Short Communication

IN VITRO PROPAGATION OF *CYMBIDIUM ELEGANS*, LINDL FROM SHOOT TIP CULTURE

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ABSTRACT

In vitro culture of *Cymbidium elegans* was carried out in 0.8 % (w/v) agar solidified MS media with or without exogenous supply of growth regulators, BAP and NAA. Most of the *in vitro* germinating seeds first developed into protocorms and then differentiated into shoots whereas some directly gave rise to shoot buds. MS media supplemented with BAP (1mg/l) and NAA (0.5mg/l) was found to be the most effective condition for proliferation of multiple shoots from shoot tip explants. MS medium supplemented with 0.5 mg/l IBA was the most effective condition for induction of multiple roots. Approximately 85% of plant survived during acclimatization in mixture of 2:1 cocopeat and moss.

Key words: *in vitro*, protocorms, shoot tip, multiplication

INTRODUCTION

Cymbidium elegans is one of the important medicinal and ornamental orchids of Nepal distributed in the central and eastern parts at an altitude ranging from 2100-2500 meters above sea level. It blooms in the month of September-November. It has high medicinal values as its pseudobulb and leaves are used as nerve tonic, and against hysteria madness, epilepsy and rheumatism (Vaidya et.al. 2000), (Pant and Raskati, 2013). It has also great importance in horticulture because of its long-lasting and beautiful flower. Due to its high horticultural and medicinal values, the plant is excessively collected from the wild for local uses and trade and its population is decreasing at an alarming rate. Natural propagation of orchid by seeds is a very slow process as it requires a mycorrihizal association for germination. Thus *in vitro* propagation of orchid by tissue culture technique is an alternative tool to obtain a large number of genetic ally pure elite populations in a short time period in an artificial nutrient medium under aseptic condition (Pant et. al. 2011). In order to meet its commercial demand and to conserve this species in its natural habitat, *in vitro* multiplication is an efficient alternative. The *in vitro* propagation of this orchid from shoot tip culture is reported here.

MATERIALS AND METHODS

Shoot tip: Shoot tips of C. elegans for culture were obtained from *in vitro* grown seedlings.

Media

MS (Murashige & Skoog's, 1962) medium either alone or in combination with different concentrations of two plant growth regulators viz; BAP (0.5 – 2.0 mg/l) & NAA (0.5mg/l) were used for present investigation (Fig 1). Media contained 30 g/l sucrose and 100 mg/l myo-inositol was adjusted at pH 5.8 before autoclaving. Media were solidified by adding agar at 8.0g/l. About 16-20ml medium was dispensed into each sterile culture tube (120mm X 25mm) and covered with aluminum foil. Media containing culture tubes were then autoclaved at 120°C for 15min. at 15lb/ inch pressure.

Inoculation of explants

About 3-5mm shoot tips were excised from 32 weeks old *in vitro* grown seedlings. Shoot tips explants were cut with the help of sterile surgical blade and inoculated on MS media alone and MS media supplemented with different hormone combinations. All the cultures were kept at 25°C (± 2°C) under 16/8 hrs photoperiod. For *in vitro* rooting, the microshoots obtained from shoot tip explants were transferred to the MS media supplemented with three different rooting hormones IAA, IBA & NAA at concentrations (0.5 – 2.0 mg/l). Growth of roots was observed regularly every week. For hardening, the mouth of culture tubes containing well developed rooted shoots were opened and kept under room temperature for one week. Rooted shoots were first washed under tap water to remove extra agar slicked on it and the plantlets were acclimatized in clay pot by using appropriate potting mixture of coco peat and sphagnum moss in the ratio of 2:1.

RESULTS & DISCUSSIONS

Shoot tip explants cultured on MS media alone and MS

media supplemented with different concentrations of hormones showed different responses. Almost all the conditions favored multiplication of shoots except MS basal media and MS+NAA (0.5mg/l). Single shoot as well as multiple shoot were obtained from shoot tip culture. In some culture condition callus and protocorms were regenerated from shoot tips before giving rise to shoots. Shoot tip explants cultured on MS basal media supplemented with different combinations of BAP (0.5-2 mg/l) and NAA (0.5 mg/l) favoured luxurious growth of protocorm like bodies (PLB's). Later these PLBs differentiated into shoots. Among the different concentrations, BAP (1.5 mg/l) + NAA (0.5 mg/l) was the most effective hormone concentration for the development of PLBs and then shoots. Protocorm growth from shoot tip in MS medium supplemented with BAP and NAA were also reported by Rajkarnikar and Niroula 1994 in *Dendrobium fimbriatum*, Shrestha and Rajbhandari 1994 in *Cymbidium longifolium*.

By changing hormone concentrations the direct induction of PLBs or indirect induction through callus growth was possible. The most effective hormone concentration for the development of callus from shoot tip explants was BAP (0.5mg/l) + NAA (0.5 mg/l). The equal proportion of two growth hormones enhances the callus regeneration rather than shoots. Shoot tip explants also produced pseudobulb on NAA (0.5 mg/l) & BAP (2mg/l) + NAA (0.5 mg/l) supplemented conditions.

Direct shoot development was observed on MS medium supplemented with BAP (1mg/l) and NAA (0.5mg/l) (Fig.1). The shoots multiplication started after 4 weeks of shoot tip culture, whereas in hormones combinations (BAP and NAA in 0.5 and 0.5 mg/l; 1.5 and 0.5 mg/l; 2.0 and 0.5 mg/l) shoot multiplied without root formation also after 6, 7 and 8 weeks respectively. The shoot development through protocorms was comparatively poor when compared to direct shoot development from shoot tip explants. The shoot tip explants showed the capacity to give rise to callus, protocorm like bodies, pseudobulb as well as direct shoot.

The enhancement in multiple shoot formation was also observed in various orchids when grown in MS medium supplemented with high concentration of BAP and low concentration of NAA (Chung et. al. 1998 in *Cymbidium forresti* and *C. kanran*; Shrestha and Rajbhandari 1988 in *Cymbidium giganteum* Wall. ex Lindl.; Basker and Bai 2006 in *Coelogyne stricta* (D.Don) Schltr; Swar and Pant 2004 in *Coelogyne cristata* Lindl; Pradhan and Pant 2008 in *Dendrobium densiflorum* Lindl.; Koirala 2007 in *Coelogyne fuscescens* Lindl.; Pongener and Deb 2009 in *Cymbidium iridioides* D.Don., Rajkarnikar 2011 in *Cymbidium aloifolium* (L.) Sw., Pant and Thapa 2012 in *Dendrobium primulinum* Lindl.).

Poor shoot multiplications were observed in media without hormone and with only NAA in comparison to media supplemented with BAP. MS media with high concentration of BAP (1mg/l) and low concentration of NAA (0.5 mg/l) was found most favorable for induction of shoot multiplication directly.

The shoots regenerated were tested for rooting in MS media with different concentration of auxin (0.5mg/l – 2mg/l) such as IAA, IBA and NAA. Microshoots of an average height of 3-5mm were taken as initial explants for rooting.

Root development was observed in all media with IAA, IBA and NAA ranging from 0.5 mg/l – 2mg/l,

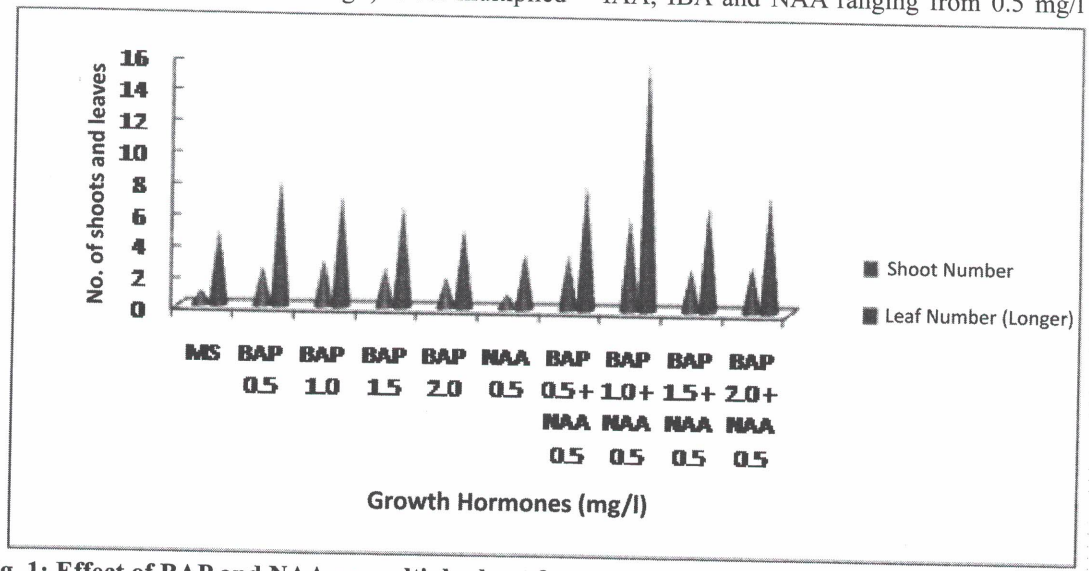

Fig. 1: Effect of BAP and NAA on multiple shoot formation and average number of leaves after 16 weeks of culture of *Cymbidium elegans* Lindl. Culture conditions: MS medium, 25 ± 2°C, 16 weeks of primary culture, 4 replicates were used in each combination

Table 1: Effect of different auxins on rooting of shoot tips of *C. elegans* Lindl.

Parameters	Concentration of different auxin hormones (mg/l)											
	IAA				IBA				NAA			
	0.5	1	1.5	2	0.5	1	1.5	2	0.5	1	1.5	2
Number of Roots (mean± SD)	1.5 ± 0.57	1.75 ± 0.95	1.5 ± 0.57	1.75 ± 0.5	3.50 ± 0.95	2.25 ± 0.57	1.75 ± 0.81	1.5 ± 0.57	2.25 ± 0.50	1.25 ± 0.50	1.5 ± 0.57	1.25 ± 0.50
Length of Roots (mean± SD)	1.31 ± 0.23	2.2 ± 0.47	2.53 ± 0.59	1.93 ± 0.42	2.73 ± 0.66	2.52 ± 0.16	2.26 ± 0.65	1.88 ± 1.03	2.12 ± 0.64	1.37 ± 0.47	0.42 ± 0.08	0.77 ± 0.24

Culture condition: - MS medium 25±20c, 12 weeks, 4 replicates were used in each combination.

MS media supplemented with IBA was the most favorable rooting condition. Rooting in this condition started after 4 weeks of culture and the roots were aerial, strong, hairy and greenish in colour. The average number of root ranged from 1.5 - 3.5 per culture and length of root ranged from 2.1-3.5cm after 12 weeks of culture (Table 1). The results are in consistent with the reports of Pant and Swar 2004, Pradhan and Pant 2008, Koirala 2007 where highest number of roots was obtained on MS medium supplemented with IBA on *Cymbidium iridiodoids* & *Coelogyne cristata*, *Dendrobium densiflorum* and *Coelogyne fuscescens* respectively.

MS medium supplemented with different concentrations of NAA showed poor result on growth of root in comparison to IBA & IAA. It took 7 weeks of culture to initiate the roots. The roots were small, thin and green in color. Rooting in other media started later and produced weaker roots. About 85% of plantlets survived when the *In vitro* grown plantlets transferred to the earthen pots with epiphytic medium containing. This result suggested that the potting mixture (coco peat and sphagnum moss in 2:1 ratio) is efficient for providing suitable nutrient environment to this orchid species in order to adjust in nature.

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