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Shoot Organogenesis and Callus Induction of Indian Herbal Plant *coleus forskohlii*

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Abstract

Coleus forskohliibriq.is an important medicinal plant with excellent export potential in herbal drug trade. It is valuable plant, member of the family Lamiaceae was investigated for callusing and shoot induction of node explants. The pharmacological properties of *Coleus forskohlii* makes it useful to cure high blood pressure spasmolysis, obesity and constipation, asthma and certain cancers. It has important medicinal as well as aromatic values. The present work is aimed to develop a protocol for propagation of complete plant through tissue culture technique. It is an endangered plant so it is necessary for its growth and development by plant tissue culture. Within 45 days using node explants; we have been developed complete plants of *Coleus forskohlii*in MS medium containing 2.0mg/l BAP through multiplication at the rate of 4-8 shoots per explants. We followed various protocols of growth hormones auxins alone and in combination of with cytokinin. We have developed callus from leaves of *Coleus forskohlii* in MS medium containing 1 mg/l 2,4-D and 0.5 mg/l BAP after 5 weeks of incubation. We have subcultured the explants and obtained more shoots using the growth hormones 1 mg/l IAA and 2mg/l KN in MS medium after the incubation of 2-3 weeks. This protocol is very efficient for the mass multiplication of *Coleus forskohlii* and is the fastest method as compare to all the reported ones.

Key words: *Coleus forskohlii*, shoot induction,callusing, MurashigeSkoog' media.

Introduction

*Coleus forskohliibriq.*is an important medicinal plant. Its belongs to the family of Lamiaceae. It grow in the sub-tropical temperate climates of India,Nepal, Srilanka and Thailand(valdes et al.,1987). Theword Coleus is derived from the Greek word 'Keleos' meaning sheath referring to a typical characteristics of Coleus plant where the filaments fuse at the bottom to from a sheath around the style (de Loureiro 1790;Peterson 1994). It is indigenous of India and is recorded in AyurvedicMatricamedica under the Sanskrit name Makandi and Mayani. The genus Coleus

belonging to the 150 species. It is small, perennial and medicinal herbs that raiseup to 1-2 feet and its leaves are teardrop shaped. The root is golden brown, thick fibrous and radially spreading. Roots of this plant are the only source of forskolin (labdadindeterpine), when the forskolin (substance) content is highest and the root colour is brightest(Bhat et al., 1997). Many member of coleus species (Lamiaceae), such as *Coleusforskohlii,coleusbulmeri* and *coleus ambinicus*,are main sources of secondary metabolites, such as forskolin, rosmarinic acid, thymol and carvacrol(bhatsv et,al.1977).This herbal plants engage on important place in human health,food and cosmatics industries all over the word. Forskolin is manily used in Ayurvedic medicine in the treatment of heart ailments,asthma, bronchitis, insomnia,epilepsy and inflammation. *Coleus forskohlii* is traditionally multiplication by means of vegetative cutting but it is time consuming and limited number propagules. *Coleus forskohlii* plant has increased market demand and population of this herb is decreasing from the natural habitat which has necessary the use of biotechnology in conservation and management of the this endangered plant species(Sahoo Y &chand P K 1998). Micropropagation method is commonly used to conserve rare and endangered species. The present study aim the developing the proper and rapid protocol for propagation of *Coleus forskohlii*.

Materials and methods

Collection site and explant preparation

Apical and axillary meristem were used excised from the plant procured from ICAR(Anand ,Gujarat) and growing in the green house of Gujarat university. Explant were washed with soap solution carried out by running tap water for 10 min to remove adhering dust particle. Surface sterilization were carried out using 0.1% Hgcl, solution for 2 min followed by 0.1% sodium hypochlorite for 2 min and periodic rinsing with sterile distilled water.

Media and culture conditions

After surface sterilization, explant were inoculated on sterilized semisolid MS (Murashige andSkoog) medium. MS medium was prepared in jam bottles using macro, micro elements, vitamins and different hormone concentration. It was sterilized by autoclaving at 121°C for 20 minutes. Each treatment consisted of 3 replicates. Incubation was carried out in culture 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 and relative humidity of 50-60%. The culturewere observed continuously.

Shoot induction and callus formation

To observe the effect of different growth hormones on shoot multiplication and callus induction. TheMS medium was supplemented with different concentration of auxins and cytokines. The MS media was supplemented with different hormone concentration of BAP (0.5-2.0 mg/l) , KN(0.5,1.5 &2.0 mg/l) and IAA (0.5,1.0&1.5mg/l).shoot regeneration response to growth hormones at different concentration were expressed as number of regenerated shoots per explant. For callus formation the leaves & node explant were cultured with different hormone concentration of BAP (0.5 - 2.0mg/l) , 2,4-D (1.0-2.0mg/l)&NAA (1.0-2.0mg/l).

Subculture

Subculturing was carried out at regular intervals of thirty days. Visual observation of the cultures

were taken for every transfer and the effect of different treatments were quantified on the basis of percentage of cultures showing the response.

Result and discussion

Different explant like leaf, node and shoot tip of *Coleusforskohlii* were cultured on different hormone concentration. It was observed that explants showed growth response like enlargement, shoot formation and initiation of callus. The advantage of node culture is that in a relativelyshort time a large number of disease free plants could be produced.The best effect of response was observed when nodeexplant were cultured in medium with 2.0mg/l BAP. And the callus was showed the best effect of hormones concentration on 1.0mg/l 2,4-D & 0.5mg/l KN(Fig G & H). The callus is the unorganized mass has localized regions of merismatic activity 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).Callus is a good source of adventitious shoot generation. The explant when treated with 0.1 % Hgcl, solution for 4-5 minutes, offered more sterile culture .when the treatment of explant is more than 5 minutes explant will be dehydrated respectively. Also when the explant were treated with 70% alcohol for any time duration the explant got dehydrated. So treatment of alcohol is avoided in the explants of Coleus forskohlii. Number of shoots initiation were observed in

the medium containing BAP 2.0mg/l . And maximum number of shoots were observed during the sub culturing of explant in hormone concentration of 1.0mg/l IAA & 2.0 mg/l KN(Table 1& Fig E,F). Higher amount Greenish callus was observed in the hormone concentration of 1.0 mg/l2,4-D &0.5 mg/l KN(Table 1). Callus formationand shoot initiation both were observed in same explant on hormone concentration of 2.0 mg/l KN. In *Coleus forskohlii* shoot multiplication was obtained maximum in presence of BAP alone.

Geetha et al. also studied high frequency shoot multiplication from the cotyledonary nodes of pigeon pea in presence of BAP alone. This protocol is very efficient for the mass multiplication of *Coleus forskohlii* and its study may be utilized for the cultivation practices of this commercially important Indian herbal plant. The main significant of shoots culture is that in a short time a large number disease free plant the explant perform better response when collected and inoculated from the month of August to March. For the multiplication of shoots from a single nodal part, the highest number shoots were obtained in the medium containing (1.0 IAA& 2.0 KN) The medium containing IAA & KN were standardized as the best media for mass production of *coleus forskohlii*. The medium containing the BAP(2mg/l) produced the shoots and callus. Shoot multiplication was observed in BAP alone. Maximum shoot production protocol developed in the study may be utilized for the cultivation practices of this commercially important herbal plant.

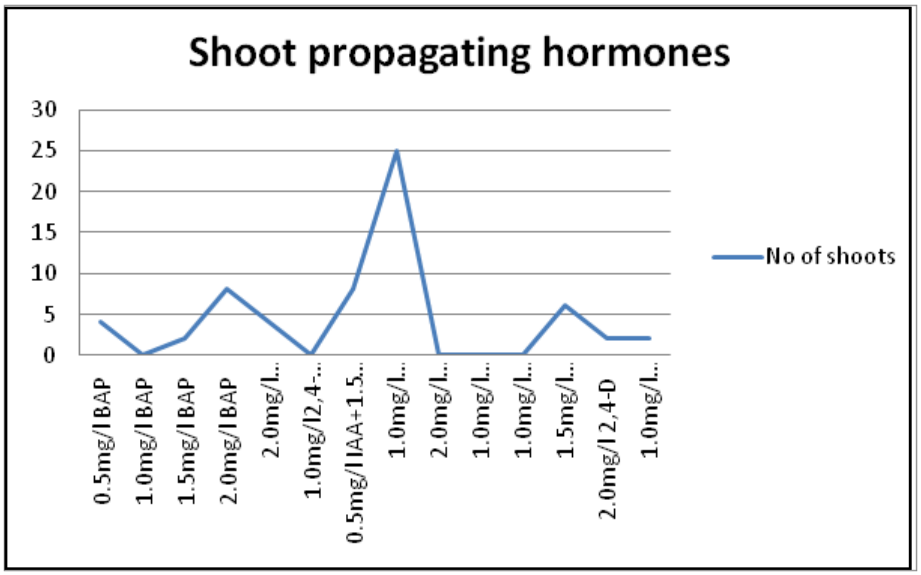
Conclusion

Coleus forskohlii is a very important herbal plant. The herbal plant contain an active substance called forskolin. The demand on this plant is increasing day by day and large multiplication of this plant is necessary. This is in vitro propagation procedure should be suitable for conservation and commercial cultivation of the *coleus forskohlii*.

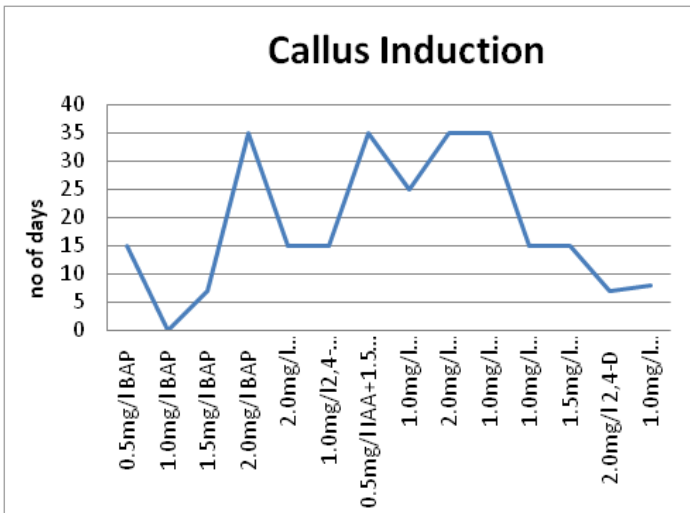
Table 1: Different hormone concentration for the growth of callus and shoots

Sr No.	Plant growth hormens (mg/l)	No of shoots	Callus formation	No of days inoculation
1	0.5mg/l BAP	4	-	15
2	1.0mg/l BAP	-	-	-
3	1.5mg/l BAP	2	-	7
4	2.0mg/l BAP	8	Greenish brown colour	35
5	2.0mg/l BAP+0.5mg/l KN	4	-	15
6	**1.0mg/l2,4-D+0.5mg/l KN**	**-**	**Green callus**	**15**
7	0.5mg/l IAA+1.5 mg/l BAP	8	-	35
8	**1.0mg/l IAA+2.0mg/l kn**	**25**	**-**	**25**
9	2.0mg/l BAP+1.0mg/l NAA	-	Greenish colour	35
10	1.0mg/l BAP+2.0mg/l BAP	-	Greenish colour	35
11	1.0mg/l NAA+0.5mg/l BAP	-	Callus	15
12	1.5mg/l IAA+1.5mg/l NAA	6	-	15
13	2.0mg/l 2,4-D	2	-	7
14	1.0mg/l IAA+1.5mg/l BAP	2	-	8

Graph1: Effect of hormone concentrations for shoot propagation



Graph 2:Effect of hormone concentrations for Callus Induction



Figures: Different developmental stage of regeneration of *Coleus forskohlii*

A. Shoot regeneration from 0.5 mg/l BAP hormone concentration

B. Shoot regeneration & Callus Induction from 2.0 mg/l BAP hormone concentration

C& D. Emergence of multiple shoots in 2.0 mg/l BAP + 0.5 mg/l KN

E. Formation of multiple shoot in 1.5 IAA + 1.5 mg/l NAA

F. Maximum shoot formation in 1 mg/l IAA + 2 mg/l KN

G. Formation of Greenish brown Callus H. Maximum formation of Greenish Callus on 1 mg/l NAA + 0.5 mg/l BAP on 1.0 mg/l 2, 4- D + 0.5 mg/l KN

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