Effect of Various Vitamins on Growth and Development of *Dendrobium chryseum* Rolfe *In Vitro* Propagation

Na Di Shwe Yee Oo[1], Tin Moe Aye[2], Yin Yin Khaing,[3] Yee Yee Nwe[4],

Abstract

In this experiment, the protocorm like bodies of *Dendrobium chryseum* Rolfe were used as plant material to observe the effects of various concentrations of vitamins and to study the growth and development of PLBs of *Dendrobium chryseum* Rolfe. The protocorm like bodies (PLBs) of *Dendrobium chryseum* Rolfe (Pinn-Daya-Pann-Shwe-Wah) were obtained from Mingalardon Orchid Garden, Mingalardon Township, Yangon Region. The experiment was carried out at the tissue culture laboratory, Department of Botany, Dagon University during from December 2022 to July 2023. Using the protocorm like bodies of *Dendrobium chryseum* Rolfe (Pinn-Daya-Pann-Shwe-Wah) were cultured in VW (Vacin and Went, 1949) medium. This media was supplemented with different concentrations (0 mg/l, 1.0 mg/l, 1.5 mg/l, 2.0 mg/l and 2.5 mg/l) of vitamin B_2, B_6 and B_9. The highest fresh weight and growth value were obtained 2.5 mg/l of vitamin B_6 followed by 2.5 mg/l of vitamin B_9 and B_2. Each experiment has five treatments with three replicates. Data collections were done on number of shoots, length of shoots, number of leaves, length of leaves, number of roots, length of roots and plant height after three months. The growth and development of protocorm like bodies (PLBs) were recorded by color photographs.

Keywords : In vitro propagation, growth and development, vitamins, Orchidaceae, *Dendrobium chryseum* Rolfe, protocorm like bodies

Introduction

The great beautiful and valuable orchids belong to the orchidaceae family which have about 28,000 currently accepted species, distributed in about 763 genera (Christenhusz *et al*., 2016).As the data recorded from Myanmar, there are 273 families,2371 genera and over 11,800 species of vascular plants, including 800 species of Orchidaceae (Ye Lwin Aung , *et al*.,2020).Orchids are top of the list among all flowering plants marketed as cut flowers and potted plants, conveying a very high price in the national and international market. Orchids have great commercial importance and this lead to their tremendous production in nowadays. Besides from their adorning values, orchids are also famous for their medicinal usages especially in the traditional system of medicine. Many orchid species are endangered by over collection and thus they are facing extinctions. At present, International Union for Conservation of Nature and Natural Resources listed many orchids in the Red data book (Ashok N.Pyati, 2022).

In the family Orchidaceae, *Dendrobiums* have becoming widely popular because of its different maleficent colors, variety sizes and shapes and long flowering life of several weeks to months. Dendrobium consists of about 85% of the total cut flower orchid species to the forticulture industry in the world. *Dendrobium chryseum*

[1] MSc Student, Department of Botany, Dagon University
[2] Dr., Professor and Head, Department of Botany, Dagon University
[3] Dr.,Associate Professor, Department of Botany, Dagon University
[4] Dr., Associate Professor, Department of Botany, Dagon University

is a sympodial, an epiphytic and lithophytic herb found throughout Bangladesh, Bhutan, Nepal, Myanmar, Thailand, Laos, China, Vietnam, and Taiwan. It grows in cold climates and imperiled by deforestation and overuse (Joshi et al., 2017). In Myanmar, native *Dendrobium* mainly grows in Southern Shan State and Mandalay Division (Seidenfaden, 1992). *Dendrobium chryseum* Rolfe is broadly used in traditional Chinese medicine for its antipyretics, and immune–modulatory effects and its benefits for the eyes (Yang *et al*., 2007). As this orchid species has great values in the forticulture industry and also has medicinal value, tissue culture should be promoted for its mass propagation and conservation (Pant, 2013).Dendrobiums, the commercially important cut flower, has been achieved a big success on in vitro propagation protocol by Martin et al.,(2005) over shoot multiplication using protocorm like bodies(PLBs).

Plant tissue culture technique can preserve and grow a plant cell throughout the year and in orchid tissue culture, they can produce orchid seeds from crosses in huge quantities in a short period (Sri Hartati et al., 2017). Generally, the choice of the explant is the most critical thing in a micropropagation of orchids (Vineet Soni et al., 2021). The most common used explants in plant tissue culture are protocorm like bodies (PLBs), callus, pollen, shoot apex, and seeds. A new plantlet regenerate from the PLBs is the beautiful one of orchid tissue cultures for the building up of orchid cultures (Zan Zan Wint Kyaw, 2017). The suitable choice of a culture medium and supplements play a critical role on the growth and development of the explants of orchids (Sri Hartati *et al*., 2017).Vacin and Went medium was commonly used in orchid tissue culture and is composed of the macroelements and microelements. The combination of vitamins with other constituents , have been got direct and indirect effects on the growth of callus, somatic growth, rooting and embryonic development (Peter et al.,2011).The higher number of shoot information have observed when vitamins were kept in the medium(S.Roest and G.S.Bokelmann , 1975).In plant tissue culture , the most used vitamins are thiamine (B_1) and Pyridoxine (B_6) and other vitamins such as riboflavin (B_2) and folic acid (B_9) are used in some culture media (John H.Dodds.*et al*.,1985).

The aims and objectives are to observe the effects of various concentrations of vitamins in vitro propagation and to study the growth and development of protocorm like bodies of *Dendrobium chryseum* Rolfe.

Materials and methods

Collection and identification

The protocorm like bodies (PLBs) of *Dendrobium chryseum* Rolfe were used as plant material in this experiment. The PLBs of *Dendrobium chryseum* Rolfe (Pinn-Daya-Pann-Shwe-Wah) were obtained from Mingalardon Orchid Garden, Mingalardon Township, Yangon Region. The morphological studies were made from the collected specimen by using available literature such as Hundley and Chit Ko Ko (1987) and Flora of China (2009).

Inoculation

The experiment was carried out at the tissue culture laboratory, Department of Botany, Dagon university during from December 2022 to July 2023).Vacin and Went(1949),VW medium is used as a basal medium. Sucrose 2%, 0.12 % (w/v) agar and various concentrations of vitamins B_2, B_6 and B_9 were added to the medium. The

pH value of the media was measured and adjusted to 5.8 with NaOH and HCL before sterilized by autoclaving at 121ºC (1.5 kg/cm$^{2)}$ pressure for 15 minutes. All culture bottles were incubated in culture room at the temperature of 24±1 ºC.The light was supplied by 4 feet fluorescent tubes and photoperiod of 16/8 (light/dark).Data collections were done by each treatment with three replicates on three months old culture in this experiment.

Table (1) Experiment 1 (Vitamin B_2)

Treatment	Explant	Medium	Supplement	Concentrations
T0(Control)	PLBs	VW	-	-
T1	PLBs	VW	Vitamin B_2	1.0 mg/l
T2	PLBs	VW	Vitamin B_2	1.5 mg/l
T3	PLBs	VW	Vitamin B_2	2.0 mg/l
T4	PLBs	VW	Vitamin B_2	2.5 mg/l

Table (2) Experiment 2 (Vitamin B_6)

Treatment	Explant	Medium	Supplement	Concentrations
T0(Control)	PLBs	VW	-	-
T1	PLBs	VW	Vitamin B_6	1.0 mg/l
T2	PLBs	VW	Vitamin B_6	1.5 mg/l
T3	PLBs	VW	Vitamin B_6	2.0 mg/l
T4	PLBs	VW	Vitamin B_6	2.5 mg/l

Table (3) Experiment 3 (Vitamin B_9)

Treatment	**Explant**	**Medium**	**Supplement**	**Concentrations**
T0(Control)	PLBs	VW	-	-
T1	PLBs	VW	Vitamin B_9	1.0 mg/l
T2	PLBs	VW	Vitamin B_9	1.5 mg/l
T3	PLBs	VW	Vitamin B_9	2.0 mg/l
T4	PLBs	VW	Vitamin B_9	2.5 mg/l

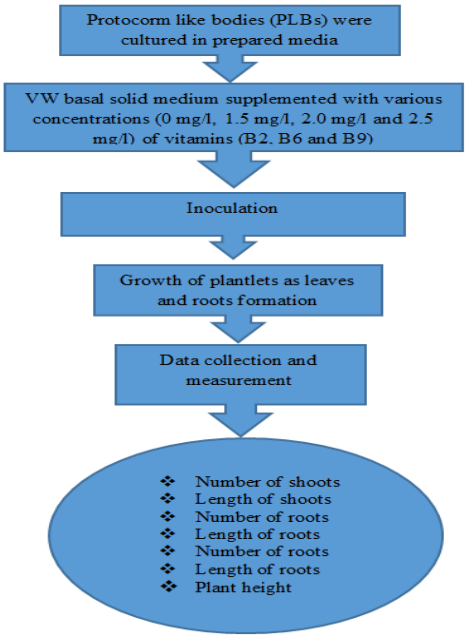

Figure (2) Word diagram of Experiment 1, 2 and 3

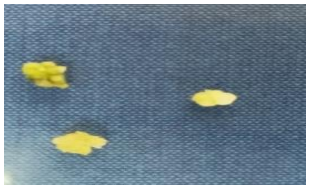

Figure (3) Initial stage of protocorm like bodies of *Dendrobium chryseum* Rolfe.

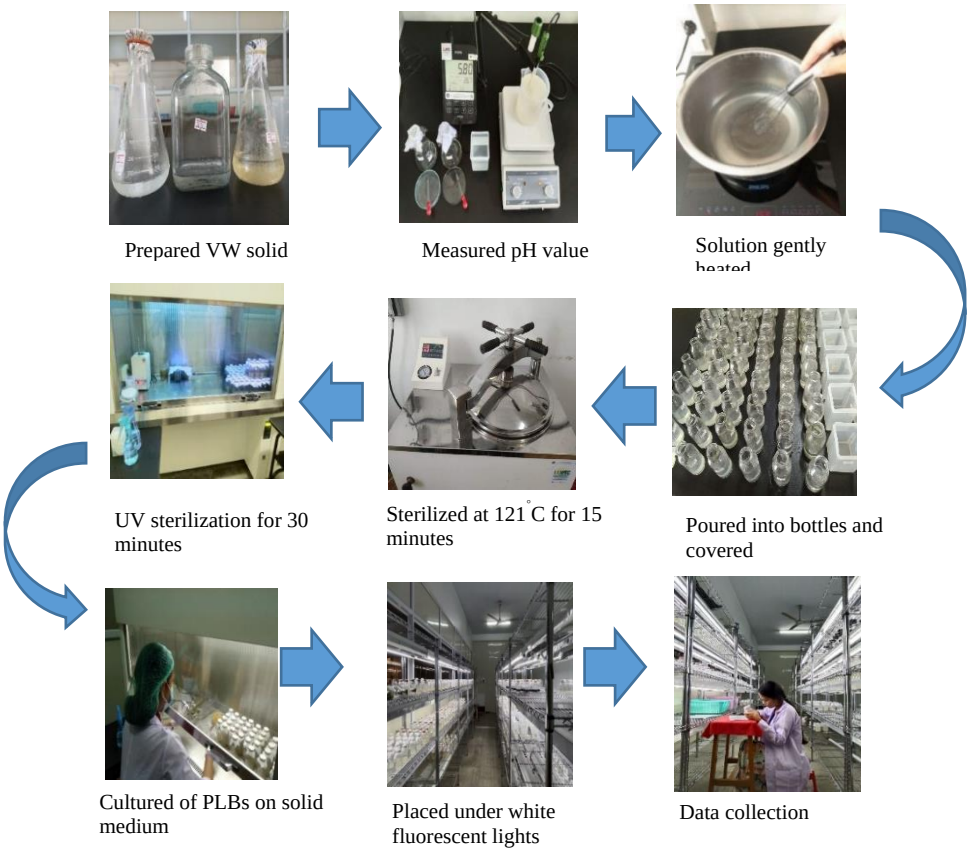

Figure (2) Procedure of the experiment 1, 2 and 3

Results

Scientific name - *Dendrobium chryseum Rolfe.*
Family - Orchidaceae
Myanmar name - Pinndaya-Pann-Shwe-Wah

Sympodial epiphytes. Pseudo-stem, slender, unbranched with many nodes, pale yellow when dry. Leaves simple, alternate, linear, leathery, base sheathing, apex obtuse, leaf sheath tightly embracing stem. Inflorescence terminal racemes. Flowers spreading, golden yellow, floral bracts pale white, complete, bisexual, irregular zygomorphic. Sepals 3, dorsal sepal oblong-elliptic, obtuse, lateral sepals slightly obliquely oblong, and apex obtuse. Petals 3, free 2 laterals similar, broadly elliptic obovate, yellow lip with soft hairs, brown spot at the inner side. Fruit a capsule with numerous minute seeds. Seeds many, minute.

Figure (1) Habit of *Dendrobium chryseum* Rolfe.

Experiment (1) Vitamin B2

Figure (5) Comparison of growth and development of protocorm like bodies of *Dendrobium chryseum* Rolfe on various concentrations of vitamin B_2

Table (4) Growth parameters of protocorm like bodies of Dendrobium chryseum Rolfe. on different concentrations of vitamin B_2

Growth parameters	Treatments (Vitamin B_2)				
	Control	1.0 mg/l	1.5 mg/l	2.0 mg/l	2.5 mg/l
Number of shoots	1.73	2.02	2.71	4.02	4.33
Length of shoots	0.18	0.21	0.22	0.24	0.32
Number of leaves	2.33	3.31	3.71	5.02	5.32
Length of leaves	0.41	0.82	1.02	1.13	1.32
Number of roots	2.31	3.02	3.73	4.71	5.02
Length of roots	0.38	0.31	0.41	0.43	0.62
Plant height	0.62	0.92	1.02	1.21	1.51

The average length of plantlets in T0 was 0.62 cm per culture and final fresh weight was 180 mg.
The average length of plantlets in T1 was 0.92 cm per culture and final fresh weight was 290 mg.
The average length of plantlets in T2 was 1.02 cm per culture and final fresh weight was 300 mg.
The average length of plantlets in T3 was 1.21 cm per culture and final fresh weight was 320 mg.
The average length of plantlets in T4 was 1.51 cm per culture and final fresh weight was 340 mg.
So,the best result was found on Treatment 4(T4) in vitamin B_2 test.

Experiment (2) Vitamin B_6

Figure (7) Comparison of growth and development of protocorm like bodies of *Dendrobium chryseum* Rolfe on various concentrations of vitamin B_6

Table (5) Growth parameters of protocorm like bodies of Dendrobium chryseum Rolfe on different concentrations of vitamin B_6

Growth parameters	Treatments (Vitamin B_6)				
	Control	1.0 mg/l	1.5 mg/l	2.0 mg/l	2.5 mg/l
Number of shoots	1.64	3.31	4.32	5.31	7.03
Length of shoots	0.17	0.23	0.24	0.27	0.33
Number of leaves	2.02	4.32	5.31	5.72	6.33
Length of leaves	0.31	1.23	1.32	1.52	1.82
Number of roots	1.73	3.32	4.72	5.32	6.03
Length of roots	0.21	0.33	0.51	0.71	0.91
Plant height	0.51	1.12	1.41	1.47	1.92

The average length of plantlets in T0 was 0.51 cm per culture and final fresh weight was 180 mg.
The average length of plantlets in T1 was 1.12 cm per culture and final fresh weight was 320 mg.
The average length of plantlets in T2 was 1.41 cm per culture and final fresh weight was 330 mg.
The average length of plantlets in T3 was 1.47 cm per culture and final fresh weight was 360 mg.
The average length of plantlets in T4 was 1.92 cm per culture and final fresh weight was 380 mg.
So,the best result was found on Treatment 4(T4) in vitamin B_6 test.

Experiment (3) Vitamin B_9

Figure (9) Comparison of growth and development of protocorm like bodies of *Dendrobium chryseum* Rolfe on various concentrations of vitamin B_9

Table (6) Growth parameters of protocorm like bodies of Dendrobium chryseum Rolfe on different concentrations of vitamin B_9

Growth parameters	Treatments (Vitamin B_9)				
	Control	1.0 mg/l	1.5 mg/l	2.0 mg/l	2.5 mg/l
Number of shoots	1.64	2.31	4.01	4.31	5.02
Length of shoots	0.16	0.21	0.23	0.31	0.34
Number of leaves	2.31	4.02	5.02	5.28	5.72
Length of leaves	0.31	0.91	1.13	1.27	1.53
Number of roots	2.04	3.03	4.32	4.72	5.68
Length of roots	0.26	0.32	0.41	0.53	0.72
Plant height	0.53	0.93	1.13	1.31	1.73

The average length of plantlets in T0 was 0.53 cm per culture and final fresh weight was 180 mg.
The average length of plantlets in T1 was 0.93 cm per culture and final fresh weight was 300 mg.
The average length of plantlets in T2 was 1.13 cm per culture and final fresh weight was 310 mg.
The average length of plantlets in T3 was 1.31 cm per culture and final fresh weight was 330 mg.
The average length of plantlets in T4 was 1.73 cm per culture and final fresh weight was 360 mg.
So,the best result was found on Treatment 4(T4) in vitamin B_9 test.

Discussions and Conclusions

The morphological characters of *Dendrobium chryseum* Rolfe are sympodial epiphytes, pseudo-stem, slender, leaves simple, alternate, inflorescence terminal racemes, flowers golden yellow, bisexual, complete, irregular zygomorphic, yellow lip with soft hairs and brown spot at the inner side . Fruit a capsule with numerous minute seeds. These findings are in agreement with Flora of Chin (2009). In this study, VW (Vacin and Went, 1949) medium supplemented with different concentrations (0 mg/l, 1.0 mg/l, 1.5 mg/l, 2.0 mg/l and 2.5 mg/l) of vitamins (B_2, B_6 and B_9).In vitamin B_2 experiment, 2.5 mg/l of supplementations got the highest growth values and PLBs increase. This results agreed with Daw Khin Myo Thant, (2018) who got the superior growth in VW (Vacin and Went, 1949) medium supplemented with 1.0 mg/l of riboflavin (vitamin B_2).In vitamin B_6 experiment, 2.5 mg/l of supplementations got the best growth values and PLBs well regenerate into plantlets. This results agreed with Myint Thawdar Naing, (2015) who said that 1.5 mg/l of vitamin B6 gave the highest fresh weight and growth in *Dendrobium wardianum*. In vitamin B_9 experiment, also 2.5 mg/l of supplementations got the highest growth values and PLBs increase. This results agreed with Daw Khin Myo Thant, (2018) who got the best growth in VW (Vacin and Went, 1949) medium supplemented with 1.0 mg/l of folic acid (vitamin B_9).

Among the all experiments, vitamin B_6 got the best growth values when compared to other vitamins and it was followed by vitamin B_9 and B_2.As the above results, it was showing that larger concentrations of vitamins achieved the better

results for the growth of PLBs of *Dendrobium chryseum* Rolfe. This result agreed with Myint Thawdar Naing (2015), who reported that higher concentrations of vitamins gave the best results for regeneration of *Dendrobium wardianum.* It is concluded that the more vitamin concentrations can get the better results and vitamins supplements are necessary in speedy growth culture of basal medium. *Dendrobium chryseum* Rolfe has not only medicinal value but also commercial values and becoming endangered species so that it should be protected by multiplication using tissue culture technique.

Acknowledgements

We would like to express appreciation to Dr, Thar Tun Maung, Reactor Pro-Reactor and Dr. Myo Min, Dr. San San Hmawe, Dr. San San Lwin, Pro-Rector of Dagon University, for permission to carry out of this research work. We also wish to express my thanks to Jeonbuk National University for giving us a chance to participate in 4th Korea-Myanmar Conference.

References

Ashhok N.Pyati. (2022).In vitro Propagation of orchid (*Dendrobium ovatum* (L.)Kraenzl.) through Somatic embryogenesis.

Chrisrenhusz, M. J. M. and J.W. Byng. (2016). The number of known plant species in the world and its annual increase. Phytotaxa. 261(3):201-217.

Hundly, H.G and Chit Ko Ko. (1987). List of Trees, Shrubs, Herbs and Principle Climbers of Myanmar. Government Printing Press, Yangon, Myanmar. Joshi, N., K .S. Dhakal, D.S. (2017).Checklist of CITES Listed Flora of Nepal .Department of Plant Resources (DPR), Thapathali, Kathmandu, Nepal.

John H.Dodds and Lorin W. Roberts. (1985).Department of biological sciences, University of Idaho, Experiments In Plant Tissue Culture, second edition, Cambridge University Press.

Khin MyoThant. (2018).Study on the micropropagation and hardening process of *Dendrobium Carniferum* Rchb.f., 1st Myanmar-Korea conference.

Myint Thawdar Naing. (2015).In Vitro Propagation of *Dendrobium Wardianum* Warner on Nutrient media. Department of Botany, Dagon University, Myanmar.

Pant, B. (2013).Medicinal orchids and their uses: Tissue culture, a potential alternative for conservation. African Journal of plant science. Plant Biotechnology and Biochemistry Laboratory, Central Department of Botany, Tribhuvan University, Kathmandu, Nepal.7 (10), 448-467.

Peter Abahamian, Arumugam Kantharajah. (2011).Effect of Vitamins on In Vitro Organogenesis of Plant. American Journal of Plant Sciences.Vol 2.

Saurabh Bhatia, Kiran Sharma. (2015).Modern Applications of Plant Biotechnology in Pharmaceutical Sciences, Pages 345-360.

Seindenfaden, G. (1992).The Orchids of Indochina, University of Copenhagen Printed in Denmark AIO print Ltd, Odense.

Sri Hartati, Retna Bandriyati Arniputri, Lili Anatus Soliah, Ongko Cahyono.2017.Effects of organic additives and naphthalene acetic acid(NAA) application on the in vitro growth of black orchid hybrid (Coelogyne pandurata Lindley).Bulgarian journal of agricultural science.23 (No 6).951-957.

S. Roes, G. S. Bokelmann. (December 1975).Vegetative propagation of *Chrysantehmum morifolium* Ram.in vitro .Volume 3, Issue 4, Pages 317-330.

Vacin. E and F Went.1949.Some pH changes in nutrient solution. Botanic Gardens Conservation News 110,605-613.

Vineet Soni, Kiran Keswani, Upma Bhatt, Deepak Kumar, Hanwant Singh.(2021).In vitro propagation and analysis of mixotrophic potential to improve survival rate of Dolichandra unguis-cati under ex vitro conditions.

Wu, Z. Y.., P. H. Raven & D. Y .Hong. (2009). Flora of China. Vol.25 (Orchidaceae).

Yang L., Wang Y., Zhang G.,Zhan f., Zhan Z.,Wang Z. & Xu, L. (2007). Simultaneous qualitative and quantitive analysis of bioactive phenols in *Dendrobium aurantiacum* var. *Denneanum* by high performance liquid chromatography coupled with mass spectrometry and diode array detection.Biomedicinal chromatography ,21:687-694.

Ye Lwin Aung ,Aye Thin Mu, Mung Htoi Aung , Qiang Liu and Xiaohua Jin.(2020,Jan 10).An annoted checklist of Myanmar orchid flora.