RESPONSES OF *Protocorm Like Bodies* HYBRID DENDROBIUM ORCHID ON VARIOUS TYPES AND CONCENTRATION OF CYTOKININ AND AUXIN ON MURASHIGE AND SKOOG (MS) MEDIUM

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ABSTRACT

The purpose of this research was to find out the best influence of type and concentration of cytokinin and auxin on *plb* of Hybrid Dendrobium orchid growth.

The Completely Randomized Design was used in this experiment with twelve treatments and replicated three times. The responses were observed including the addition of *plb* number, the sum of shoots, roots and leaves.

The result showed that the type and concentration of Plant growth regulator of cytokinin and auxin on MS medium there was significant effect on characters sum of *plb* and shoots, but was not significant effect on characters sum of roots and leaves.

The treatment of BAP 4 mg/L+ NAA 1 mg/L was the efficient treatment on the characters addition sum of *plb* and the treatment TDZ 0.02 mg/L + NAA 1 mg/L on shoots and leaves and the treatment BAP 2 mg/L + NAA 1 mg/L on the sum of roots.

Keywords: *Plb*, BAP, TDZ, NAA, MS

INTRODUCTION

Orchid is a horticulture which has esthetics and economic values higher than other decoration plants, such as rose, gladiolus or krishan, this case because the form, color and flower characters that unique also its long lasting flowers. Nowadays this orchid that dominant favors by public is Dendrobium (34%), then Oncidium Golden Shower (26%), Cattleya (20%) and Vanda (17%) also other orchids (3%) (Litbang Departement Pertanian, 2005). The development of orchid production in this country is relatively still low because limited supply of qualify seed. One method used to solve this by tissue culture or in vitro culture.

In vitro culture is a method to isolate the part of the plant such as protoplasm, cells, group cells, tissue, organ, also growing in aseptic condition, thus the parts of it can make copies and regenerate to be a complete plant (Gunawan, 1995).

The succeed to copies plant by in vitro culture is particularly by better knowledge on the medium used and explant cultured. According to Arditti and Ernst (1993) MS medium is a media that often uses for various cultures because having complete nutrition content. The next factor is the explant use. Explant is the part of plant that uses as the material to initiate a culture. In micro copies of orchid plant can be done by using explant *protocorm like bodies (plb).*

The research results from various orchids by multiply *plb* using the combination of Plant growth regulator such as : 2,4-D + NAA + BAP by concentration each of it between 0.1-10 mg/L (Arditti dan Ernst, 1993), TDZ as the replacer of BAP between 0.01-0.1 mg/L (Shankhla dkk., 1994). Therefore need to do a research to know the responses of *protocorm like bodies* of Hybrid Dendrobium orchid towards the type and concentration of cytokine and auxin in MS media.

MATERIALS AND METHOD

Materials used in this experiment were explant of *protocorm like bodies* Dendrobium which was from the seedling of seed crossing result with Dendrobium Bandung White (putih) with Dendrobium Anching Lubag (putih), the composing of MS medium, the Plant growth regulator : BAP (6- Benzyl Amino Purine), TDZ (N-haraenyl-N-1,2,3-thidiazol-5-haraenylurea), NAA (Naharatalene Acetic Acid), 2,4-D (2,4 Dichloroharaenoxy Acetic Acid), alcohol 70% and 95%, spirtus, tablet formalin, Clorox 5%, aquades, bayclean, tissue, rubber and aluminium foil.

Experimental design used was Completely Randomized Design with twelve treatments and three replications. The treatments were BAP concentration 2, 4, 6 mg/L, TDZ concentration 0.02, 0.04, 0.06 mg/L that combined by NAA concentration 1 and 3 mg/L. All treatments gave addition 2,4-D 0.5 mg/L The observation did toward : the addition sum of *plb*, shoots, roots, and leaves. The observation was used to monthly.

RESULTS AND DISCUSSIONS

At this experiment formed two process of growth those were embryogenesis and organogenesis. The forming of plb at this experiment was the form of embryogenesis at this experiment could be seen from the form of organ either shoots, roots or leaves. Explant which had able to form plb, most of them able to result seeds, roots and leaves however there was also which able to form one organ only.

This experiment result showed that the type and concentration of Plant growth regulator of cytokinin and auxin in MS medium was signicant effect to the sum of plb and shoots but showed was not significant effect to the sum of roots and leaves.

The Addition Sum of *Protocorm Like Bodies (Plb)* Averaged Per Explant.

The using of BAP cytokine BAP result the addition sum of *plb* average per explant more many than the using of cytokine TDZ (Table 1). This case caused by cytokine TDZ had ability to stimulate biosynthesis etilen which can hamper the growth of *plb* orchid Dendrobium Hibrida. Khalafalla and Hattori (2000) stated that TDZ can produce and higher the content of ethylene, then distributed to hamper embryogenesisgenesis somatic. Based on it, assumed the low value number addition of *plb* because TDZ increased ethylene production in high sum, thus somatic embryo, in this case *plb* become hampered.

Cytokinin (BAP and TDZ) which combined by NAA 3 mg/L resulted the addition of plb that was higher than NAA 1 mg/L. This case based on the Pierik's statement (1987) that the process of somatic embryo induction stimulated by the increasing of auxin and amino acid. Gunawan (1992) stated that the influence of auxin toward the growth of plant tissue assumed by two ways, those were : (a) auxin induced secretion of ion H^+ cells out through cells wall. Acidification of cells wall caused the composed of matrix wall cells wide aparat. As the cause of wide apart the composing of matrix wall cell, water into the cells thus the cells bigger, (b) influenced the methabolism of RNA which meant protein methabolism, probably through molecule transcription of RNA.

The J treatment showed that the using of TDZ by low concentration (TDZ 0.02 mg/L + NAA 3 mg/L) had able to result the sum of *plb* higher than the treatment of other TDZ. Victor, et al (1999) stated that cytokinin TDZ had good activity in induce embryo somatic to very low concentration also in the short using period and the giving of auxin to high concentration can stimulate the induction of embryo somatic (Pierik, 1987).

On 8 and 12 WAI, the treatments of B, E, and F resulted the sum of more *plb* than other treatments. Cytokinin BAP was fair to induce the growth of *plb*. The behavior of cytokinin BAP and its auxin NAA averaged increased proliferation of protocorm and induced *plb*. The balance

concentration of cytokinin and auxin to the treatments of B, E, and F were based on the growth of *plb*.

Table 1. The Effects of Various Types and Concentration of Cytokinin and Auxin in MS medium Toward the Addition Sum of *Plb* Averaged Per Eksplant (piece)

Code	Treatments	The Sum of Addition *Plb* (pieces)		
		4 WAI	8 WAI	12 WAI
A	BAP 2 mg/L+NAA 1 mg/L	1.90 a	2.60 a	3.30 b
B	BAP 4 mg/L+NAA 1 mg/L	3.97 c	5.23 b	5.23 c
C	BAP 6 mg/L+NAA 1 mg/L	2.83 c	3.30 a	3.30 b
D	BAP 2 mg/L+NAA 3 mg/L	2.83 c	3.57 a	3.57 b
E	BAP 4 mg/L+NAA 3 mg/L	3.50 c	4.23 b	4.23 c
F	BAP 6 mg/L+NAA 3 mg/L	4.07 c	4.50 b	4.50 c
G	TDZ 0.02 mg/L+NAA 1 mg/L	2.60 b	3.10 a	3.10 b
H	TDZ 0.04 mg/L+NAA 1 mg/L	2.33 b	3.07 a	3.07 b
I	TDZ 0.06 mg/L+NAA 1 mg/L	1.77 a	2.30 a	2.30 a
J	TDZ 0.02 mg/L+NAA 3 mg/L	3.13 c	3.40 a	3.40 b
K	TDZ 0.04 mg/L+NAA 3 mg/L	2.40 b	2.67 a	2.67 a
L	TDZ 0.06 mg/L+NAA 3 mg/L	1.70 a	2.13 a	2.13 a

Information: the number marked by the same letter showed insignificant difference based on Scott-Knott's test at standard 5%

To the treatment used cytokinin BAP, generally the growth occured at 12 WAI, more to the larger direction size of *plb*, while the treatment used cytokinin TDZ, the growth occurred more to the direction of induced shoots and leaves. Therefore, assumed the nutrition available and the source of rest energy divided to shoots, roots and leaves, thus the energy for growing *plb* decreased.

From the outline above, the treatment of B was the most efficient treatment to increase the sum of *plb* Hybrid Dendrobium Orchid. The balance concentration of BAP 4 mg/L + NAA 1 mg/L was fair in induced *plb.*

The Sum of Shoots and Roots Averaged Explant

Organogenesis is a proccess of induced the forming of tissue, cells or kalus into shoot and perfect plant (Kartha, 1991). This process started by the growth hormone. The Balance and Interaction of ZPT endogen and exogen will determine the direction and form of growth, such as: forming shoots, roots or plantlet.

Table 2 showed that the treatment used in the experiment able to stimulate the regeneration of *plb* into sheeds. Morfology direction of a culture was very influenced by the balance of Plant growth regulator contain in the medium and explant itself. The growth of shoots and leaves particularly stimulates by the balance of cytokinin concentration higher than auxin, but in this case other medium substration such as amino acid, macro nutrient elements, micronutrient elements, and vitamin also has a role to form shoots.

Table 2 showed that the treatment used cytokinin TDZ resulted the sum of shoots averaged higher than cytokinin BAP. The treatments of G, H, I, J, and K resulted the sum of shoots higher than treatments of A, B, C, D, E, F, and L.. TDZ knew can hamper cytokinin oxidize more effective compare than other cytokinin (Pierik, 1987). The Research result of Chen and Piluek (1995) stated that TDZ was more effective than BAP in stimulate the forming of axilarry's shoots. The same also stated by Shankhala et al. (1994) that TDZ was more effective in shoots proliferation than adenine cytokinin.

Table 2. The Effects of Various Types and Concentration of Cytokinin and Auxin in MS medium toward the Sum of Shoots Averaged Per Explant (pieces)

Code	Treatments	Sum of Shoots (pieces)		Sum of Roots (Pieces)	
		8 WAI	12 WAI	8 WAI	12 WAI
A	BAP 2 mg/L+ NAA 1 mg/L	0.33 a	1.23 a	1.67 a	1.77 a
B	BAP 4 mg/L+ NAA 1 mg/L	0.67 a	0.83 a	1.37 a	1.43 a
C	BAP 6 mg/L+ NAA 1 mg/L	0.67 a	1.00 a	0.87 a	1.20 a
D	BAP 2 mg/L+ NAA 3 mg/L	0.83 a	1.60 b	0.83 a	1.00 a
E	BAP 4 mg/L+ NAA 3 mg/L	0.00 a	0.00 a	0.33 a	1.60 a
F	BAP 6 mg/L+ NAA 3 mg/L	0.00 a	0.33 a	1.27 a	1.57 a
G	TDZ 0.02 mg/L+ NAA 1 mg/L	1.57 b	1.90 b	1.43 a	1.73 a
H	TDZ 0.04 mg/L+ NAA 1 mg/L	1.68 b	2.57 b	0.73 a	0.77 a
I	TDZ 0.06 mg/L+ NAA 1 mg/L	2.27 b	2.73 b	0.67 a	0.83 a
J	TDZ 0.02 mg/L+ NAA 3 mg/L	1.67 b	2.17 b	1.00 a	1.03 a
K	TDZ 0.04 mg/L+ NAA 3 mg/L	1.93 b	2.33 b	1.00 a	1.27 a
L	TDZ 0.06 mg/L+ NAA 3 mg/L	1.07 a	1.40 a	1.27 a	1.05 a

Information: the number marked by the same letter showed insignificant difference based on Scott-Knott's test at standard 5%

From all experimental combination, either at 8 or 12 WAI the treatment of G, that was 0.02 mg/L TDZ + 1 mg/L NAA was efficient treatment for the character sum shoots of Hybrid Dendrobium Orchid, because by the concentration of TDZ and NAA that was low had able to result the sum of optimal shoots.

Cytokinin and auxin in the right balance influenced toward organogenesis process. Organogenesis is the process of forming plant organt such as shoots or roots. Cytokinin, either itself or combined by auxin will cause differentia and the forming of shoots or roots. The forming of shoots will occur at once or induced after the forming of shoots (Kartha, 1991). The forming of shoots is one form of morfogenesis somatic cell.

Table 2 also juga showed that the treatment of type and cytokinin concentration combined by auxin concentration was not gave significant difference toward the sum of roots, thus to form roots just enough to use BAP 2mg/L + NAA 1 mg/L or TDZ 0,02 mg/L with NAA 1 mg/L.. Khalafalla and Hattori (2000) stated that such as etilen that consider as the hamper in forming roots, the ability of TDZ stimulated etilen biosynthesis become the cause of difficulty to form roots than shoot induction. Chen and Chang (2000) stated the same that TDZ hampered the initiation of rooted. Therefore to induce the forming of shoots better to use BAP.

The Sum of Leaves Averaged Per Explant

On Table 3 can be seen that the type and concentration of cytokinin and auxin was not significant effect toward the sum of leaves, but the type of cytokinin TDZ inclined resulted the sum of leaves averaged per explant more than cytokinin of BAP. This case related with TDZ ability in forming axillary shoots (Chen and Piluek, 1995)

On 8 WAI various types and cytokinin and auxin concentration in MS media had not able to induce leaves. On 12 WAI, had occured the forming of leaves, however it had not perfect. The leaves formed still in the form of primodia leaves. Generally, although *plb* had formed and resulted shoots and roots, however its not yet fullfilled the criterion as leaves and resulted shoots and roots, but it had not fulfill the criterion, thus treatments medium had not fulled criterion as previous leaves,

giving criterion as leaves, thus previous media treatment had not able to give influence to the forming of leaves

Table 3. The Effect of Various Types and Concentration of Cytokinin and Auxin in MS Medium toward the Sum of Leaves Averaged Per Explant (Pieces).

Code	Treatments	The sum of leaves (pieces)	
		8 WAI	12 WAI
A	BAP 2 mg/L+ NAA 1 mg/L	0.00 a	0.00 a
B	BAP 4 mg/L+ NAA 1 mg/L	0.00 a	0.00 a
C	BAP 6 mg/L+ NAA 1 mg/L	0.00 a	0.06 a
D	BAP 2 mg/L+ NAA 3 mg/L	0.00 a	0.11 a
E	BAP 4 mg/L+ NAA 3 mg/L	0.00 a	0.11 a
F	BAP 6 mg/L+ NAA 3 mg/L	0.00 a	0.00 a
G	TDZ 0.02 mg/L+ NAA 1 mg/L	0.33 a	0.44 a
H	TDZ 0.04 mg/L+ NAA 1 mg/L	0.16 a	0.23 a
I	TDZ 0.06 mg/L+ NAA 1 mg/L	0.00 a	0.34 a
J	TDZ 0.02 mg/L+ NAA 3 mg/L	0.00 a	0.11 a
K	TDZ 0.04 mg/L+ NAA 3 mg/L	0.57 a	0.78 a
L	TDZ 0.06 mg/L+ NAA 3 mg/L	0.00 a	0.11 a

Information: the number marked by the same letter showed insignificant difference based on Scott-Fisher's test at standard 5%

Table 3 showed that the treatment of G (TDZ 0.02 mg/L + NAA 1 mg/L) was the better treatment to form leaves, because the concentration of cytokinin and auxin higher would not cause the increase sum of leaves. On the treatment of G, the giving of TDZ and NAA by low concentration had able to induce the forming of leaves.

CONCLUSIONS

Conclusions

1. The type and concentration of Plant growth regulator of cytokinin (BAP and TDZ) and auxin (NAA) in the medium of Murashige and Skoog (MS) gave significant difference toward the growth of *plb* Hybrid Dendrobium orchid to the growth characters sum of pada *plb* and the sum of shoots but was insignificant toward the characters sum of roots and sum of leaves.
2. The treatments of BAP 4 mg/L + NAA 1 mg/L is the better treatment to increase the sum of *plb*, the treatment of TDZ 0.02 mg/L + NAA 1 mg/L for the sum of shoots and leaves, and the treatment of BAP 2 mg/L + NAA 1 mg/L for the sum of roots.

Suggestion

1. To result perfect plantlet, needed to do a subculture of explant to a new medium by considering the balance of ZPT give based on the aim of cultured activity of Hybrid Dendrobium orchid.

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