Commercial Propagation of *Dendrobium pulchellum*

Yin Yin Sint[1], Myat Myat Moe[2]

Abstract

Dendrobium pulchellum Roxb.ex Lindl. (Sin-ma-myet-kwin) is one of the flowering plants belongs to the family Orchidaceae. It is cultivated for its ornamental and medicinal purposes. Immature seeds of Dendrobium pulchellum Roxb.ex Lindl were cultured on Vacin and Went (1949) medium. NAA, IAA, banana and coconut water were supplement to basal medium. Of these 0.1mg/l NAA resulted in the highest growth plantlet. Two year old plantlets raised from *in vitro* culture were planted in community pots to acclimatize in nursery. Then, plantlets were transplanted to thumb pots and finally repotted to individual pots. Both in community pots and thumb pots, three types of potting media (tree bark, charcoal husk) were used. Among these, coconut husk gave the best result. Thus, it was chosen as potting medium in individual pot for further work.

Key words: *Dendrobium pulchellum*, IAA, NAA, coconut water

Introduction

Being attractive beauty and distinguished structure, orchids are popular around the world. Orchidaceae is the largest family of the flowering plant. There are about 660 genera and 25,000 species of orchids in the world (Teoh, 1985). In Myanmar, there may be 84 genera and about 600 species (Grant, 1966). It exhibits a vast diversity in vegetative and floral characteristics and is of considerable interest due to its broad geographic distribution and high value of hybrids as a floricultural commodity (Hawkes, 1970; Jones *et al.*, 1988). Among them, *Dendrobium* is the second largest genus of the family Orchidaceae. *Dendrobium* are widely distributed throughout the Asia and South Pacific, tropics and subtropics, from low and warm regions in Northern Australia, Thailand and Himalayan mountains (Seidenfaden, 1992). In Myanmar, the majority of native *Dendrobium* grows in Southern Shan State and Mandalay Division (Seidenfaden, 1992). *Dendrobium pulchellum*, is widely distributed from the Himalayas to Myanmar, Thailand, Indochina and Malaysia. The pseudobulds are thick and erect. The leaf sheaths are characteristically striped with purple. Flowering period is from March to April, pendulous inflorescences are produced on the apical portion of mature leafless stems. Orchids are well known for being major trade plants in developed countries (Sagawa & Kunisaki, 1984). *In vitro* culture has proved particularly useful with groups of plants, which are difficult to propagated using conventional techniques (Fay, 1994). When mass propagation of a new hybrid or varieties is needed with a short time, tissue culture is the only method (Goh *et al*., 1992). In the present work, *in vitro* propagation by using green pod culture was applied and suitable media was investigated for the growth and development. And then, the seedlings were also cultured in selected media supplement with the various concentrations of growth promoting substance (undefined additives). Growth regulators (growth hormones or phytohormones) are generally used in the culture medium to improve and regulate growth development of tissues and organs in culture. They can promote cell expansion and division, growth of tissue and regulate morphogenesis. Growth regulators widely used in culture are auxins and cytokines.

[1]Lecturer, Dr, Botany Department, Dagon University

[2]Professor ,Dr, Head of the Botany Department, Dagon University

The micropropagates have poorly developed cuticle, stomata apparatus, photosynthetic ability and conducting tissues, and they fail to withstand direct exposure to harsher climates outside the *in vitro* regimes until or unless they are properly acclimatized prior to transplanting (Hegde, 2001). The collection of wild *Dendrobium* continues at levels ranging from hobbyist to large-scale illegal trade. Satisfying the interest of the hobbyist and demand by the traders through large-scale micropropagation is one of the preferable options to prevent illegal collection from wild (Sunitibala & Rajikumar, 2009). Orchid stalks can be used in the treatment of liver, blood and cancer diseases. It was used as treat night sweats, fortify a person's body, to cure kidneys and impotent, antipyretic, tonic and peptic in Taiwan (Yen, 1980). Thus, this kind of orchid is being used for the benefit of people as medicinal purpose.

Material and Methods

Plant material

The green pod (capsules) of *Dendrobium pulchellum* Roxb. ex Lindl. were collected from Myanmar Agriculture Service. The specimens were collected during the flowering and fruiting periods from the months of March to May.

Culture medium

Vacin and Went (1949) medium was used for the initial culture of seed and a modified Vacin and Went (1949) medium was used for the propagation of protocorms. The pH of all media was adjusted to 4.8 ±5.2 before autoclaving. Then, the media were autoclaved at 121°C for 15 min.

Culture condition

Cultures were kept at a temperature of 24° to 28°C for 24 hours under illumination with 4 feet fluorescent tube which yielded an intensity of approximately 2000 lux.

Procedure

Mature green capsules were harvested and washed with detergent liquid under running tap water. After that, they were dipped in 70% ethanol and for a few seconds followed by sodium hypochlorite solution for 20 min, then flamed rapidly in a laminar flow cabinet. Then the surface of sterilized capsules were opened by sterile knife and young mature embryos were inoculated into modified Vacin and Went medium (Arditti, 1979). Reflasking or subculture was carried out every 4 or 5 months.

Measurement and Recording of Growth

Growth of plantlets was measured by recording fresh weight and dry weight, number of PLBs, shoots and plantlets.

Growth value

The growth value is the ratio of final fresh weight of PLBs to initial fresh weight of inoculum.

Initial fresh weight of inoculated 39 ± 3 PLBs was 215 ± 5 mg that is 21 ± 1 mg dry weights per flask.

Deflasking

To pull out plantlets from the flask, cover was removed and small amount of water was poured into the flask. Then the flask was shaken to dislodge the agar. After that the plantlets were taken out by bent wire.

Potting of plant

Clay pots having 15 cm in diameter x 5 cm in depth were used. The bottom (1/3) of the pot was filled with coarse chips of charcoal (1-1.5 cm^3). Coconut husk was cut into 1 cm long fiber and filled in the middle (1/3) of the pot. Then, plantlets were planted on the potted medium.

$$\text{Survival rate (\%) of platntlets} = \frac{\text{Total number of survive plants}}{\text{Total number of cultivated plants}} \text{ x } 100$$

Table. 1. Composition of Vacin and Went medium (1949)

Component	Amount
Tricalcium phosphate, $Ca_3(PO_4)$	200 mg/l
Potassium nitrate, KNO_3	525 mg/l
Potassium phosphate, KH_2PO_4	250 mg/l
Magnesium sulfate, $MgSO_4.7H_2O$	250 mg/l
Ammonium sulfate, $(NH_4)_2SO_4$	500 mg/l
Ferric tartrate, $Fe_2(C_4H_4O_6)_3$	28 mg/l
Manganese sulfate, $MnSO_4.4H_2O$	7.5 mg/l
Sucrose	20 mg/l

Results

Botanical Studies

Scientific name - ***Dendrobium pulchellum* Roxb.ex Lindl**.
Vernacular name - Sinma-myet-kwin

Outstanding Features

Habit - Sympodial epiphyte
Stem - Erect, cylindrical, oblong
Leaves - Elliptic-oblong, the tips acuminate, the margins entire, both surfaces glabrous
Inflorescences - Axillary racemes, several flowers pendulous
Flowers - Pink with reddish purple, scented and showy
Flowering period - March to May

Fig.(1) Habit

Propagation

Mature seeds of *Dendrobium pulchellum* Roxb. ex Lindl. was sown in Vacin and Went (1949) medium. One month after sowing the seeds (embryos) turned green then developed into protocorms. After five months, *Dendrobium pulchellum* seedlings were transplanted in Vacin and Went (1949) medium supplemented with different concentration of IAA, NAA, and banana and coconut water. Two months after transplanting, the growth of seedlings was measured for fresh weight, dry weight, number of PLBs, shoots and plantlets.

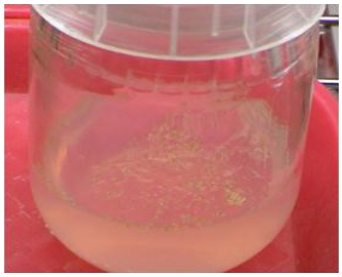


Fig.(2-a) One month after sowing the seeds (embryo) turned green

Fig.(2-b) developed into protocorms

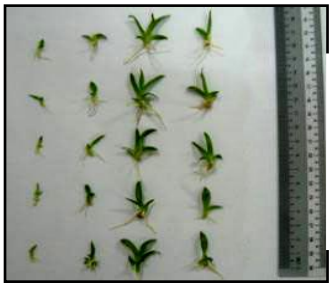
Fig.(2-c) 4 months-old culture

The effect of NAA

In this experiment, 0.01 mg/l, 0.1 mg/l and 1.0 mg/l of NAA were added to the control medium (VW). Control medium (VW) with 0.01 mg/l NAA yielded 1777 mg fresh weight, 113 mg dry weight, 6.39 % of dry weight per unit fresh weight, a growth value of 8.27 and 306 PLBs. Control medium (VW) with 0.1 mg/l NAA yielded 2126 mg fresh weight, 123 mg dry weight, 5.79% of dry weight per unit fresh weight , a growth value of 9.89 and 365 PLBs. Control medium (VW) with 1.0 mg/l NAA yielded 1482 mg fresh weight, 103 mg dry weight, 6.95% of dry weight per unit fresh weight , a growth value of 6.89 and 246 PLBs.

Table. 2. Yields of PLBs on the addition of various levels of NAA

Growth	NAA levels			
	Control	**0.01 mg/l**	**0.1 mg/l**	**1.0 mg/l**
Fresh weight, mg/flask	705	1777	2126	1482
Percentage of yield increase in fresh weight	-	152.06	201.56	103.12
Dry weight, mg/flask	55	113	123	103
Percentage of dry weight per unit fresh weight	7.80	6.39	5.79	6.95
Growth value	3.28	8.27	9.89	6.89
Number of PLBs	120	306	365	246
Number of shoots	18	30	35	35
Number of plantlets	-	-	-	-



a-control
b-0.01mg/l
c-0.1mg/l
d-1.0mg/l

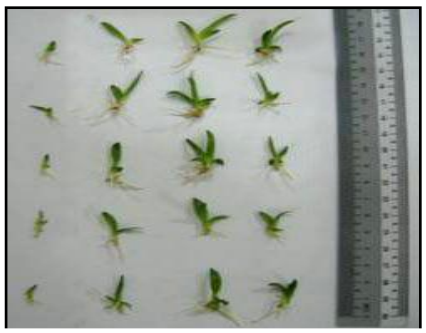
Fig. (3) Germination of plantlets on various level of NAA treatments (a) (b) (c) (d)

The effect of IAA

In this experiment, 0.01 mg/l, 0.1 mg/l and 1.0 mg/l of IAA were added to the control medium (VW). Control medium (VW) with 0.01 mg/l IAA yielded 1346 mg fresh weight, 98 mg dry weight, 7.28% of dry weight per unit fresh weight, a growth value of 6.26 and 235 PLBs. Control medium (VW) with 0.1 mg/l IAA yielded 1866 mg fresh weight, 114 mg dry weight, 6.11% of dry weight per unit fresh weight, a growth value of 8.68 and 221 PLBs. Control medium (VW) with 1.0 mg/l IAA yielded 1434 mg fresh weight, 103 mg dry weight, 7.18% of dry weight per unit fresh weight, a growth value of 6.67 and 249 PLBs.

Table. 3. Yields of PLBs on the addition of various levels of IAA

Growth parameter	IAA levels			
	Control	0.01 mg/l	0.1 mg/l	1.0 mg/l
Fresh weight, mg/flask	705	1346	1866	1434
Percentage of yield increase in fresh weight	-	90.92	164.68	103.4
Dry weight, mg/flask	55	98	114	103
Percentage of dry weight per unit fresh weight	7.80	7.28	6.11	7.18
Growth value	3.28	6.26	8.68	6.67
Number of PLBs	120	235	221	249
Number of shoots	18	35	30	30
Number of plantlets	-	-	-	-



a-control
b-0.01mg/l
c-0.1mg/l
d-1.0mg/l

(a) (b) (c) (d)

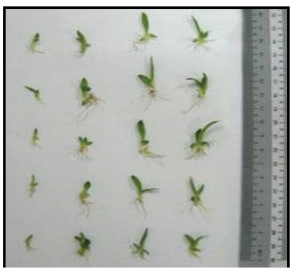
Fig. (5) Germination of plantlets on various level of IAA treatments

The effect of coconut water

In this experiment 50 ml/l, 100 ml/l and 200 ml/l of coconut water were added to the control medium (VW). Control medium (VW) with 50 ml/l coconut water yielded 858 mg fresh weight, 73 mg dry weight, 8.51% of dry weight per unit fresh weight, a growth value of 3.99 and 151 PLBs. Plantlets and roots were not observed. Control medium (VW) with 100 ml/l coconut water yielded 997 mg fresh weight, 81 mg dry weight, 8.12% of dry weight per unit fresh weight, a growth value of 4.64 and 176 PLBs. Each replicate consisted of 2-5 plantlets with 2-6 leaves and 1-2 roots. The leaves were 5-15 mm long and 2-4 mm wide and the roots were 3-10 mm long. Control medium (VW) with 200 ml/l coconut water yielded 890 mg fresh weight, 75 mg dry weight, 8.43% of dry weight per unit fresh weight, a growth value of 4.14 and 150 PLBs. Each replicate consisted of 3-6 plantlets with 2-6 leaves and 1-3 roots. The leaves were 5-15 mm long and 2-4 mm wide and the roots were 3-10 mm long.

Table. 4. Yields of PLBs on the addition of various levels of coconut water

Growth parameter	Coconut water levels			
	Control	50 ml/l	100 ml/l	200 ml/l
Fresh weight, mg/flask	705	858	997	890
Percentage of yield increase in fresh weight	-	21.70	41.42	26.24
Dry weight, mg/flask	55	73	81	75
Percentage of dry weight per unit fresh weight	7.80	8.51	8.12	8.43
Growth value	3.28	3.99	4.64	4.14
Number of plbs	120	151	176	150
Number of shoots	18	35	40	40
Number of plantlets	-	-	2-5	3-6



a-control
b-50 m/l
c-100 m/l
d-200 m/l

Fig. (6) Germination of plantlets on various level of coconut water

(a) (b) (c) (d)

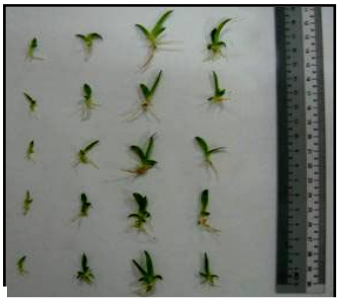
The effect of banana

In this experiment, 25g/l, 50 g/l and 100 g/l banana pulp were added to the control medium (VW). Control medium (VW) with 25 g/l banana yielded 1049 mg fresh weight, 83 mg dry weight, 7.91% of dry weight per unit fresh weight, a growth value of 4.88 and 185 PLBs. Control medium (VW) with 50 g/l banana yielded 1151 mg fresh weight, 87 mg dry weight, 7.56% of dry weight per unit fresh weight, a growth value of 5.35 and 195 PLBs. Each replicate consisted of 4-6 plantlets with 2-5 leaves and 2-4 roots. The leaves were 5-15 mm long and 2.0-4.0 mm wide and the roots were 3-10 mm long. Control medium (VW) with 100 mg/l banana yield 1019 mg fresh weight, 82 mg dry weight, 8.05% of dry weight per unit fresh weight, a

growth value of 4.75 and 180 PLBs. Each replicate consisted of 2-5 plantlets with 2-5 leaves and 1-3 roots. The leaves were 5-15 mm long and 2-4 mm wide and the roots were 3-10 mm long.

Table.5. Yields of PLBs on the addition of various levels of banana

Growth parameter	Banana pulp levels			
	Control	**25 g/l**	**50 g/l**	**100 g/l**
Fresh weight, mg/flask	705	1049	1151	1019
Percentage of yield increase in fresh weight	-	48.79	63.26	44.54
Dry weight, mg/flask	55	83	87	82
Percentage of dry weight per unit fresh weight	7.80	7.91	7.56	8.05
Growth value	3.28	4.88	5.35	4.75
Number of PLBs	120	185	195	180
Number of shoots	35	35	40	40
Number of plantlets	-	-	4-6	2-5



a-control
b-25 g/l
c-50 g/l
d-100 g/l

Fig. (6) Germination of plantlets on various level of bannan

(a) (b) (c) (d)

Table (6) Survival rate (%) of *Dendrobium pulchellum* plantlet in community pot.

Treatment	Percentage of survival rate in each month				
	1	2	3	4	5
Tree barks	68.00	88.23	94.44	99.41	99.42
Coconut husk	86.66	92.30	98.33	99.57	100.00
Charcoal	65.33	91.83	96.66	97.70	99.41

Initial plantlet = 150 plantlet per pot

Table (7) Survival rate (%) of *Dendrobium pulchellum* plantlet in thumb pot.

Treatment	Percentage of survival rate in each month				
	1	2	3	4	5
Tree barks	88.00	89.00	94.11	96.87	96.77
Coconut husk	89.13	92.00	97.56	97.50	100.00
Charcoal	72.00	83.33	90.00	96.29	96.30

Initial plantlet = 25 plantlet were arranged

Discussion and Conclusion

In the present research, small plantlets of *Dendrobium pulchellum* Roxb. ex Lindl. were cultured on basal Vacin and Went medium supplemented with various concentration of plant growth hormones such as NAA and IAA for more PLBs produced and undefined substances coconut water and banana for germination. The growth value was found to increase gradually at different concentaration of NAA, where the highest growth value was obtained from 0.1mg/l NAA and followed by 0.01mg/l and 1.0mg/l (Table-2). NAA is synthetic plant hormone in the auxin family and is an ingredient in many commercial plant rooting horticultural products. The results from these experiments agree with those of some investigators. Kano (1965) reported that 0.1 mg/l NAA gave germination and seedling growth in *Dendrobium.* The addition of undefined substances 100 ml/L of coconut water was promoted rapid germination (Table 4). Coconut water is rich in potassium, hormones (auxins and cytokinins), various minerals and antioxidants which alone is totally responsible for growth promoting qualities (Molnar *et al*., 2011). After deflasking two years old plantlets were planted in community pot and thumb pot, containing mixture of moss ,charcoal chips and dry coconut husk powder. In the present s study, three kinds of potting media use in community pot to grow plantlets of *Dendrobium pulchellum.* Among the potting media, coconut husk enhanced the relatively high percentage of survival rate. The result showed that finding agrees with other workers Teoh (1995) reported that coconut husk is maintaining humidity around the plantlets. From this study, it was observed in thumb pot stage. Tree bark, coconut husk and charcoal are used. Among of them, coconut husk was yielded relatively higher percentage of survival rate, it is a fact that coconut husk has more ability of maintaining humidity which is essential for growing of orchid than charcoal and tree barks. *Dendrobium pulchellum* should be grown and propagated by using tissue culture technique in Myanmar for the benefit of getting numerous amount of plants within a short period. In future research programs, Sin-ma-myet-kwin plant may be propagated by tissue culture (*in vitro*) and keiki (*in vivo*) for large amounts of plants and the production of pure compounds. In addition to the above results, there are numerous recorded of remarkable used to bioactive effect on human health as antioxidant activity, anti-spasmodic and anti-inflammatory activity. So it should be very interesting for further research and which should be implemented.

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