# The Effect of NAA and BAP in Induction of Protocorm Like Bodies (PLB) *Cattleya* sp. Orchid In Vitro

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**Abstract.** *Cattleya* sp. seeds do not have an endosperm that cause orchid is threatened with extinction. Forming Protocorm-Like Bodies (PLB) that capable of producing large quantities of orchid seeds quickly. The aim was to determine the best interaction of NAA and BAP on growth induction of PLB. The research method used random design with NAA: 0; 0.5; 1; 1.5 ppm and BAP: 0; 0,5; 1 ppm. The data were analyzed with ANAVA and using the colorgrab application. The result showed there is significantly effect of NAA and BAP interaction on the time PLB was formed and there is no significant interaction on the number of PLB. PLB formed the fastest in week 3 with MS + NAA 1.5 ppm + BAP 1 ppm, the highest number of PLB was found in MS + NAA 1.5 ppm + BAP 1 ppm which was 6,67 PLB. The best color of PLB being green.

Keywords: Protocorm-Like Bodies , *Cattleya* sp. , In vitro

## 1 Introduction

Orchid is a plant that has high aesthetic value and economic value. Orchid is a type of ornamental plant whose existence in the wild has decreased and is threatened with extinction [1][2]. Orchid seeds do not have an endosperm as a food reserve, so they need nutrients to germinate to help seed growth. Therefore, for the survival of this plant, it is necessary to cultivate orchids, one way is in vitro cultivation [3][4][5]. In tissue culture, orchid cultivation can be done by taking young plant parts (meristem) and then growing them in agar media in a sterile environment [1-6]. One type of orchid is the *Cattleya* type. The *Cattleya* orchid has a special feature, namely that it has large, beautiful flowers, bright flower colors and smells good. *Cattleya* orchids generally have larger flower sizes compared to other orchids, so *Cattleya* is nicknamed The Queen of Orchid. *Cattleya* is one of the orchids that has the potential to continue to be developed because it has a variety of shapes, colors and sizes. In addition, *Cattleya* orchids can also be used as cut flowers or as pot flowers[6]

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Tissue culture, also known as in vitro culture, is a technique of separating parts of a plant such as terminal shoots, axillary shoots, leaves, stems or embryos and growing them in artificial media under aseptic conditions to form complete plants. This is based on the presence of cell totipotency. The formation of complete plants from explants of cut plant parts is influenced by many factors, including: physiological conditions of the explants, explant genotype, basic media, growth regulators and the culture environment such as lighting or humidity and room temperature. Vegetative propagation can be done by splitting (separating tillers), cutting young plants that come out of the stem (cuttings), and cutting children that come out of flower stalks (keiki). However, vegetative propagation is less profitable because the amount of propagation produced is very limited [7]. Efforts to propagate plants using in vitro techniques (tissue culture) on selected cultivars by forming Protocorm-Like Bodies (PLB) or somatic embryos through somatic embryogenesis is a method capable of producing large quantities of orchid seeds quickly [7] [8].

In general, PLB is widely used as an explant because it has a high ability to regenerate. Propagation using PLB is able to produce complete plantlets in a short time. PLB that regenerates is characterized by swelling of the PLB and is followed by the emergence of shoots [9][10][11]. This process is initiated by growth hormones, namely auxins and cytokinins. The composition between auxin and cytokinin hormones can cause the growth of Protocorm-Like Bodies (PLB) with the aim of research [12]. Induction of Protocorm-Like Bodies (PLB) from several orchid explants such as pieces of leaves, stems, root tips and seeds in various compositions of ZPT (Growth Regulatory Substances) and media is not easy, and the same thing was also reported by Hardjo et al (2016) who successfully induced PLB from stem and leaf explants of Vanda tricolor Lindl.var pallida using NAA 1.0 ppm + BAP 0.5 ppm after 4 weeks of culture, but there is no data on PLB regeneration.

This study used *Cattleya* sp. young stem explants. in vitro. Using young stem explants is a good step because this section contains young tissue and is easy to grow. The addition of ZPT with the right concentration will encourage the growth of Protocorm-Like Bodies and other organs in plants. PLB induction media for *Cattleya* sp. in the form of MS media treated with ZPT.

## 2 Research Method

### 2.1 Materials and Research Tools

The study was conducted at the YAHDI Tissue Culture Laboratory in Medan from January 2023 to July 2023. The research sample used young stem explants of *Cattleya* sp. In vitro from YAHDI Medan's plant tissue culture laboratory. The media used is the basic medium of Murashige-Skoog (MS).

The tools used are autoclave, culture bottle, rubber, plastic, aluminum foil, beaker glass, spatula, petri dish tweezers, Bunsen lamp, Laminar Air Flow Cabinet (LAFC), handsprayer, volume pipette, refrigerator, heater (stove), pH meters, measuring cups, scalpels, measuring kettles, funnels, analytical balances, stirring rods, heating pans, tissues, label paper, pens, rulers and culture racks. The materials used were young orchid stems derived from in vitro,

96% alcohol, 70% alcohol, distilled water, detergent, MS media, growth regulators Naphthaleine Acrytic Acid (NAA) and Benzyl Amino Purine (BAP).

### 2.2 Working Method

In vitro cut stem explants grown on MS basic medium were given various treatments of a combination of NAA (Naphthaleine Acrytic Acid) growth regulators 0 ppm, 0.5 ppm, 1 ppm, 1.5 ppm and BAP (Benzyl Amino Purin) 0 ppm, 0.5 ppm and 1 ppm. as shown in table 1 below:

**Table 1.** Combinations of growth regulators in MS media

| NAA (ppm) / BAP (ppm) | **0** | **0,5** | **1** | **1,5** |
|---|---|---|---|---|
| **0** | $N_0B_0$ | $N_{0,5}B_0$ | $N_1B_0$ | $N_{1,5}B_0$ |
| **0,5** | $N_0B_{0,5}$ | $N_{0,5}B_{0,5}$ | $N_1B_{0,5}$ | $N_{1,5}B_{0,5}$ |
| **1** | $N_0B_1$ | $N_{0,5}B_1$ | $N_1B_1$ | $N_{1,5}B_1$ |

### 2.3 Research design

The research design used a completely randomized design with 2 treatment factors, namely: (a) The first factor was the concentration of NAA (Naphthaleine Acrytic Acid) consisting of 4 levels (0; 0.5; 1; 1.5 ppm). (b) The second factor is the concentration of BAP (Benzyl Amino Purine) consisting of 3 levels: (0; 0.5; 1 ppm). Growth regulator composition (12 combinations of growth regulators) with 3 x replicates (explants) for each treatment combination. Observational data were analyzed using statistical tests, namely the analysis of variance (ANAVA). If the test results show significant differences, further testing will be conducted using Duncan's Multiple Range Test (DMRT).

The parameters observed in this study were the time PLB was formed directly from the explants, the number of PLBs per explant and the PLB color. The PLB color observation was carried out by observing the color change on the *Cattleya* sp. orchid Protocorm Like Bodies (PLB). using the colorgrab application. Observations are made every week. Observations and measurements were made from outside the bottle, while the plantlet was still inside the culture bottle. Data collection was carried out every week until PLB was formed from the explants.

## 3. Result and Discussion

Parameters of observation of PLB formation time directly from explants, number of PLB and PLB color in the combination of NAA (0; 0.5; 1; 1.5 ppm) and BAP (0; 0.5; 1 ppm) treatments with MS base media can be seen in table 2.

**Table 2.** Average Time of formation of PLB, Number of PLB and Color of PLB *Cattleya* sp. at 12 Weeks After Planting

| Combination | PLB formation time (week) | Amount of PLB | Color of PLB (Classification) |
|---|---|---|---|
| $N_0B_0$ | $5,67^{d}$ | 2,67 | Green:Yellow (#C8E75B) |
| $N_0B_{0,5}$ | $4,33^{abc}$ | 5 | Green:Yellow (#C8E75B) |
| $N_0B_1$ | $4,33^{abc}$ | 3,33 | Green (#8BBA2B) |
| $N_{0,5}B_0$ | $4^{ab}$ | 5 | Green:Yellow (#8BAA32) |
| $N_{0,5}B_{0,5}$ | $3,67^{a}$ | 6 | Green (#80B22A) |
| $N_{0,5}B_1$ | $5,33^{cd}$ | 2,33 | Green (#669B1D) |
| $N_1B_0$ | $4,00^{ab}$ | 4,33 | Green (#7BA243) |
| $N_1B_{0,5}$ | $4,33^{abc}$ | 5 | Dark Green (#5D891E) |
| $N_1B_1$ | $5,00^{bcd}$ | 5,33 | Green (#7AA131) |
| $N_{1,5}B_0$ | $6^{d}$ | 3 | Green (#739930) |
| $N_{1,5}B_{0,5}$ | $5,33^{cd}$ | 2 | Dark Green (#59852c) |
| $N_{1,5}B_1$ | $3,33^{a}$ | 6,67 | Dark Green (#519817) |

A high concentration of auxin is needed to induce the formation of protocorm-like bodies [13], the combination of auxin with cytokinins accelerates the growth rate of PLB so that it forms more quickly [14]. The result of the analysis of variance showed that the combination of NAA and BAP have a significant effect on the time PLB was formed. The fastest Protocorm-Like Bodies was formed in the MS + NAA 1.5 ppm + BAP 1 ppm treatment, which was 3 weeks after planting. Embryogenesis somatic auxin plays a crucial role in inducing cell polarity and asymmetric cell division. After the somatic embryo induction stage, it is important to reduce or decrease the concentration of auxin to initiate bilateral symmetry and expression of somatic embryos [14]. It is known that NAA at 1.5 ppm combined with BAP at 1 ppm is the fastest and most suitable combination for inducing PLB. In this type and concentration, there is believed to be a change in endogenous auxin concentration, thus achieving a relative balance for PLB induction. Inducing PLB in Orchidaceae, as observed by several researchers, also requires the addition of auxin combined with cytokinin, as seen in Cymbidium PLB explants, leaf explants, and stems[8][22].

The result of the analysis of variance showed that the combination of NAA and BAP did not have a significant effect on the number of PLB of *Cattleya* sp. orchids. Based on table 2 above, the highest average number of PLB was in the treatment medium MS + NAA 1.5 ppm + BAP 1 ppm, which was 6,67. The optimum concentration of NAA and BAP for Protocorm Like Bodies induction in stem explants in this study was higher than other previous researchers, Hardjo et al 2016 study used the best treatment NAA 1.0 ppm + BAP 0.5 ppm to induce PLB in stem explants Vanda tricolorvar. pallida. Based on research results, high concentrations of auxin combined with lower cytokinins are effective in the PLB induction process in orchids [15].

The results of observations of PLB color of *Cattleya* sp. at 8 weeks after the planting period showed 3 color variations namely Green, Green: Yellow, Dark Green (Fig. 1). The best PLB color is green [16].

The green PLB has the most meristematic cells and indicates PLB which is rich in nutrients so that it can produce PLB multiplication and is able to regenerate to form organs [16]. On the other hand, the addition of auxin NAA alone without the cytokinin BAP only produced a yellowish PLB, and such PLB was apparently not able to regenerate to form organs or embryos, and over time the PLB even browned. The green PLB color occurs due to the addition of a combination of NAA and BAP, the cytokinin effect of BAP growth regulators promotes the formation and development of chlorophyll in two ways, namely to stimulate the further development (in light conditions) of the etioplast into a chloroplast, especially by encouraging the formation of grana, and to increase the rate of chlorophyll formation by increasing the synthesis of glutamate [17] [18].

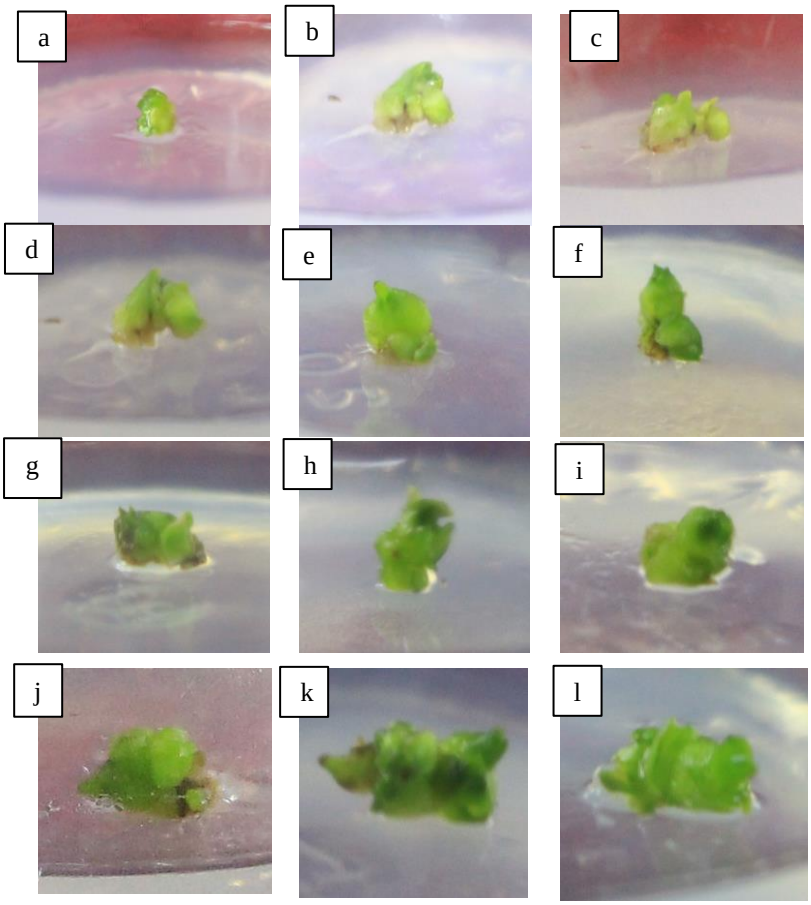


**Fig 1.** Research Result of Protocorm-Like Bodies Induction in *Cattleya* sp. Orchid with NAA and BAP :a. $N_0B_0$,b. $N_0B_{0,5}$, c. $N_0B_1$, d. $N_{0,5}B_0$, e. $N_{0,5}B_{0,5}$, f. $N_{0,5}B_1$,g. $N_1B_0$, h. $N_1B_{0,5}$, i. $N_1B_1$, j. $N_{1,5}B_0$, k. $N_{1,5}B_{0,5}$, l. $N_{1,5}B_1$

Using the strong auxin 2,4-D at 4mg/l combined with kinetin at 2 mg/l to induce PLB formation from the hypocotyl section of *Grammatophyllum scriptum*, it was observed that this treatment was capable of inducing and accelerating the growth of PLBs in G. scriptum orchids. However, there hasn't been PLB regeneration [19]. The synthetic auxin 2,4-D is resistant to degradation through enzymatic reactions and photooxidation. This proves that the type and concentration of auxin required for PLB induction varies for different types of explants and different genotypes [20]. Similarly, it's proposed that each phase of somatic embryogenesis requires different concentrations and combinations of plant growth regulators, ultimately leading to embryo formation [21]. The results of this PLB study also align with the fact that different combinations of plant growth regulators can alter the differentiation process. High auxin concentration combined with low cytokinin concentration effectively differentiates the monopodial orchid *Rhynchostylis retusa* [15].

## 4 Conclusion

The combination of NAA and BAP give a significant influence on the time PLB was formed and did not give a significant influence on the number of PLBs *Cattleya* sp. PLB formed the fastest in week 3 with the media treatment MS + NAA 1.5 ppm + BAP 1 ppm, the highest number of PLB was found in MS + NAA 1.5 ppm + BAP 1 ppm. Three PLB of *Cattleya* sp. color variations were produced, with the best color being green.

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