Utilization of Shoot Multiplication Medium for In-Vitro Conservation of *Vanda tricolor*

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**Abstract.** *Vanda tricolor* is an orchid endemic to Mount Merapi in Yogyakarta Indonesia, whose existence is threatened due to the eruption and exploitation of its original habitat. This orchid conservation effort can be done through in vitro culture. Multiplication is one of the in vitro culture techniques used for propagation of the *Vanda tricolor* orchid by growing explants on various types of multiplication medium. This study aims to determine the effect of medium types on the multiplication of *Vanda tricolor* orchids. The method used in this research is a laboratory research method prepared with a completely randomized design, with the treatment of the growth medium consisting of 5 types, namely New Dogashima Medium, Murashige and Skoog, Vacint and Went, leaf fertilizer and liquid organic fertilizer medium. All mediums were supplemented with 150 ml / l coconut water, 150 g / l banana extract, and 0.2 g / l activated charcoal. Each treatment was repeated 3 times, each replication consisted of 3 samples. The results showed that liquid organic fertilizer medium is the best medium for multiplication of *Vanda tricolor* orchids as indicated by the addition of the shoots.

**Keywords:** *Vanda tricolor*, Multiplication, Medium, Shoot

1. **Introduction**

*Vanda tricolor* is an endemic orchid species located on the hillside of Mount Merapi, Sleman Regency, Yogyakarta, Indonesia. This orchid has white flowers with reddish-purple spots. The *Vanda tricolor* orchid flower has a diameter of 10 cm and has a distinctive aroma (fragrant), this aroma is related to the height of the orchid plant. The higher the altitude where the orchid grows, the more fragrant the orchid will be [1]. The population of *Vanda tricolor* orchids on the slopes of Mount Merapi is decreasing and is almost extinct, this is due to hot cloud bursts from Mount Merapi. This hot cloud burst caused 80% of the original habitat of the *Vanda tricolor* orchid to be damaged and scorched by the hot cloud burst. In addition, there is a lack of public awareness in the Mount Merapi area about the importance of preserving orchids, so that many people take these plants to sell to customers and as a result, the population of *Vanda tricolor* orchids is decreasing and it is increasingly difficult to find them. The dwindling existence of this orchid has prompted means to restore the plant which has become an ornamental plant typical of Mount Merapi [1]. Several ways have been done to re-develop *Vanda tricolor* orchid plants, one of which is using conventional propagation. However, traditional proliferation methods performed by farmer cohorts in the Mount Merapi area have not been effective, moreover, this plant is needed in very large quantities. Therefore, it is necessary to have a *Vanda tricolor* orchid propagation technique, one technique that can be used is in vitro propagation.
Multiplication of *Vanda tricolor* has been carried out by Ningsih [2] namely New Dogashima Medium with the addition of 150 ml/l coconut water + 150 g/l banana extract to encourage the growth of new shoots, increase in shoot height, emergence of roots, and a high percentage of explant life in this medium. However, New Dogashima Medium is quite expensive, so it is necessary to try using another medium that is cheaper and easier to obtain, such as Growmore™ Leaf Fertilizer and DI Grow Liquid Organic Fertilizer. In addition, other synthetic mediums such as Murashige & Skoog and Vacint & Went should also be tried for shoot multiplication of *Vanda tricolor*. Alternative media such as Growmore™ Leaf Fertilizer and DI Grow Liquid Organic Fertilizer are used to minimize costs for in vitro culture media. Kharisma [3] stated that the use of Growmore™ leaf fertilizer influenced the growth of the number of roots, increasing the number of seedlings for black orchids. Meanwhile Yusuf and Indrianto [4] stated that the use of Liquid Organic Fertilizer on Vanda limbata Blume x *Vanda tricolor* Lindl orchids resulted in a greater number of roots, number of shoots, and number of leaves compared to the use of VW medium. Purwanto et al. [5] stated that the use of liquid organic fertilizer in coconut plant increase leaf chlorophyll and nitrogen content which affects the photosynthesis rate. Adequacy of N in the leaves will sustain the synthesis of protein and amino acids which important for growth of vegetative and generative. This study aims to determine the best type of medium for multiplication of *Vanda tricolor* orchids in vitro.

2. Materials and Methods

This study used laboratory research methods arranged in a completely randomized design (CRD), with 5 types of growing medium, namely New Dogashima Medium (NDM), Murashige and Skoog (MS) medium, Vacint and Went (VW) medium, Growmore™ leaf fertilizer medium and DI Grow Liquid Organic Fertilizer medium. All treatment mediums were supplemented with 150 ml/l coconut water, 150 g/l banana extract, and 0.2 g/l activated charcoal. Each treatment combination was repeated 3 times, and each replication consisted of 3 samples.

2.1. Medium Preparation

The medium was prepared by mixing basic medium (NDM, MS, VW, Foliar fertilizer-Growmore™ or Liquid Organic Fertilizer-DI Grow), sucrose, vitamins, activated charcoal, Plant Preservative Medium. All treatment mediums were supplemented with 150 ml/l coconut water, 150 g/l banana extract, and 0.2 g/l activated charcoal. The pH of the medium was adjusted, then Pythagel was used as a solidifier in solid medium and without Phytagel for liquid medium. The medium was sterilized using an autoclave at a temperature of 121°C and a pressure of 1 psi for 20 minutes. The sterile medium was placed in the incubation room until used.

2.2. Explant Preparation and Inoculation

This research used sterile 1 year old shoots of *V. tricolor* as explants. The inoculation was carried out by taking the shoots from the explant source bottle and placing them in a petri dish, then separating the shoots to be used. After that, the shoots to be used were sterilized using 1% sodium hypochlorite by shaking for 3 minutes. After that, the orchid shoots were sterilized again using distilled water 3 times. The orchid shoots were then drained using a petridish that had been coated with filter paper. Each bottle was planted with one explant. Furthermore, the bottle is covered with aluminum foil, tied with a rubber band and covered with a wrapper. Then the culture bottles containing the explants were placed on the incubation rack, in the incubation room at a temperature of 20-28°C.

2.3. Parameter Observed and Data Analysis

Observations were made from the beginning to the 8th week after inoculation. Parameters observed were: Percentage of viable explants (%), percentage of contaminated explants (%), percentage of vitrified explants (%), percentage of browning explants (%), percentage of explants sprouting (%), time of shoot emergence, increase in number of shoots, increase in shoot height and shoot morphology. Data were analysed using analysis of variance with an error rate of 5%.
3. Results and Discussion
The success of subculture on orchid explants was indicated by the parameters of the percentage of viable explants, the increase in shoot height and the emergence of new shoots.

3.1. Percentage of viable explants, contaminated explants, and vitrified explants
Explants were declared alive if they did not experience vitrification, browning, and contamination. Meanwhile, explants that experienced vitrification, browning, and contamination would cause the explants not to grow or cause the explants to die. The results of observations on the percentage of viable explants, vitrification and browning are presented in Table 1.

| Treatment                                | No. of Viable Explant (%) | No. of Browning Explant (%) | No. of Vitrified Explant (%) |
|------------------------------------------|---------------------------|-----------------------------|-----------------------------|
| New Dogashima Medium                     | 33.33 ± 19.24             | 44.44 ± 11.11               | 22.22 ± 22.22               |
| Murashige & Skoog Medium                 | 33.33 ± 0.00              | 33.33 ± 19.24               | 33.33 ± 19.25               |
| Vacint & Went Medium                     | 55.56 ± 11.11             | 22.22 ± 11.11               | 11.11 ± 11.11               |
| Growmore™ Foliar Fertilizer Medium       | 22.22 ± 11.11             | 22.22 ± 11.11               | 55.56 ± 22.22               |
| DI Grow Liquid Organic Fertilizer Medium| 55.56 ± 11.11             | 22.22 ± 11.11               | 22.22 ± 11.11               |

Note: The data displayed is the mean value ± Standard Error (SE). All mediums were supplemented with 150 ml/l coconut water, 150 g/l banana extract, and 0.2 g/l activated charcoal.

Based on Table 1, the percentage of viable explants on VW medium and DI Grow liquid organic fertilizer was relatively the highest at 55.56%, compared to other treatments. Explants in other medium treatments had a viable percentage of less than 50% because the explants experienced browning and vitrification, causing the explants to die. The explant size affects the success of in vitro culture. Smaller explants will affect the physiological function of the explants, so that the explants cannot survive and die [6]. The small size of the explants had metabolites that could not match the growth regulators given to the treatment medium, so that the explants did not respond to growth and died. This is also in accordance with the statement Fadhilah et al. [7] that viable explants were influenced by the type, age and size of the explants used. Contamination only occurred in the VW medium treatment of 11.11%. The contamination is very low because the explants used are sterile explants.

The data on Table 1 showed that the highest relative browning percentage of 44.44% occurred in NDM medium, while the VW medium treatment, Growmore™ Leaf Fertilizer and POC DI Grow medium treatment were the treatments with the lowest browning percentage at 22.22%. Browning in explants started from the appearance of brown color on the edges of the explants which were injured during the explant inoculation process. This is in accordance with the statement of Hutami [8] that the occurrence of browning is a process of adaptive changes in explants that often occur due to physical and biochemical influences. Several studies also mentioned that browning in explants was triggered by phenolic compounds followed by oxidase enzyme activity and polymerization [8,9]. Phenylalanine ammonia lyase is one of the phenylpropanoid enzymes that affects browning. Wounds on the explants will trigger stress on the explants, causing Phenylalanine ammonia lyase activity to increase, followed by the production of phenylpropanoids.

Growmore™ Leaf Fertilizer Medium was the treatment with the highest percentage of vitrified explants at 55.56%. Changes in the color of the explants to a transparent white color indicated that the explants lost leaf green matter which was probably caused by growth regulators and explants adaptation factors to the medium. This is also explained by Ajijah et al. [10] which states that one
form of adaptation between explants and the medium is the loss of chlorophyll. Adaptation between explants and the medium is thought to be the physiological response of the explant to survive or the explant’s response to death. In this study, the process of explants vitrified was associated with changes in explants that initially experienced browning or browning. These explants initially browned and then slowly turned vitrified.

3.2. Percentage of explants sprouting
The results of the data analysis of the percentage of explants sprouting *Vanda tricolor* are presented in Figure 1.

![Figure 1](image.png)

**Figure 1.** The effect of medium type on the percentage of explants sprouting *Vanda tricolor* at 8 weeks after planting

The data on Figure 1 showed that the DI Grow liquid organic fertilizer medium treatment resulted in the highest relative percentage of explants sprouting at 22.22%. This is due to the content of endogenous cytokinins in DI Grow liquid organic fertilizer which can stimulate and increase the growth of new shoots quickly. DI Grow liquid organic fertilizer also contains nitrogen which can stimulate the growth and vegetative growth of explants. The percentage of explants sprouted was also influenced by the addition of 150 ml/l coconut water and 150 g/l banana extract in the treatment medium, because 150 ml/l coconut water and 150 g/l banana extract contained growth regulators in the form of cytokinins that can induce shoots. Based on the results of research from Seswita [11], the addition of coconut water as a substitute for synthetic growth regulator Benzyl Adenine resulted in the best multiplication of *Curcuma zanthorrhiza* shoots in vitro within 2 months.

3.3. Time of shoot emergence
The time of shoot emergence is one of the parameters that shows the speed at which shoots appear since the beginning of planting. The time of emergence of shoots was observed every week. The results of the analysis of the emergence of shoots are presented in Figure 2.

Based on Figure 2, the relatively fastest shoot emergence time occurred 2 weeks after planting on NDM medium. According to Fadhilah et al. [7], NDM contains vitamins and complex organic materials such as amino acids and other organic materials that promote the growth of explants sprouting on orchid shoots. The emergence of shoots is indicated by the growth of buds on the side of the explant. The speed of the emergence of shoots is also influenced by the growth regulators used in the treatment medium. This is in accordance with the statement of Watimena [12] that the use of growth regulators affects cell division. 150 ml/l coconut water and 150 g/l banana extract were added to the treatment medium as exogenous growth regulators because both compounds contained cytokinins and auxins. Cytokinins interact with auxin in stimulating cell division, thereby accelerating shoot growth in explants [13]. The difference in the time of shoot emergence is also influenced by the different conditions and sizes of different explants (large and small), so that each explant has a
different response in absorbing nutrients for the regeneration process. The smaller the explant used, the longer it takes for the shoots to initiate new shoots [14].

![Figure 2](image1.png)

**Figure 2.** The effect of medium on the time of shoot emergence of *Vanda tricolor* at 8 weeks after planting

3.4. *Increase in number of shoots*

The increase in the number of shoots is a parameter that shows the number of new shoots that appear in each treatment medium. Observation of the number of shoots was carried out to see the effect of the medium used in the process of shoot formation on *Vanda tricolor* explants. The increase in the number of shoots is one of the successes of plant multiplication. The increase in the number of *Vanda tricolor* shoots is shown in Figure 3.

![Figure 3](image2.png)

**Figure 3.** The effect of medium on the increase in the number of shoots of *Vanda tricolor* at 8 weeks after planting

The data in Figure 3 showed that the relatively highest increase in the number of *Vanda tricolor* shoots of 0.33 was obtained in the DI Grow liquid organic fertilizer medium. This is because the DI Grow liquid organic fertilizer medium contains nitrogen as well as endogenous and exogenous cytokinins derived from the addition of 150 ml/l coconut water. Tuhuteru et al. [15] stated that cytokinins in coconut water promote cell division thereby inducing the formation of lateral shoots. Coconut water also affects nucleic acids that affect protein synthesis and enzyme activity in cell differentiation for shoot formation of D. anosmum orchids. However, MS and VW medium did not increase the number of shoots up to 8 MST. This was because MS medium and VW medium did not contain endogenous
growth regulators in the medium. The addition of exogenous growth regulators in the form of 150 ml/l coconut water has not been able to increase the number of shoots on MS and VW medium. DI Grow liquid organic fertilizer medium also contains the elements N, P, K, Fe, S, and Zn which are needed in the process of forming new shoots in explants [16]. Meanwhile, Sahtiana et al. [17] stated that DI Grow liquid organic fertilizer can replace Vacin and Went medium as an alternative medium for increasing the number of shoots in *Vanda tricolor*.

3.5. Increase in shoot height

The results of the analysis of the increase in shoot height of *Vanda tricolor* are presented in Figure 4. Based on Figure 4, Growmore™ foliar fertilizer medium resulted in the highest relative shoot height increase (4.56 mm) compared to other treatments. Growmore™ foliar fertilizer contains nitrogen, phosphorus, and potassium with a composition of 32:10:10 or a percentage of 32% nitrogen, 10% phosphorus, and 10% potassium. Growmore™ foliar fertilizer contains nitrogen elements needed by explants. The nitrogen content in Growmore™ leaf fertilizer can stimulate the growth of explant shoot height. In addition, the content of cytokinins and auxins in coconut water and banana extract can stimulate plant cell division. The addition of banana extract and coconut water to all treatment mediums could increase shoot height growth. Banana extract contains a high source of carbohydrates used as a source of carbon and energy in explants can influence the growth of shoot height [18]. Meanwhile, according to Djajanegara [19], the addition of 150 ml/l of coconut water to the treatment medium stimulated the growth of stems and roots of *Phalaenopsis* orchid explants.

![Figure 4](image_url)

*Figure 4.* The effect of medium on the increase in shoot height of *Vanda tricolor* at 8 weeks after planting

3.6. Shoot morphology

Observations on the development of shoot morphology were carried out to see the development of explants, whether the explants could adapt, grow, and develop on the tested treatment medium. Observations on the development of explants were carried out using a microscope at 4 and 8 weeks after planting. The results of observations of the development of shoot morphology are presented in Fig. 5.

Based on Fig. 5, each medium caused different responses to explants during 8 weeks of observation. At the 4th week of observation, the explants on NDM medium changed color to brownish until the 8th week. Color changes in explant leaves also occurred on MS and VW medium. This change was caused by browning of explants due to the presence of phenolic compounds. Phenol compounds that accumulate in cells cause damage and cell death so that explants browning. This is also because orchids are one of the plants that easily release phenolic compounds, so that orchid explants are susceptible to browning. Meanwhile, explants on Growmore™ leaf fertilizer medium and DI Grow liquid organic fertilizer medium did not change color for 4 weeks of observation.
Figure 5. Morphological development of *Vanda tricolor* orchid shoots on all mediums at 4 and 8 weeks after planting

In the 8th week of observation, explants on Growmore™ leaf fertilizer medium changed color to yellowish green. Changes in leaf color to yellowish green indicate damage to the explant tissue, so that the ability of chlorophyll to carry out activities decreases, and will cause the death of the explants. Meanwhile, the explants in the DI Grow liquid organic fertilizer medium increased, the number of shoots and root size increased. This is because the content of cytokinins and auxins in the liquid organic fertilizer medium DI Grow stimulates shoot formation, cell enlargement, growth and root formation in explants. The results of this study are also supported by the results of research by Isnawan et al. [20] that the root length is influenced by the chlorophyll content in rice.

4. Conclusion

Based on the results of the study, DI Grow liquid organic fertilizer was the best medium for multiplication of *Vanda tricolor* orchids as indicated by the increase in the number of shoots.

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