Growth and Development of *Dendrobium spectabile* Orchid Protocorm to Various Combination Alternative Media *In Vitro*

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**ABSTRACT.** The use of alternative materials in culture media on *Dendrobium spectabile* orchid *in vitro* propagation was applied to deal with reducing the high operational cost in that propagation. The aim of this research was to obtain the composition of *in vitro* alternative media that give the best effect to the growth of *Dendrobium spectabile* orchid protocorm. The experiment was carried out at Tissue Culture Laboratory of Seed Technology, Faculty Agriculture, Padjadjaran University from November 2010 till January 2011. *Dendrobium spectabile* orchid protocorms were used as culture material in this experiment. The treatments consisted of various combinations between red hyponex foliar fertilizer media at concentration 1 g/l and 2 g/l and organic materials, which are yeast extract at concentration 1 g/l; 1.25 g/l; and 1.5 g/l and sweet potato at concentration 50 g/l; 100 g/l; and 150 g/l. The experimental design that was used was complete randomized design with 12 treatments and 3 replications. The result of the experiment showed that hyponex foliar fertilizer media at concentration 1 g/l combined with yeast extract at concentration 1 g/l and 1.25 g/l indicated higher number of leaves (6 sheet), plantlet height (15.76 mm), numbers of roots (2.22 pcs), root length (6.22 mm), and plantlet fresh weight (37.33 mg) than other treatments of media.

Keywords: Protocorm, *Dendrobium spectabile*, hyponex, yeast extract, and sweet potato
INTRODUCTION

*Dendrobium spectabile* orchid protocorm is one of culture material in *in vitro* propagation that yielded from seed germination. This seed germination and later, its growth and development can be very important tool in the conservation of wild orchids and for that, the growth of the protocorm to form the complete organ is surely need to be assessed. There are many factors that effect the growth of the protocorm. One of the factors is the culture media. The success of *in vitro* propagation methods depends on the media used but, there is a high cost to be incurred. One solution in dealing with this problem is to use alternative materials in tissue culture.

Basically, alternative materials have potential to supporting the growth of explants. Only, the dose and modification remains to be investigated to obtain a suitable composition, so it can be seen what kind of the composition of alternative materials that can support plant growth. Foliar fertilizer is known to be utilized in tissue culture as a replacement for MS and VW basic media. Ramadiana et al. 2008, stated that the basic media Hyponex and Growmore can be used as an alternative to substitute VW or ½ MS in seedling growth of *Dendrobium* orchid hybrid. Other research indicates that the use of fertilizers Hyponex and MS media were not significantly on dendrobium orchid culture (Alam et al., 2002).

Research on the effect of organic materials which were bananas extracts, potatoes, sweet potato, soybean, and coconut water in combination with NAA on the growth of black orchid show that the use of organic materials provides a significant effect on explant growth variables (plant height, leaf number, root length, and roots number) (Untari et al., 2006). According to this experiment Media Vacin & Went with the addition...
of sweet potato extract 150 g/l without NAA was the best media for optimal growth of seedlings of black orchid *Coelogyn pandurata* results *in vitro* (Untari et al., 2006). Other research about organic material showed that the use of yeast extract about 1.2 g/l produces the best of plantlets height, leaf area, root length and root number (Widiastoety, D. and S. Kartikaningrum 2003).

The aim of this experiment was to test the effect of foliar fertilizer and organic material combinations on the growth of *D. spectabile* protocorm. It is expected there is one of the alternative media would give the best effect on the growth of *Dendrobium spectabile* orchid protocorm.

**MATERIALS AND METHODS**

The study was conducted in laboratory tissue culture, Faculty of Agriculture Padjadjaran University, Jatinangor, from November 2010 till January 2011. Plant material or explants used in this study is *Dendrobium spectabile* orchid protocorm, germination resulting from in vitro aged 7 months. Materials used in culture media, are Hyponex, red foliar fertilizer, coconut milk, pureed sweet potatoes and yeast extract. Other materials used in the media is, sugar, aquades, and agar. The treatment consists of a combination of fertilizer with organic material which were sweet potato porridge or yeast extract. 12 kinds of treatment used in the experiments are Hyponex 1 g/L + sweet potato 50 g/L (M1); Hyponex 1 g/L + sweet potato 100 g/L (M2); Hyponex 1 g/L + sweet potato 150 g/L (M3); Hyponex 2 g/L + sweet potato 50 g/L (M4); Hyponex 2 g/L + sweet potato 100 g/L (M5); Hyponex 2 g/L + sweet potato 150 g/L (M6); Hyponex 1g/L + yeast extract 1 g/L (M7); Hyponex 1 g/L + yeast extract 1.25 g/L (M8); Hyponex 1 g/L + yeast extract 1.5 g/L (M9); Hyponex 2 g/L + yeast extract 1 g/L (M10); Hyponex 2 g/L + yeast extract 1.25 g/L (M11); and Hyponex 2 g/L + yeast extract 1.2 g/L (M12).

The design used was completely randomized design consisting of 12 treatments, 3 replications, and each replication consists of 10 units, so the overall total there are 360 experimental units. Observations started 1 week after incubation and then the object was continuously observed per week.
Observed variables were number of leaves, plantlets height, root number, root length, and fresh weight of plantlets. The data that had been collected was tested using SPSS 17.0 and Duncan test at level 5 % was used to test significances.

**RESULTS AND DISCUSSION**

At 1 week after incubation the explants showed develop from protocorm which has globular form, become bud. This development later continued with the leaf initiation at 2 week after incubation. This leaf initiation occurred in all media treatments. The growth speed seen different after couple weeks later. At 6 week after incubation the growths of explants planted on media consist of yeast extract showed faster growth of bud and leaf, and faster root initiation, meanwhile the explants planted on media consist of sweet potato porridge showed slower growth. This different growth speed occurred till last observation conducted or till at 12 week after incubation.

![Figure 1. The Average Leaves Number at Each Treatment Media at 12 Week after incubation](Image)

Media treatment gave a good effect on the average number of leaves per explant was all over the media treatment containing yeast extracts.
Those media were M7, M8, M9, M10, M11, and M12. These were presumably because the elements that play a role in the leaves formation are on the appropriate amount on these media. Important elements in the formation of leaves were calcium, phosphorus, iron, thiamin, and riboflavin (Untari and Puspitaningtyas, 2006).

In the media containing sweet potatoes, only media M2 that gave the effect as good as the media of M7, M8, M9. These showed that the nutrient in media M2 which play an important role in the formation of the leaf is at the right amount that required by the explants. Loebeinstein and Thottappilly (2009), mentioned that about 80-90% of the total dry matter consists of carbohydrates sweet potato. Therefore the higher concentration of sweet potato which was 150 g/L in the media M3 and M6 caused an imbalance of nutrients in the media, due to increased concentrations of carbohydrates that were too much. Similarly, the higher concentration of Hyponex fertilizer which was 2 g/L only contributed to the increase in the concentration of macro elements N, P, and K and some other elements such as boron and magnesium. The lower concentration of sweet potato was actually causing the least amount of vitamin in the media. Concentrations of vitamins that are too low would inhibit leaves formation, because the vitamin is needed in the process of cell division (George, et al., 2008).

![Planlet height](image)

Figure 2. The Average Planlet Height at Each Treatment Media at 12 Week After Incubation
Treatment media that gave a good effect on the average plantlets height are M$_8$ and M$_9$. Widiastoety and Nurmalinda (2010), mentioned that the plantlet height increase occurred mainly due to an increase of biosynthesis in endogenous growth hormone. Production of endogenous plant growth hormones is influenced by the provision of additional substances in the media. One of the additional substances that play a role in the biosynthesis of endogenous hormones was carbohydrates. High amount of carbohydrate contained on the media treatments which were containing sweet potatoes. While the carbohydrate content in media containing yeast extract is lower. Carbohydrate content on yeast extract only 20% of the total dry ingredients. Low carbohydrate content that is actually a positive impact on increase of plantlet height. In media containing sweet potatoes, the high concentration of carbohydrates causes the inhibition of explant growth due to disruption of the process of absorption of nutrients by the explant.

![Number of roots](image.png)

**Figure 3.** The Average Root Number at Each Treatment Media at 12 Week After Incubation

Media treatment that gave a good effect on the average number of roots was a medium M$_7$ and M$_8$, because both media containing yeast extract organic material that were a source of amino acids and vitamins, especially vitamin inositol and thiamin or B1 (George, et al., 2008). These elements were very involved in the growth of root explants. Hyponex content on both media were at a concentration of 1 g/L. This concentration
is suspected in accordance with that required by the explants. At higher concentrations of Hyponex which were 2 g/L, causes inhibition of root growth due to increased concentrations of nitrogen and boron element.

The effect of media containing sweet potato was different to media containing yeast extract. This is presumably due, the high concentration of carbohydrates in the media containing the sweet potato, because the inhibition of the absorption of nutrients by the explant. The explants could not obtain the nutrients, especially thiamin in the appropriate concentration to root growth.

![Root Length Diagram]

Figure 4. The Average Root Length at Each Treatment Media at 12 Week After Incubation

Treatment media that gave a good effect on the average of root length per explant is M7, M8, and M9. Widiastoety, et al. (2009), mentioned that the length of the roots due to the process of cell division in root tip meristem, followed by the process of cell elongation and enlargement.

Untari and Puspataningtyas (2006), mentioned that the elements required in the root elongation process are calcium, niacin, riboflavin, and especially thiamin. These elements found in yeast extract organic materials which were added in the media. As for sweet potato organic ingredients, nutritional content of the most prominent is carbohydrates. It is thought to cause the explants lacked vitamin needed for root growth.
The average fresh weight per explant at each treatment media were M₁, M₇, M₈, and M₉. Aktar et al. (2007 and 2008), mentioned that the fresh weight of explants is affected by the availability of sucrose in the media. Sucrose is an important component of the media in vitro that serves as a source of carbon (C) and energy of the explants (Faria et al., 2004).

Sucrose contained in the treatment media sweet potatoes comes from the addition of sugar and complex organic material that is sweet potato itself. In media containing yeast extract, sucrose only comes from sugar, because yeast extract did not contain sucrose. The highest fresh weights explants took place in the treatment of media containing yeast is M₈. This shows that sucrose content in that media was in the right amount corresponding to the required explants. The content of sucrose in the media containing high concentrations of sweet potato with suspected it causes disturbances in osmotic potential between the media and the tissue explants that resulted in delays in the process of absorption of nutrients.

**CONCLUSION**

Treatment media which gave a good effect on the growth of *Dendrobium spectabile* orchid protocorms are media M₇ containing...
Hyponex 1 g/L in combination with yeast extract 1 g/L and M₈ containing Hyponex 1 g/L in combination with yeast extract 1.25 g/L. The variables observed were number of leaves (6 sheet), plantlet height (15.76 mm), numbers of roots (2.22 pcs), root length (6.22 mm), and plantlet fresh weight (37.33 mg).

REFERENCE


