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Formation of Multiple Shoots and Regenerated Plantlets by Culture of Pseudobulb Segment in Nobile Type Dendrobium

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Dendrobium is a genus having numerous species including one of the most popular orchids such as the nobile type used in this study. Although shoot tip culture is one of the most popular methods to propagate orchids, it has some problems to be solved such as seasonal limitation, sacrifice of new shoots, being technically difficult and not being possible in some orchids.

Hence, explants from many other parts of the plant, such as flower stalk[1], root[2] and leaf[3] have been utilized. Axillary buds excised from pseudobulbs of *Cattleya* (Scully, 1967)[4] and from bulbs of *Dendrobium phalaenopsis* (Kim *et al.*, 1970)[5] were cultured in liquid media. When pseudobulb segments of *Dendrobium crysanthum* were cultured on MS media, only a single shoot was obtained from each node (Vij *et al.*, 1989)[6].

The purpose of this study is to show the possibility of culturing pseudobulb segments in nobile type *Dendrobium* Malones 'Hope' in order to induce multiple shoots and regenerate plantlets.

Pseudobulbs (about $\phi 2.5 \times 50$ mm) of *Dendrobium* Malones 'Hope' were excised from the meri-clone plantlets grown *in vitro* for one year, and cut into two parts: the upper (including 2nd node) and the lower (including 4th node) parts. After removing leaves, pseuobulbs of each plant were cut into 5 mm-long segments, each with one node. To examine the effects of growth regulators on the shoot and root formation, each pseudobulb segment was cultured in a scintillation vial ($\phi 25 \times 50$ mm) containing the basal MS medium supplemented with 3% sucrose, 3 g/*l* gellan gum at pH 5.8, and a combination of NAA and BA at 0 and 0.1 mg/*l* levels (4 different kinds of media in total) under fluorescent light with a 12 hour-photoperiod (2500 lux) at 23°C. After 8 weeks of culture, each segment was transferred into a 350 m*l* glass bottle containing the same medium for each treatment. In the present study, each treatment consisted of 4 replicates, and each replicate consisted of 5 pseudobulb segments. The axillary bud of psedobulb segment began to develop after 1 week of culture. The axillary buds did not develop into a protocorm like body (PLB) but directly into shoots. After 4 weeks of culture, shoots and roots were observed. The number of shoots and roots developing from one explant of pseudobulb segment after 8 weeks of culture are shown in **Table 1**. The number of shoots depended on the plant growth regulators added to the medium. This fact was observed in cultures of both the upper and the lower segments, and was also confirmed statistically (**Table 1**).

The greatest number of shoots were induced on a medium containing 0.1 mg/*l* NAA and 0.1 mg/*l* BA in both parts of segment culture. Moreover, the maximum multiple shoots (5 shoots) were induced from a single upper segment of pseudobulb on the same medium after 8 weeks. Multiple shoots were observed in all treatments except the controls (without plant growth regulators) in cultures of both the upper and the lower segments.

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Table 1. Effect of NAA and BA combinations on shoot and root formations from the upper and lower segments of pseudobulb after 8 weeks of culture.

NAA (mg/*l*)	BA (mg/*l*)	Number*1 of shoots±sd*5	Growth of shoot in length (mm) *2	Number of roots*3	Growth of root in length (mm) *4
Culture of the upper segment of pseudobulb					
0	0	0.3±0.4^{a}*6	8.3±14.3	4.8±1.3^{a}	10.8±0.6
0.1	0	1.5±0.6^{a}	26.1± 1.8	8.8±1.1^{b}	4.4±1.6
0	0.1	1.8±0.8^{a}	20.7± 9.7	8.7±0.5^{b}	8.9±1.6
0.1	0.1	2.5±0.7^{b}	20.6± 9.5	5.8±1.7^{a}	7.3±3.7
Culture of the lower segment of pseudobulb					
0	0	1.6±0.5^{ab}*6	29.7± 6.6	6.0±3.5^{a}	8.2±3.5
0.1	0	1.7±0.8^{bc}	33.9± 4.4	6.3±2.1^{a}	7.7±2.0
0	0.1	1.2±0.4^{a}	28.9± 9.2	11.3±3.8^{a}	6.6±2.5
0.1	0.1	2.3±0.4^{c}	26.3±10.9	9.0±5.3^{a}	5.3±3.9

Each treatment consisted of 4 replicates (A replicate consisted of 5 segments).

*1 Mean number of shoots induced from a single pseudobulb segment.

*2 Mean growth in length of shoot induced from a single pseudobulb segment.

*3 Mean number of roots induced from a single pseudobulb segment.

*4 Mean growth in length of root induced from a single pseudobulb segment.

*5 sd; standard deviation.

*6 Values within columns followed by different letters are significantly different by Duncan's multiple range test at the 5% level.

Multiple shoots were not obtained in either the culture of a shoot with detached leaves in *Den. antennathum*[7] or in the culture of pseudobulb segment in *Den. chrysanthum*[6]. Peptone was required to induce shoots in *Den. antennathum*[7], and yeast extract or urea should be supplemented into the media to induce shoots in *Den. chrysanthum*[6]. BA promoted the development of shoots from an axillary bud, but NAA+IBA delimited it in the culture of a shoot with detached leaves in *Den. antennathum*[7]. If the medium contained yeast extract, kinetin promoted shoot formation through callusing in the culture of pseudobulb segment of *Den. crysanthum*[6]. In the present study, organic nutrients were not required to induce shoots in *Den.* Malones 'Hope' although a combination of NAA and BA promoted shoot formation from a pseudobulb segment (**Table 1**). Therefore, the degree of apical dominance might not be so intense that axillary buds could sprout only by cutting a pseudobulb into segments in *Den.* Malones 'Hope'.

Shoots and roots began to be formed from the same single segment of pseudobulb in all treatments including control within 4 weeks. Transfer of explants onto the media containing auxins was required to induce roots in *Den. antennathum* and in *Den. crysanthum*, but not required in this study. Hence, the variation, caused by culturing *in vitro* and using plant growth regulators, might be reduced to the minimum. After 6 months 543 plantlets proliferated through this culture of pseudobulb segments. Of these 9 exhibited morphological variation. Those variations were observed in two treatments: control (without any plant growth regulator) and 0.1 mg/*l* NAA.

The maximum number of plantlets per single segment of pseudobulb obtained after 6 months of culture was 37.3 in the treatment of 1 mg/*l* NAA+1 mg/*l* BA. Various stages of plantlet regeneration from pseudobulb segments after 8 weeks of cullture were shown in **Fig. 1 (A~D)**.

Consequently, this research confirms that the culture of pseudobulb segment is a very simple and suitable method for propagation in *Dendrobium* Malones 'Hope' in order to get multiple shoots and plantlets *in vitro*.

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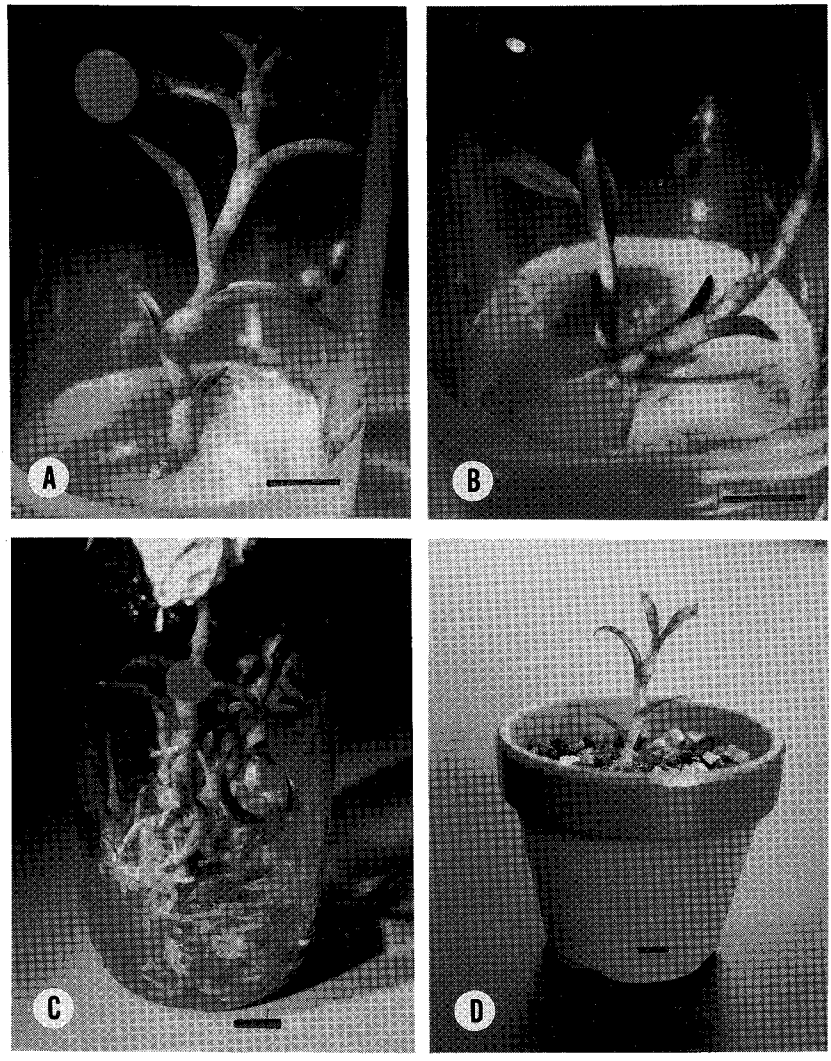

Fig. 1 Regenerated shoots and plantlets from pseudobulb segments after 8 weeks of culture at 23°C under 12L/12D condition.
A: A shoot regenerated from the upper segment of pseudobulb, which was cultured on a control medium(without any plant growth regulator).
B: Two shoots with roots regenerated from the lower segment cultured on a medium supplemented with 0.1 mg/*l* BA.
C: Multiple shoots and roots regenerated from the upper segment cultured on a medium supplemented with 0.1 mg/*l* NAA and 0.1 mg/*l* BA.
D: An acclimatized plantlet regenerated from the upper segment cultured on a medium supplemented with 0.1 mg/*l* NAA and 0.1 mg/*l* BA. Bars in all pictures indicate 1 cm.

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