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Short note [Nota corta]

MICROPROPAGATION OF *Coelogyne stricta* (D.Don) Schltr. VIA PSEUDOBULB SEGMENT CULTURES

[MICROPROPAGACIÓN DE *Coelogyne stricta* (D.Don) Schltr. VIA CULTIVO DE SEGMENTOS DE PSEUDOBULBOS]

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SUMMARY

Reproducible protocols for micropropagation of *Coelogyne stricta via* pseudobulb segments were developed. *In vitro* grown pseudobulb segments responded positively by induction shoot buds on half strength Murashige and Skoog's (MS) medium supplemented with growth regulators α-naphthalene acetic acid (NAA) and Benzyl Adenine (BA) individually and in combinations. The combination of NAA (1.0 mg/l) + BA (2.0mg/l) and BA individually induced maximum number of multiple shoots. Root number proved to increase with NAA (1.0 and 2.0 mg/l) in the MS medium. The well developed rooted plantlets were hardened successfully in the potting mixture containing coconut husk, perlite, charcoal, broken tiles, brick pieces in the ratio of 2:1:1:1:1.

Key words: *Coelogyne stricta,* pseudobulb segments, micropropagation, growth regulators, hardening.

RESUMEN

Se desarrollaron protocolos para la micropropagación de *Coelogyne stricta* emplenado segmentos de pseudobulbos. Los segmentos respondieron positivamente (*in vitro*) en medio Murashige y Skoog (MS) suplementado con los reguladores de crecimiento α-naphthalene acetic acid (NAA) y Benzyl Adenine (BA), individualmente y en combinación. La combinación de NAA (1.0 mg/l) + BA (2.0mg/l) y BA sólo, indujeron un número máximo de crecimientos. El número de raíces se incremento con NAA (1.0 y 2.0 mg/l) en el medio MS. Las plántulas obtenidas fueron exitosamente transferidas a macetas conteniendo cáscara de coco, carbón, perlite, ladrillos y bloques rotos en una proporción 2:1:1:1:1.

Palabras clave: *Coelogyne stricta,* segmentos de pseudobulbo, micropropagación, reguladores de crecimiento.

INTRODUCTION

Orchids are one of the unique plant groups and famous for their diverse and complex flower characteristics, *in vivo*. Uncontrolled collection and habitat destruction have led to drastic reduction in number of orchids in India (Pradhan, 1985). They are generally propagated through pseudobulbs. Methods for rapid multiplication of orchids are essential to meet the commercial demand and conserve the wild orchid population in their natural habitat. *In vitro* propagation is valuable tool for orchids in this context. Previous works on *in vitro* culture include propagation from explants such as shoot tips (Morel, 1960) stem discs of *Dendrobium moschatum* (Kanjilal *et al.,* 1999), *Cymbidium ensifolium* rhizomes (Chang and Chang, 2000), nodal, leaf explants of *Paphiopedilum* (Chen *et al*., 2004), foliar meristem of *Vanda spathulata* (Decruse *et al.,* 2003).

Coelogyne is a large genus comprising of more than 200 species. India is known to have 34 species of which *Coelogyne stricta* is endemic to pan Himalayas distributed in North East India, Uttar Pradesh, Sikkim, Bhutan, Myanmar and Nepal. The pseudobulbs of *C. stricta* are used by rural Khasi and Jainitia tribes of Meghalaya (India) as source of medicine (Kharkongor and Joseph, 1981). Orchids require a combination of multiplicity of factors for continued reproduction in nature. The seeds are unique and are poorly developed even at maturity. They lack endosperm and require a suitable fungal stimulant for germination in nature; the fungus is believed to augement the carbohydrate, auxin and vitamin transport in the orchid (Arditti *et al.,* 1982). In nature, only 0.2 to 0.3 % of seeds germinate

(Singh, 1992) even if they do so, the seeds take a long time for their germination and any disturbance in the habitat or physical environment destroys the whole population. Also the seedlings take up to 12 years to become an adult plant. They are highly heterozygous and their vegetative propagation through division is rather slow. This difficulty in natural population drives some of the indigenous species to extinction. Pseudobulb segment culture is an efficient system for the production of large number of plantlets in a short time. Regeneration potential of pseudobulb explants has been successfully tested in several orchids including *Cattleya, Miltonia, Cymbidium, Phaius* (Morel, 1964), *Arundina* (Mitra, 1971), *Dendrobium* (Vij and Sood, 1982; Vij and Pathak, 1989), *Bletilla* (Vij and Dhiman, 1997) and *Malaxis* (Vij and Kaur, 1998). In the present work, the method of micropropagation *via* pseudobulb segment has been adopted for regeneration of *C. stricta* (D.Don) Schltr.

MATERIAL AND METHODS

The undehisced capsules of *Coelogyne stricta* (D.Don) Schltr. were collected from the National Orchidarium, Botanical Survey of India, Yercaud (1500 MSL) in the Servarayan Hills (Tamil Nadu), Southern India. The freshly collected capsules were thoroughly washed with running tap water and then with the detergent teepol (0.1%) followed by mercuric chloride solution (0.1%) for 3 minutes and were subsequently rinsed in sterilized double distilled water. The capsules were dipped in 80% ethyl alcohol for a minute and flamed. The sterilized capsules were cut longitudinally and the seeds were extracted into MS basal medium under aseptic conditions. The pseudobulbs developed from the plantlets grown on MS medium for one year was used as explant.

Pseudobulbs of *C. stricta* were cut longitudinally into two equal segments under sterile conditions and inoculated on a medium supplemented with half strength MS salts (Murashige and Skoog, 1962) with 30g/l sucrose and plant growth regulators such as α-naphthaleneacetic acid (NAA), and N^6 Benzyl Adenine (BA) individually and in combinations. The medium were dispensed into 15 x 2.5 cm (Borosil) test tubes and capped with a layer of aluminum foil. The pH of the medium was adjusted to 5.8 before adding agar (0.8%) and sterilized at 121^0C for 20 minutes. After 45 days of culture, the regenerated plantlets were subcultured on the same medium for further growth.

The cultures were incubated in 12/12 hours of light/dark cycles at 25 ± 2^0C at 50-60% relative humidity. Observations were made every 15 days and five replicates were maintained for each treatment and data were recorded and analyzed according to Duncan, (1955). For acclimatization, the regenerated shoots with roots after 90 days were transplanted to the potting medium containing coconut husk, perlite, charcoal (3 × 2 cm), broken tiles (3 × 2 cm) and brick pieces (3 × 2 cm) in the ratio of 2:1:1:1:1.

RESULTS AND DISCUSSION

The regeneration competence of the pseudobulbs seems to be markedly influenced by physiological age of the mother plant, position of donor and growth stimulus in nutrient pool (Vajrabhaya, 1978). In the present investigation, regeneration capacity of pseudobulbs was tested for *C. stricta* (Table 1). The pseudobulb segments obtained from one year old *in vitro* cultures, responded readily on half strength MS medium supplemented with growth regulators. The results of the study demonstrated that pseudobulb segments proved a better alternative for multiplication of orchids. The pseudobulb explants from split halves produced shoot buds (Figure 1A) without any intervening callus or PLB formation in culture. After regeneration the explants turned brown whereas, the newly formed shoot buds grew as normal plants. The results were in agreement with the earlier observations in *Ascofinetia* (Intwong and Sagawa, 1973), *Dendrobium crepidatum* and *Dendrobium pierardianum* (Vij *et al.,* 1991) and *Neostylis* (Sagawa and Kunisaki, 1982). Addition of growth regulators was essential for the differentiation of plantlets. Among the different combinations tried in this study NAA (1.0mg/l) and BA (2.0mg/l) were most effective for the plantlet development (see table 1). In the present study, the combination of NAA and BA showed significant increase in the production of the higher number of plantlets. As the concentration of BA from 0.5 to 2.0mg/l considerably increased the multiple shoot production. This revealed that the concentration of BA higher than 2.0mg/l might be effective in production of multiple shoots further. The results agree with the findings of Roy and Banerjee (2003) in *Dendrobium oculatum*. However, root number proved to be effective in MS along with NAA (1.0 and 2.0 mg/l). Individual treatment with BA (1 and 2 mg/l) and combined treatment with NAA resulted in production of direct development of shoot buds (Figure 1B and C). The results were in agreement with the results of *Cattleya* (Mauro *et al.,* 1994); shoot tips and flower stalk buds of *Phalaenopsis* and *Doritaenopsis* (Tokuhara and Mii, 1993) and foliar meristems of *Vanda coerulea* (Seeni and Latha, 2000).

Table 1. Effect of plant growth regulators on growth and differentiation of pseudobulbs excised *in vitro* in 1/2 strength MS medium

S. No.	Medium (mg /l) (Half strength)	Mean shoot length (cm)	Mean multiple shoots	Mean root number	Mean root length (cm)	Morphogenic Response
1.	Basal	1.28 [a]	3.0 [a]	2.0 [a]	1.40 [a]	+
2.	NAA (0.5)	1.98 [b]	3.6 [ab]	4.4 [d]	2.28 [cd]	+
3.	NAA (1.0)	2.76 [cd]	4.0 [abc]	6.6 [e]	2.50 [d]	+ +
4.	NAA (2.0)	4.40 [f]	4.8 [cd]	6.2 [e]	3.20 [e]	+ + +
5.	BA (0.5)	2.06 [b]	4.6 [bcd]	1.6 [a]	1.60 [ab]	+
6.	BA (1.0)	2.60 [bcd]	6.6 [e]	2.4 [ab]	2.06 [bcd]	+
7.	BA (2.0)	2.80 [cd]	6.4 [e]	3.0 [bc]	2.58 [bcd]	+
8.	NAA (0.5) + BA (0.5)	2.16 [bc]	4.6 [bcd]	3.0 [bc]	1.78 [abc]	+
9.	NAA (0.5) + BA (1.0)	2.76 [cd]	4.8 [cd]	3.8 [cd]	1.92 abc	+
10.	NAA (0.5) + BA (2.0)	3.20 [de]	5.4 [de]	4.0 [d]	1.38 [a]	+
11.	NAA (1.0) + BA (0.5)	3.48 [e]	5.6 [de]	4.4 [d]	2.20 [cd]	+ +
12.	NAA (1.0) + BA (1.0)	3.52 [e]	5.8 [de]	4.6 [d]	3.26 [e]	+ + +
13.	NAA (1.0) + BA (2.0)	3.78 [ef]	6.4 [e]	4.6 [d]	3.54 [e]	+ + +

+ + + Best + + Good + Moderate

The regenerative pathway and differentiation varied with quality, quantity and combination of growth regulators BA and NAA in the medium. In *Bletilla*, the pathway of regenerants was markedly influenced by the level of BA; 1 mg/l favoured callus mediated PLB development whereas, 2 mg/l favoured the development of multiple shoots. A combination of BA (1 mg/l), NAA (1 mg/l) and Caesin hydrolysate (1g/l) favoured PLB's in *Malaxis acuminata.* In *C. stricta,* irrespective of concentration of either BA or NAA favoured the development of multiple shoots in *Malaxis acuminata* (Vij and Kaur, 1998). The well developed plantlets along with two to three roots (Figure 1D) were survived well (70%) in the potting mixture containing coconut husk, perlite, charcoal, broken tiles and brick pieces in the ratio of 2:1:1:1:1 (Figure 1E). In the present study, the regeneration potential of the plantlets greatly depend on the growth regulators in the medium and the presence of growth regulators proved significant increase in the development of the plantlets than control. The ability of pseudobulb segments in orchids to regenerate multiple shoot buds and or/PLBs suggest that pseudobulb culture can be successfully employed for rapid multiplication by suitably adjusting the nutrient environment. The present study will add a new dimension in rapid micropropagation of this wild orchid *C. stricta.*

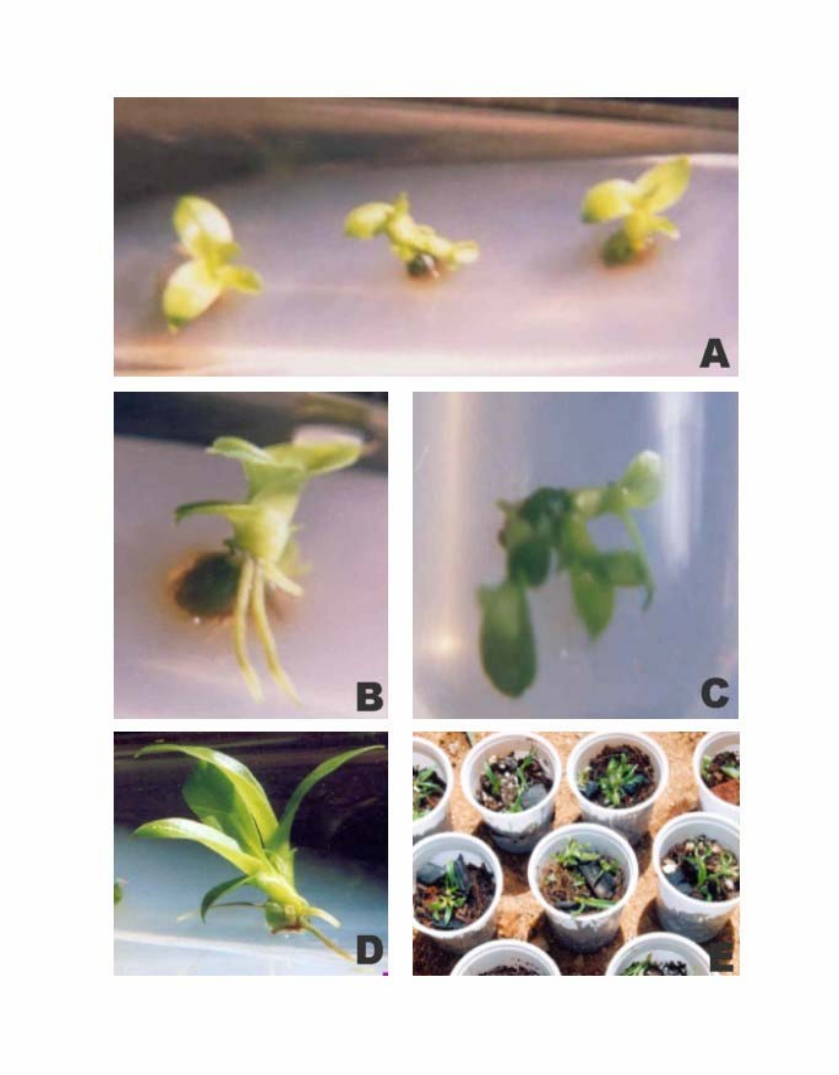

Figure 1. *In vitro* multiplication of pseudobulb segemnts of *coelogyne stricta.*
A. Shoots arising from pseudobulb segments
B. Formation of roots on newly formed shoots
C. Multiple shoots developed from pseudobulb segments
D. Shoots along with well developed roots
E. Hardening of plantlets.

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