Utilization of foliar fertilizer as an alternative medium for enlargement of Vanda orchid plantlets before acclimatization

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Abstract: This study aims to obtain an appropriate and inexpensive alternative media combination for the growth of Vanda hybrid orchids prior to acclimatization. Experiment using Factorial Randomized Block Design. The first factor is the type of media (Vacin - Went and foliar fertilizer ) and the second factor is the addition of organic matter (coconut water composition, sweet corn composition, tomato composition, BAP, NAA and without organic matter or PGR). The results showed that the interaction of treatment on plant height was only seen at 18 weeks after treatment, while the number of leaves could be seen earlier at 16 weeks after treatment. The independent effect of organic matter and PGR on the number of leaves has been seen since the age of the plant 10 weeks after treatment, while the plant height was only seen 16 weeks after treatment. Independent treatment of growing media had no effect on height and number of leaves up to 18 weeks after planting. The use of foliar fertilizer media with the type of organic material composition being tested can be suggested as an alternative growth medium for plantlet growth, not significantly different from vacin-went with BAP media.

1. Introduction

The orchid (Orchidaceae) business is a solution that should be considered because it is a plant that has high demand. Market demand is never empty, one of the favorite types of orchids in Indonesia is Vanda which has 5000 species with a variety of flower shapes and colors [1]. Based on data from the Central Statistics Agency for ornamental plants, orchid production in Indonesia has fluctuated, orchid production was 20,777,071 stalks (2013), 19,739,627 stalks (2014), 21,514,789 stalks (2015), in 2016 as many as 19,978,078 stalks, in 2017 as many as 20,045,577 stalks, in 2018 24,717,840 stalks, and in 2019 as many as 18,608,657 stalks.

The solution to increase the production of Vanda orchids is still more appropriate through in-vitro propagation, growing explan ts from one part of the plant under aseptic and accentic conditions. The main need for in vitro propagation is the presence of a growth medium equipped with complete nutrition, growth regulators and controlled lighting. This means that the determinants of the success of tissue culture techniques are the suitability of the explant characters with the nutrient composition of the culture media, aseptic/acsenic environmental conditions and the presence of growth regulators. The use of growth regulators (PGR) in tissue culture is very important, namely controlling the morphogenesis of the formation and development of shoots, roots, and callus. The formation of shoots generally uses cytokinins, while the formation of roots or callus uses auxin. [2]. PGR consists of natural and synthetic PGR. Natural ZPT can be used by Ambon banana, sweet corn, tomatoes, coconut water, and potatoes, replacing the role of synthetic ZPT Benzyl Aamino Purine (BAP) and Naphthalene Acetic Acid (NAA).
VW media (Vacin-Went) formulated by E. Vacin and F. Went in 1949 consisted of macro and micro nutrients in the form of inorganic salts suitable for the growth and development of orchids in vitro. The high cost of chemicals spurs the idea of using alternative materials to develop, the use of foliar fertilizers on the market as a nutrient source, for example Gandasil D. Consideration by taking into account that in the vegetative growth phase a lot of fertilizers with nitrogen content are needed as a protein constituent for cell division. Utilize organic materials in the manufacture of media compositions. Alternative substitute for synthetic growth regulators (auxins/cytokinin's) using tomato water (Solanum lycopersicum) contains the hormone auxin which functions to accelerate shoot growth in the process of cell division. According to [3] the content of auxin in tomato extract can stimulate organogenesis, somatic embryogenesis and shoot growth in micropropagation in various plant species. The content of auxin in tomatoes in some plants needs to be balanced with the provision of synthetic cytokinins (BAP). Cytokinins function to stimulate shoot growth, cell metabolism, cell division, reduce apical dominance, and encourage lateral shoot initiation. Based on the results of research [4] on the number of shoots given BAP 3 ppm + 100 ml of tomato extract in Vanda orchid culture media showed a positive response. Likewise, coconut water and sweet corn in several studies were able to affect cell elongation, tissue differentiation and initiation of root formation, while potatoes and bananas, apart from containing carbohydrates, also contributed some suitable vitamin content to support plantlet growth and development, so these alternatives were tried in this research.

2. Methods
The experiment was conducted at the Laboratory of Culture in Vitro at the State Polytechnic of Jember Laboratory in Jember (80 m above sea level). Planting material is plantlets from the propagation of Sanderiana vanda orchid (hybrid) 1 month old in media 0. Vacin Went media materials, foliar fertilizer (Gandasil D), BAP, NAA, sugar and culture glass The environmental design used was Random Group Design, consisting of two factors. The first factor is the type of planting media (M1=Vacin-Went and M2=Gandasil D). The second factor is six organic matter composition (Z1. Potato 150gr/L+ Banana 150gr/L+Coconut Water 150ml/L, Z2= Potato 150gr/L+ Banana 150gr/L+ Sweet Corn 100ml/L, Z3= Potato 150gr/L+ Banana 150gr /L+Tomato 100ml/L, Z4 = BAP 1 ppm, Z5= NAA 0.1 ppm, Z6 = media without organic matter or PGR). The experiment was repeated 3 times. Each trial unit consists of 3 bottle, each bottle consists 5 eksplan. Variables observed included survival (%), plant height (cm), number of leaves (strands), number of shoots. Data were analyzed using the F test at 5% with the BNJ test at 5%. The research was started by preparing all the ingredients, plant in media 0 for a month before entering the treatment media.

3. Result and Discussion
3.1. Plant height
The increase in plant height describes the pattern of growth rate during the culture period. The growth and development of vanda orchid seedlings depends on the ability of these plants to respond to the treatment of planting media. The interaction of treatment types of media (composition of nutrient and hormone) in this experiment was observed at week 18, while the role of a single factor hormone had actually been seen since the observation at week 16. VW media with BAP remained a good composition of media which had a positive effect on growth. tall. Plant height growth using organic matter composition of sweet corn on VW media had the same effect as the treatment without PGR and synthetic PGR (BAP and NAA) (see Table 1). Treatment of coconut water, sweet corn and BAP, gave no significant difference in height growth on Gandasil media. Tomato extract gave the lowest height growth not significantly different on VW and Gandasil media (Table 1). In Table 2, the single effect without ZPT had no significant effect on growth height with synthetic PGR (NAA and BAP). The best plant height variable in the 1 ppm BAP treatment was 1.47 cm, while the lowest plant height variable was in the tomato composition treatment (Potato 150 gr/L + Banana 150 gr/L + Tomato 100ml/L. At 18 WAP observation, VW media showed that media is suitable for orchid growth because it has been formulated for orchid explants, while Gandasil D media is an alternative medium used for orchid culture. According to research from [5] the use of VW media and several growth regulators showed different results. on the
formation of organogenesis of Dendrobium orchid explants. Plantlet responses tended to show fresh green leaves and wider growth of shoot organs, leaves and roots that grew more, while the roots grew longer.

Table 1. Plant height in the treatment of media and growth regulators

| Treatment | Plant height (cm) |
|-----------|-------------------|
|           | (week after planting) |
|           | 2     | 4     | 6     | 8     | 10    | 12    | 14    | 16    | 18    |
| M1Z1      | 0.51  | 0.54  | 0.55  | 0.57  | 0.59  | 0.66  | 0.71  | 0.74  | 0.76 a |
| M1Z2      | 0.48  | 0.51  | 0.54  | 0.56  | 0.58  | 0.60  | 0.81  | 1.21  | 1.35 bcd |
| M1Z3      | 0.67  | 0.67  | 0.68  | 0.69  | 0.73  | 0.73  | 0.73  | 0.74  | 0.84 ab |
| M1Z4      | 0.74  | 0.82  | 0.89  | 0.98  | 1.02  | 1.06  | 1.30  | 1.70  | 1.78 e  |
| M1Z5      | 0.37  | 0.56  | 0.67  | 0.82  | 1.10  | 1.14  | 1.21  | 1.37  | 1.50 cde |
| M1Z6      | 0.55  | 0.61  | 0.70  | 0.86  | 1.07  | 1.33  | 1.40  | 1.51  | 1.67 de |
| M2Z1      | 0.51  | 0.57  | 0.63  | 0.66  | 0.75  | 0.79  | 0.93  | 1.07  | 1.14 abc |
| M2Z2      | 0.51  | 0.58  | 0.61  | 0.64  | 0.69  | 0.74  | 0.94  | 1.11  | 1.15 abc |
| M2Z3      | 0.48  | 0.50  | 0.52  | 0.54  | 0.59  | 0.65  | 0.79  | 0.98  | 1.01 abc |
| M2Z4      | 0.50  | 0.55  | 0.58  | 0.62  | 0.74  | 0.85  | 1.03  | 1.10  | 1.15 abc |
| M2Z5      | 0.55  | 0.64  | 0.69  | 0.71  | 0.78  | 0.84  | 1.04  | 1.22  | 1.39 cde |
| M2Z6      | 0.49  | 0.54  | 0.57  | 0.60  | 0.64  | 0.68  | 0.88  | 1.04  | 1.07 abc  |

Note: Numbers followed by different letters in one column show a significant difference based on the DMRT test at the -5% level. M1 (Media VW), M2 (Media Gandasil D 2gr/L), Z1 (Potato 150gr/L+ Banana 150gr/L+Coconut Water 150ml/L), Z2 (Potato 150gr/L+Banana 150gr/L+Sweet Corn 100ml/L), Z3 (Potato 150gr/L+ Banana 150gr/L+Tomato 100ml/L), Z4 (BAP 1 ppm), Z5 (NAA 0.1 ppm), Z6 (Control)

Table 2. Plant height in the single treatment of growth regulators and media treatment

| Treatment | Plant height (cm) |
|-----------|-------------------|
|           | (week after planting) |
|           | 2     | 4     | 6     | 8     | 10    | 12    | 14    | 16    | 18    |
| Z1        | 0.51  | 0.56  | 0.59  | 0.62  | 0.67  | 0.73  | 0.82  | 0.95  | 0.95 a |
| Z2        | 0.50  | 0.54  | 0.58  | 0.60  | 0.63  | 0.67  | 0.87  | 1.25  | 1.25 ab |
| Z3        | 0.58  | 0.59  | 0.60  | 0.61  | 0.66  | 0.69  | 0.76  | 0.92  | 0.92 a |
| Z4        | 0.62  | 0.69  | 0.74  | 0.80  | 0.88  | 0.95  | 1.17  | 1.47  | 1.47 b  |
| Z5        | 0.46  | 0.60  | 0.68  | 0.76  | 0.94  | 0.99  | 1.13  | 1.44  | 1.44 b  |
| Z6        | 0.52  | 0.57  | 0.64  | 0.73  | 0.85  | 1.01  | 1.14  | 1.37  | 1.37 b  |
| M1        | 0.55  | 0.62  | 0.67  | 0.75  | 0.85  | 0.92  | 1.03  | 1.21  | 1.32   |
| M2        | 0.51  | 0.56  | 0.60  | 0.63  | 0.70  | 0.76  | 0.93  | 1.09  | 1.15   |

Note: Numbers followed by different letters in one column show a significant difference based on the DMRT test at the -5% level. M1 (Media VW), M2 (Media Gandasil D 2gr/L), Z1 (Potato 150gr/L+ Banana 150gr/L+Coconut Water 150ml/L), Z2 (Potato 150gr/L+Banana 150gr/L+Sweet Corn 100ml/L), Z3 (Potato 150gr/L+ Banana 150gr/L+Tomato 100ml/L), Z4 (BAP 1 ppm), Z5 (NAA 0.1 ppm), Z6 (Control)
3.2. Number of leaves
Leaves are vegetative organs, their growth is influenced by the nitrogen content in the media. In addition, leaves are the site of photosynthesis which is important in plant growth, namely the process of forming carbohydrates from CO2 and H2O with the help of sunlight. The large number of leaves indicates better growth of explants [6]. The increase in the number of leaves is influenced by genotype factors and the environment itself. Plant growth and development is supported by various external and internal plant factors that work together in a harmonious balance [7].

| Treatment  | 2     | 4     | 6     | 8     | 10    | 12    | 14    | 16    | 18    |
|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| M1Z1      | 2.37  | 2.37  | 2.37  | 2.43  | 2.56  | 2.70  | 2.74  | 2.85  | 3.07  |
| M1Z2      | 2.15  | 2.30  | 2.44  | 2.74  | 3.20  | 3.55  | 3.74  | 6.11  | 7.67  |
| M1Z3      | 2.72  | 2.72  | 2.72  | 2.81  | 2.85  | 2.85  | 2.85  | 2.85  | 2.85  |
| M1Z4      | 2.65  | 2.85  | 3.24  | 3.65  | 5.02  | 5.69  | 6.61  | 9.28  | 10.61 |
| M1Z5      | 1.94  | 2.33  | 2.67  | 2.91  | 3.54  | 3.74  | 4.02  | 4.72  | 6.33  |
| M1Z6      | 3.72  | 3.81  | 4.07  | 4.85  | 5.94  | 6.36  | 6.67  | 7.42  | 8.50  |
| M2Z1      | 2.07  | 2.28  | 2.80  | 3.24  | 3.70  | 3.96  | 4.98  | 6.74  | 7.43  |
| M2Z2      | 2.07  | 2.20  | 2.50  | 2.69  | 3.43  | 3.63  | 4.72  | 5.33  | 6.02  |
| M2Z3      | 1.89  | 2.02  | 2.13  | 2.31  | 3.02  | 3.22  | 3.41  | 4.57  | 4.60  |
| M2Z4      | 2.52  | 2.78  | 3.41  | 4.00  | 4.02  | 4.48  | 5.11  | 6.56  | 7.07  |
| M2Z5      | 2.31  | 2.50  | 3.00  | 3.37  | 4.11  | 4.46  | 4.89  | 8.00  | 8.20  |
| M2Z6      | 2.26  | 2.37  | 2.76  | 2.93  | 3.81  | 4.30  | 5.43  | 7.48  | 7.72  |

Table 3 Number of leaves in the interaction of media and growth regulators

Based on Table 3 the interaction of media and growth regulators showed significantly different results at 16 WAP and 18 WAP. The best number of leaves was treated with VW media and 1 ppm BAP as many as 10.61 leaves, while the lowest number of leaves was found in VW media treatment and tomato composition was 2.85 leaves. The increase in plant height was followed by an increase in the number of leaves. According to research from [8] giving BAP in low concentrations (1 ppm) can accelerate the growth of Vanda orchid shoots compared to high concentrations (See Table 3). Exogenous PGR is used to provide a balance for endogenous hormones so that they can affect the dedifferentiation of meristematic cells again, influence the response of shoot morphogenesis and root formation, and determine the physiological response as a driver of cell division and elongation during multiplication, and during ([9])

Based on Table 4. the number of leaves in the treatment of growth regulators showed results that were not significantly different at 2 WAP - 8 WAP, significantly different at 10 WAP - 18 WAP. The best treatment for the variable number of leaves was Z4 (BAP 1 ppm) which was 8.84 strands, while the lowest treatment was the coconut water composition treatment of 5.25 strands. BAP is a growth regulator of the cytokinin group, where one of the purposes of using cytokinins is to induce the formation of the main organs. Observation of the number of leaves variable with the media factor showed results that were not significantly different from 2 WAP - 18 WAP. In the vegetative phase, it is necessary to provide fertilizer with nitrogen (N) content, because the element N is the main ingredient for compiling proteins needed for cell division. Young orchid plants fed fertilizer with N content showed better and faster growth. Elemental N is the main ingredient of amino acids, proteins, nucleic acids, various
enzymes and as a greening agent [10]. One of the fertilizers containing N is Gandasil D. Leaf fertilizer Gandasil D contains nutrients N (20 %), P (15 %), K (15 %) and additional micro elements Mg, Mn, B, Cu, Co, and Zn

### Table 4. Number of leaves on single treatment of growth regulators and media treatment

| Treatment   | Number of leave (sheet) (week after planting) |
|-------------|-----------------------------------------------|
|             | 2    | 4    | 6    | 8    | 10   | 12   | 14   | 16   | 18   |
| Z1          | 2.22 | 2.32 | 2.58 | 2.83 | 3.13 a | 3.33 a | 3.86 a | 4.80 ab | 5.25 ab |
| Z2          | 2.11 | 2.25 | 2.47 | 2.71 | 3.31 ab | 3.59 a | 4.23 ab | 5.72 abc | 6.84 bc |
| Z3          | 2.31 | 2.37 | 2.43 | 2.56 | 2.94 a | 3.04 a | 3.13 a | 3.71 a | 3.73 a |
| Z4          | 2.58 | 2.81 | 3.32 | 3.82 | 4.52 bc | 5.08 b | 5.86 bc | 7.92 c | 8.84 c |
| Z5          | 2.13 | 2.42 | 2.83 | 3.14 | 3.82 abc | 4.10 ab | 4.45 abc | 6.36 bc | 7.27 bc |
| Z6          | 2.99 | 3.09 | 3.42 | 3.89 | 4.88 c | 5.33 b | 6.05 c | 7.45 c | 8.11 c |
| M1          | 2.59 | 2.73 | 2.92 | 3.23 | 3.85 | 4.15 | 4.44 | 5.54 | 6.51 |
| M2          | 2.19 | 2.36 | 2.77 | 3.09 | 3.68 | 4.01 | 4.76 | 6.45 | 6.84 |

Note: Numbers followed by different letters in one column show a significant difference based on the DMRT test at the -5% level. M1 (Media VW), M2 (Media Gandasil D 2gr/L), Z1 (Potato 150gr/L+Banana 150gr/L+Coconut Water 150ml/L), Z2 (Potato 150gr/L+Banana 150gr/L+Sweet Corn 100ml/L), Z3 (Potato 150gr/L+Banana 150gr/L+Tomato 100ml/L), Z4 (BAP 1 ppm), Z5 (NAA 0.1 ppm), Z6 (Control)

#### 3.3. Percentage of life

Based on Figure 1, the live percentage variable at 18 WAP showed the highest percentage of survival in the M1Z3 treatment (VW Media and Potatoes 150 gr/L + Bananas 150 gr/L + Tomatoes 100 ml/L) of 89%. According to research from [11] giving 100 ml/L tomato extract added to the media can meet the needs of explants to form shoots in a fast time and a high percentage of life. Tomato extract contains the hormone auxin which plays a role in the formation of primordial shoot cells which causes cell elongation. Auxin hormone produced from tomato extract causes primary cell wall material to be produced and transferred to both cell walls, then the cell structure is stretched so that it will form more cell walls. Tomato extract contains the IAA auxin hormone so that it can accelerate plant growth and increase growth potential.

**Picture 1.** The percentage of explant life due to the influence of media and growth regulators

Note: M1 (Media VW), M2 (Media Gandasil D 2gr/L), Z1 (Potato 150gr/L+Banana 150gr/L+Coconut Water 150ml/L), Z2 (Potato 150gr/L+Banana 150gr/L+Sweet Corn 100ml/L), Z3 (Potato 150gr/L+Banana 150gr/L+Tomato 100ml/L), Z4 (BAP 1 ppm), Z5 (NAA 0.1 ppm), Z6 (Control)
4. Conclusion
The real interaction between the media and the best growth regulators on plant height variables was in the VW and BAP 1 ppm media treatment with an average plant height of 1.78 cm. The variable number of leaves was in the treatment of VW and 1 ppm BAP media as many as 10.61 strands, single VW and Gandasil D media were not significantly different.

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