International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Shoot Regeneration from Leaf Derived Callus of *Coleus forskohlii* Briq

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Abstract: ***Coleus forskohlii Briq. a valuable medicinal important plant, member of the family lamiaceae was investigated for callusing and shoot induction of shoot explants. Leaf was studied using various growth harmones. Auxins (2,4-D) alone and in combination with cytokinines (BAP) induced green colour, light green colour, cremish, green fragile, brown colour was formed. Best Shoot induction was achieved from the callus using BAP (0.5 mg/l) and NAA (0.1 mg/l) after 2 weeks of inoculation. Where highest no. of shoot induced (3.00 ± 0.28) and with maximum frequency (80 ± 0.56) was regenerated.***

Keywords: *Coleus forskohlii*, callusing, *in vitro* shoot induction, Murashige and Skoog's (MS) media

1. Introduction

Coleus forskohlii Briq. is a member of the mint family, Lamiaceae. It grows in the subtropical temperate climates of India, Nepal, Sri lanka and Thailand (**Valdes *et.al*.,1987**). It is indigenous to India and is recorded in Ayurvedic Matrica Medica under the Sanskrit name Makandi and Mayani. All the species of Coleus have four didynamous, dedinate stamens, and the filaments of the stamens unite at their base to form a sheath around the style. The genus Coleus consist of 150 species. It is a perennial herb that grows upto 1-2 feet and its leaves are teardrop shaped. The root stock is typically golden brown, thick, fibrous and radially spreading. The root is harvested in fall, when the forskolin content is highest and the root colour is brightest (**Bhat *et.al* .,1997**).It is mostly used in Ayurvedic medicine in the treatment of heart ailments, asthma, bronchitis, insomnia, epilepsy and inflammation. Due to its unique pharmacological industry. Thus demand led to rapid depletion of wild populations resulting in its listing as a plant under vulnerable to extinction category in India (**Gupta *et.al* 1988**). This situation encouraged the cultivation of *Coleus forskohlii* on vast areas. Micropropagation is one of the powerful tools used to conserve elite, rare and endangered species. The present study aims at developing a simple rapid, economical and high frequency regeneration protocol from leaf explants of *Coleus forskohlii* so as to give rise to true - to - type clones for potential application in large - scale propagation.

2. Material and Methods

2.1 Collection of plant Material

Young branches of *Coleus forskohlii* were collected in the month of July 2014 from Barkhera pathani Bhopal and was identified by Taxonomist of these centre.

2.2 Preparation and Surface Sterilization of Explants

Leaf explants was collected from potted plants and processed for aseptic culture. Explants were surface sterilized by cleaning thoroughly under running tap water for 5 min. The explants were then surface sterilized with an antifungal agent (Bavistin) for 1 hours and are further with detergent for 10 min. and rinsed 4-5 times tap water. Further sterilization procedure was carried out inside Laminar air flow chamber. The cleaned explants were finally treated with $HgCl_2$ (0.1%) for 1 min.under aseptic condition and washed 3-4 times sterile distilled water to remove traces of $HgCl_2$. After surface sterilization the explants were cut into small pieces and inoculated in MS supplemented with harmones 2,4-D (0.0, 0.01, 0.02, 0.03, 0.04, 0.1, 0.5, 1.0, 2.0, 2.5 mg/l), BAP (0.0, 0.01, 0.02, 0.03, 0.04, 0.1, 0.5, 1.0, 1.5 mg/l) in different concentration and combination for the production of callus

2.3 Media and Culture Conditions

After surface sterilization, sterile explants were inoculated on sterilized solid Murashige and Skoog's media fortified with 3% sucrose. Nutrient media was dispensed in autoclaved culture bottles and test tubes. The $_pH$ was maintained between 5.4-5.7 which was adjusted with 1N NaOH or 1N HCl before gelling with 0.8% (w/v) agar. All cultures were maintained at 22 ± 2^0 C and 16 h photoperiod (light intensity 40-80 µmol m^{-2} s^{-1}) provided by cool white fluorescent tubes, Philips India). All the subsequent sub culture were done at 2 weeks interval.

2.4 In vitro culture induction of callus and shoots:-

The leaf segments were cultured on agar solidified MS media supplemented with different concentration of growth harmones 2,4-D (0.0 - 2.5 mg/l) and BAP (0.0 – 1.5 mg/l) for callus induction. Callus is sub culturing was carried out different concentrations of growth harmones BAP (0.0 – 2.0 mg/l) and NAA (0.0-1.0 mg/l) at regular intervals of 2 weeks to promote no. of shoots formation.

3. Result

The characteristic trait of a callus is that the unorganized mass has localized regions of merismatic activity and rudimentary cambial regions with zones of vascular differentiation which have the potential to produce normal shoots, roots and embryoids that ultimately from plantlets (**Dodds and Roberts., 1982**). Moreover, callus is a good source of genetic variability and adventitious shoot formation.

For the induction of callus and multiple shoots from the leaf explants of *coleus forskohlii*. Explants were inoculated on MS medium supplemented with various concentration of 2,4-D ranging from(0.0 - 2.5 mg/l) and BAP (0.0-1.5 mg/l) were used (Table 1). Callus produced from leaf explants were light green, dark green, creamish and brown within 2 weeks of inoculation (figure). MS supplemented with BAP and 2,4-D was more effective for inducing callus from leaf explants than supplementation with NAA and BAP.

The callus was subcultured on to MS medium supplemented with various concentration and combination of auxins (NAA) and cytokinine (BAP) showed an organic response and produced multiple shoots (Table2).

4. Discussions

Callus induction and proliferation systems are known to be very useful for the study of Bio-synthesis of natural products of *Coleus forskohlii*. This approach of synthesis of forskolin from callus has been used (**Sen *et.al*.,1992; Mukherjee *et.al*.,1996; Reddy *et.al*.,2001**). Hence the optimization of cellular proliferation is the first essential step to establish cultures from plant tissues for the production of natural products. The leaf explants were capable of directly regenerating large no.of plantlets in standard MS medium cytokinin and an auxin with a maximum frequency of response and produced no.of shoots.

Callus induction is a prerequisite for shoot formation and also for the other in vitro genetic improvement among the various growth harmones tested using BAP+NAA induced maximum frequency of shoot induction.When Callus culture were established on MS media and further cultivated on white;s medium (**White, 1963)** with 1 mg/l BAP and 1mg/l NAA they became friable, whitish and rhizogenic. A high frequency shoot organogenesis and plant establishment protocol has been developed for *Coleus forskohlii* from leaf derived callus cultures (**Malathy and Pai, 1999; Reddy et.al., 2001**).

Similar result were documented in leaf explants of *coleus forskohlii* at different concentration of 2,4-D induced the highest growth increment and formation of callus by **Praveena, Pandian ,Jegadessan 2012.** They reported that light green to dark green colour, nodular to fragile was formed. Similar callusing response was noted in several medicinal plants such as *Catalpa ovate* (**Lisowska and Wysokinska.,2000**) and *P.vulgaris* (**Kwapata *et.al*.,2010**). **Sreedevi *et.al* 2013** showed that various explants such as leaf, petiole, node, internode explants when implemented on MS medium fortified with 2mg/l 2,4-D + 0.5 mg/l BAP resulted in good proliferation of callus.

Table 1: Effect of different concentration of auxin (2,4-D) alone in combination with BAP in MS media on callus induction from leaf explants of Coleus forskohlii . Observation :- After 2 weeks.

Plant growth regulators (mg/l)		Media	Intensity of callus formed	Nature of Response
2,4-D	BAP			
0.0	0.0	M1	-	No response
0.01	0.0	M2	-	No response
0.02	0.01	M3	+	Creamish
0.03	0.02	M4	+	Creamish
0.04	0.03	M5	++	Yellow
0.05	0.04	M6	+++	Light green
0.5	0.1	M7	+++	Dark green
1.0	**0.5**	**M8**	**++++**	**Light brown fragile**
2.0	1.0	M9	++	Dark green
2.5	1.5	M10	++	Light brown

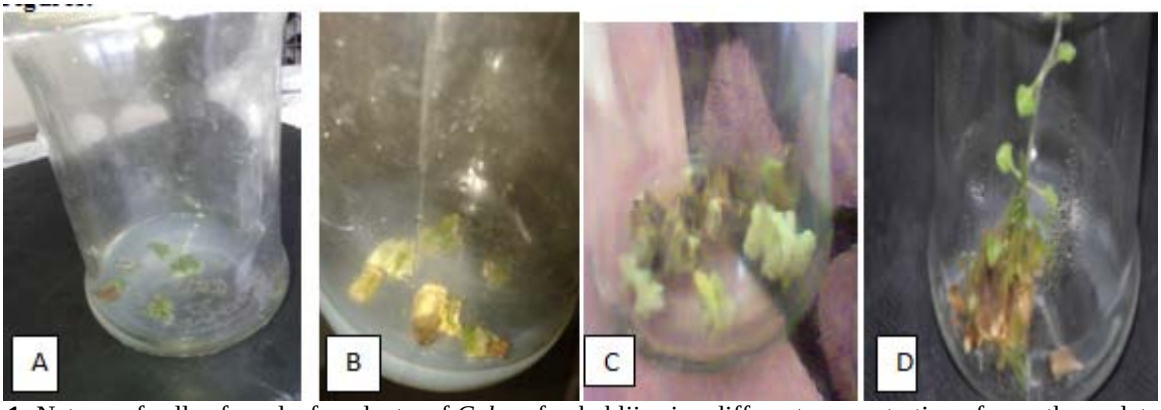
-No response, + little amount, ++ medium, +++ profuse proliferation.

Table 2: Effect of BAP and NAA individually or in combination on shoot regeneration from leaf explants of Coleus forskohlii in MS media. Observation: After 2 weeks.

Plant growth regulators (mg/l)		*Media*	*Frequency of shoot regeneration*	*No. of shoot/explants Mean±SE*	*Length of shoot (cm) Mean±SE*
BAP	*NAA*				
0.0	0.0	M1	-	-	-
0.01	0.0	M2	5 ± 0.08	1.0 ± 0.04	0.2 ± 0.06
0.02	0.0	M3	10 ± 0.04	1.5 ± 0.44	0.5 ± 0.09
0.03	0.0	M4	35 ± 0.45	2.0 ± 0.33	1.0 ± 0.07
0.04	0.0	M5	40 ± 0.45	1.6 ± 0.02	2.1 ± 0.11
0.05	0.01	M6	50 ± 0.25	1.05 ± 0.25	3.1 ± .21
0.1	0.02	M7	40 ± 0.04	2.05 ± 0.28	3.5 ± 0.34
0.5	**0.1**	**M8**	**80 ± 0.56**	**3.00 ± 0.28**	**3.6 ± 0.12**
1.0	0.5	M9	55 ± 0.54	2.00 ± 0.12	2.2 ± 0.15
2.0	1.0	M10	60 ± 0.21	1.15 ± 0.48	0.1 ± 0.26

Mean ± S.E. for three replication (10 culture for each replication)

Figures:-



Figures 1: Nature of callus from leaf explants of *Coleus* forskohlii using different concentration of growth regulators using 2,4-D and BAP(A-C.). (A) light green callus,(B) yellow,greenish callus, (C) Creamish brown callus, (D) Shoot induction.

5. Conclusions

In conclusion, an efficient and simple protocol for *in vitro* shoot induction from callus has been described. As callus culture can be obtained as rapid plant regeneration system which could be used for the somaclonal variation for producing transgenic plants. Hence, in vitro callus method developed in this study has greater potential for producing forskolin in larger quantities on an industrial scale.

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