA promotes the proliferation and elongation of shoots in *Petasites hybridus* (Wldi *et ah*, 1998), *Eucalyptus grandis* (Luis *et ah*, 1999) and *Hybanthus enneaspermus* (Prakash, 1999). In the present study, higher concentration of NAA suppressed the rate of shoot regeneration (Table 2). Addition of lower concentration of IAA along with optimal concentration of kinetin enhanced the production of callus instead of organogenesis. Results here showed uniformity with other studies where the addition of NAA promotes the proliferation and elongation of shoots in *Petasites hybridus* (Wldi *et al.*, 1998), *Eucalyptus grandis* (Luis *et al.*, 1999) . This was considered as the optimal growth regulator combination for shoot regeneration in *C. forskohlii*. For further multiplication and continuous induction of shoot buds, the regenerated callus was sub cultured continuously onto the fresh regenerating medium. Formation of fresh shoot buds from callus surface was noticed in subculture.

Rooting

Well-developed shoots were transferred to MS basal medium for rooting and one week after inoculation, root development was observed from the shoots .Decrease the MS salts to half-strength enhanced the root development from the shootlets. The

maximum frequency of root development, number and the length of the roots were achieved in half-strength MS medium without growth regulators (Table -4 and Fig-1f fig-3 f). Whereas 1/4 strength MS nutrient medium showed low response of root formation. However, adding of IAA and IBA suppressed the root development. In dissimilarity to the current study, it has been reported that auxin is required for the stimulation of roots in *C. forskohlii* (Sharma *et al* 1991).

Table-3 Effect of cytokinins and auxins supplement to MS medium on shoot regeneration from leaf derived callus of *C. forskohlii*

Hormones	Treatment (mg/l)	Leaf		
		Percentage of regeneration	Average number of shoots/callus	Average length of shoot (cm)
BAP+IAA	1+0.1	70.2 ^g	13.8 ^f	1.4 ⁱ
	1+0.2	72.2 ^f	11.8 ^g	1.5 ^h
	1+0.3	73.4 ^{ef}	8.6 ^{gh}	1.8 ^f
	1+0.4	75.4 ^e	5.6 ^h	1.9 ^e
BAP+NAA	1+0.1	68.2 ^g	16.6 ^e	1.2 ^k
	1+0.2	64.6 ^h	13.8 ^f	1 ^l
	1+0.3	63.2 ^{hi}	8.8 ^h	1.3 ^j
	1+0.4	61.2 ^j	4.6 ⁱ	1.6 ^h
KN+IAA	1+0.1	82.2 ^d	12.8 ^f	1.8 ^f
	1+0.2	83.8 ^d	8.4 ^{gh}	1.7 ^g
	1+0.3	86.6 ^c	6.8 ^h	1.9 ^e
	1+0.4	87.4 ^c	5.8 ⁱ	1.8 ^f
KN+NAA	1+0.1	95.8 ^a	72.6 ^a	2.5 ^a
	1+0.2	91.6 ^b	68.6 ^b	2.1 ^d
	1+0.3	89.6 ^b	45.6 ^c	2.3 ^c
	1+0.4	86.6 ^c	14.6 ^d	2.4 ^b

Means within a column followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.

Table-4 Influence of MS medium concentrations on root induction from *in vitro* raised shoots in *C.forskohlii*

MS medium (mg/l)	Rooting (%)	Roots /shoots	Root length(cm)
MS BASAL	72	12 .4 ^b	3.2 ^b
½ MS	95	18.2 ^a	6 .8. ^a
¼ MS	48	8. 3 ^c	4 .2 ^b

Means within a column followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.

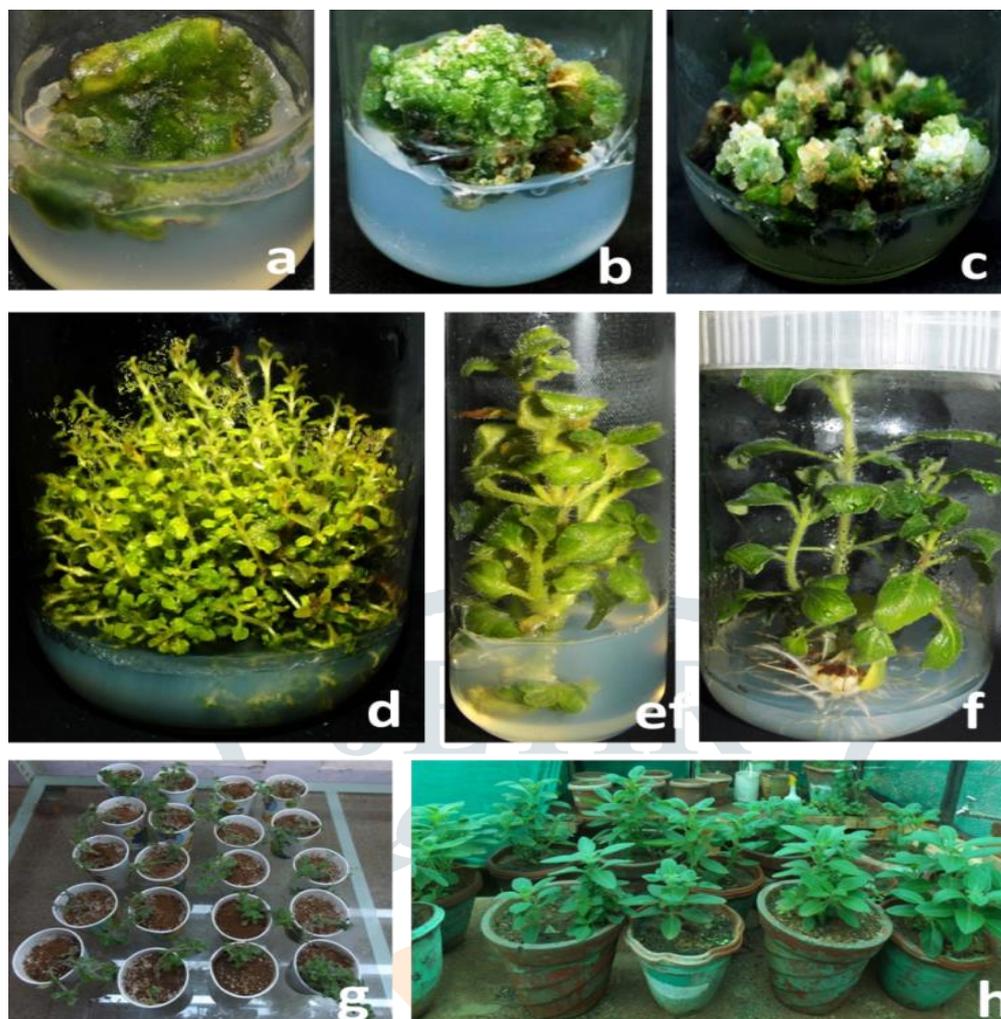


Fig-3. Mass Shoot propagation from the leaf derived organogenic callus of *C.forskholli*

a.leaf explant; b. organogenic callus from leaf explant in KIN 1.0mg/l; c.Shoot bud induction from leaf derived callus; d. mass multiplication in KIN 1.0 mg/l +NAA 0.5mg/l ; e.elongated shoots ; f. Rooting of regenerated shoot on $\frac{1}{2}$ MS media ; g. *In vitro* Accamalization and hardening; h. Plants transferred in green house condition

Hardening

Well-developed plantlets with long roots were removed separately from the culture tubes. After washing the roots carefully with sterilized tap water, they were transferred to fresh culture tubes containing sterilized tap water and maintained at culture room conditions. After weeks of incubation, plantlets were transplanted to paper cups, plastic pots containing autoclaved soil mixture containing equivalent quantities of sand: red soil: vermiculite (1:1:1) (Fig-1g,h fig-3g) and covered with polythene bags. Plants were watered every three days with half strength MS salt solution for 20 days. Polythene bags were removed after two weeks and in directive to adapt them to the greenhouse condition.(Fig-1h Fig-3 i) Fully adapted plants were transferred to field with 100% survival rate. The maximum survival of *in vitro* raised plantlets was achieved by transferring them to the potting mixture. The perfect hardening environment was covering the plantlets with punched polythene covers and keeping under moist condition exerted greater survival percentage of plantlets (Velmurugan, 2007 and Velmurugan *et al.* 2008.).

Conclusion

In conclusion, the present study grants an efficient protocol for the large-scale mass propagation of *C. forskohlii* through direct and indirect organogenesis using leaf explants. This *in vitro* propagation procedure should be suitable for conservation and large-scale commercial cultivation of the threatened *C. forskohlii*. Direct shoot regeneration methods suitable for large-scale multiplication of plantlets in *in vitro* by preventing clonal variation as opposed to regenerated from calli alone, which frequently leads to somaclonal variation.

Referances

- Abraham Z, Srivastava AK, Bagchi GD (1988). Cytoplasmic vesicles containing secondary metabolites in the root of *Coleus forskohlii* (Willd.) Briq. *Curr. Sci*
- Balasubramanya S, Rajanna L, Anuradha M (2012). Effect of plant growth regulators on morphogenesis and forskolin production in *Plectranthus barbatus* Andrews. *In vitro cell Dev. Biol.* -Plant 48:208-215.. 57:1339-1377.
- Bhat SV, Bajwa BS, Dornauer H, De Souza and Fehlhaber HW (1977) Structure and stereochemistry of new labdane diterpenoid from *Coleus forskohlii* Briq. *Tetrahedron Lett.* 19: 1669-1672.
- De Souza NJ, Dohadwalla AN, Rupp RH (eds.) (1986). Forskolin - its chemical, biological and medicinal potential. Hoechst India Ltd. Bombay.
- Dubey MP, Srimal RC, Nityananda S, Dhawan BN (1981). Pharmacological studies on coleonol, a hypotensive diterpene from *Coleus forskohlii*. *J. Ethnopharmacol.* 3:1-13.
- Lata H, Bedin E, Horsik A, Ganzera M, Khan I, Moraes RM (2002). *In vitro* plant regeneration from leaf derived callus of *Cimicifuga racemosa*. *Planta Med.* 68:912-915.
- Ling, JT, Iwamasa, M (1997). Plant regeneration from embryogenic calli of six *Citrus* related genera. *Plant Cell Tissue Organ Cult.* 49:145-148.
- Luis, P.B.C., Adriane, C.M.G.M., Silveira, B.R.C.C., and Ana Christina, M.B. (1999). Plant regeneration from seedling explants of *Eucalyptus grandis* X *E. urophylla*. *Plant Cell Tiss. Org. Cult.*, 56: 17-23.
- Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant.* 15:473-497.
- Mukherjee, S., et al (1996). *Plant Cell Rep.*, 15: 691-694.
- Goh CJ, Sim GE, Morales CL, Loh CS (1995). Plant regeneration through different morphogenic pathways in pommelo tissue culture. *Plant Cell Tissue Organ Cult.* 43:301-303
- Prakash, E., Sha Valli Khan, P.S., Sairam Reddy, P., and Rao, K.R. (1999). Regeneration of plants from seed-derived callus of *Hybanthus enneaspermus* L. Muell., a rare ethnobotanical herb. *Plant Cell Rep.*, 18:873-8
- Rupp RH, De Souza NJ and Dohadwalla AN (eds) (1986) Proceedings of the International Symposium on Forskolin, Bombay. January 1985 pp 28-29.
- Rao PS, Narayanaswamy S, Benzamine BD (1970). Differentiation *ex vitro* of embryos and plantlets in stem tissue cultures of *Tylophora indica*. *Physiol. Plant.* 27:271-276.
- Sen, J. and Sharma, A. K. (1991). *Plant Cell Rep.*, 9: 696-698
- Sharma N, Chandel KPS and Srivastava VK (1991) *In vitro* propagation of *Coleus forskohlii* Briq., a threatened medicinal plant. *Plant Cell Rep.* 10: 67-70
- Shrivastava S and Banerjee M (2008). *In vitro* clonal propagation of physic nut (*Jatropha curcas* L.): Influence of additives. *Int. J. Integrative Biol.* 3: 73-79.
- Reddy PS, Rodrigues R and Rajasekharan R (2001) Shoot organogenesis and mass propagation of *Coleus forskohlii* from leaf derived callus. *Plant Cell Tiss. Org. Cult.* 66:183-188.
- Velmurugan, M. (2007). Ph.D. Thesis Tamil Nadu Agricultural University, Coimbatore.
- Velmurugan, M. et al (2008). In. Proc. National Conference on Modern Trends in Plant *in vitro* Biology.
- Wldi, E., Schaffner, W., and Berger, K.B. (1998). *In vitro* propagation of *Petasites hybridus* (Asteraceae) from leaf and petiole explants and from inflorescence buds. *Plant Cell Rep.*, 18: 336-340.