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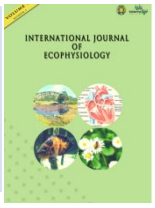

Retardan Variation of Paclobutrazol in VW (Vacin & Went) Media Towards the Artificial seeds Viability of the *Dendrobium* sp.

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Abstract. The research of the variation of paclobutrazol retardant in VW (Vacin & Went) media toward the viability of artificial seeds of *Dendrobium* sp. had been carried out at the Laboratory of Physiology and Plant Tissue Culture, Department of Biology, Faculty of Mathematics and Natural Sciences, University of North Sumatra, Medan. The purpose of the study was to obtain the best combination of paclobutrazol treatments and the storage time on the viability of artificial seeds of Dendrobium sp. This study used a factorial completely randomized design by two factors, which were six levels of paclobutrazol 0; 1; 2; 3; 4; 5 ppm, and 2 levels of storage duration times (0; 2; 4 weeks). The statistical analysis of the data showed that the combination of paclobutrazol treatments and storage times significantly affected the growth time of *plb Dendrobium* sp. as well as the wet weight and the dry weight ($P < 0.05$). Paclobutrazol concentration of 4 ppm and storage period of 4 weeks (P4L2) gave the best effect in delaying the growth of synthetic seeds of *Dendrobium* sp. with an average growth of 21.6 days. The 2 ppm treatment of paclobutrazol and the 4 weeks storage time (P2L2) gave the highest wet weight and dry weight by an average of 0.198 grams and 0.007760 grams, respectively.

Keywords: *Artificial seed, Paclobutrazol, Storage period*

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1. Introduction

Dendrobium is one of the large groups of Orchids in the world. This Orchid presumed consists of 1.600 species (Lestari, 1985). The beauty of orchid attracts the people to breed them in order to sell or just to nurture them. The breeding of The Dendrobium Orchid can be done with conventional method (Syammiah, 2006). The weakness of the conventional method is, it takes a long time in order to get good results. This problem can be tackled by using in vitro technique, such as tissue culture technique. According to Krisnanta (2013), in vitro tissue culture technique allows the orchid to be bred massively even though it just comes from few broodstocks. However, not everyone understands the concept of breeding plants using tissue culture techniques, this vitro technique needs to be introduced to other people.

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Artificial seeds have the physical appearance that's reminiscent of the zygotic seeds. In the making of artificial seeds, there are two mains nutrition needed in the growth media. These nutrition are regularly used for tissue culture, those being in the Vacin & Went Media. Growth regulator substance is used to regulate the growth of explants, protecting the embryo from dry conditions. One of the growth regulators often used to prevent the embryo from drying is Paclobutrazol. The research of artificial seed had been done before, one of them by making of pineapple's artificial seed (by Roostika *et al.* (2012). The breed of this plant could endure for one month. The research about the usage of Paclobutrazol also had been done before by Aridha *et al* (2009) on banana plantlet (*Musa paradisiaca* L). Therefore, the research of paclobutrazol adding in the VW Media to produce artificial seeds of the *dendrobium sp* was conducted to increase the viability and the endurance of the embryo of the seed in the future. The goal of this research was to obtain the treatment combination of paclobutrazol and the storage time that gave the best growth to produce the *Dendrobium* sp. synthetic seeds.

2. Materials and Methods

Material research of Protocorm-like body (plb) *Dendrobium* sp. obtained from the Lab. Cell Culture and Micropropagation, Indonesian Center for Biotechnology and Bioindustry Research. This research designed by Complete Random Design within factorial with two factors; the concentration of paclobutrazol with six levels which are 0, 1, 2, 3, 4, and 5 ppm; and the storage period which were consist of no storing, two weeks storing, and four weeks storage period. The amount of treatment combinations was 18, and it used five repetitions on each treatment, therefore, the total of samples was 90 units.

Mechanism Encapsulation of Matrix Nutrition

The media was made carefully by measuring the weight of each chemical substances, macros, micros, and sucrose based on the composition needed for 1 liter of the Vacin and Went (V and M) Media. Those ingredients would be diluted with aquadest, then stirred by magnetic stirrer until homogeneous and then inserted into an erlenmeyer that's already had the labels for each usage.

The nutrition were measured by using a pH meter 5.6 ± 0.2. pH adjusted by HCl 0.1 N or NaOH 0.1 N. The nutrition stock was used as a solvent for making the matrix encapsulation. The matrix calcium hydrogel capsule was prepared in two separate solutions, which were contained of 3% alginate solution and 75 Mm $CaCl_2.H_2O$. Each of the solutions was mixture by the media solvent above. The variation of paclobutrazol treatments in the matrix encapsulation were prepared by adding few volumes of paclobutrazol on 3% stock solvent until the levels of concentration 1, 2, 3,4, and 5 ppm obtained.

Alginate Calsium Capsule (Artificial Seed) making

The Artificial Seed capsules were made by dipping the *plb Dendrobium* sp explants in the VW Media solvent contained of the 3% Alginate solution combined by paclobutrazol for 1 minute

duration. The explant that had been dipped with alginate and the nutrition taken by using the dropping pipette and was given to the 75 mM of $CaCl_2.2H_2O$ solution. After 30 minutes, the artificial seeds were strained and dried until the volume reduced into 50% from the initial weight. The artificial seeds were stored in a dry sterile storage (*in vitro*) for 2 weeks and 4 weeks. Then. it was compared by the artificial seed dried regularly. The whole procedures were done under laminar air flow (Muliawati *et al*., 2016).

Germination of The artificial Seed

The dried artificial seeds were rehydrated by dipping the seed capsules into warm water (50^0 C) until the volume swell back to the original size and ready for all treatments. All artificial seeds dehydrated according to the duration time of treatments. The artificial seeds were put in a culture bottles to examine the growth capabilities and their viabilities.

Parameter Monitoring

This research were observed based on some parameters, which were Protocorm-like body of the artificial seeds; capsulized and stored artificial seeds shape;. Duration time of sprouting; Wet Weight of the coated the artificial seed and the dry weight. The result of the *Dendrobium* sp. germination that had been measured then covered by the aluminium foil and kept in an oven at 60^0C for 5 hours. To observe the dried weight, the artificial seeds kept in a 60^0C oven again until the weight became constant, then the weight measured by using the analytic scale.

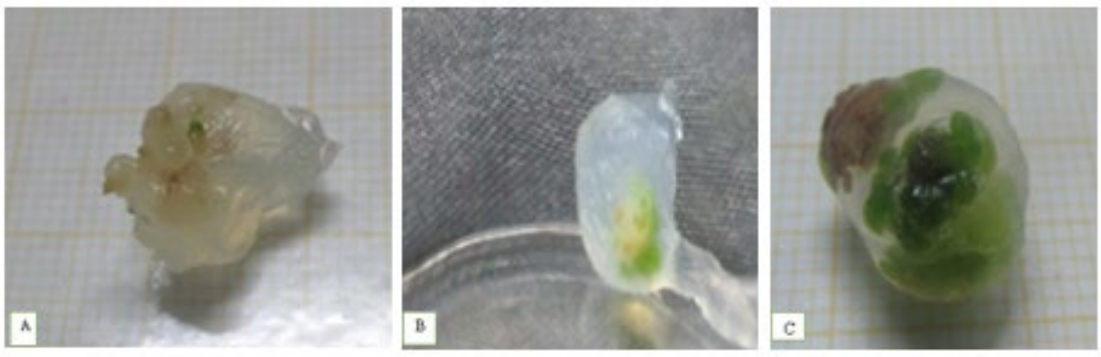
Data Analysis

The data obtained was analyzed by using the bootstraping SPSS 22 method which had the variant analysis (ANOVA) in it, then proceeded by the Duncan New Multiple Range Test (DMRT) at 5% significant level towards the treatment factor.

3. Result and Discussion

The Artifcial Seed Profiles

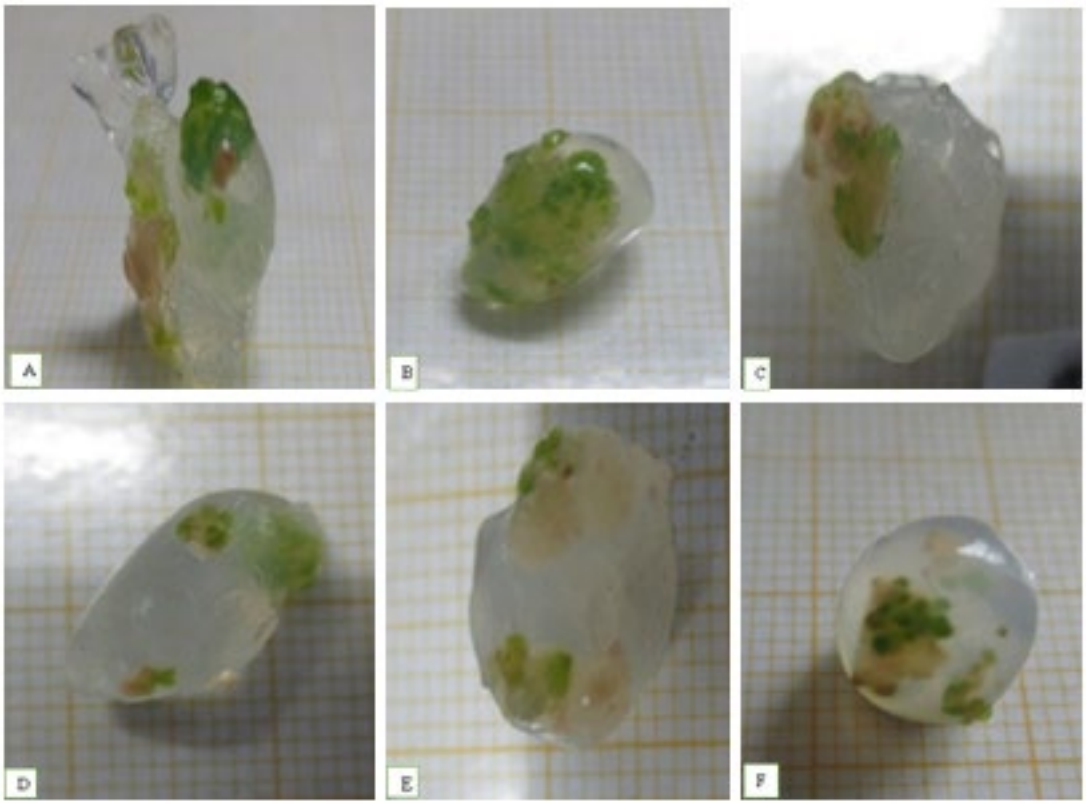
The Artificial Seeds *form* the *plb Dendrobium* sp. were shaped and coated with a mantle of alginate, as show at the Picture 1.



Picture 1. The view of artificial Seeds of the *Dendrobium* sp. Orchid. A. The artificial Seed after the 4 weeks storage time. B. The rehydrated artificial seed. C. Artificial seed after kept to 4 weeks of storage time.

Base on the Picture 1, the artificial seeds after rehydration and germination look fresher and their size were bigger. The green *plb* was fresher compared to the artificial seeds that already kept for 4 weeks of storage times which had wrinkled shape and pale in color. According to Muliawati *et al.* (2016), the change of characters of the capsulized seed after the dry storing process happened due to the difference of water availability inside the artificial seeds and also due to the environment outside of the capsule. On the other hand, the state of the artificial seed changed after going through rehydration happened because of the imbibiton process that happened fastly from small water to the sufficient water. The process of water insertion into the artificial seed identified by the fact that the alginate calcium hydrogel capsules got bloated and made the artificial seeds became shiny.

The viability of the artificial seed coated by alginate on *plb Dendrobium* sp. could growth well, so it produced the *Dendrobium* sp., as shown below.



Picture 2. The germination of the artificial seed of the *Dendrobium* sp. on some treatments of paclobutrazol after 30 days of the storage time. A. Artificial seed with 0 ppm concentration, B. Artificial seed with 1 ppm concentration, C. 2 ppm treatment of Artificial seed, D. Artificial seed with 3 ppm concentration, E. Artificial seed with 4 ppm concentration treatment, F. Artificial seed with 5 ppm concentration treatment.

The *plb* growth in the alginate continued for a long time. Based on the picture 2, *plb* that grew was not tall. This could be caused by paclobutrazol acting as an inhibitor of growth. The presence

of paclobutrazol in a media cause the breed will become shorter, wider, denser and has thicker and greener leaf. The breed had paclobutrazol became shorter because it inhibited the biosynthesis of gibberelin (Methouachi *et al*, 1996; Pinhero and Fletcher, 1994). According to Chaney (2004), the inhibition of growth that was caused by paclobutrazol happened because the chemical components that were present inside the paclobutrazol inhibit the production of gibberelin on a terpenoid way by preventing the enzyme to catalyze the metabolism reaction.

The Germination and Storage Time of The Artificial Seed of Dendrobium sp. by Paclobutrazol Treatments

The sprouting period was counted manually since the coating of alginate until the protocorm-like body bloated and grew well. The statistics analysis toward the time of of protocorm-like body the Dendrobium sp. growth showed that the combination of paclobutrazol and storage time treatments indeed caused the growth inhibition of the artificial seed of the *Dendrobium* sp. ($p<0,05$). The average range of sprouting time of *plb* happened on the $11,8^{th}$ until the $21,6^{th}$ days after the separation of alginate. The interaction of treatments and sprouting times can be seen in the table below.

Table 1. Sprouting Time of *plb Dendrobium* sp. by Paclobutrazol Treatments and the Storage Periods

Storing Period	Paclobutrazol					
	0 PPM	1 PPM	2 PPM	3 PPM	4 PPM	5 PPM
0 weeks	$11,8^{a}$	$14,2^{ab}$	$16,4^{bcd}$	$14,8^{bc}$	$16,4^{bcd}$	$17,4^{cdef}$
2 weeks	$16,2^{bcd}$	$17,8^{defg}$	$18,2^{defg}$	$20,4^{gh}$	$19,4^{efgh}$	20^{fgh}
4 weeks	14^{ab}	$16,6^{bcde}$	$14,8^{bc}$	19^{defgh}	$21,6^{h}$	$19,8^{fgh}$

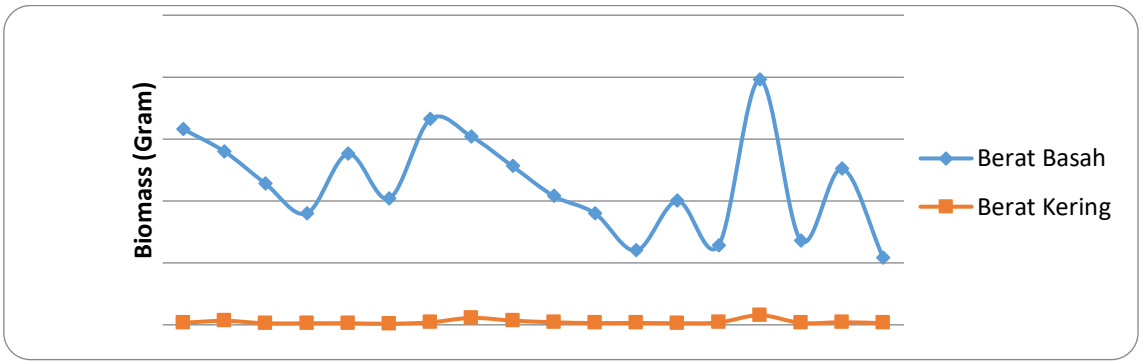
Description: The values followed by the same letters not differ significantly on 5% DNMRT test ($p<0,05$).

The table above shows the 4 ppm paclobutrazol treatment and the storage period of 4 weeks (P_4L_2), gave different realistic effects towards the control treatment of the artificial seed of *Dendrobium* sp. It shows that 4 ppm paclobutrazol treatment and the storing period of 4 weeks (P_4L_2) was the optimum treatment for inhibiting the growth of the artificial seed of the *Dendrobium* sp. The decreasing and increasing of paclobutrazol treatments had the tendency of triggering the growth of the artificial seed of *Dendrobium* sp.The longest period growth of the artificial seeds happened on the 4 ppm paclobutrazol treatment combined with 4 weeks of storing period (P_4L_2) by the average times of 21,6 days whereas the growth artificial seeds of the *Dendrobium* sp. happened on the control treatment (P_0L_0) by the average of 11,8 days. According to Lakitan (1996), the plant regulator substance will induce the growth when it is on the optimal

concentration. The treatment of 5 ppm paclobutrazol combined with 4 weeks of storage period (P_5L_2) degrades the inhibition of the growth of the *Dendrobium* sp's. artificial seed.

The Relation of the Wet and Dry Weight of *Dendrobium* sp. at the Paclobutrazol and Storage Period Treatments

The growth can be determined by measuring the increase of the plant's biomass.



Picture 3. Wet and Dry Weight of *Dendrobium* sp. by Treatments of Paclobutrazol and Storing Period.

P0L0: 0 ppm paclobutrazol without storing period; P1L0: 1 ppm paclobutrazol without storing period; P2L0: 2 ppm paclobutrazol without storing period; P3L0: 3 ppm paclobutrazol without storing period; P4L0: 4 ppm paclobutrazol without storing period; P5L0: 5 ppm paclobutrazol without storing period; P0L1: 0 ppm paclobutrazol and 2 weeks storing period; P1L1: 1 ppm paclobutrazol and 2 weeks of storing period; P2L1: 2 ppm paclobutrazol and 2 weeks of storing period; P3L1: 3 ppm paclobutrazol and 2 weeks of storing period; P4L1: 4 ppm paclobutrazol and 2 weeks of storing period; P5L1: 5 ppm paclobutrazol and 2 weeeks of storing period; P0L2: 0 ppm paclobutrazol and 4 weeks of storing period; P1L2: 1 ppm paclobutrazol and 4 weeks of storing period; P2L2: 2 ppm paclobutrazol and 4 of weeks storing period; P3L2: 3 ppm paclobutrazol and 4 weeks of storing period; P4L2: 4 ppm of paclobutrazol and 2 weeks of storing period,; and P5L2: 5 ppm of paclobutrazol and 4 weeks of storing period.

The approach was done was by measuring the wet and dry weight of the plant. Based on that previous statement, it is necessary to view the relation of wet and dry weight of artificial seeds of *Dendrobium* sp. with the treatment of paclobutrazol and storage time. The relation of wet and dry weight graph with paclobutrazol and storing period can be seen on Picture 3.

Based on the picture above, the treatment of 2 ppm paclobutrazol and the 4 weeks (P_2L_2) storage time had the highest wet and dry weight. Hypothetically, it can be concluded that this happened because of the rehydration process which allowed the water to be inserted into the cell therefore

the enzymes that were necessary for metabolism can be activated and increased the growth of the plant. Lakitan (2006) , water can be activate the enzymes that were in the cell, those enzymes could provide substances that could be utilized in the development processes, and they can also affect the growth of the cells themselves. On the wet weight observation, the lowest wet weight was on the 5 ppm paclobutrazol and 4 weeks storing period treatment (P_5L_4) with the average of 0,054 grams. However, the lowest dry weight was produced by the 5 ppm paclobutrazol treatment which was no storage time treatment (P_5L_0) by the average of 0,00088 gram. This presumed because the 5 ppm paclobutrazol without storage period (P_5L_0) had a lot of water content compared to the 5 ppm paclobutrazol and 4 weeks of the storage period (P_5L_4). According to Hardiyanto *et al*. (2004), it caused by the wet weight already obtained the higher water content then others. Therefore, during long storage time, there were plenty of water evaporated on the drying process and caused it lost a lot of water, and in the end made the dry weight lower than others.

4. Conclussion and Suggestion

Conclusion:

4 ppm paclobutrazol concentration and 4 weeks storing period (P_4L_2) was the best combinations treatment in producing artificial seeds of *Dendrobium* sp. It gave the longest storage period of 21,6 days and the treatment of 2 ppm paclobutrazol and 4 weeks storage period (P_2L_2**)** produced the best artificial seed of *Dendrobium* sp. growth.

Suggestion:

Based on the result of the research, it is suggested to further the research of paclobutrazol by more variation concentrations should have done, such as 0-4 ppm and also longer storage period in order to gain the best time of viability and the longevity of artificial seeds of *Dendrobium* sp.

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