In vitro propagation of *Coleus forskohlii*, an important medicinal plant

Deok-Chun Yang[1], Manju Meluttu George[1] and Jong-Seong Jeon[2]

[1] Department of Oriental medicinal materials & processing.
Kyung Hee University, Suwon 449-701, Korea

[2] Graduate School of Biotechnology & Plant Metabolism Research Center,
Kyung Hee University, Suwon 449-701, Korea

ABSTRACT

For mass multiplication of an important medicinal plant *Coleus forskohlii*, a procedure for the high frequency regeneration of *Coleus forskohlii* has been developed using leaf explants via callus culture. Callus formation occurred in MS medium supplemented with 1-2 mg/L each of NAA and BAP. A large number of shoots were formed on MS + 1 mg/L BAP from 50-60 days old greenish calli. Rooting of healthy shoots occurred on 0.1-0.4 mg/L NAA. The protocol described could be useful in future for genetic manipulation of this plant species.

INTRODUCTION

The demand for the plant based 'herbal' medicines is increasing every day due to their fewer side effects in comparison to synthetic chemical drugs. It is therefore very important to conserve and propagate medicinal plants of any country. *Coleus forskohlii* Briq. of the family Lamiaceae, is an aromatic herb 30-60 cm high with dull orange tuberous roots. It grows wild in the sub-tropical Himalayas, distributed from the Kumaon hills to Nepal ascending up to 2000 m, and in Bihar and Gujarat (Anonymous, 1950). The plant yields a valuable secondary metabolite known as "forskolin" which is a labdane diterpenoid. Though forskolin is found in all parts of the plant, roots are the main source of the compound (Shah *et. al.*, 1980). *Coleus forskohlii* is the only known source of this compound.

Forskolin is used in medicine for the treatment of glaucoma, congestive cardiomyopathy and asthma (Valdes *et. al.*, 1987). It is characterized by hypertensive property, inhibitory action on thrombocyte aggregation and reduction of intra ocular pressure (Shah *et. al.*,1980; Messinger *et. al.*,1988). In addition, forskolin is used in purification of adenylate cyclase and in receptor binding assays (Sukhdev,1997).

Indiscriminate and large-scale collection of this plant from forest and other natural sources coupled with insufficient attempts to either allow its replenishment or its cultivation have lead to the rapid depletion of its resources. Gupta (1988) has listed this plant as one of the plant species in India vulnerable to extinction. Micropropagation is a useful tool for rapid and large-scale clonal multiplication of economically useful plants. In the present work, we report a protocol for the high frequency regeneration of *Coleus forskohlii* using leaf explants via callus culture.

Corresponding author : **Deok-Chun Yang**, E-mail : dcyang@khu.ac.kr

MATERIALS AND METHODS

Leaves of approximately 5 cm length from one-year old *Coleus forskohlii* plants, collected as explants. The leaves were surface sterilized with 0.1% $HgCl_2$ for 2 min. and then thoroughly washed with sterile distilled water (4-5 times) and cut into smaller pieces before inoculation.

The leaf explants were inoculated on MS basal medium supplemented with 3% sucrose, 0.8% agar and Naphthalene Acetic Acid (NAA), 6- Benzyl aminopurine (BAP), 6-Furfuryl aminopurine (KN) and 3-Indole butyric acid (IBA) in various combinations for callus induction, KN or BAP for shoot induction and NAA or IBA for rhizogenesis. The medium was buffered to pH 5.8 and dispensed to culture vessels before autoclaving at 121℃ for 15 min. All cultures were maintained in a 12 hour photoperiod at 25±2℃.

RESULTS AND DISCUSSION

Exuberant callus formation occurred in MS medium supplemented with 1-2 mg/l each of NAA and BAP after 10 days of culture (Fig. 1A; Table 1) and these calli after 30-40 days were transferred to shoot induction media. The present observations with callus induction in *Coleus forskohlii* closely resembles a report on *Salvia officianalis*, another medicinal plants where equimolar concentrations of auxin and cytokinin (BA+NAA) stimulated callus formation from leaf explants (Kintziose *et. al.*, 1999). Callus initiation from the leaf disc explants has been reported from various other Lamiaceae members (Julian *et. al.*, 1998).

The subculturing of 50-60 days old greenish calli (Fig. 1B) on MS basal medium and with KN or BAP at varying concentrations showed that MS + 1 mg/L BAP produced maximum number of shoots (Table 2). Besides, higher concentrations of BAP were inhibitory for shoot differentiation and kinetin was found less effective for shooting. Similar results are also reported (Sharma *et. al.*, 1991) using shoot tip culture (Maximum shooting at MS + 2 mg/L IBA) and nodal segment culture (Maximum shooting at MS + 1 mg/L KN + 1 mg/L IAA) of *Coleus forskohlii*. In a number of medicinal plants, BAP has been found to be more effective in shoot induction than KN.

Rooting of healthy shoots was tried on half-strength as well as full strength MS basal medium supplemented

Table 1. Media combinations tried for callus induction from leaf explants of *C. forskohlii*

Media Combination	Response
MS + 0.5 mg/l NAA + 0.5 mg/l BAP	+
MS + 1.0 mg/l NAA + 0.5 mg/l BAP	+
MS + 1.5 mg/l NAA + 0.5 mg/l BAP	+
MS + 2.0 mg/l NAA + 0.5 mg/l BAP	+
MS + 0.5 mg/l NAA + 1.0 mg/l BAP	+ + +
MS + 1.0 mg/l NAA + 1.0 mg/l BAP	+ + +
MS + 1.5 mg/l NAA + 1.0 mg/l BAP	+ + +
MS + 2.0 mg/l NAA + 1.0 mg/l BAP	+ + + +
MS + 0.5 mg/l NAA + 1.5 mg/l BAP	+ +
MS + 1.0 mg/l NAA + 1.5 mg/l BAP	+ +
MS + 1.5 mg/l NAA + 1.5 mg/l BAP	+ +
MS + 2.0 mg/l NAA + 1.5 mg/l BAP	+ +

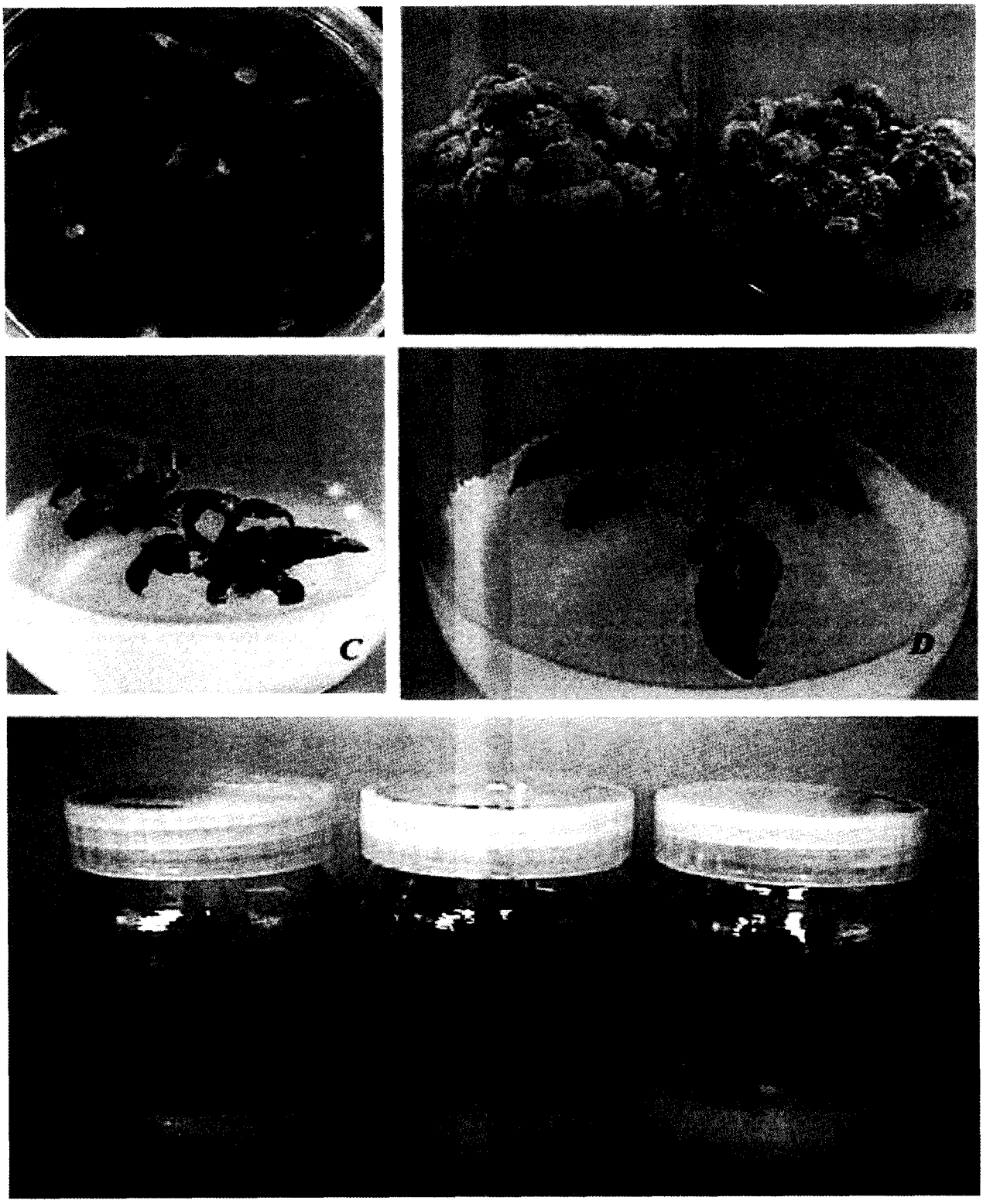

Fig.1. Callus formation and Shoot regeneration of *Coleus forskohlii*. (A, B) Callus induction and growth; (C) Shoot regeneration; (D, E) Root growth.

Table 2. Combination tried for shoot induction from leaf derived calli of *C. forskohlii*

Media Combination	Observation	Response
MS	Shoot initiation	+
MS + 0.5 mg/l BAP	Shoot initiation	+ + +
MS + 1.0 mg/l BAP	Shoots formed	+ + + +
MS + 1.5 mg/l BAP	Greening of calli + shoots	+ +
MS + 2.0 mg/l BAP	Greening of calli + shoots	+ +
MS + 0.5 mg/l KN	Callus remained green	-
MS + 1.0 mg/l KN	Callus remained green	-
MS + 1.5 mg/l KN	Callus remained green	-
MS + 2.0 mg/l KN	Callus remained green	-

Table 3. Media combinations tried for rhizhogenesis in *C. forskohlii*

Media combinations	Response
MS half-strength	+ + ++
MS full-strength	+ +
MS + 0.1 mg/l NAA	+
MS + 0.2 mg/l NAA	+
MS + 0.4 mg/l NAA	+
MS + 0.5 mg/l NAA	Callus induction from base of the shoots
MS + 1.0 mg/l NAA	Callus induction from base of the shoots
MS + 0.2 mg/l IAA	-
MS + 0.5 mg/l IAA	-
MS + 1.0 mg/l IAA	-
MS + 0.2 mg/l IBA	-
MS + 0.5 mg/l IBA	-
MS + 1.0 mg/l IBA	-

with auxins (NAA, IAA, IBA) at varying concentrations. NAA at lower concentrations (0.1-0.4 mg/L) produced roots and MS medium augmented with IAA or IBA did not induce any rooting response. In half-strength and full-strength MS basal medium also, shoots produced roots with maximum number if healthy roots in the former (Fig. 1D-E; Table 3).

The protocol described could prove to be useful for mass multiplication of this threatened medicinal plant. It could also prove useful in future for genetic manipulation of this plant species through 'Genetic engineering' techniques.

REFERENCES

Anonymous. 1950. The Wealth of India. Raw material Vol. 2. Council of Scientific and Industrial Research (CSIR) India pp. 308-309.

Gupta R. 1988. The endangered Indian medicianl plants. Indian Journal of Plant Genetic Resources 1: 98-102.

Jullien F., Diemer F., Colson M. Faure O. 1998. An

optimizing protocol for protoplast regeneration of three peppermint cultivars (Mentha Piperita). Plant Cell Tissue Organ Culture 54: 153-159.

Kintziose S., Nikolaou A., and Skoula M. 1999. Somatic embryogenesis and *in vitro* rosmarinic acid accumulation in *Salvia officinalis* & S. *fruticosa leaf* callus culture. Plant Cell Reports 18: 462-466.

Mersinger R., Dornauer H., and Reinhard. 1978. Economic importance of Foskolin extracted from *Coleus forskohlii*. Planta Medica 34: 200-204.

Shah V., Bhat S.V., Bajwa B.S., Dornauer H. and de Souza N.J. 1980. Planta Medica 39: 183-185.

Sharma N., Chandel K.P.S and Srivastava V.K. 1991. In vitro propagation of Coleus forskohlii Briq. - A threatened medicinal plant. Plant Cell Reports 10: 67-70.

Sukh Dev. 1997. Ethnopharmaceutics and modern drug development: The potential of Ayurveda. Current Science 73: 909-928.

Valdes L.J., Mislanker S.G. and Paul A. 1987. Economic Botany 41: 474-483.

(Received Aug. 2, 2003)
(Accepted Aug. 15, 2003)