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Study on Multiplication and Germination of Protocorm-like Bodies (PLB) in *Phalaenopsis* sp.

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ABSTRACT— Phalaenopsis*, one of plant genus in Orchidaceae, is commercially produced as pot plant and cut flower because it has high economical value in world market, including Indonesia. Several micropropagation methods have been developed for* **Phalaenopsis***, but not all of these methods can be used for commercial micropropagation because of differences in survival rate, PLB formation and plantlet regeneration. Therefore, continued studies about the most important stages in* **Phalaenopsis** *micropropagation, which are multiplication and germination stages, are needed. Combination of NAA and BAP produce PLB formation and multiplication. Objectives of this study were to determine the effect of NAA and BAP concentrations in PLB multiplication, and germination of PLB from this multiplication results grown in medium without plant growth regulators. Effects of NAA and BAP combinations, which are 0.1 ppm NAA and 1 ppm BAP; 0.1 ppm NAA and 5 ppm BAP; 0.1 ppm NAA and 10 ppm BAP; 0.1 ppm NAA and 20 ppm BAP; 1 ppm NAA and 1 ppm BAP; 1 ppm NAA and 5 ppm BAP; 1 ppm NAA and 10 ppm BAP; 1 ppm NAA and 20 ppm BAP; with control without NAA and BAP, in multiplication medium were observed and compared with the results of PLB germination. PLB multiplication observed on ten weeks of incubation showed that multiplication of* **Phalaenopsis** *PLB was optimum in 0.1ppm NAA and 5 ppm BAP medium (20.6 PLBs per PLB and has green color). Statistical analysis one-way ANOVA with post hoc Tukey test (p <0.05 showed that results of PLB multiplication between control medium and all treatment medium was significantly different, except for 0.1 ppm NAA and 5 ppm BAP, 0.1 ppm NAA and 10 ppm BAP, 0.1 ppm NAA and 20 ppm BAP treatments. In 0.1 ppm NAA treatment was resulted more PLB than 1 ppm NAA treatment. Observation of PLB germination was performed after PLB was incubated in germination medium for 42 weeks. This observation showed germination of* **Phalaenopsis** *PLB optimally occurred in PLB that has previously cultured in medium multiplication with combination of 1 ppm NAA and 10 ppm BAP (108 plantlets).*

Keywords— BAP, NAA, plantlet, micropropagation, PLB, Phalaenopsis

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1.INTRODUCTION

Phalaenopsis, one of known plant genera in Orchidaceae, is commercially produced as pot plant and cut flower because it has high economical value in world market, including Indonesia [1].

Several micropropagation methods have been developed for Phalaenopsis sp, including the culture of flower stalks with axillary buds, meristems, shoot tips of flower stalk buds, internodal segment of flower stalks, leaf segments and root tips. However, not all of these methods can be used for commercial micropropagation because of response differences in survival rate, PLB formation and plantlet regeneration. Moreover, PLB obtained through some of these methods did not proliferate readily and their viability was low [2]. Therefore, further studies about the most important stages in *Phalaenopsis* micropropagation, which involving multiplication and germination stages are needed. First objective of this study was to determine effect of NAA and BAP concentration combinations on the multiplication of PLB. Second objective of this study was to determine effect of NAA and BAP when in PLB multiplication medium on germination of PLB in medium without plant growth regulator.

2.MATERIALS AND METHODS

2.1 Materials

Only one genotype of *Phalaenopsis* sp were used in this study. PLB samples were extracted, from this genotype, then prepared and used as explants source.

2.2 Methods

PLB specimens were cultured on multiplication medium supplemented with various concentrations of NAA and BAP (Table 1). Cultures were incubated in 16 hr of photoperiodic cycles and 25°C for 10 weeks. Ten weeks old PLBs were subcultured onto germination medium and incubated in 16 hr photoperiod and 25°C for 42 weeks.

Table 1. Composition of multiplication and germination medium for PLBs of *Phalaenopsis* sp

No.	Stage	Basal salt Medium	Plant Growth Regulator		Addition component	Treatment code
			NAA (ppm)	BAP (ppm)		
1.	Multiplication	NDM	0	0	*Phytagel* 0,25% Sucrose 2%	N0B0
			0,1	1		N0,1B1
				5		N0,1B5
				10		N0,1B10
				20		N0,1B20
			1	1		N1B1
				5		N1B5
				10		N1B10
				20		N1B20
2.	Germination	½ NDM	0	0	Banana juice 10% Active charcoal 0,01% *Phytagel* 0,43% Sucrose 2%	--

The observation of the effect of NAA and BAP treatment on PLB multiplication was performed by counting of the number of PLB samples and visualizing the colour of PLB on the 10th week. The data was then subjected to one-way ANOVA incorporating the *post hoc test Tukey HSD*. In germination process, the observation of plantlets formation was conducted on the 42th week.

3.RESULTS AND DISCUSSIONS

3.1 Multiplication of PLB

After ten weeks of incubation (Fig. 1) more PLBs were produced on 0.1 ppm NAA than 1 ppm NAA. The highest number of PLB was obtained on control medium (without plant growth regulator) (29.7 secondary PLB per primer PLB) but the colour of PLB was yellow greenish. According to ANOVA with *post hoc tukey test* ($p<0.05$) the result of multiplication was significantly different for control and all treatment except for N0,1B5; N0,1B10; and N0,1B20.

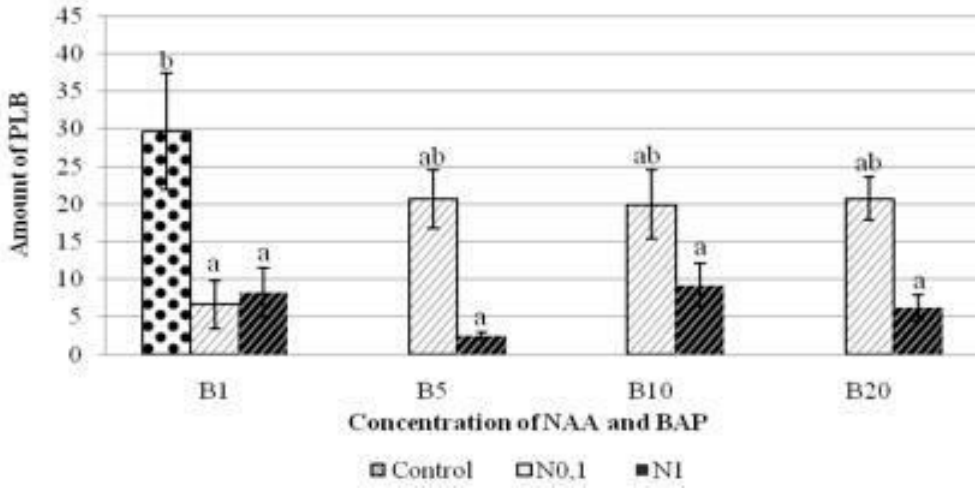

Figure 1: Effect of NAA and BAP concentration on the amount of *Phalaenopsis* PLB formed after 10 weeks of culture (N = NAA, B = BAP; ppm)

In Table 2 it was shown that on NDM medium supplemented with low concentration of BAP, the PLB was green. This result supports view of [2], who stated that combination of low concentration of NAA (0.1 ppm) with low

concentration of BAP (1-5 ppm) caused turning green of PLBs. Green PLB indicating that PLB was healthy [3]. Meanwhile, PLBs on control medium, N0,1B20; N1B10; and N1B20 were yellow greenish. Therefore, NDM medium with the combination of N0,1B5 was considered as a suitable medium for multiplication of *Phalaenopsis* PLB with a multiplication rate of 20.6.

Table 2: Effect of NAA and BAP concentration on the PLB colour

No.	NAA (ppm)	BAP (ppm)	PLB colour
1	0	0	Yellow greenish
2	0,1	1	Green
3	0,1	5	Green
4	0,1	10	Green
5	0,1	20	Yellow greenish
6	1	1	Green
7	1	5	Green
8	1	10	Yellow greenish
9	1	20	Yellow greenish

3.2 Germination of PLB

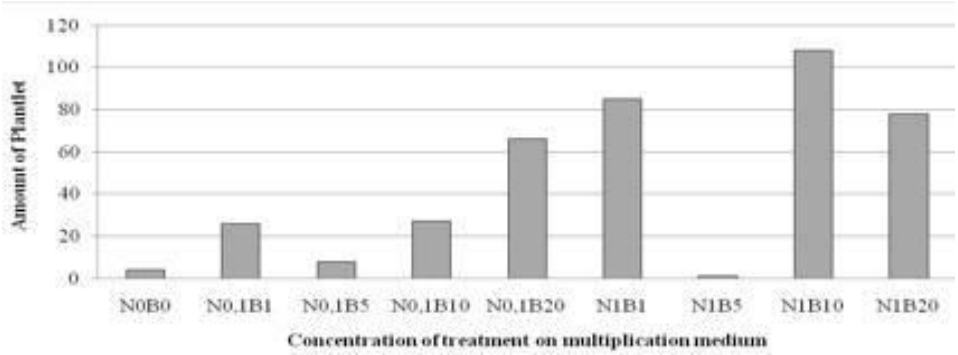

Figure 2: Germination of *Phalaenopsis* plb after 42 weeks of culture (N = NAA, B = BAP; ppm)

After 42 weeks of culture, effect of NAA and BAP supplemented in multiplication medium on germination of PLB in medium without plant growth regulator could be observed. Figure 2 showed pattern of the increment of plantlet amount derived from PLB on N0.1B5 up to N1B10. However, PLB resulted from N1B5 multiplication medium did not follow the pattern. Figure 2 also showed that secondary PLB as the result of multiplication on medium with high concentration of NAA (1 ppm) formed a few PLBs but resulted in more plantlet compared to that of low concentration of NAA (0.1 ppm).

Table 3: Comparison of multiplication and germination result of *Phalaenopsis* PLB

PLB from treatment	PLB multiplication	PLB germination
Control (N0B0)	Amount of PLB: 29.7 secondary PLB/primary PLB Colour of PLB : Yellow greenish	Amount of plantlet: 4
N0,1B5	Amount of PLB: 20.6 secondary PLB/primary PLB Colour of PLB: Green	Amount of plantlet: 8
N1B10	Amount of PLB: 9.25 PLB secondary PLB/primary PLB Colour of PLB: Yellow greenish	Amount of plantlet: 108

Table 3 showed that PLB from N0.1B5 treatment grew optimally (many PLBs and the colour was green) on multiplication medium, but formed only 8 plantlets on germination medium. This indicated that nutrients provided in the medium was used for multiplication process, which was the PLB devided continuously. This process was also seen in control treatment, which resulted in many PLBs but formed only 4 plantlets on germination medium. Meanwhile, N1B10 treament resulted in a few PLBs (the colour was yellow greenish) on multiplication medium but could form many plantlets (108) on germination medium (Fig. 3). This indicated that nutrients provided in the medium was used more for shoot and root differentiation so that germination process was optimal. Germinated PLB was illustrated (Fig. 4).

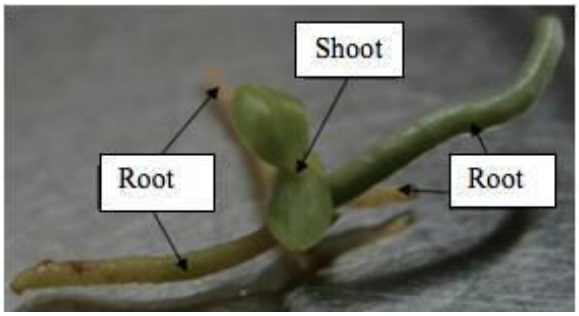

Figure 3: Germinated plantlet of *Phalaenopsis* PLB after 42 weeks of culture derived from N1B10 treatment

Figure 4: Germination of *Phalaenopsis* PLB after 30 weeks of culture

It was possible that habituation process happened in the medium, especially with auxin (NAA) and cytokinin (BAP). Habituation was a phenomena when an *in vitro* culture after a period (after subculture was performed), no need plant gorwth regulator anymore [4]. The habituated culture usually enable to renew themself (*self-perpetuating*) from one vegetative generation to next generation, but the condition could be reversible [5].

According to George *et al*. (2008), eventhough the habituation means as a change in the culture needs, i.e. previously need exogenous substance until self-sufficiency culture, the definition could be wider until included all cell or tissue which was self-sufficient. The tissue that was habituated through the production of certain substance (auxin and cytokinin for instance) called autotrophic to these substances. Whereas the tissue that was not habituated called heterotrophic [5].

PLBs were cultured on multiplication medium supplemented with NAA and BAP became habituated with that condition so that PLBs were selected. When PLB were germinated, the selected PLB were later developed into plantlets. Every PLB will respond differently [6].

CONCLUSION

This research concluded that multiplication medium with the combination of 0.1 ppm NAA and 5 ppm BAP resulted in optimal multiplication of *Phalaenopsis* PLB is about 20.6 secondary PLB per PLB and the colour was green. Then combination of 1 ppm NAA and 10 ppm BAP on multiplication medium resulted in optimal quality in the germination process of *Phalaenopsis* PLB producing about 108 plantlets.

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