



## Acclimatization of Pencil Orchid (*Papillionanthe hookeriana* Rehb.f) as Affected by Different Types of Planting Media and Fertilizing Frequency

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### ABSTRACT

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Acclimatization is a process of an environmental adaptation from heterotrophic conditions to autotrophic conditions. In order to be successful in acclimatization, it requires proper planting media and enough nutrition supply. The objective of this experiment were to determine the best planting media and the best spraying frequency of leaf fertilizer on Pencil orchid growth during acclimatization period. The experimental design used was Randomized Complete Block Design, arranged in factorial (2 factors, 3 replications). The first factor was type of planting media: Coconut Coi, Rockwool, Wood Shavings, and Fern Roots. The second factor tested was fertilizing frequencies: every 2 days, every 3 days, and every 4 days. The results showed that the best media for acclimatization of Pencil orchid was fern root fertilized every four days, the best media for growing Pencil orchid was fern root and Wood Shavings, and the best fertilizing frequency to promote Pencil orchid growth were every 3 days or every 4 days.

### INTRODUCTION

Pencil orchid (*Papillionanthe hookeriana* Rehb.f) a local-exotic orchid found in Bengkulu Province, Indonesia, and protected by Governmental Decree (Peraturan Pemerintah) No.7/1999 under the subject of conservation of wild species for animal and plants (Romeida *et al.*, 1996). In Bengkulu, this orchid is found at Dusun Besar Natural Conservation area (*Cagar Alam Dusun Besar*), Bengkulu City. The existence of this orchid was firstly reported by the British colonial government and called “the queen of orchid” in 1882. Recently, this orchid was awarded as “The First Class Certificate” by the environmental agency authority (BKSDA Bengkulu Province, 2015). In 2017, Pencil

orchid was registered by the Bengkulu Province authority as the local seed orchard named “Semarak Bengkulu Orchid”.

Pencil orchid has been successfully micropropagated via *in vitro* technique by the University of Bengkulu scientists. Any crop propagated via tissue culture has to be acclimated or adapted through very rigorous technique and effort in order for the plant to be grown at the new environment like in the greenhouse, at nursery, or in the field. This process of adaptation is called acclimatization, in which freshly taken out plantlets are put at the controlled environment, supplied with all growth resources needed by the crop so that the crop will be strong enough to be transplanted to the field. Acclimatization is a changing process from heterotroph phase of the plant to autotroph

phase (Basri, 2004). Fail to proceed this phase, there will be no crops. Therefore, this process is very critical for tissue culture procedure. It starts with providing proper planting media, plant nutrition, and proper maintenance of the crops (Wattimena, 1997). Acclimatization of orchid plantlets have been done for many kind of orchid, such as Black Orchid (Adi et al., 2014), Dendrobium orchid (Astuti, 2006), and Catleya Archid (Sulistiana and Sukma, 2014).

A good medium for acclimatization has to have the following criteria: good water holding capacity, good drainage and aeration, not easy to decompose, its pH reaction around 5-6, high cation exchange capacity, and not source of pathogen, environmentally friendly, and locally available (Budisantoso, 2013; Iswanto, 2002). In the meantime, the commonly used material for orchid planting media are charcoal, fern, moss, chopped wood, chopped brick, pine bark, wood shavings, Coconut Coir, coconut palm fiber, and lichen (Ginting, 2008). A planting medium needed by the Pencil orchid is to support the plant so that it can stand erectly. Choosing a proper planting medium for orchid is somewhat tricky. (Suradinata *et al.* 2012), acclimating Black orchid, reported that when they used moss, most all of the orchid roots got rotten and the crops died. However, when fern was used the orchid grew very well.

In addition to suitable planting media, Pencil orchid also needs to get enough supply of plant nutrition to support its growth, which, in this respect, can be provided by spraying leaf fertilizer. A good leaf fertilizer has to provide enough amount of macro nutrients (NPK, Ca, Mg) and micro nutrient (Cu, B, Co, Mo, Mn, and Zn provided that the media used for growing the orchid has no nutrient contents whatsoever. Many leaf fertilizers only provide NPK with different ratios of each, such as NPK 20-20-20, NPK 32-20-20, and NPK 20-15-15. Which NPK suitable for the crops and what is the proper dosage depend very much on the crops and how often the crops will be sprayed. Damanik *et al.* (2011) have explained that perfect timing for fertilizing the crops is very important. Furthermore, Sukma and Setiawati (2010) reported that spraying Dendrobium orchid with leaf fertilizer ( $2 \text{ gl}^{-1}$ ) every 3 days,

compared to every 6 days, gave the best growth, indicated by the longest leaf blade.

The objective of this experiment were to determine the best planting media for growing Pencil orchid, to determine the best spraying frequency of leaf fertilizer on Pencil orchid growth, and to evaluate whether there was interaction effects between planting media and spraying frequency of leaf fertilizer on Pencil orchid growth.

## MATERIALS AND METHODS

The experiment was carried out from January to May 2017 at the Screen House located at the “*Dendam Tak Sudah*” lake area, about 50 m above sea level. The experimental design used in this experiment was Randomized Complete Block Design, arranged in factorial (2 factors, 3 replications). The first factor was type of planting media (M), consisting of four levels: Coconut Coir (M1), Rockwool (M2), Wood Shavings (M3), and Fern Roots (M4). The second factor tested was fertilizing frequencies (HS), consisting of three levels: every other day (2HS), every three days (3HS), and every four days (4HS).

**Media Preparation.** Coconut coir and fern roots were cut off in 4cm x 5cm. These media and Wood Shavings were rinsed in water for 24 hours. The water was replaced with Mankozep solution 80% for the next 24 hours. The media were ready to use after air dried. The last media, rock-wool, was ready to use without additional treatment.

**Plantlet Preparation.** The plantlets were taken out of the bottles, washed under running water, and rinsed in fungicide solution for 5 minutes. Before planting, the plantlets were measured for plant height, number of leaf, and number of root and followed by spraying vitamin (B1) and fertilizer (NPK, 32-10-10) for  $1 \text{ gl}^{-1}$ . Represented the adult Pencil Orchid and ready acclimated planted was displayed in Figure 1.

**Variable Measured.** At the end of the experiment, we measured plant height, leaf number, stem diameter, root number, and root length. The data were analyzed for variance at 5% level followed by comparing the mean values with Duncan’s Multiple Range Test.



Figure 1. Adult Pencil orchid (left) and plantlet ready for acclimatization (right).

## RESULTS AND DISCUSSIONS

The results of analysis of variance showed that there was no significant effect of the interaction between planting media and fertilizing frequency on observed variables, except for stem diameter. Furthermore, the planting media significantly affected all of observed variable while fertilizing frequency significantly affected leaf number, root number, and root length (Table 1).

When transferred to Coconut Coir media (M1), the best growth was found when the

Table 1. Results of analysis of variance (ANOVA) at  $\alpha=5\%$

Variable Measured	Calculated F Values		
	Planting Media	Fertilizing Frequency	Interaction
Plant height	11.97*	2.50ns	0.96ns
Leaf number	9.98*	3.94*	2.23ns
Root number	5.31*	8.01*	0.71ns
Stem diameter	11.60*	2.96ns	2.74*
Root length	5.78*	4.59*	0.51 ns

Note: \* = significantly different; ns = not significantly different at 5%

plantlets were fertilized every 3 days. In Rockwool (M2), there was no significant different whether the plants were fertilized every 3 days or every 4 days. Furthermore, fertilizing frequency did not really matter when the plantlets were transferred to Wood Shavings (M3). Finally, when Fern Root (M4) was used as the media for Pencil Orchid acclimatization, the best growth showed when the plants were fertilized every 4 days, resulting in 2.24 cm in stem diameter (Table 2).

However, one should find the alternative for Fern Root as this plant have been becoming endanger species. The best choice might be Coconut Coir giving 2.02 cm of stem diameter (Table 2) (Fig. 2). Saputra (2013) explained that large diameter of orchid stem would support good plant growth. The stem of Pencil orchid was green indicating the high content of chlorophyll. Suradinata *et al.* (2012) reported that black orchid grown on Fern root gave the highest plant height. Moreover, Punami *et al.*

Table 2. Effects of interaction between Planting media and fertilizing frequency on the stem diameter of Pencil Orchid (Anggrek Pensil).

Planting Media	Fertilizing frequency		
	2 HS	3 HS	4 HS
Coconut Coir (M1)	1.86 ab (A)	2.02 a (AB)	1.78 b (B)
Rockwool (M2)	1.32 b (B)	1.79 a (C)	1.81 a (B)
Wood Shavings (M3)	2.02 a (A)	1.85 a (BC)	1.91 a (B)
Fern roots (M4)	1.99 b (A)	2.15 ab (A)	2.24 a (A)

Note: \* = 2HS = every 2 days, 3HS = every 3 days, 4HS = every 4 days. Non-capitalized letter was to compare the effect of fertilizing frequency. Capitalized letter was to compare the effect of planting media.



Figure 2. Acclimated Pencil orchid using different planting media.

(2014) reported that the best fertilizing frequency for orchid was every 4 days. Previous results showed that Coconut Coir media was the best one for acclimatization of *Dendrobium* orchid (Wardani *et al.*, 2011), due to its high content of potassium, magnesium, nitrogen, and phosphor, which significantly support the growth of orchid root and leaf (Raffli and Zulman, 2011). Other orchid needed different media as reported by Diaz *et al.* (2010) using a mixed of perlite and fern for acclimatization of *Cattleya* and *Phalaenopsis*. Gutierrez-Miceli *et al.* (2008) reported that the best growth of *Guarenthe skinnerii* orchid was found when fern media was inoculated with *Trichoderma harzianum*.

Even though it was not significantly different from other media for root length, Fern Root media (M4) showed the best growth, indicated by plant height (2,94 cm), leaf number (6.22

Table 3. Effect of planting media on plant height (TT), leaf number (JD), root number (JA), and root length (PA) of Pencil Orchid

Planting Media	Variable Measured			
	Plant height (cm)	Num. leaf	Suck-ers	Root length (cm)
Coconut Coir (M1)	2.09 b	5.41 a	5.55 ab	3.14 b
Rockwool (M2)	1.89 b	4.11 b	4.55 b	2.99 b
Wood Shavings (M3)	3.60 a	5.89 a	6.52 a	4.95 a
Fern roots (M4)	2.94 a	6.22 a	5.44 a	4.29 ab

blades), and root number (6.44 cm), as presented at Table 3. Pencil orchid grown in Rockwool media and Coconut Coir was unable to stand straight, because of which affected plant height, resulting in less ability to harvest sunlight for photosynthesis. Furthermore, orchid plants that were not firmly attached to the media would not be able to absorb nutrient effectively because of which would result in less growth in general. Adi *et al.* (2014) reported that black orchid grown in fern media was found to be the tallest plant. On the other hand, when transplanted to moss media during acclimatization, *Dendrodium* orchid showed rotting symptom like browning leaf and root, which might be attributed to the high water

holding capacity of the media.

Rockwool media (M2) produced the least number of leaf blade while the rest of the media produced more leaf number (Table 3). This experiment was carried out during the rainy season which resulted in poor rooting system of the Pencil orchid for some of the media, especially Rockwool (M2) as this media held too much water so that some roots got rotten. These results confirmed previous findings reported by Astuti (2006) and Adi *et al.* (2014). Astuti (2006) reported that fresh Coconut Coir gave better growth (more leaf number) for orchid than composted Coconut Coir and composted fern. Furthermore, Adi *et al.* (2014) found that the highest number of leaves was produced by fern media while the least one was found in charcoal media.

The highest number of root (6.52) was found in Wood Shavings media (M3) while the least one (4.55) was found in Rockwool media (M2) even though it did not significantly differ from Coconut Coir media (M1), as presented in Table 3. Again, Wood Shavings showed the best root growth (4.95 cm root length) although it was similar to Fern root producing 4.29 cm of root length (Table 3). It suggested that Wood Shavings was suitable for acclimatization media of Pencil orchid. These findings were confirmed previous results reported by Yosefa *et al.* (2013). Yosefa *et al.* (2013) found that moss, coconut-husk, chopped fern, rice charcoal, bark of *trembesi*

Table 4. Effect of fertilizing frequency on leaf number, root number, and root length of Pencil Orchid.

Fertilizing frequency	Variable measured		
	Leaf number (blade)	Root number (#)	Root length (cm)
Every 2 days	4.94 b	4.86 b	3.05 b
Every 3 days	5.94 a	6.81 a	4.48 a
Every 4 days	5.33 ab	5.84 b	4.19 a

stem showed the same results for percentage of surviving plants, plant height, leaf number, tiller number, root number when used for *Dendrodium* acclimatization.

Fern root (M4) and Wood Shavings (M3)

showed superior performance compared to the others (Table 3). Yet, one should kept in mind that fern has become endangered and protected by law. It therefore, the authors suggested to use Wood Shavings (M3) for transferring plantlets of Pencil orchid during acclimatization, as this media are locally available.

Fertilizing acclimated Pencil orchid plants every 3 days and 4 days gave the highest number of leaf, the highest number of root, and the longest root (Table 4). Previous research by Sutinah and Mutryarny (2013) reported that fertilizing orchid crops every 3 days promoted shoot growth, leaf area, and leaf length of *Dendrobium* orchid. Moreover, *Dendrobium* orchid crop sprayed with leaf fertilizer (NPK 20-20-29) every 10 days at 2 g<sup>-1</sup> produced 3,38 leaf blades while the control treatment only produced 1,25 blades, suggesting that the nutrients sprayed to the crops promoted orchid growth. Sukma and Setiawati (2010) also reported that spraying leaf fertilizer Grow Quick LB every 3 days was promote better vegetative growth than spraying the orchid every 6 days.

## CONCLUSIONS

The best media for acclimatization of Pencil orchid was fern root fertilized every four days. However, if one wish to fertilize the plantlets every 3 days, the best media would be Coconut Coir and Wood Shavings..The best media for growing Pencil orchid was fern root and Wood Shavings. The best fertilizing frequency to promote Pencil orchid growth were every 3 days or every 4 days.

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