vitro and that this effect does not diminish over a 6 months culture period. The study suggests use of ABA to increase *in vitro* conservation period of kiwifruit.

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In Vitro Conservation and Encapsulation of *Coleus forskohlii*

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Key Words: Conservation, *Coleus*, Tissue culture, Encapsulation

Coleus forskohlii Briq. (Lamiaceae) is an important plant in Indian Ayurvedic medicine because its roots produce a labdane diterpenoid, forskolin (Bhat *et al.*, 1977) which has a positive intropic effect on heart action, lowers blood and intra ocular pressure and is an anti-inflammatory (Mukherjee *et al.*, 1996). Forskolin is commonly purified from tuberous roots of wild or cultivated *C. forskohlii* plants. Indiscriminate collection of *C. forskohlii* has led to rapid depletion of wild populations listing it as vulnerable plant in India. Therefore, the conservation of such rare and endangered plant species has become imperative. *C. forskohlii* is mainly propagated vegetatively to maintain clonal genotype. At present, the most common method to preserve the genetic resources of vegetatively propagated plant is, as whole plant in the field. But there are several serious limitations with field genebanks *(Withers and Engels, 1990)* mainly due to attacks by pests and pathogens, exposure to natural disasters, *etc.* In addition distribution and exchange from field genebank is difficult because of the vegetative nature of the material and the greater risks of disease transfer.

The present work aims at detecting the most suitable way of conservation and recovery of true-to-type elite *Coleus forskohlii* plant through the use of *in vitro* culture and encapsulation technique.

Shoot tip (0.5 cm) and nodal explants were collected from micropropagated *C. forskohlii* plants maintained in laboratory condition. Murashige and Skoog (1962) medium (MS) was used as basal medium. Explants were cultured in 25 x 150 mm culture tubes (single explant/ tube) containing 15 ml of semisolid 1/4 MS, 1/2 MS 2/3 ms (where concentration of the macro and micro salts were 1/4, 1/2, 2/3, respectively of the original concentration of MS) and MS medium supplemented

with 1% sucrose. No growth hormones were added in any of the media. The pH of the medium was adjusted to 5.6-5.8 before addition of agar and were autoclaved at 121°C and 103.4 kPa for 15 min. Cultures were incubated under 18 h light provided by cool-white fluorescent tubes (41.76 μ mol/m^{2}s^{-1}) at 25±2°C. After 6 months data were recorded on total length of shoot, number of nodes and number of days to induce rooting. Shoot tips from cultures with considerably reduced growth were recultured on MS with Kn and IAA (both at 0.1 mg/l) to observe their regeneration efficiency. The matrix for encapsulation was prepared by mixing liquid MS medium with 3% sodium alginate. pH of the matrix was adjusted at 5.8 before autoclaving. Shoot tip and nodal explants (0.3-0.4 cm) excised from the source as mentioned above, were mixed with encapsulating matrix and dropped (single explant/drop) in a beaker containing the solution of a mixture of MS medium and 50 mM $CaCl_2$. $2H_2O$with gentle stirring to form the alginate beads. Beads were left in the solution for at least 30 min for complete complexation, after which the solution was decanted. The beads were washed with sterile distilled water twice, dried with sterilized filter paper and lastly transferred to petridish containing MS medium with 3% sucrose and without growth hormones. After sealing with parafilm petridishes were stored at 4°C, -20°C, -70°C in dark for different periods (7, 15, 30, 45, 60 days). The viability was tested by germinating them in medium exactly similar to that used during storage.

In the first part of the present experiment on medium term *in vitro* storage of *C. forskohlii*, effect of the composition of the culture medium (MS) was only considered. The modifications made to reduce growth rate of the cultures were, reduction of sucrose to 1% level, complete avoidance of growth hormones and lowering of mineral concentrations of MS long with normal MS minerals in the medium. It was observed that elongation of the cultured shoot was inversely proportional to the concentration of the salts (Table 1). For *C. forskohlii* MS (with 1% sucrose) proved to be the most suitable for medium term conservation. It slowed down growth rate effectively. Even after 6 months, shoots grew only about 1 cm (0.5 to 1.55 cm) without developing any new nodes. Least reduction in growth rate was noted in 1/4 MS where shoots increased up to a length of 5.8 cm and differentiated 5-6 nodes (Table 1). Contrasting to this result is the report of Kartha *et al.* (1981) where low mineral elements (1/2 of the basal medium) was successfully used to conserve coffee plantlets rather than using medium with normal salt concentration.

Table 1. Effect of salt concentration of MS medium on *in vitro* conservation of *C. forskohlii*.

Medium	Length of explants after 2 months (cm) ± S.E.	No. of node differentiated	Days of rooting
MS	1.55 ± 0.72	1-2	100-110
½ MS	3.00 ± 0.291	1-2	60-70
2/3 MS	4.05 ± 0.27	3-4	45-50
¼ MS	5.8 ± 0.74	5-6	20-30

In the present experiment roots were differentiated from shoots in all media even in the absence of growth hormones. Early rooting was found to be associated with better growth in low salt medium – 1/4, 1/2 or 2/3 MS and proved to be unsuitable for reaching the goal whereas root differentiation at later part of the culture period as in MS, didn't interfere slow growth of the cultures. Advantage of rooting in *in vitro* conservation was discussed by Chandel and Sharma (1997). It was pointed out that lately differentiating rooted shoot cultures had an added advantage in conservation of being able to absorb nutrients from the medium especially at the later stage when water and nutrients were nearly exhausted as compared to the shoot cultures without roots.

Regarding the second part of the experiment, short term storage of encapsulated shoot tips (EST) and nodal segments (ENS) of *C. forskohlii* were carried out at temperatures *viz.* -70°, - 20° and 4°C and their post storage viability was examined. An encapsulated matrix of 3% sodium alginate from Sigma, USA and 50 mM $CaCl_2$, $2H_20$ was most suitable for formation of firm and spherical beads (Fig. A) with diameter (in case of isodiametric beads) ranging from 0.4-0.5 cm. Encapsulation of different plant parts like shoot tip, nodal segment, root parts, protocorms in addition to embryo/ somatic embryoides is now considered to be a useful technique in conserving genetic resources (Uozumi and Kobayashi, 1995; Datta *et al.* 1999). In the present experiment, storage of both EST and ENS below 4°C showed drastic loss of viability even after the shortest storage period (7 days). Therefore, results on preservation at 4°C only were taken into consideration (Table 2). Storage of cultures at non-freezing temperatures (2°-8°C) has been successfully applied in several other

Table 2. Viability percentage of the encapsulated explants of *C. forskohlii* at different time intervals (storage temp. 4°C)

Type of explant encapsulated	Time interval	% of regeneration ± S.E
Shoot tip	7	100 ± 0.00
	15	92 ± 1.076
	30	90 ± 1.074
	45	88 ± 0.286
	60	85 ± 0.291
Nodal Part	7	100 ± 0.00
	15	95 ± 0.079
	30	92 ± 0.421
	45	85 ± 1.05
	60	70 ± 1.52

plant species (Malemnganba *et al.*, 1996 Nayak *et al.*, 1998).

Considering germination percentage, there was no major difference between EST and ENS (Table 2). On immediate transplantation on basal MS medium enriched with 3% sucrose and without any growth hormone, 100% germination was noted. In this medium, induction of growth was observed within 9-10 days. The first sign of growth was emergence of green shoot tip through one pole of the bead and subsequently fine thread like roots through another. Fig. B shows 2-week-old *C. forskohlii* growing on the substrate, leaving aside the alginate matrix. High viability percentage (85% and 70% respectively) for 60 days stored EST and ENS may be due to the availability of nutrients within the gel matrix. Thus, longer storage period over 2 months could be planned for future experiments. Individual plantlets after growing up to a height of 3-4 cm (Fig. C) on the same medium in separate culture tubes, were transferred to small plastic cups containing soilrite: soil (1:1) (Fig. D). Cups with plants were initially covered with thin polythene sheets for 10 days to avoid dessication, after which the polythene sheets were removed and the plants were kept in the culture room for another 15 days before transfer to the greenhouse and establishment in the field (Fig. E).

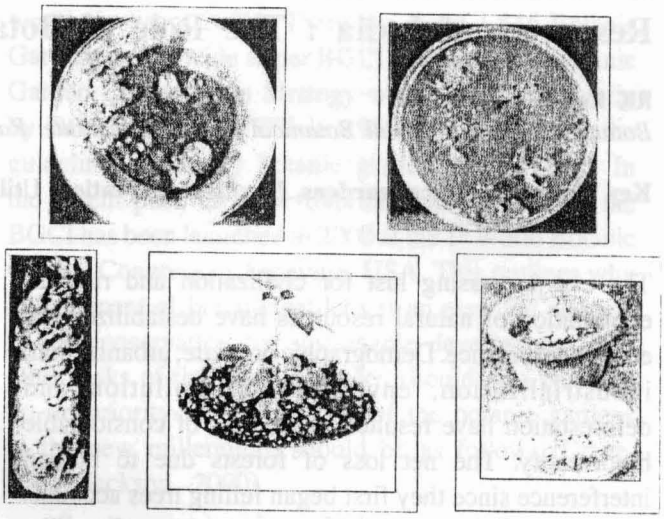

Fig. 1

a. Encapsulated shoot tip and nodal segment of C. forskohlii
b. 2-weeks-old plantlets regenerated from ruptured beads
c. Proliferation of C. forskohlii after germination from encapsulated shoot tip
d. A plant in soilrite:soil mixture; e. Field trial plants

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