In vitro propagation of *Coleus forskohlii* Briq., a threatened medicinal plant

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**ABSTRACT**

In vitro clonal multiplication of *Coleus forskohlii* Briq., a threatened plant, has been achieved on MS medium supplemented with Kn (2.0 mg/l) and IAA (1.0 mg/l) using nodal segments as explants. Shoots multiplied at a rate of 12-fold every six weeks. Rooting was achieved upon transfer of shoots onto MS medium containing IAA (1.0 mg/l). The micropropagated plants were successfully established under field conditions. Forskolin content in tubers of plants obtained by micropropagation was found to be 0.1%, the same as that found in wild plants. This micropropagation procedure should be useful for conservation as well as production of this important plant.

**ABBREVIATIONS**

BAP, 6-benzylaminopurine; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; Kn, Kinetic; MS, Murashige and Skoog (1962) basal medium; NAA, α-naphthalene acetic acid.

**INTRODUCTION**

*Coleus forskohlii* Briq. (Lamiaceae) is an aromatic herb, 30-60 cm high with tuberous roots. It grows wild in the subtropical Himalayas, distributed from the Kumaon hills to Nepal ascending up to 2000 M, and in Bihar, Deccan, Peninsular and Gujarat (Anonymous, 1950). The plant is valued for production of the forskolin drug used for treatment of glaucoma, congestive cardionmyopathy and asthma (Vaidya et al., 1987). *C. forskohlii* is the only known source of this compound. Though forskolin is found in almost all parts of the plant, roots are the main source of the compound (Shah et al., 1980). The pharmaceutical industry is largely dependent upon wild populations for supply of plant material for forskolin extraction.

Due to large scale and indiscriminate collection of wild material from forests, and insufficient attempts either to allow its replenishment or its cultivation, *C. forskohlii* is rapidly disappearing. In fact, it is now listed as one of the plant species in India vulnerable to extinction (Gupta, 1988). Thus, it is considered urgent to develop methods for the conservation of this threatened species. In vitro micropropagation techniques offer powerful tools for plant germplasm conservation. To our knowledge, however, there are no reports on micropropagation or in vitro culture of *C. forskohlii* except for an attempt at cell suspension culture (Mersinger et al., 1988).

The present work reports a reliable and efficient procedure for rapid clonal propagation through in vitro culture of *C. forskohlii*, a threatened plant species in India.

**MATERIALS AND METHODS**

**In vitro multiplication**

Plants of *C. forskohlii* collected from the wild were maintained ex situ. Nodal stem segments, 1.0-1.5 cm long from 6-month-old potted plants were used as explants to initiate the cultures. Small cut twigs were brought to the laboratory in water. Expanded leaves were removed and the stems were washed in tepal detergent for 15 min at slow speed on a magnetic stirrer, and later washed thoroughly under running water for 2 h. Surface sterilization was carried out with 0.1% HgCl₂ (BDH) for 10 min. These stems were then thoroughly rinsed with double distilled sterile water and were implanted vertically onto the nutrient medium. Murashige and Skoog (1962) basal medium with 3% sucrose, 100 mg/l myo-inositol and 1% agar was used. MS was supplemented with Kn, BAP, IAA, NAA.
or IBA in various combinations. The pH of the media were adjusted to 5.8 before dispensing in 150 mm x 25 mm rimless culture tubes. Media were autoclaved at 1.08 kg/cm² (121° C) for 15 min. At least 24 cultures were raised for each experiment and all experiments were repeated at least once. Cultures were maintained at 25 ± 3°C with 16 h photoperiod (2800-3000 lux) and subcultured every 4-6 weeks.

Establishment of plants in soil

Six to eight week-old regenerated plantlets were removed from culture tubes and washed free of agar. The roots were dipped in 0.2% Bavistin fungicide for 5-10 min, and plantlets were potted in a sterilized mixture of soil and sand (1:1). They were initially irrigated with half strength MS solution for one week, and subsequently with water. They were acclimatized at 25 ± 3°C with 16 h photoperiod for 3-4 weeks. Established plantlets were then transplanted to the field in earthen pots along with a ball of soil adhering to roots, and watered regularly.

Estimation of forskolin

The re-established in vitro propagated plants were harvested after six months of growth in the field, and the total production of tuberous roots was shade dried and powdered. The forskolin content of these samples was estimated using HPLC following the procedure of Inamdar et al. (1984), and compared to samples obtained from wild plants.

RESULTS AND DISCUSSION

Explants from mature potted plants were cultured on MS media supplemented with various growth regulator treatments (Table 1). On a medium supplemented with BAP (0.1-1.0 mg/l) and NAA (0.1-1.0 mg/l), callusing was observed (data not shown). Single shoot proliferation could be attained in the presence of BAP and IAA (Table 1). Kinetin (0.5-2.0 mg/l) used in combination with IAA (0.5-2.0 mg/l) resulted in multiple shoot production. The number of shoots produced per culture was maximal in a medium supplemented with 2.0 mg/l Kn and 1.0 mg/l IAA. Single node explants cultured in this medium showed 50% bud break during the first passage. Upon transfer of these sprouted buds to fresh medium, the shoots attained a length of ca. 4.3 cm with 4-5 nodes within 6 weeks of total time of culture (Fig. 1), thus providing at least five cuttings per shoot. Following subculture of such individual cuttings, an average of 12 new shoots were produced every 6 weeks (Figs. 2, 3).

For rooting, individual shoots were placed on MS medium supplemented with various auxins (Table 2). Rooting did not occur without an auxin, and of various auxins, IAA and IBA readily induced rooting. Maximal root induction was observed using IAA (1.0 mg/l), where root initiation took place within one week of transfer and ca. 35 roots were produced in four weeks.

In vitro produced plants were established in soil with almost 100% survival (Fig. 4). The plants grew