Response of the Vanda Planlet (Vanda sanderiana) to the Addition of Guano and Mycorrhizal Fertilizers in the Acclimatization Stadia

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Abstract. Acclimatization is a critical point for the success of tissue culture propagation. In this phase, in addition to maintaining the condition of the growing environment, accuracy of media composition is required. This study aims to determine the growth response of the Vanda orchid planlet (Vanda sanderiana) acclimatization phase to the dose of guano and mycorrhizal fertilizers. The design used in this study was a Factorial Randomized Block Design (RBD) with 2 factors. The first factor is the dose of guano fertilizer (without guano, 4g/plant and 8 g/plant), the second factor is mycorrhizal dose (without mycorrhizal, 10 g/plant and 20 g/plant), repeated 3 times. Growth observation variables include number of leaves, leaf length, leaf width and life percentage. The experimental results were analyzed using Analysis of Variance and followed by 1% BNJ test. The results showed that the interaction of the two treatment factors did not significantly affect all parameters. Conclusions guano and mycorrhizal fertilizers are not needed in the acclimatization phase of the Vanda orchid planlet.

1. Introduction

Orchids for the community are still the Queen of flower. Indonesia has a diversity of orchids totalling around 5,000 species [1], more than half are found in Papua and 2,000 species are found in Kalimantan and other islands [2]. Its beauty and uniqueness lead to the existence of many forest hunted people, increasingly threatened its sustainability, including one of them is Vanda (Vanda sp).

Orchid production in Indonesia is still experiencing fluctuations, monitored in 2015 as many as 21,514,789 stalks while in 2016 as many as 19,978,078 stalks, there was a value of a decrease of 7.14%. One of the production fluctuations is constrained by the availability of seedlings. Naturally the breeding of orchids lies in the character of the seeds, has a perfect embryo but does not have endosperm, so the growth of sprouts depends on the environment. This condition is a rare cause of its existence, so that the greatest potential for preserving is only through seed propagation techniques in vitro. Propagation technology is able to provide seeds in large enough quantities and is able to maintain sustainability. Constraints that need to be considered in the process are the adjustment of environmental transition from heterotrophic conditions in vitro to the autotrophic environment of the planlet obtained, among others, to some climate components (light, temperature, and humidity), planting media and nutrition (fertilization).

In nature, many orchids grow attached (epiphyte) on the branches of forest trees that contain lots of humus. In this case organic materials are naturally overhauled by microorganisms into humus that is rich in nutrients, then utilized by orchids. Guano fertilizer derived from bat manure is beneficial in
repairing and enriching soil structure because 40% contains organic material, rich in bacteria and microbiotic flora, besides being beneficial for plant growth it is also able to play a role as a natural fungicide controlling harmful nematodes in the soil [3]. According to research conducted by Yuliani and Fitrihidayati [4], administration of guano, at a dose of 3.96 g/plant, has a better effect on the growth of peanut plants compared to other compost doses. Another study conducted by Herliana et.al [8], the addition of mycorrhizal biological fertilizer at a dose of 10 g/plant was able to increase the growth and development of Dendrobium orchids. According to Rungkat [5], mycorrhizal biofertilizers are a form of mutualism symbiosis between fungi and high-level crop firing systems [6]. Mycorrhiza can produce hormones (auxin, cytokinin, gibberellins) for its host so that it can stimulate plant growth faster and plant yields will be maximal [7]. Based on this description, the experiment was carried out by adding guano and mycorrhiza to the acclimatization planting media and observing the growth and development response of the Vanda orchid plantlet.

2. Methods
This research activity was carried out in January to September 2019 at the Plant Laboratory - State Polytechnic of Jember, Jember, East Java, Indonesia at an altitude of 90 m above sea level. The material used is Vanda sp. plantlet three months old from regions in Indonesia, media (kadaka fern root), guano and mycorrhizal, pesticides and vitamins. The design used in this study was a factorial Randomized Block Design (RBD) with 2 factors. The first factor is the dose of guano fertilizer (without guano, guano 4g/plant and guano 8 g/plant), the second factor is mycorrhizal dose (without mycorrhizal, 10 g/plant and 20 g/plant), repeated 3 times. Growth observation variables include number of leaves, leaf length, leaf width and life percentage. The experimental results were analyzed using Analysis of Variance and followed by 1% BNJ test. The research was started by preparing all the ingredients, then removing the plantlet from the bottle, washing the remaining media agar, soaking it with pesticides for 10 minutes and draining it, then planting it in a media pot that had been prepared according to treatment.

3. Results and Discussion
Growth response (increase in number of leaves, length and width of orchid leaves) to the application of guano, mycorrhizae in growing media until 9 weeks after planting was not significantly different (Table 1). The growth character of the Vanda orchid plant is monopodial, growth growth is indeed slow, the type of growth on one stem is straight up, the flower comes out from the side of the stem between the two leaves. The condition of the age of 9 weeks after planting in the acclimatization phase of seedling growth still exceeds the phase of slow or unmeasured growth.

The acclimatization process is a very complex condition for plantlets against changing conditions from completely available (heterotroph), must be able to adapt in an autotroph environment. Factors that must be considered are the environment (temperature, humidity and light) and the media where it grows (water and nutrition). The humidity is cultivated between 65% - 75% so that the reduction is not too drastic compared to when in a bottle that is between 90% - 100%. In addition to environmental factors, nutrient-borne planting media also have an important influence in the acclimatization stage. The function of the planting medium conditions the growth of new roots, because the roots formed in the bottle do not function properly. The orchid growing media must meet the requirements of having good water holding capacity, good aeration and drainage. Fertilizer functions as a source of nutrients needed by plants to overcome nutritional deficiencies, especially the elements of nitrogen, phosphorus and potassium.
Tabel 1. F-test recapitulation on the growth parameters of the vanda orchid (*Vanda sanderiana*) against the dose of organic guano and mycorrhizal fertilizers at the acclimatization stage

| Observation Parameters | Treatment: | M (Mikoriza) | G (Guano) | M x G |
|------------------------|------------|--------------|-----------|-------|
| Number of Leaves (strands) 1 WAP | 1,17 ns | 0,67 ns | 0,58 ns |
| Number of Leaves (strands) 2 WAP | 1,17 ns | 0,63 ns | 0,22 ns |
| Number of Leaves (strands) 3 WAP | 2,94 ns | 1,53 ns | 0,35 ns |
| Number of Leaves (strands) 4 WAP | 3,44 ns | 3,44 ns | 3,01 ns |
| Number of Leaves (strands) 5 WAP | 3,44 ns | 3,59 ns | 1,38 ns |
| Number of Leaves (strands) 6 WAP | 1,05 ns | 0,85 ns | 0,66 ns |
| Number of Leaves (strands) 7 WAP | 1,82 ns | 0,14 ns | 0,70 ns |
| Number of Leaves (strands) 8 WAP | 0,31 ns | 0,57 ns | 0,24 ns |
| Number of Leaves (strands) 9 WAP | 0,83 ns | 2,48 ns | 2,48 ns |
| Number of Leaves (strands) 10 WAP | 2,22 ns | 7,35** | 2,40 ns |
| Number of Leaves (strands) 11 WAP | 1,46 ns | 4,28* | 0,40 ns |
| Leaf length (cm) 27 MST | 0,1 ns | 0,001 ns | 0,08 ns |
| Leaf width (cm) 27 MST | 0,08 ns | 0,009 ns | 0,04 ns |
| Life percentage (%) 12 MST | 0,30 ns | 0,08 ns | 0,08 ns |

| F Table Level | 5% | 1% |
|---------------|----|----|
|               | 3,63 | 3,63 | 3,01 |
|               | 6,23 | 6,23 | 4,77 |

Note: (ns) No significant different, (*) significant different at 5%, (**) Significant different at 1%; WAP = week after planting

Growth in the number of new leaves showed a difference after growth of 10-11 weeks after planting, but the addition of guano fertilizer remained unable to show its response, apparently the best growth in the treatment without guano fertilizer (Table 1 and Table 2). Leaves that have been fully opened each cell experiences three phases namely division, enlargement and differentiation so that the aged seedlings begin to respond to growth even though growth has not been able to support to form maximum leaves.

The effectiveness of mycorrhize is strongly influenced by the environment (abiotic factors) such as nutrient concentration, pH, water content, temperature, media and fertilizer / pesticide use as well as biotic factors such as microbial interactions, host plant root types and composition between mycorrhizal fungi [9]. Therefore, it is suspected that the level of infection from mycorrhiza tends not to be able to significantly increase plant growth. This is also supported by the research of Munir and Zulman [10], that orchids are very young, their conditions are still vulnerable to be planted independently, while Lucia [11] states, the effectiveness of mycorrhizae varies in giving an effect on growth, and not all species mycorrhizae are effective and match the characteristics of plants. Increased mycorrhizal infections are caused by an increasing number of spores formed around the root of the seedlings [12]. Because plant roots are also not maximal so the effect of mycorrhizal on growth has not been able to show a real and measurable influence, even though mycorrhiza has an important role in helping the absorption of nutrients such as P, N, K and Ca on the leaves and helps in the absorption of water by infecting the plant's root system host through expansive growth of external hyphal tissue [13]. According to Pranata [14], the lack of interaction between the two treatments is caused by the two factors that try to provide the needs of plants individually and do not synergize between one factor with another.
Table 2. Response of leaf growth of Vanda orchid (*Vanda sanderiana*) to the dosage of guano fertilizer

| Treatment   | G1 | G2 | G3 |
|-------------|----|----|----|
| Week after planting |   |    |    |
| 1           | 14<sup>a</sup> | 13<sup>a</sup> | 14<sup>a</sup> |
| 2           | 14<sup>a</sup> | 13<sup>a</sup> | 13<sup>a</sup> |
| 3           | 14<sup>a</sup> | 14<sup>a</sup> | 13<sup>a</sup> |
| 4           | 13<sup>a</sup> | 15<sup>a</sup> | 12<sup>a</sup> |
| 5           | 14<sup>a</sup> | 15<sup>a</sup> | 12<sup>a</sup> |
| 6           | 14<sup>a</sup> | 15<sup>a</sup> | 13<sup>a</sup> |
| 7           | 15<sup>a</sup> | 14<sup>a</sup> | 14<sup>a</sup> |
| 8           | 15<sup>a</sup> | 14<sup>a</sup> | 13<sup>a</sup> |
| 9           | 14<sup>a</sup> | 13<sup>a</sup> | 12<sup>a</sup> |
| 10          | 15<sup>a</sup> | 13<sup>b</sup> | 11<sup>b</sup> |
| 11          | 14<sup>a</sup> | 11<sup>b</sup> | 11<sup>b</sup> |

Note: Numbers followed by the same letter in the same line shows no real difference. G1 = without guano, G2 = guano 4g / plant and G3 = guano 8 g / plant

4. Conclusion
Application of guano and mycorrhizal fertilizer doses has not been able to increase the growth of Vanda orchids (*Vanda sanderiana*), namely the number of leaves, leaf length, leaf width and life percentage. Therefore it is necessary to do a re-study in the use of guano and mycorrhizal fertilizers using different doses supported by the appropriate environment at the orchid acclimatization stage so that later it will be able to increase the growth of orchid seedlings.

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