

# Protocorm Like Bodies (PLBs) dendrobium orchid “Gatton Sunray”

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**Abstract.** The Dendrobium Orchid Gatton Sunray is a hybrid orchid of *Dendrobium pulchellum* × *Dendrobium illustre*. A study was aimed to find the best type of medium and type of banana for the multiplication of Protocorm-Like Bodies (PLBs) of this orchid. This research was conducted from February to September 2020 at the Plant Tissue Culture Laboratory, Faculty of Agriculture, The University of Bengkulu. Treatments included type of media and cultivar of ripe banana fruit, added as source of organic complex, consisted of 4 cultivars, namely Ambon Curup, Kepok, Tanduk and Mas. Ripe bananas were mashed and added to the *in vitro* medium as much as 50 g.L<sup>-1</sup>. Types of media consist of Murashige and Skoog (MS), ½ Doses of Murashige and Skoog (MS) media, and Complex of fertilizer media. The results showed that the ½ MS medium added with 50 g.L<sup>-1</sup> cv. Kepok fruit produced the greatest multiplication (11.8 PLBs per jar), the best growth of PLBs, including the greatest PLBs number (28.5 PLBs per jar), the heaviest fresh weight (1.30 g per jar) and the longest PLBs (2.56 cm).

## 1 Introduction

Dendrobium Gatton Sunray is a hybrid orchid from a cross between *Dendrobium pulchellum* × *Dendrobium Illustre* conducted by Colman in 1919 and has been registered in the Royal Horticultural Society (RHS) in England. The advantages of Dendrobium Gatton Sunray orchids include having very attractive flower shapes and colours, long blooming time of up to 2 months or more, and can flower throughout the year. The petals and sepals are light yellow which blends beautifully with the dark red labellum at the base, with the apices are wavy with fine yellow velvety hairs [1, 2].

Mass orchid propagation using *in vitro* using newly emerged shoots of about 5 cm in size, called Mericlone propagation [3] has successfully to overcome lengthy and a very slow number of new plants produced through conventional method by dividing bulbs. The type and composition of the growing medium is one of the factors that determine the success of *in vitro* propagation of orchids. Murashige and Skoog (MS) medium, both in full strength [4-6] or in half strength [7] performed as the best medium for different orchids. In addition, compound foliar fertilizers can be used for the propagation of orchid plants, such as

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*Dendrobium antennatum* [8] and Vanda orchid plantlets [9]. The results may be comparable to Murashige and Skoog media [10, 11].

The use of complex organic compound also plays a very important role in the in vitro propagation of orchids. Coconut water, bananas, yeast extract, and malt extract are commonly used. Addition of 15% coconut water in Vacint and Went (VW) increased the development of PLBs to form plantlets of *Dendrobium lasianthera* JJSm. orchids as compared to those on medium VW + 150 gL<sup>-1</sup> bananas [12]. Enrichment with 0.6 gL<sup>-1</sup> coconut water, 30 gL<sup>-1</sup> banana homogenate, and 10 gL<sup>-1</sup> apple-homogenate into MS medium very effectively stimulated the regeneration of orchid PLBs *Sedirea japonica* and increasing plantlet fresh weight. The results of this study have been standardized as a medium for mass production and conservation of this rare epiphytic orchid [13, 14]. The use medium NP + 100 gL<sup>-1</sup> banana in in vitro culture media increases the growth rate of *D. lineale* orchids [15]. For the *Dendrobium* Gatton Sunray orchid plantlet, the addition of 75 mL.L<sup>-1</sup> coconut water and 75 gL<sup>-1</sup> banana extract had a good support their growth [16]. The interaction of the media with organic banana organic on MS media showed a significant influence on the growth and development of PLBs and plantlet regeneration of *Dendrobium* orchids in vitro [17].

The aim of this study was to obtain the best type of medium and type of banana for the growth and multiplication of PLBs *Dendrobium* Gatton Sunray Orchid.

## 2 Material and methods

### 2.1 Media preparation

An experiment was carried out at the Plant Tissue Culture Laboratory, Faculty of Agriculture, Bengkulu University under a factorial completely randomized design (CRD) design. Treatments included the type of planting media, i.e., Murashige and Skoog (MS), ½ Murashige and Skoog (½MS), and complex fertilizer media (Growmore 32:10:10), and the ripe pulp banana cultivar as a source of complex organic matter, i.e., Ambon Hj. Kuning, Kepok, Tanduk, and Pisang Mas. Ripe banana fruit pulps are weighed 50 gL<sup>-1</sup> and mashed until smooth, put into the growing medium according to the treatment. A total of 12 treatment combinations were repeated 5 times, each consisted of 3 jar of culture as a sample. Total experimental units were 180 jars of culture.

MS medium and 1/5MS medium were prepared as standard. Complex fertilizer medium was prepared by weighing the Growmore fertilizer 2 gL<sup>-1</sup> media. All types of media were added 0.5 mgL<sup>-1</sup> NAA + 1.0 mgL<sup>-1</sup> BAP + 2% activated charcoal, 2 mgL<sup>-1</sup> Calcium Pantothenate, 30 gL<sup>-1</sup> sucrose + 6 g L<sup>-1</sup> Agar, and adjusted the pH 5.0. Media that has been heated until boiling then poured 20 ml per bottle. The culture Jars were covered with clear plastic and tied with rubber bands. The growing media were sterilized using an autoclave under a temperature of 121°C, pressure of 15 psi, for 20 minutes. Before the planting medium was used, incubation was carried out for 7 days to ensure the planting medium was completely sterile.

### 2.2 Planting and maintenance of Protocorm Like Bodies (PLBs)

The planting materials of PLBs aged 8 weeks after planting were obtained at the Plant Tissue Culture Laboratory of Taman Anggrek Indonesia Permai (TAIP) Taman Mini Indonesia Indah (TMII) Jakarta. Using standard in vitro technique, each culture jar was planted 3 PLBs mass consisting of 5 PLBs per mass. The jars containing PLBs are incubated in a culture room with the temperature ± 22 °C, the irradiation time is 16 hours using an 18 Watt of LED lamp (2000 lumen) for 12 WAP.

Data were collected at the start of the study for the number of PLBs planted, PLB colour, and initial PLB weights. Weekly observations were made for the number of new PLBs formed and the colour of the PLBs. At the end of the study (12 WAP), data were collected on the number of PLBs, PLB mass diameter, final PLBs weight, PLBs colour, PLBs development to form plantlets and multiplication rate. Observational data were analyzed using the F test at 5% level. If significant was existence in any of the observed variable, Scott-Knott were used for further grouping the mean values that are significantly different. Data analysis used the CoStat Version 6400 program and Microsoft Excel 2016. Qualitative data on callus colour was displayed visually in the form of photos of research results.

### 3 Results and discussion

The results of the analysis of variant using the 5% level F test showed that there was no interaction between the treatment factors of the type of media and the cultivar of banana used for all the variables observed (Table 1). However, the single factor of the type of planting medium or the cultivar of banana used affected all the variables tested, except PLBs germinated until at 12 WAP (Table 1).

**Table 1.** Analysis of variant test on the effect of media type and banana cultivar on multiplication and growth of PLBs of the dendrobium orchid gatton sunray.

No	Variable	F-count		
		Interaction	Media Type	Banana Cultivar
1	PLBs Germinated	0.31 ns	0.01 ns	0.13 ns
2	Number of PLBs	0.45 ns	4.61 *	6.46 *
3	Multiplication Rate	0.91 ns	4.84 *	6.64 *
4	Diameter Mass PLBs	0.93 ns	3.49 *	3.65 *
5	Final Fresh Weight of Research	0.39 ns	7.61 *	5.46 *

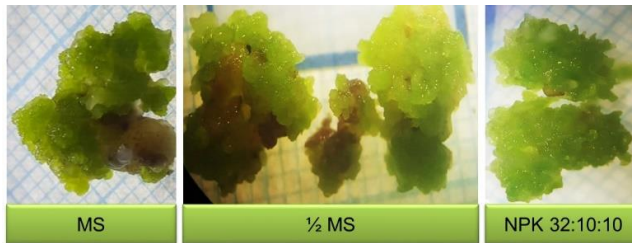
Note: ns = not significantly different at the 5% F test level, \* = significantly different at the 5% F test level

The type of medium significantly affected multiplication and growth of PLBs of *Dendrobium Gatton sunray*, with exception of time of the PLBs to grow. The results of the Scott-Knott test at 12 WAP showed that the Half-MS media type produced the best number of PLBs, multiplication speed, callus diameter and final result weight compared to other types of media, i.e., full MS or NPK (Table 2). Appearance and color of PLBs of *Gatton sunray* orchids were presented in Table 2, Figure 1. The best performance of *Gatton Sunray* orchids PLBs were produced on Half-MS medium. The growth and development of PLBs planted on MS medium was as good as those on compound fertilizer NPK 32:10:10. For callus size, the use of NPK medium resulted in a bigger diameter of the callus as compared to those plated on MS medium. The resulting PLBs already contained high enough chlorophyll so that PLBs which is about 8 WAP is already dark green. Such PLBs usually will develop into plantlets more quickly.

**Table 2.** The effect of planting media type on the growth of PLBs of *dendrobium* gatton sunray orchid (12 WAP).

Media Type	Number of PLBs per Jars	Multiplication Speed (plb per period)	PLB Diameter (mm)	PLBs Weight (g)	Dominant Callus Color
MS	21.3b	7.7b	1.02c	1.04b	5 GY 7/6
Half-MS	28.5a	11.8a	2.76a	1.38a	5 GY 6/6
NPK 32-10-10	22.3b	8.4b	1.44b	1.12b	5 GY 7/4

Note: The numbers followed by the same letter in the same column did not significantly different at the Scott-Knott test. MS=Murashige & Skoog.



**Fig. 1.** PLBs growth and multiplication of gatton sunray dendrobium orchid under different of base medium at 12 weeks after plating (WAP).

The results of this study indicate that orchid seeds can grow and develop to form PLBs and then will develop into plantlets, not requiring high concentrations of nutrients. The use of Growmore compound fertilizer has been able to grow PLBs as good as those the use of MS medium, a complete medium which contains 19 types of chemicals composed of macro nutrients, micro nutrients, vitamins, myoinositol and very complete sugars. As Growmore is much cheaper and easy to prepare than MS medium, the use Growmore shows more advantage. Similar results have been reported regarding the success of using of compound fertilizers. For examples, NPK 10-55-10 media plus 10% coconut water for in vitro seed germination of *Dendrobium antennatum* [8], NPK 32-10-10 NPK medium containing micro nutrients for the growth and development of PLBs of *Eulophia graminea* orchid [9]. On the other hand, the use of Half-MS strength medium also resulted in the best growth and development of PLBs in *Dendrobium* var. Sonia [6], *Cattleya bicolor orchid* [7], *Sedirea japonica orchid* [13], and *Dendrobium* seedlings [10]. The use of Half-MS strength medium also proves that for the growth and development of PLBs, several types of orchids do not require high concentrations. What is needed is the right chemical composition and the chemicals used are complete according to the needs of orchid growth and development.

Our results showed that addition of Ambon Curup banana resulted in the best growth and development of Gatton Sunray PLBs as compared to the other three types of bananas. Under addition of Ambon banana, the number of PLBs up to 12 WAP reached 21.3 PLBs per jars, 24.88% higher than those with the addition of Golden bananas, 25, 82% with Kepok bananas and 42.25% with Tanduk bananas. Ambon banana addition also resulted in the best multiplication speed (10.8 times), callus diameter (2.58 cm) and final fresh weight of PLBs (2.38 g). On the other hand, the use of Tanduk bananas gave the worst response for the growth and development of PLBs Gatton Sunray orchids up to 12 WAP (Table 3). The dominant PLBs formed were Yellowish Green with different brightness - in each type of banana. Under media with the addition of Ambon Curup bananas and Emas bananas, The dominant colour of PLBs was dark green which indicated the formation of perfect chlorophyll grains. Brownish green PLBs were produced on PLBs grown on medium with the addition of Tanduk bananas.

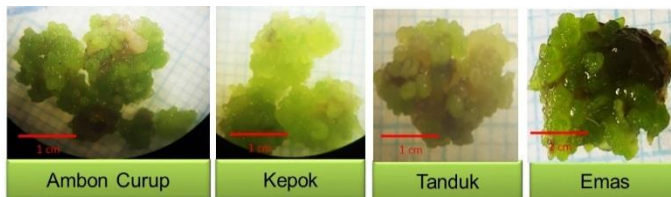
**Table 3.** The effect of banana fruit cultivar on the growth of PLBs orchid *dendrobium gatton sunray* at 12 weeks after plating.

Banana cultivar as a source of organic complex	Number of PLBs per Jar	Multiplication Speed	PLB Diameter	PLBs. weight	Dominant Callus Colour
Ambon HJ-Kuning	21.3a	10.8a	2.58a	1.38a	5 GY 7/ 4
Kepok	15.8b	7.4b	1.56b	1.10b	5 GY 6 / 6
Tanduk	12.3c	4.8c	1.44b	1.08b	2.5 GY 7/4
Emas	16.b	8.7b	1.89b	1.18b	5 GY 7/6

Note: The numbers followed by the same letter in the same column are not significantly different at the Scott-Knott test.

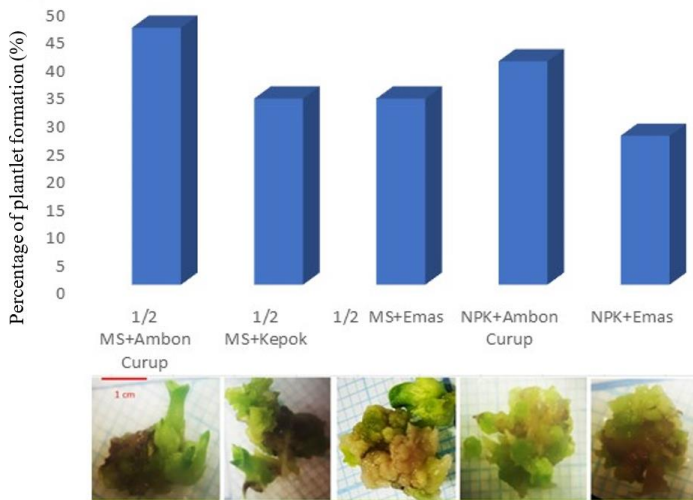
The nutritional content contained in ripe bananas is potassium, which is 373 mg per 100 g bananas, vitamin A 250-335 g per 100 g bananas. The nutritional content of Ambon banana per 100 g is energy 102.89 kcal, water 72.28 g, carbohydrates 24.72 g, protein 1.02 g, fat 0 g, and potassium 217 mg [17-19]. The starch yield and resistant starch content of Ambon banana (8.58%; 29.37%), Kepok Kuning (22.01%; 27.70%), and Tanduk (2.07%; 29.60%). Water absorption and swellability of Ambon banana (1.44 ml/g; 2.53 g/g), Kepok Kuning (1.49 ml/g; 2.58 g/g), and Tanduk (1.32 ml/g) g; 2.23 g/g) [20, 21].

The results of this study indicate that the high starch content is found in the Kepok Kuning banana and Ambon banana. Meanwhile, the carbohydrate content in Ambon banana (25.80 g per 100 g) is lower than those in Emas banana (33.6 g per 100 g), but the content of Vitamin A and Vitamin C being higher. Based on the results of this study, we found that the good growth of PLBs in media with the addition of Ambon HJ-Kuning bananas is due to the nutritional composition contained in the fruit which is in agreement with the needs of complex organic matter desired to stimulate the growth and development of PLBs of Gatton Sunray orchids, especially the carbohydrate content as an energy reserve. Meanwhile, vitamin A and vitamin C contained in bananas can prevent the aging of PLBs and can maintain the freshness of PLBs for a long time, so that it can accelerate the multiplication of PLBs in Gatton Sunray orchids.

**Fig. 2.** Effect of banana type on growth and multiplication of PLBs orchid *dendrobium gatton sunray* (12 WAP).

The PLBs developed to form plantlets could be observed on Half-MS medium with the addition of Banana Ambon Curup, Kepok and Emas, as well as on complex fertilizer medium with the addition of Ambon Curup and Emas bananas, but those with Tanduk (Figure 3). The best plantlets that showed normal growth to form prospective leaves and roots was produced in Media MS + Banana Ambon Curup. In this media, plantlets had started to form at 10 WAP;

whereas those on Media MS + banana Kepok and banana Emas began to form at 11 MS. Under 32:10:10 NPK media with the addition of Ambon banana or those with Emas bananas, platelets were detected at 12 WAP. None of the PLBs planted on MS media developed to form plantlets up to 12 WAP. Much of the PLBs continue to multiply which form PLBs again.



**Fig. 3.** Combination of treatments that can stimulate the growth of PLBs into *Dendrobium Gattton Sunray* Orchid Plantlets (12 WAP). a. Media MS+Pisang Ambon, b. Media MS+Pisang Kepok, c. Media MS+Banana Gold, d. Media NPK 32:10:10+Banana Ambon, and e. Media NPK 32:10:10+Golden Banana.

The development of PLBs to form plantlets requires a suitable medium. Stimulation of plantlets formation can be done by modifying the planting media in several ways, such as changing the carbon source, adding growth regulators or elicitors. The requirements for growth regulators vary from species to species and explants to explants [22]. The lower ratio of NAA and BA may result in slower plantlet development of PLBs [23]. The development of PLBs to form plantlets in this study was due to the addition of BAP which had a higher concentration than NAA, as well as complex organic matter from bananas containing energy sources in the form of carbohydrates and fats. In spite of that, there were also several natural growth regulators that could stimulate the regeneration of PLBs into plantlets.

## 4 Conclusion

Based on the results of experiments that have been carried out for 12 WAP, the following conclusions can be drawn. The best growth and multiplication of PLBs of *Gattton Sunray* orchids was produced on a medium Half-MS+ 50 gL<sup>-1</sup> Banana Ambon, with the criteria for the highest number of PLBs 28.5 PLBs per bottle, the heaviest fresh weight 1.38 g per bottle and the greatest PLBs diameter 2.56 cm. Half-MS media enriched with organic complex of Ambon HJ-Kuning, Kepok and Emas bananas as well as complex fertilizer media with the addition of Ambon and Emas bananas were able to stimulate the development of PLBs into plantlets of *Dendrobium Gattton Sunray* orchids at 12 WAP.

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