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In Vitro Culture Studies on Medicinal Herb - *Coleus forskohlii* Briq

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Abstract: *Coleus forskohlii* Briq. (Lamiaceae) is an important medicinal plant with excellent export potential in herbal drug trade. The root of this plant is medicinally useful for high blood pressure, spasmolysis, obesity and constipation. A diterpene compound, forskolin obtained from tuberous root of this plant has been used for glaucoma congestive, asthma and certain cancers. The present work was aimed at evolving a protocol for rapid multiplication using culture technique. Tissue explants from leaf lamina, node and shoot tip were cultured on MS medium supplemented with different concentrations (0.5 - 2.0 mg/l) of IBA, 2, 4-D and BAP and their growth responses like callusing and shooting and rooting were elucidated. It was observed that shoot tip elicited maximum callusing, shooting and rooting response than that of leaf lamina and node. Multiple shoots were obtained from leaf lamina explants. This indicates the possibility of using tissue culture protocol for commercial scale of production.

Key words: *Coleus forskohlii* % Leaf lamina % Node % Shoot tip

INTORDUCTION

Recently medicinal plants occupy an important place in health care, cosmetics and food industries throughout the world. Herbal drugs are more preferred than allopathic drugs because of higher efficacy, affordability, easy availability and causing less or nil side effects. Even western world begin to use herbal drugs and herbal formulations described n traditional medicines like Chinese traditional medicines and Indian traditional medicines like Ayurveda and Siddha literature for curing various diseases.

In order to as certain quality of herbal drugs, their identity, quality, efficacy and safety are to be established standardization of herbal drugs thus include establishment of botanical identity, cultivation or collection, harvesting, processing, storage, preservation, formulation and packaging, therapeutic efficacy of herbal drugs depend upon the quality and quantity of biological active compounds.

Plant tissue culture is a modern tool available to rapidly propagate plants. It can be successfully used to conserve rare and endangered medicinal plants and multiply them in a short duration. Recent spurt in demand for herbal raw drugs can be met wholly or supplemented by tissue culture derived plants. The quality of herbal raw drug produced through tissue culture will be fairly uniform in terms of active principle. Tissue culture technique is potentially valuable for studying the biosynthesis of secondary products and may also eventually provide an efficient means of producing commercially important plant products.

Coleus forskohlii Briq. (Lamiaceae) is a species native to subtropical and warm temperate habitats. It grows at 600-1800m elevation on sun-exposed hill slopes and plateaus in arid and semiarid climatic zones. In India it occurs at high altitude up to the height of 2500 meters in Himalayas in the regions from Garhwal kumanon to Nepal. It also occurs in Decan Peninsula and Parasnath hills in Bihar. It is an herbaceous plant with annual stem and perennial root stock. The root material may be tuberous, semi-tuberous or fibrous depending upon the growth conditions. Approximately 1-500g of root material can be obtained from a single plant. The forskolin content of the roots varies from 0.07% to 0.58% of dry matter [1]. Dube *et al*. [2] elucidated an efficient method of *ex situ* conservation of germplasm and sustainable management of this medicinally important plant.

Tissue explants from different parts of an important Indian medicinal plant, *Coleus forskohlii* were cultured *in vitro* and their growth responses like callusing, shooting and rooting were elucidated in the present

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investigation. Explants from leaf lamina, node and shoot tip were cultured on Murashigee and Skoog medium supplemented with different concentration and combination of plant hormones of auxins like IBA, 2,4-D and BAP.

MATERIALS AND METHODS

Materials: *Coleus forskohlii* Briq. is the plant material used for the present study. High yielding *C. forskohlii* Salem variety were procured from Ariyalur, Tamil Nadu and India. Explants of leaf lamina, node and shoot tip were excised.

Surface Sterilization: The explants were treated with 0.1% mercuric chloride for 1-2 minutes and washed twice with sterile distilled water. Then the materials were rinsed in 50% alcohol for 2-3 minutes. The explants were thoroughly washed twice with sterile distilled water.

The composition of MS basal medium used in this study was similar to the composition of hormones free MS medium used by Murashige and Skoog [3] (Table 1).

The pH of the medium was adjusted to 6.5 using 0.1N NaOH or 0.1N HCl. Then appropriate quantity of agar agar was added. The medium was heated until the agar dissolved. Then the medium was poured into the culture tubes (15ml of the medium in 25 x 150mm culture tube) and the tubes tightly were plugged with absorbent cotton. The plugged culture tubes were sterilized in a pressure cooker at 121°C for 20 minutes and cooled to room temperature.

Culture Room: The culture room was maintained at a temperature of 25±2°C. The cultures were kept under the light intensity of 2,000 Lux at the level of culture tubes, using white fluorescent lamps. Photoperiod of 12hrs per day was maintained. The relative humidity of the room was maintained at 70 percent. Ten replicates were kept for treatment and this experiment was repeated at least once to confirm the results.

Sub Culture: Explants and calli were sub cultured every 4-5 weeks interval. The tubes containing culture material were externally sterilized with 50% alcohol. The materials were transferred to culture tubes containing fresh medium with the help of sterile forceps in the inoculation chamber. After subculture they were transferred to the culture room.

Growth Measurement: Fresh and dry weights of a few explants before and after culture period were measured. Regular observation at an interval of two days was made for the formation of callus, change of color and initiation of the root or shoot.

Table 1: Preparation of stock solution for MS medium

Stock solution	Constituents	Concentration of stock solution gm/ 100ml	Volume of solution in final medium ml/l.
A	NH_4NO_3	8.25	20
B	KNO_3	9.25	20
C	H_3BO_3	0.124	5
	KH_2PO_4	3.4	
	KI	0.0166	
	$NaMoO_4$ $2H_2O$	0.005	
	$CoCl_2$ $6H_2O$	0.0005	
D	$CaCl_2$ $2H_2O$	8.8	5
E	$MgSo_4$ $7H_2O$	7.4	5
	$MnSo_4$ $4H_2O$	0.446	
	$ZnSo_4$ $5H_2O$	0.172	
	$CuSo_4$ $5H_2O$	0.0005	
F	Na_2EDTA	0.745	5
	$FeSo_4$ $7H_2O$	0.5571	
G	Thiamine HCl	0.01	1
	Nicotinic acid	0.05	
	Pyridoxine HCL	0.05	
	Glycine	0.2	
	Myo-inositol	10.0	

RESULTS

Different explants like leaf lamina, node and shoot tip of *C. forskohlii* (Lam) were cultured on different concentration of auxins (IBA and 2, 4-D) and cytokinin (BAP). It was observed that explants showed growth responses like enlargement, initiation of callus and formation of shoot and root. Effects of different auxins and cytokinin on growth of different explants of *C. forskohlii* were observed.

When IBA was supplemented in different concentrations to MS basal medium, all the explants showed maximum growth increment at 1mg/l conc (Table 2). Among explants, shoot tip explants registered the highest growth (898.04 mg fr. Wt.) at 1mg/l IBA conc. followed by leaf lamina explants (575.85 mg fr. Wt.). Thus, at IBA 1mg/l conc. shoot tip and leaf lamina explants showed more than twenty four fold growth increment after 4 weeks culture period, no callus or morphogenesis observed in all the concentrations of IBA.

2, 4-D at different concentrations induced the highest growth increment and formation of callus in node and leaf lamina explants (Table 3). Callus was initiated in all the explants after 2 weeks in culture. Shoot tip explants were more proliferative and registered the highest growth (1951.94 mg fr. Wt.) at 1.5 mg/l 2, 4-D concentration. At this concentration all other explants also showed a maximum growth. MS medium supplemented with 1 mg/l 2, 4-D also induced good growth on all the explants. Nodal explants showed the least growth in all the 2, 4-D concentrations.

Table 2: Effect of different concentrations of IBA on growth of *C. forskohlii* explants

	Leaf base		Leaf lamina		Node		Shoot tip	
Concentration of IBA (mg/l)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)
Basal	133.29±1.211	1.33±0.066	127.37±1.129	1.26±0.072	138.41±2.061	1.35±0.065	141.08±1.413	1.40±0.042
0.5	658.57±1.549	6.57±1.565	536.37±2.015	5.35±2.775	432.57±2.754	4.31±3.302	769.25±0.811	7.68±1.476
1.0	712.84±1.269	7.12±1.941	575.45±2.710	5.75±2.811	305.38±2.969	3.04±2.171	898.28±1.768	8.97±1.457
1.5	206.74±1.967	2.06±1.152	198.72±1.925	1.98±0.095	109.26±1.759	1.09±0.049	290.37±0.490	2.19±1.102
2.0	101.49±0.634	1.01±0.099	95.36±1.25	0.95±0.072	108.12±2.06	1.04±0.053	179.85±1.215	1.79±0.091
Medium:	MS Basal							
Age of culture:	4 Weeks							

	Leaf base (mg)	Leaf lamina (mg)	Node (mg)	Shoot tip (mg)
Initial fresh weight	38.50±3.25	22.0±2.45	12.0±0.90	37.9±2.85
Initial dry weight	6.3±0.050	1.1±0.04	1.0±0.03	22.0±2.45

Table 3: Effect of different concentrations of 2, 4-D on growth of *C. forskohlii* explants

	Leaf base		Leaf lamina		Node		Shoot tip	
Concentration of 2,4-D (mg/l)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)
Basal	133.29±1.211	13.42±0.068	127.37±1.291	12.61±0.074	138.41±1.062	13.52±0.061	141.08±1.341	14.02±0.042
0.5	210.6±0.7720	21.06±2.125	114.0±1.8400	11.40±1.862	106.3±1.8810	10.63±2.712	212.10±2.018	21.20±1.139
1.0	532.6±52.390	53.26±1.656	340.0±1.4360	34.00±3.103	237.2±2.1530	23.72±2.113	1007.0±0.9180	100.52±1.270
1.5	867.8±1.1190	86.78±1.382	469.8±1.2150	46.98±2.041	258.9±2.9150	25.88±2.238	1951.9±1.2210	194.10±1.923
2.0	746.58±1.357	74.6±1.0260	541.94±1.025	54.11±1.724	315.49±2.875	31.42±0.081	840.28±2.715	74.40±7.050
Medium:	MS Basal							
Age of culture:	4 Weeks							

	Leaf base (mg)	Leaf lamina (mg)	Node (mg)	Shoot tip (mg)
Initial fresh weight	38.50±3.25	22.0±2.45	12.0±0.90	37.9±2.85
Initial dry weight	6.3±0.050	1.1±0.04	1.0±0.03	22.0±2.45

Table 4: Effect of different concentrations of BAP growth of *C. forskohlii* explants

	Leaf base		Leaf lamina		Node		Shoot tip	
Concentration of BAP (mg/l)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)
Basal	133.29±1.311	13.33±0.046	127.37±1.229	12.62±0.079	138.41±1.206	13.51±0.061	141.08±1.341	14.10±0.004
0.5	846.01±1.264	74.61±1.280	625.45±0.214	62.06±1.567	51.5±1.570	5.13±1584	1009.28±1.605	101.07±1.981
1.0	702.37±1.515	70.22±1.851	675.28±1.521	67.4±1.4500	63.5±1.694	6.24±1.415	891.38±1.831	89.30±1.764
1.5	591.38±1.588	59.32±1.450	507.57±1.451	50.64±1.710	40.6±1.362	4.05±0.315	784.37±1.764	78.32±1.693
2.0	601.73±1.207	60.02±1.225	571.85±1.563	57.41±1.457	15.9±1.452	1.58±0.050	651.57±1.349	65.03±1.513
Medium:	MS Basal							
Age of culture:	4 Weeks							

	Leaf base (mg)	Leaf lamina (mg)	Node (mg)	Shoot tip (mg)
Initial fresh weight	38.50±3.25	22.0±2.45	12.0±0.90	37.9±2.85
Initial dry weight	6.3±0.050	1.1±0.04	1.0±0.03	22.0±2.45

The different concentration of BAP induced more growth, compared to other auxins like IBA and 2, 4-D (Table 4). Profuse growth (1009.75 mg fr. Wt.) was induced by the 0.5 mg/l of BAP in shoot tip explants. At the above concentration of BAP, nodal explants showed minimum growth. The growth of explants at different concentration of BAP was in the following order 0.5>1.0>1.5>2.0. In this medium also shoot tip explants showed the best growth response.

The organogenetic pattern of different explants (leaf lamina, node and shoot tip) on MS medium supplemented with different concentrations and combinations of auxins and cytokinin was observed. In leaf lamina explants cultured on MS medium with BAP 2mg/l + 2, 4-D 1.5mg/l produced callus, shoot and root and the frequency was 90, 71 and 90% respectively (Table 5).

The nodal explants produced the least amount of growth. The medium with BAP 1mg/l + 2, 4-D 1.5mg/l induced white and friable callus in nodal explants. Inductions of multiple shoots are absent in nodal explants. Rooting is induced on nodal explants, cultured containing medium BAP 1.5mg/l + 2, 4-D 2mg/l.

Shoot tip of *C. forskohlii* is suitable for induction of morphogenesis in culture. The shoot tip explants were cultured on different concentration of IBA, 2, 4-D and BAP induced profuse growth. Among the three explants (leaf lamina, node and shoot tip) shoot tip showed best response.

DISCUSSION

Among different explants of *C. forskohlii* Briq. (Lamiaceae) shoot tip explants showed more growth response in culture. Shoot tip of *C. forskohlii* contain more meristematic tissues than matured leaves. Even though role explants containing auxillary buds proliferated very well in culture, shoot tip explants are more ideal for induction of callus and multiple shoots.

Out of two auxins used in the present study (IBA and 2, 4-D) explants of *C. forskohlii* preferred 2, 4-D for profuse callusing. Callus inducing ability of the two auxins on explants of *C. forskohlii* was in the following order 2, 4-D >IBA.

Shoot tip explants of *C. forskohlii* have more callusing potential on medium supplemented with 2, 4-D 1.5mg/l + BAP 0.5 mg/l. Different factorial combination of hormones elicited different growth response on leaf and shoot tip explants. Since auxin and cytokinin ratio determines differentiation in culture tissues. Endogenous hormone level of the explants or organs alters the exogenous requirement of plant hormones. The role of growth regulators in growth of plant tissue cultured in *in vitro* was known after the pioneering work of Skoog and Miller [4]. Morphogenetic potential of tissue explants is also altered by genetic and physiological age of the mother plant [5, 6, 7].

Rooting on multiple shoots was made easy when sub cultured on rooting medium containing different concentrations of IBA. MS basal + IBA 0.5 and1mg/l were ideal for roots and shoots formed from medium with BAP were separated and rooted on rooting medium. Since, many plantlets are formed from multiple shoots, for mass propagation in shorter period multiple shoots are preferred.

Asamenew and Narayana Swamy [8], worked on *C. forskohlii* obtained proliferation of callus from medium containing 1mg/l IAA and 1.5mg/l BAP and also adventious shoots were obtained from compact green callus and medium containing various concentration and combination of IAA and Kinetin.

Table 5: Effect of Factorial combination of auxins and cytokinin on organogenesis of *C. forskholii*

				Oraganogenesis					
S.No	IBA mg/l	2,4-D mg/l	BAP mg/l	Formation of Callus	Frequeny of Callusing (%)	Formation of Shoot	Frequency of Shooting (%)	Formation of Root	Frequency of Rooting (%)
1.	-	-	0.5	++++	95	++++	95	++++	91
2.	0.5	-	-	++++	90	++++	96	++++	91
3.	0.5	-	0.5	+++	79	++++	91	-	-
4.	0.5	-	1.0	+++	80	++	74	++	65
5.	0.5	-	1.5	++	37	+++	64	+++	75
6.	0.5	-	2.0	++++	95	++++	97	++	49
7.	1.0	-	-	97	++++	98	++++	97	-
8.	1.0	-	0.5	++++	96	++++	91	-	-
9.	1.0	-	1.0	++	55	+	21	+	17
10.	1.0	-	1.5	+++	61	++	53	++	49
11.	1.0	-	2.0	++++	88	+++	81	++++	87
12.	1.5	-	-	+	11	+	10	+	8
13.	1.5	-	0.5	++++	90	++++	89	++++	92
14.	1.5	-	1.0	++++	95	+++	78	+++	75
15.	1.5	-	1.5	++	32	++	30	+	19
16.	1.5	-	2.0	++++	44	+++	49	++	29
17.	2.0	-	-	-	-	-	-	-	-
18.	2.0	-	0.5	++++	73	+++	59	++++	58
19.	2.0	-	1.0	++	21	+	16	+	18
20.	2.0	-	1.5	++++	85	+++	81	++++	90
21.	2.0	-	2.0	++++	96	++++	95	++	40
22.	-	0.5	-	++	31	-	-	-	-
23.	-	0.5	0.5	++	24	+	13	+	15
24.	-	0.5	1.0						
25.	-	0.5	1.5	++++	77	-	-	-	-
26.	-	0.5	2.0	++++	84	-	-	-	-
27.	-	1.0	-	++++	96	++++	94	++++	93
28.	-	1.0	0.5	++++	49	-	-	-	-
29.	-	1.0	1.0	+++	41	-	-	++	36
30.	-	1.0	1.5	++++	90	+++	80	++++	89
31.	-	1.0	2.0	++++	89	++++	92	+++	74
32.	-	1.5	-	++++	91	++++	92	++++	89
33.	-	1.5	0.5	++++	41	-	-	++	15
34.	-	1.5	1.0	+++	33	+++	57	-	-
35.	-	1.5	1.5	++++	89	++++	90	++	27
36.	-	1.5	2.0	++++	90	+++	71	++++	90
37.	-	2.0	-	++++	74	-	-	-	-
38.	-	2.0	0.5	++	20	+	13	+	15
39.	-	2.0	1.0	+++	46	+	11	+	10
40.	-	2.0	1.5	++++	79	++++	88	+++	68
41.	-	2.0	2.0	++++	66	++++	83	++	49

Medium: MS Basal

Age of culture: 4 weeks

In the present study multiple shoots were obtained on medium with 1mg/l IBA and 0.5mg/l BAP. The difference in the responses of explants from the same species might be due to genetic seasonal variation physiological age. Malathy and Pai [9] observed, *forskolin* production in callus culture from leaf, stem and root origin as well as roots *in vitro* grown plants.

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