STUDY ON THE MICROPROPAGATION AND HARDENING PROCESS OF *DENDROBIUM CARINIFERUM* RCHB.F.

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Abstract

In vilro culture of *Dendrobiumcariiniferum*Rchb.f. (Mahadawai) was carried out in Tissue Culture Laboratory, University of Yangon. Plant specimens were collected from Taungyi Township, Southern Shan State. The collected plants containing flowers and small pods were grown in Botanical garden, University of Yangon. The fruits were mature at four months after pod setting. The seeds from the collected pods were cultured on Vacin and Went (1949)media. Six months old plantlets from seed culture were used in continuous ***in vitro*** culture. The control treatment was used as the basal medium and other treatments were supplemented with different concentration of Vitamin B_1, B_2, B_{12}& Folic acid. The result of the experiments showed that T_{15} (folic acid 1.0 mgl^{-1}) treated plant possessedsuperior growth 17 numbers of plantlets, 1.4cm in length, 4.0 numbers of leaves with the length of 0.6cm and 3.2numbers roots. The plants were maintained in *in vitro* culture for 12 months. Then these plants were acclimatized inthe field. The first stage of hardening, different potting media were tested. Among potting media, T_4 (2 ch :2 br :1 lm:1 coh) produced number of plantlets growth. Thumb pots were used in the second hardening process. Each plantletwas grown in a thumb pot. Seven additives were used to treat the plantlets of thumb pot as treatments. Among themT_4 (NPK, 2O: 20: 20) produced the best plantlets growth.

Key words: Micropropagation and hardening process

Introduction

One of the popular orchids, *Dendrobiurn cariniferum* belongs to the family Orchidaceae. *Dendrobium* is the second largest genus of the family Orchidaceae .*Dendrobium* are widely distributed throughout the Asia and South Pacific, tropics andsubtropics, from low and warm regions in Northern Australia, Papua New Guinea toThailand and Himalayan mountains. In Myanmar, the majority of native *Dendrobium* grows in Southern Shan State and Mandalay Division (Seidenfaden, 1992).In the

early 1900's, Bernard, Burgeff andKnudson introduced the methods of culture that are used for growing orchid seedlingsin large quantities by germination of seed on nutrient media under aseptic conditions.(VitaminB_1) is essential and riboflavin(VitaminB_2) is active in metabolism metabolism especially cellular respiration. Folic acid showed coenzymes- activity (Kyte&Kleyn, 1999). Vitamin B_{12} was beneficial in establishing tissue and cell culture (Reinert& White, 1956). The plantlets were established under natural conditions using simple pottingmediasuch as dried roots and leaves of water hyacinth and *Salvinia*, charcoal, brick chips, sand, bark powder, dried rice straw, fern roots, mosses, vermiculite, farm yardmanure (FYM) and coconut husks etc. (Roy &Sarma, 1999 and Baruah, 1996). Epiphytesrequire an open, porous medium for the best root action (Edward, 1946).The bestpotting medium are brick chips, charcoal, leaf mould, coconut husk (2:2: 1: 1) (Hazarika & Sarma, 1995). are toinvestigate the proper medium for hardening plantlets of *Dendrobium cariniferum*. The aim of this study are to multiplicate of the *Dendrobium cariiniferum* in *in vitro* propagation, to grow the micropropagated plants in the natural environmentandto find out the suitable fertilizer for acclimatization as well as field condition of *Dendrobiumcariniferum*.

Materials and Methods

Collection, Identification and Inoculation

The plants were collected from Taungyi Township, Southern Shan State. Thecollected plantscontaining flowers and small pods were grown in green house of Botanical garden, Botany Department, University of Yangon.The plant identification was made by Holttum (1964), Davis (1966) and Grand (1966). Culture media was basal Vacin and Went (1949) (VW) media. The media were autoclaved at 1.5 kg cm^{-2} and 121ºC for 15 minutes.The temperature of the culture room was ranged from 23 - 25 ºC and 50% of relative humidity. Four feet white fluorescent tubes were equipped on the shelves for the light source. Seed culture process was done inside the laminar flow cabined. Obtained the seed culture plantlets were maintained on basal medium for 12 week in culture room. The seed culture were followed the method of Arditti (1967).

Experiment (1) The effect of different kinds of vitamin and folic acid on growth of plantlets

Approximately equal size of *Dendrobiumcariniferum* were selectedfrom basal medium of previous culture. Then five plantlets were cultured in a containing basal medium with different supplements (Vitamin B_1, B_2, B_{12} and folic acid). Different concentrations of supplements (0.1, 0.5, 1.0 and 1.5 mgl^{-1}) were used.

Fig. 1 Flower of *Dendrobiumcariniferum*

Fig. 2Source of stock plantlets

Experiment (2) Hardening of plantlets in baskets

The media preparation for acclimatization were followed the methods of Sarmaand Kalita (2001) and Sarma and Kaur (2001). The composition of preparation media were T_1 (1 charcoal: 1 brick chips; 3 sand; 2 leaf mould), T_2(2 charcoal: 2 brick chips; 1 dried water hyacinths), T_3 (3 charcoal: 2 brick chips: 2 sand) and T_4 (2 charcoal: 2 brick chips: 1 leaf moulds: 1 coconut husk). Healthy and strong twelve months old selected plantlets were grown in the prepared media. They contained two and more green leave and two stout roots. Then they were surface sterilized with $2mgl^{-1}$Orthocide fungicide solution for 2 minutes.

Experiment (3) Transplant the partial acclimatized plantlets into thumb pots (3cm in diameter)

Two cubic centimeter-sized coconut husks were cut and these cubes were rinsed intap water. The water was decanted in everyday until to obtain the clear water. A plantlet was grown in one pot. The fertilized solutions (T_0 - Control (pure water), T_1- Pigeon dung (1g 1^{-1}), T_2- Cow dung (1g 1^{-1}), T_3- Fish emulsion (10ml 1^{-1}), T_4- NPK_1 20:20:20 (10ml 1^{-1}), T_5- NPK_2 21:21:21 (10ml 1^{-1}) and T_6- NPK_3 30:20:20 (10ml 1^{-1}) were used in this stage. The pots weremaintained under the shade. They were sprayed with water twice in a day to preventdry condition of

media. The plants were sprayed fertilized solutions once in a week. The procedure was followed by Rao and Hedge (2001).

Results

Experiment (1) The effect of different kinds of vitamin and folic acid on growth of plantlets

The results of the experiment showed that among different concentrations of vitamin and folic acid treatments, T_{15} (folic acid 1.0 mg l^{-1}) treated plants possessed superior growth 17.0 numbers of plants, 1.4 cm in length, 4.0 number of leaves with the length of 0.6 cm and 3.2 number of roots. All of these treatments showed new plantlets formation bat the number were different. Some had plant like bodies formation at 0.25 mm in diameter.The statistical results showed that growth of *Dendrobiurncariniferum*in VW medium supplemented with 1.0 mg l^{-1} folic acid was highest in plant growth and 0.1 mg l^{-1} vitamin B_1 was the least. However, the platelets growth from vitamin B_1 media was superior to the control (Table 1a andb).

Table 1(a)Growth results of different kinds of vitamin and folic acid

Treatments	Fresh weight (mg)*	Dry weight (mg)*	Percentageof Dry weight/ Fresh weight	Growth Value
T_0 (Control)	67.1	3.9	5.9	1.5
T_1 (B_1 0.1 $mg1^{-1}$)	43.1	3.3	7.5	1.0
T_2 (B_1 0.5 $mg1^{-1}$)	15.7	1.3	8.3	0.4
T_3 (B_1 1.0 $mg1^{-1}$)	95.6	8.2	8.5	2.1
T_4 (B_1 1.5 $mg1^{-1}$)	26.5	1.9	7.2	0.6
T_5 (B_2 0.1 $mg1^{-1}$)	92.0	8.4	9.1	2.1
T_6 (B_2 0.5 $mg1^{-1}$)	88.0	7.8	8.9	2.0
T_7 (B_2 1.0 $mg1^{-1}$)	110.0	9.2	8.4	2.5
T_8 (B_2 1.5 $mg1^{-1}$)	80.4	7.0	8.7	1.8
T_9 (B_{12} 0.1 $mg1^{-1}$)	63.7	5.4	8.5	1.4
T_{10} (B_{12} 0.5 $mg1^{-1}$)	69.6	6.0	8.6	1.6
T_{11} (B_{12} 1.0 $mg1^{-1}$)	60.6	5.1	8.4	1.4
T_{12} (B_{12} 1.5 $mg1^{-1}$)	56.1	4.7	8.2	1.3
T_{13} (Folic acid 0.1 $mg1^{-1}$)	140.1	13.6	9.7	3.1
T_{14} (Folic acid 0.1 $mg1^{-1}$)	150.8	14.8	9.8	3.4
T_{15} (Folic acid 0.1 $mg1^{-1}$)	176.9	16.5	9.3	4.0
T_{16} (Folic acid 0.1 $mg1^{-1}$)	160.3	15.1	9.5	3.6

* Data presented in the table the mean value of 5 replicates

Table 1(b)Growth results of different kinds of vitamin and folic acid

Treatments	No. of Plantlet*	No. of Leaf per Plantlet*	No. of Root per Plantlet	Length of Plantlet (cm)*	Length of Root (cm)*
T_0 (Control)	6.8	1.8	1.8	0.7	0.3
T_1 (B_1 0.1 mgl^{-1})	8.2	2.4	2.4	0.6	0.4
T_2 (B_1 0.5 mgl^{-1})	8.0	2.2	1.4	0.7	0.4
T_3 (B_1 1.0 mgl^{-1})	10.2	3.0	2.6	0.7	0.5
T_4 (B_1 1.5 mgl^{-1})	8.4	2.6	2.4	0.7	0.5
T_5 (B_2 0.1 mgl^{-1})	7.4	2.0	2.2	0.6	0.4
T_6 (B_2 0.5 mgl^{-1})	7.6	2.6	2.4	0.6	0.4
T_7 (B_2 1.0 mgl^{-1})	13.0	4.0	1.4	0.9	0.5
T_8 (B_2 1.5 mgl^{-1})	6.8	1.8	1.8	0.7	0.3
T_9 (B_{12} 0.1 mgl^{-1})	8.4	3.0	2.4	0.8	0.4
T_{10} (B_{12} 0.5 mgl^{-1})	8.6	2.4	2.2	0.8	0.4
T_{11} (B_1 1.0 mgl^{-1})	8.0	2.2	1.2	0.7	0.4
T_{12} (B_{12} 1.5 mgl^{-1})	6.8	1.8	1.8	0.7	0.3
T_{13} (Folic acid 0.1 mgl^{-1})	12.4	3.0	2.6	1.0	0.6
T_{14} (Folic acid 0.1 mgl^{-1})	14.4	4.0	3.4	1.1	0.6
T_{15} (Folic acid 0.1 mgl^{-1})	17.0	4.0	3.2	1.4	0.6
T_{16} (Folic acid 0.1 mgl^{-1})	14.4	3.4	3.2	1.0	0.5
F-test	**	**	**	**	ns
5% LSD	1.8	1.3	0.4	0.3	1.2
cv%	14.6	38.5	43.2	33.0	30.0

T = Treatment, B_1 = Vitamin B_1, B_2 = Vitamin B_2, B_{12} = Vitamin B_{12}

** = Highly significant ns = Non significant ,

* = Data presented in the table were the mean value of 5 replicates

Experiment (2) Hardening process (Community pots)

The results showed that among different treatments T_4 (2 ch:2 br: 1lm: 1coh) obtained the number of survived plantlets 19.7%, number of leaves 5.1and number of roots 4.7(Table 2).

Table 2 Growth result of hardening process (Community pots)

Treatment	No. of Survived Plantlets •	Survived Plantlets (%) •	No. of Leaves •	No. of Roots•	Length of Plantlets (cm) •	Length of Root (cm) •
T_1 (ch: br: s: lm)	39.2	17.4	3.9	3.9	2.8	3.7
T_2 (ch: br: dwh)	25.0	10.1	3.6	2.4	2.5	3.1
T_3 (ch: br: s)	31.5	14.0	3.2	3.1	2.6	3.5
T_4 (ch: br: lm: coh)	44.4	19.7	5.1	4.7	3.4	4.6
F-test	**	*	**	*	**	**
5% LSD	8.8	6.3	0.7	1.1	0.4	0.6
cv %	5.8	3.4	5.6	2.7	4.9	4.9

ch = charcoal, br = brick chips, s = sand, lm = leaf mould, dwh = dried water hyacinths, coh = coconut hask, * = significant, ** = highly significant, ● = data presented in the table were the mean value of 5 replicates

Experiment (3) Hardening process (Thumb pots)(3cm in diameter)

The results of T_4 (spraying of P $NPK_1$20:20:20) plantlets had averagenumbers of plantlets 19.0 were observed. Numbers of leaves were 2.6 and that ofroots were 6.4 in average. Mean length of the plantlet was 4.3 cm and that of the rootwas 7.2 cm in six months old culture (Table 2.3 and Fig. 2.9-2.10).When compared the nutrients between pure water, pigeon dung, cow dung, fish emulsion, NPK nutrients. Among them, NPK_1 (20:20:20) nutrient had superiorresults than other nutrients and followed by NPK_3(30:20:20), NPK_2(21:21:21), pigeon dung, fish emulsion, cow dung and pure water respectively (Table 3 and Fig. 3).

Table 3Growth result of hardening process (Thumb pots)

Treatment	Total Plantlets*	Number of Survival Plantlets*	Number of Non-survival Plantlets*	Percentage of Survival Plantlets*	Percentage of Non-survival Plantlets*
T_0 (Control)	20	6	12	40	60
T_1(Pigeon dung)	20	17	3	85	15
T_2(Cow dune)	20	8	14	70	30
T_3 (Fish emulsion)	20	15	5	75	25
T_4(NPK_1 20:20:20)	20	19	1	95	5
T_5(NPK_2 2l:21:21)	20	18	2	90	10
T_6(NPK_3 30:20:20)	20	17	3	85	15

* = Data presented in the table were the mean value of 5 replicates

Fig.3 Six months old NPK_1 (20: 20: 20) treated plantlets in thumb pots

Discussion and Conclusion

In this research, T_{13}, T_{14}, T_{15}, T_{16} (folic acid 0.1, 0.5, 1.0, 1.5 mgl^{-1}) treated plants is the best plant growth rate. So these result agreed Aye Aye Cho (1993), Aye AyePyone (1993) and San San Aye (1986). Max (2014) reported that vitamin, niacin, thamine, riboflavin and folic acid help for orchid growth. In first stage of hardening, the highest survival rate was observed fromT_4(2 Charcoal+ 2 brickchip + 1 leaf mould

+ 1 coconut husk) medium. The low cost of potting media can be used with success fortransplanting the plants has been established by Roy (1994) and Baruah (1996). The rate of survival (70%) and growth of theplantlets was better in the medium containing charcoal, brick chips, leaf mould andcoconut husks (2:2:1:1) were reported by Hazarika and Sarma (2001). Tucker (2013) reported that charcoal is filter toxin, helping trapping waste and anti-bacteria benefits. Lee (2017) reported that leaf moulds helps retain moisture and slowly over long period of fine.

In second stage of hardening, the highest survival rate was observed fromT_4 (NPK_1 20: 20: 20) fertilized solution. These pots were placed on the shelf under opened field condition. Hazarikaand Samar (2001) experimented that the potted plantlets were firstly kept at ambientroom temperature for hardening.Sinha and Hegde(2001) and Ketchum (2003) reported that coconut husk is hydroponic growing. It features a high cation exchange capacity, a meaturement of media to root nutritional transfer. Fibrous coir does not compact easily which leaves space for healthy root system and promotes a strong aerobic rhizosphere for nutrients and water uptake, fully saturated coir retains 20% of its air capacity. The medium's natural hormones promotes root growth and root protection.Bay (2016) reported that Nitrogen help with plant growth above ground, phosphorus support the plants growth below the grown. It will help for strong root system, flower and fruit production. Potassium supported the overall health of the plants by helping build strong in the plant tissue. Therefore it can be concluded that folic acid had effect on multiplication of *Dendrobiumcariniferum* plantlets. NPK_1 (20: 20: 20) solution was suitable for hardening process of *Dendrobiumcariniferum*plantlets.

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