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Potential of Foliar Fertilizer and Organic Additives for *Vanda tricolor* Protocorm Regeneration

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ABSTRACT

Vanda tricolor orchid is propagated by in vitro culture using Vacin & Went (VW) media. The price of this media is quite high, so research is needed on alternative media that can replace VW media. The objectives of this study were to (1) determine the effect of foliar fertilizer concentration and addition of organic materials on in vitro protocorm regeneration in to planlets, (2) obtain a combination of foliar fertilizer concentration and organic materials that can be used to subtitude VW as a medium for Vanda tricolor protocorm regeneration. The treatments tested were the concentration of NPK foliar fertilizer (21:21:21), namely: 1 g/L, 1.5 g/L, 2 g/L, and 2.5 g/L and various organic materials, namely: no organic material, banana, potato, banana+potato. VW was use as a control. Data analysis to compare VW control with all treatments used Orthogonal Contrast further test, while to compare between treatment combinations using Analysis of Variance (ANOVA) and DMRT further test at the 5% level. The results showed that leaf fertilizer at a concentration of 2.5 g/L was able to accelerate the formation of shoots and roots without the addition of organic material. In addition, leaf fertilizer media plus potatoes can be used to replace VW as a protocorm regeneration media at affordable cost.

1. INTRODUCTION

Vanda tricolor orchid is one of the endemic species in the slopes of Mount Merapi which has exotic flowers with white color variations, brown spotted petals and purple labellum (Kasutjianingati & Firgiyanto, 2018). *Vanda tricolor* is widely used as a parent in crossbreeding, especially to produce purple spots, reddish purple on the labellum, long flower arrangements, hybrid offspring with many flowers, and a fragrant aroma (Purba & Saptadi, 2019). This causes orchid to be hunted and taken from its natural habitat in the forest, so that its existence becomes rare (Hardjo, 2018).

According to Taryono (2013), *Vanda tricolor* can be commercialized in the second and subsequent generations generatively using seeds (seed culture). The shape of Vanda orchid seeds resembles powder and does not have endosperm, so they cannot be sown in ordinary media such as soil, moss, ferns, or husks. According to Darmono (2005), orchid seeds without endosperm must be grown using *in vitro* culture techniques. After the orchid embryo forms a protocorm, the protocorm regeneration will be carried out into seedlings until plantlets are formed.

Vacin and Went (VW) media is commonly used for orchid propagation by *in vitro* culture (Ashar *et al*., 2023). The price of this media is quite expensive, so it is an obstacle in cultivating Vanda orchids at the farmer level (Andiani, 2008). Therefore, it is necessary to find alternative orchid propagation media that support the growth and development of Vanda orchids *in vitro* well. NPK leaf fertilizer (21:21:21) can be used as an alternative medium for cultivating Vanda orchids *in vitro*, because leaf fertilizer generally contains complete macro and micro nutrients. The use of leaf fertilizer as a source of nutrients for protocorm regeneration media into plantlets must pay attention to the right concentration. According to Kasutjianingati *et al*. (2022), nutrient concentrations that are too high cause plant

poisoning and interfere with the absorption of other nutrients, resulting in slow protocorm growth. Moraes *et al*. (2020) stated that concentrations that are too low do not support growth and development in protocorm regeneration.

NPK leaf fertilizer (21:21:21) contains macro elements N, P, K, Ca, Mg and micro elements Na, Fe, Mn, Cu, and Zn. Media with VW formulation contains macro elements N, P, K, S, Ca, Mg, and micro elements Na, Fe, Mn, and vitamins. VW media does not contain Cu and Zn elements like in leaf fertilizer (21:21:21). Leaf fertilizer contains these elements, but does not contain S nutrients with a percentage of Ca, Mg, Mn, Fe, vitamins smaller than VW media. According to Hapsoro *et al*. (2018), the absence of these nutrients in leaf fertilizer and the low percentage of macro and micro nutrient content of leaf fertilizer can be supplemented by the addition of organic materials. According to Putri *et al.* (2024), organic materials such as bananas and potatoes can be added to orchid culture media.

Moraes *et al*. (2020) stated that organic materials as a complement to leaf fertilizer for culture media also contain other compounds that affect protocorm regeneration. The addition of organic materials can stimulate growth in the regeneration of moon orchids. According to Putri *et al*. (2024), bananas and potatoes are organic materials that can be added to the *Dendrobium* orchid culture media. Nuryadin *et al*. (2020) in their research stated that the addition of banana extract to the culture media with a concentration of 20 g/L accelerated the time in the growth phase of the *Phalaenopsis amabilis* orchid embryo. Murdad *et al*. (2010) stated that the addition of potatoes to the culture media can increase leaf area and root length in *Phalaenopsis gigantea* orchids.

Darmono (2005) stated that bananas contain 25.80 g of carbohydrates; 0.20 g of fat; 1.60 g of protein; 146.00 IU of vitamin A; 72.00 mg of vitamin C, 0.08 mg of thiamine; 0.073 mg of riboflavin; niacin 0.665 mg, pantothenic acid 0.334 mg; pyridoxine 0.367 mg, folate 20.00 mg; calcium 8.00 mg; sodium 18.00 mg; iron 0.50 mg; phosphorus 32.00 mg; magnesium 27.00 mg; and growth regulators in the form of auxin and gibberellin that support protocorm growth. Potatoes contain 2.00 g protein; fat 0.10 g; carbohydrates 19.10 g; calcium 11.00 g; phosphorus 56 mg; iron 0.70 mg; thiamine 0.09 mg; riboflavin 0.03 mg; vitamin C 16.00 mg; niacin 1.40 mg. According to Ulia *et al*. (2023), the elements contained in bananas and potatoes can support growth, development and morphogenesis in the regeneration of *Bulbophyllum* orchid protocorms.

The use of leaf fertilizer with the addition of organic materials as a medium for the regeneration of Vanda orchid protocorms needs to be studied. This study aims to (1) determine the effect of the concentration of NPK leaf fertilizer (21:21:21) and the addition of organic materials on protocorm regeneration, (2) obtain a combination of NPK leaf fertilizer concentration (21:21:21) and organic materials that can replace VW media as a medium for protocorm regeneration to become plantlets in *Vanda tricolor* orchids at an affordable cost.

2. MATERIALS AND RESEARCH METHODS

The research was conducted at the Tissue Culture Laboratory of the Agriculture and Food Service of Magelang City in June-September 2023. The environmental temperature of the incubation room of the plant tissue culture laboratory was kept at 22°C by using an AC (air conditioner) system with fluorescent lighting (Phillips, cool-white florescence light, 40 W), which is approximately 2000 lux in intensity, which is turned on for 24 h continuously.

2.1. Materials and Tools

The tools used in the study were Laminar Air Flow Cabinet (LAFC), autoclave, hot plate magnetic stirrer, measuring cup, beaker, Bunsen burner, digital scale, stationery, plastic wrapping, label paper, notebook, RHS color chart, culture bottle and dissection equipment (large tweezers, small tweezers, scalpel, and cloth). The materials used were planting materials in the form of Vanda orchid protocorm aged 8 weeks after sowing (MSS), leaf fertilizer with NPK ratio (21-21-21), banana, potato, sugar, agar, activated charcoal, distilled water, universal pH indicator paper Merck, 70% alcohol, 95% alcohol, spirits, 1 N HCl, and 1N NaOH.

The explants used in this experiment were protocorms from *in vitro* germination of *Vanda tricolor* seeds aged ± 8 weeks after sowing (MSS). The seeds were sown on VW media to form protocorms, on. The term protocorm is given to the round and green structure resulting from the germination of orchid seed embryos. Protocorm explants were selected that were healthy, uniform, and not contaminated by contaminant sources such as fungi and bacteria.

The foliar fertilizer used as a treatment in the study was foliar fertilizer with an NPK ratio (21-21-21) containing 2 sources of N (NH_4 and NO_3), while the organic materials were bananas, potatoes, and both. The bananas used as a treatment were the Ambon variety with a perfect level of ripeness, characterized by a yellow color and a soft texture of 25 g/L. The potatoes used were the Granola variety of 50 g/L. Agar-agar was added to the media, both control and treatment media as a media compactor of 6.5 g/L. Activated charcoal was added to the control and treatment media of 0.5 g/L. The pH of the control and treatment media of 5.8 was measured using Merck universal pH indicator paper.

2.2. Experimental Design

The experiment used a randomized block design (RBD) factorial pattern (4×4). The group acts as a replication. The first factor is the concentration of leaf fertilizer (K) with 4 levels, namely: (K1) 1 g/L, (K2) 1.5 g/L, (K3) 2 g/L and 2.5 g/L (K4). The second factor is the type of organic material with 4 levels, namely: (B0) without organic material bananas or potatoes, (B1) bananas, (B2) potatoes, (B3) bananas + potatoes. There are 16 treatment combinations.

The positive control used the basic media of orchid tissue culture Vacin and Went (VW) without additional organic materials. Each treatment was repeated 3 times, so there were 51 experimental units. To anticipate contamination, each experimental unit consisted 10 bottles containing 1 protocorm per bottle. There were a total of 510 experimental bottles. Data processing to compare VW control with all treatments used advanced analysis of Orthogonal Contrast, while to compare between treatment combinations with analysis of variance (ANOVA) followed by DMRT if there is a significant difference. Statistical analysis of research data using the SAS Version 9.0 program.

2.3. Observation Variables

Observations in this study were carried out up to 12 weeks of age, which was when subculture one was approaching, but subculture was not carried out. Observation variables included the time of shoot emergence with the criteria of shoot length of ±1 mm, the time of root emergence with the criteria of root length of ±1 mm. Observations of the percentage of the number of plantlets formed, and the fresh weight of the plantlets were carried out at the end of the study. The plantlets counted in the observation variables of the percentage of plantlets formed, and the fresh weight of the plantlets were those with strong yellow green C (light green) and deep yellowish green B (dark green) according to the color on the RHS color chart

3. RESULTS AND DISCUSSION

Based on Table 1 Analysis of Variance (ANOVA) between treatment combinations, it is known that the concentration of leaf fertilizer and the addition of organic materials have a significant effect on the time of shoot emergence, the time of root emergence, the percentage of plantlet formation, and the fresh weight of plantlets. If there is a significant difference, it will be continued with testing using DMRT. In the variable of root emergence time, there is an interaction between treatments, while other variables studied do not show any interaction between the concentration of leaf fertilizer and the addition of organic materials for bananas and potatoes.

3.1. Time of Shoot Emergence

Based on the results of further DMRT tests on the time of shoot emergence (Table 2), it shows that leaf fertilizer with a concentration of 2.5 g/L can stimulate shoot formation in protocorm regeneration. This is thought to be caused by the

Table 1. Analysis of variance (ANOVA) for foliar fertilizer concentration and organic material addition

Treatment	Shoot Emergence (DAP)	Root Emergence (DAP)	Plantlet Formation (%)	Weight of Plantlet (g)
Block	0.3417 ns	0.1892 ns	0.323 ns	0.0072*
K (Fertilizer Concentration)	0.0354*	0.067 ns	0.3588 ns	0.5722 ns
B (Organic Material)	0.0001*	0.0001**	0.0004**	0.0001**
K×B	0.0651 ns	0.0405*	0.096 ns	0.8467 ns
CV (%)	13	14	28	27

Notes: * = significant at 5% level, ** = highly significant at 1% level, ns = not significant. CV = Coefficient of Variation.

Table 2. Effect of foliar fertilizer concentration and organic material addition on shoot emergence time (DAP)

Treatment	K1	K2	K3	K4	Mean B
B0	37.33	44.33	35.00	30.33	36.75A
B1	35.00	37.33	39.67	32.67	36.17A
B2	25.67	23.33	30.33	28.00	26.83B
B3	32.67	37.33	32.67	30.33	33.25A
Mean K	32.67ab	35.58a	34.42a	30.33b	
CV = 13.26%					

Notes: Means followed by the same letter in the same column indicate no significant difference at a 5% level based on the DMRT test. Foliar fertilizer concentration (K) consists of K1 (1 g/L); K2 (1.5 g/L); K3 (2 g/L); K4 (2.5 g/L); Organic Mmaterial (B) consists of B0 (No organic material); B1 (Banana); B2 (Potato); B3 (Banana + Potato); CV (Coefficient of Variation).

nutrient content can stimulate the formation of new cells and shoot formation. Moraes *et al*. (2020) in their research stated that shoot growth is influenced by the availability of nutrients and nitrogen sources. Increasing fertilizer concentration can stimulate shoot formation.

Table 2 shows that the addition of bananas to the foliar fertilizer medium has no effect on the time of shoot emergence. This is likely because the bananas added to this experimental medium are 25 g/L. Moraes *et al*. (2020) in their research stated that 50 g/L of bananas can stimulate shoot formation in *Catasetum* orchids. Salsabila *et al*. (2023) added that culture media enriched with 30 g/L of banana extract can stimulate the formation of *Phalaenopsis amabilis* shoots. The addition of potatoes to the culture medium can stimulate the formation of *Vanda tricolor* orchid shoots. This is thought to be because potatoes contain more protein and vitamins than bananas. Salsabila *et al*. (2022), stated that the addition of protein to the culture medium can increase the activity of new cell formation in young plants which can stimulate leaf formation. According to Purnamasari *et al*. (2020), the addition of vitamin B1 can stimulate leaf growth in *Dendrobium nobile* orchids.

Giving bananas + potatoes to the culture medium did not result in a faster shoot formation time compared to giving potatoes alone. Zhan *et al*. (2022) stated that bananas contain the hormones auxin and gibberellin. According to Raspor *et al*. (2020), potatoes contain the hormones auxin, cytokinin, and abscisic acid (ABA). According to (Taiz *et al*., 2003), ZPT at excessive concentrations can inhibit cell division and enlargement.

3.2. Root Emergence Time

Based on the results of statistical analysis of the Root Emergence Time parameter, it shows that there is an interaction between the concentration of foliar fertilizer and the addition of organic matter. In Table 3, the concentration of 1 g/L is suspected to be low and requires the addition of organic matter to complete the nutrient content of the culture media in order to accelerate root formation in *Vanda tricolor*. According to Sarief (2015), the N element, if not available in sufficient quantities, can inhibit the vegetative growth of plants, including inhibiting root formation. Phosphorus plays a role in the formation of proteins for meristem cell division and growing new cells to form roots. Potassium acts as an enzyme activator in metabolic reactions and protein synthesis, so it is important for root formation.

Bananas added to the culture media are suspected to be at a low concentration, so they are unable to stimulate root formation. Moraes *et al*. (2020) stated that bananas with a concentration of 50 g/L can stimulate root formation. Putri & Sukma (2021), in their research on *Dendrobium* sp. orchids also stated that a banana extract concentration of 60 g/L is good for supporting root formation, while in sugarcane orchids banana extract with a concentration of 100 g/L is able to induce roots (Sulichantini *et al*., 2021).

Potatoes contain more protein and vitamin B1 than bananas. According to Salsabila *et al*. (2022), protein supports cell differentiation and development into part of the tissue that makes up leaves and roots. Protein contains the amino acid tryptophan which is an auxin precursor. Krisdianto *et al*. (2020) added that the addition of peptone to moon orchids can support root growth. Giving bananas and potatoes together has been studied by Putri *et al*. (2024), on *Dendrobium* sp. orchids and Sulichantini *et al*. (2021) which gave better results when given potatoes alone than when combined. This is because there is an accumulation of auxin ZPT with a higher concentration which can slow down the emergence of roots.

Increasing the concentration of leaf fertilizer to 1.5 g/L is thought to be still low in N, P, and K, so additional organic matter is needed to stimulate root formation. Low levels of NPK macronutrients can inhibit root growth. Sarief (2015), stated that the N element plays a role in cell division and enlargement, thus affecting the growth rate of plant vegetative organs, such as root formation. According to Sarief (2015), the P element plays an important role in cell division and the development of meristem tissue for root formation in young plants. The potassium element plays a role in the enzymatic process of helping cell differentiation and supporting root formation.

In the 1.5 g/L fertilizer treatment, the addition of bananas is suspected to be too low in concentration, so it is unable to stimulate root formation compared to the addition of potatoes. This was studied by Moraes *et al*. (2020), which stated that bananas are able to stimulate root formation at a concentration of 50 $g.L^{-1}$. Research conducted by Stefano *et al*. (2022), using 10 g/L banana powder was unable to stimulate root formation. Bananas as an additional supplement but had no effect on root formation were also studied by Rahmah *et al*. (2021).

The addition of potatoes is able to stimulate root formation because they contain higher protein and vitamin B1 (thiamine) than bananas. Research on the addition of potatoes was reported by Moraes *et al*. (2020), whose results were significant in root formation. Giving bananas and potatoes together did not have a significant impact on root emergence. This is because of the high accumulation of ZPT which can inhibit root formation, but there has been no research explaining the reasons for the antagonistic relationship between bananas and potatoes when given together in the culture medium.

Table 3. Effect of foliar fertilizer concentration and organic material addition on root emergence time

Treatment	K1	K2	K3	K4
B0	58.33 abc	60.67 ab	44.33 de	49.00 bcde
B1	65.33 a	51.33 bcde	53.67 abcd	44.33 de
B2	39.67 e	39.67 e	44.33 de	39.67 e
B3	46.67 cde	42.33 de	46.67 cde	46.67 cde
CV = 14%				

Notes: Means followed by the same letter in the same column indicate no significant difference at a 5% level based on the DMRT test. Foliar fertilizer concentration (K) consists of K1 (1 g/L); K2 (1.5 g/L); K3 (2 g/L); K4 (2.5 g/L); Organic material (B) consists of B0 (No organic material); B1 (Banana); B2 (Potato); B3 (Banana + Potato); CV (Coefficient of Variation).

In the treatment of fertilizer concentration 2 g/L (K3) with the addition of organic banana material tends to slow down root formation, allegedly because fertilizer with a concentration of 2 g/L (K3) provides sufficient nutrients to stimulate root formation in Vanda orchids. Purnamasari *et al*. (2020), in their research explained that a fertilizer concentration of 2 g/L can stimulate the growth of *Dendrobium nobile* roots. The treatment of fertilizer 2.5 g/L (K4) with the addition of various organic materials studied did not significantly affect the time of root emergence. However, the time of root emergence tended to be faster in the potato addition treatment. According to Moraes *et al*. (2020), leaf fertilizer without additional other materials with high concentrations was able to stimulate root formation in protocorm regeneration. This further strengthens that the provision of organic materials at high fertilizer concentrations does not show significant results because the nutrient content in leaf fertilizer is sufficient for root formation. In the treatment without the addition of organic materials, the protocorm regeneration media requires a high concentration to stimulate root formation. Research conducted by Purnamasari *et al*. (2020), supports this statement, that leaf fertilizer of 2 g/L affects the number of roots. The low concentration of fertilizer used will have an impact on the lack of nutrients available in the culture media for root formation, and vice versa.

The addition of bananas in this study is suspected to be too low at a fertilizer concentration of 1 g/L which indicates low nutrient content in the culture media. This can slow down the emergence of roots. Research on the use of bananas with leaf fertilizer as a culture medium for orchid regeneration has been studied by Stefano *et al*. (2022), which states that the addition of bananas is less effective when given at low concentrations. Increasing fertilizer concentration by addition of bananas can stimulate root formation. It is suspected that at high fertilizer concentrations, the available nutrients are already able to stimulate root formation, so that the banana addition treatment is not visible. Research by Moraes *et al*. (2020) stated that commercial fertilizers at high concentration (5 g/L) can be used as culture medium for the regeneration of *Catasetum fimbriatum* and *Catasetum macrocarpum* orchids.

The addition of potatoes can stimulate root formation, allegedly because they contain more protein and vitamin B1 than bananas. According to Krisdianto *et al*. (2020), protein is used to form new cells that can stimulate morphogenesis in protocorms. Protein contains amino acids that function as a source of carbon and energy for cell growth. Puspasari *et al*. (2018) added that the provision of peptone can stimulate morphogenesis in protocorms. Zahra *et al*. (2023) stated that vitamins in the form of thiamine are involved in the synthesis of phytohormones such as auxins which play a role in root growth and development. Research conducted by Ningrum & Isda (2022) stated that the provision of boiled potato water is good for stimulating protocorm morphogenesis. The addition of bananas + potatoes tends to slow down root formation. It is suspected that the addition of bananas and potatoes together at various fertilizer concentrations causes an antagonistic reaction, making it less suitable for root formation. However, until now there has been no research on this antagonism in root formation.

3.3. Percentage of Plantlets Formed

Based on the results of statistical analysis of the Percentage of Plantlets Formed parameter, it shows that increasing the concentration of leaf fertilizer does not show a significant difference in the percentage of plantlet formation (Table 4). According to Ashar *et al*. (2023), planlets are micro-sized plants that have formed complete organs from tissue culture. The formation of planlets is influenced by the activity of cell division and elongation which can stimulate the formation of vegetative plant organs such as leaves and roots. The use of leaf fertilizer as a culture medium was also studied by Moraes *et al*. (2020), on two types of *Catasetum* orchids which showed that leaf fertilizer was less effective in increasing the percentage of plantlet formation. This is suspected to be due to the low concentration of nutrients in leaf fertilizer, which inhibits the formation of vegetative organs in protocorm regeneration.

Table 4. Effect of foliar fertilizer concentration and organic material addition on the percentage of formed plantlets (%)

Treatment	K1	K2	K3	K4	Mean B
B0	26.19	41.67	44.44	44.44	39.19B
B1	63.81	59.52	62.77	44.07	58.42A
B2	83.79	85.93	57.57	55.15	70.61A
B3	75.95	59.26	48.61	67.88	62.93A
Mean K	62.44a	61.59a	54.22a	52.90a	
CV = 29%					

Notes: Means followed by the same letter in the same column indicate no significant difference at a 5% level based on the DMRT test. Foliar fertilizer concentration (K) consists of K1 (1 g/L); K2 (1.5 g/L); K3 (2 g/L); K4 (2.5 g/L); Organic material (B) consists of B0 (No organic material); B1 (Banana); B2 (Potato); B3 (Banana + Potato); CV (Coefficient of Variation).

The addition of bananas to the culture medium is thought to be too low, so it does not affect the percentage of planlet formation. According to Putri *et al*. (2024), bananas with a concentration of 60 g/L are good for protocorm regeneration which is indicated by the successful formation of leaves and roots (formation of planlets), so bananas with low concentrations can be said to reduce the percentage of planlet formation. Potatoes contain more protein and thiamine than bananas. According to Salsabila *et al*. (2022), who stated that the addition of protein can increase the percentage of planlet formation in moon orchids. Zahra *et al*. (2023) added that the addition of thiamine to orchid culture media can increase the percentage of planlet formation.

According to Utami & Hariyanto (2020), potatoes contain carbohydrates which are used as energy for the formation of new cells for the vegetative growth of plants. Ulia *et al*. (2023) in their research stated that the addition of potato extract can increase the growth of protocorms to form planlets in *Bulbophyllum* orchids. Ningrum & Isda (2022) stated that adding boiled potato water to the culture medium can increase the percentage of protocorm-like bodies formation to 100%. Bananas and potatoes, if both are added together to the culture medium, will have a more complete nutrient content. Although giving both together is slower to form plantlets compared to giving potatoes alone, it is better than giving bananas. According to Kuang *et al*. (2021), bananas contain auxin, gibberellin, and ethylene hormones. The auxin plays a role in cell differentiation, while gibberellin stimulates cell division and elongation. The ethylene plays a role in the growth of thick stems. Raspor *et al*. (2020) found auxin and cytokinin during study on endogenous hormones in potatoes. Cytokinin together with auxin plays a role in cell division.

3.4. Fresh Weight of Planlets

Based on the results of the statistical analysis of the Fresh Weight of Plantlets parameter in Table 5, it shows that the treatment of leaf fertilizer with increasing concentrations did not significantly increase the fresh weight of the plantlets. The use of leaf fertilizer has been studied by Dwiyani *et al*. (2022), which stated that leaf fertilizer alone was unable to increase the fresh weight of the plantlets in the Indonesian Black Orchid. This is suspected because the leaf fertilizer given had a low concentration, so it was unable to increase the fresh weight of the plantlets.

Table 5. Effect of foliar fertilizer concentration and organic material addition on fresh weight of plantlets (g)

Treatment	K1	K2	K3	K4	Mean B
B0	0.02	0.02	0.02	0.02	0.02BC
B1	0.02	0.03	0.02	0.02	0.02C
B2	0.03	0.03	0.03	0.04	0.03A
B3	0.03	0.02	0.03	0.03	0.02B
Mean K	0.02a	0.02a	0.03a	0.03a	
CV = 27%					

Notes: Means followed by the same letter in the same column indicate no significant difference at a 5% level based on the DMRT test. Foliar fertilizer concentration (K) consists of K1 (1 g/L); K2 (1.5 g/L); K3 (2 g/L); K4 (2.5 g/L); Organic Material (B) consists of B0 (No organic material); B1 (Banana); B2 (Potato); B3 (Banana + Potato); CV (Coefficient of Variation).

The addition of organic material in the form of bananas did not increase the fresh weight of the plantlets. According to Deng *et al*. (2020), bananas contain endogenous hormones in the form of auxin and gibberellin. The function of these two hormones can stimulate cell division. It is suspected that the addition of bananas at a low concentration, so it did not have a significant effect on the fresh weight of the plantlets. Putri *et al*. (2024) in their research stated that the addition of bananas with the appropriate concentration can increase the growth and development of *Dendrobium* orchids. In a study conducted by Moraes *et al*. (2020), the addition of bananas to various culture media as much as 60 g/L was able to increase the fresh weight of plants.

The addition of potatoes to the culture medium can increase the fresh weight of the planlets. This is thought to be because the protein and vitamin B1 content in potatoes is greater than in bananas. Krisdianto *et al*. (2020) in their previous study explained that peptone contains amino acids that can synthesize auxin to stimulate leaf and root growth in orchids which has an impact on increasing the fresh weight of the planlets. Saidi & Hajibarat (2021) added that there is a Gibberellin hormone content in potatoes which stimulates cell enlargement. According to Kasutjianingati *et al*. (2022), vitamin B1 can increase cell differentiation for leaf and root growth. The addition of thiamine has been studied by Zahra *et al*. (2023), which states that the addition of thiamine to the regeneration of moon orchids increases the fresh weight of the planlets.

Research conducted by Kasutjianingati *et al*. (2022), states that bananas contain endogenous hormones in the form of auxin and gibberellin, while potatoes contain auxin and cytokinin. These three types of hormones play a role in cell differentiation. Until now, there have been no research results that can explain the antagonistic effect between bananas and potatoes which causes a decrease in the fresh weight of orchid plantlets when given together and is more effective in increasing fresh weight when given separately.

Table 6 shows that VW media significantly gave a higher percentage of plantlet formation compared to all treatments, except for the leaf fertilizer plus potato organic matter (PBOK) media. VW media compared to treatment media increased the fresh weight of plantlets, except for the fertilizer plus organic matter and leaf fertilizer plus potato treatments. This shows that the fertilizer plus organic matter treatment can be an alternative to VW media in the regeneration of protocorms into plantlets.

According to Moraes *et al*. (2020), organic matter can be added to the culture medium to add nutrients needed by orchids. Potatoes contain higher protein and vitamin B1 than bananas. According to Puspasari *et al*. (2018) protein added to the culture medium can stimulate cell division and cell elongation. Zahra *et al*. (2023) stated that Vitamin B1 (thiamine) stimulates the production of endogenous auxin in plants which can stimulate cell division in orchid morphogenesis.

Table 6. Comparison of VW medium and treatments on the percentage of plantlet formation and fresh weight of plantlets

Contrast	Percentage of Plantlet Formation (%)		Fresh Weight of Plantlets (g)	
VW Medium vs. all Alternative Media				
VW	92.86	A	0.0332	A
All Media	57.78	B	0.0246	B
VW Medium vs. Foliar Fertilizer (PD)				
VW	92.86	A	0.0332	A
Leaf Fertilizer (PD)	39.72	B	0.0202	B
VW Medium vs. PD+B0				
VW	92.86	A	0.0332	A
B0	63.98	B	0.0253	B
VW Medium vs. PD+B1				
VW	92.86	A	0.0332	A
PD+B1	58.42	B	0.0186	B
VW Medium vs. PD+B2				
VW	92.86	A	0.0332	A
PD+B2	70.61	A	0.0317	A
VW Medium vs PD+B1+B2				
VW	92.86	A	0.0332	A
PD+B1+B2	62.92	B	0.0256	A

Notes: Means followed by the same letter in the same column indicate no significant difference at a 5% level based on the orthogonal contrast test. VW (Vacin & Went Medium); PD (Foliar Fertilizer); B0 (No Organic Material); B1 (Banana); B2 (Potato).

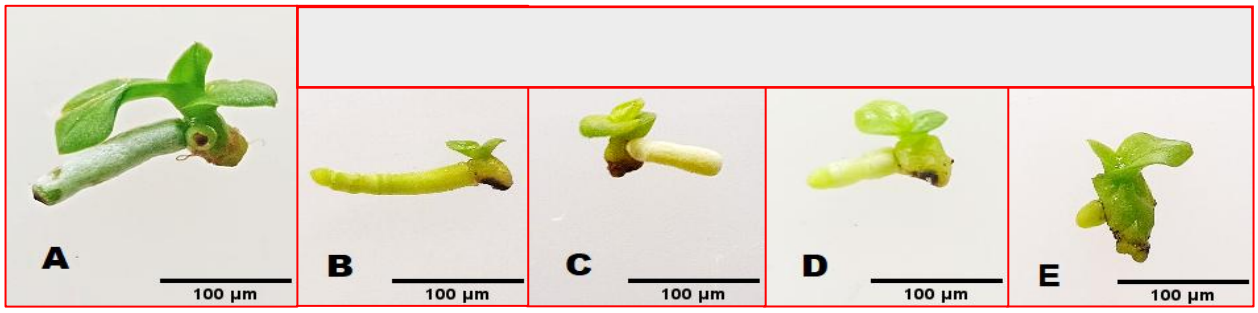

Figure 1. Plantlets resulted from treatments of VW, foliar fertilizer, and potato: (A). VW media (1.0095 cm); (B). Plantlet media foliar fertilizer 1 g/L + Potato (0.0787 cm); (C). Plantlet media foliar fertilizer 1.5 g/L + Potato (0.0544 cm); (D). Plantlet media foliar fertilizer 2 g/L + Potato (0.5630 cm); (E). Plantlet media foliar fertilizer 2.5 g/L + Potato (0.3107 cm).

The media that can replace VW media as a protocorm regeneration medium is leaf fertilizer + potato organic material as shown in Figure 1, although the picture shows that the plantlets in VW media are more vigorous. Research conducted by Ambarwati *et al*. (2021), stated that potatoes are a good source of organic material added as an orchid culture medium. Potatoes have a higher protein and vitamin content than bananas. According to Salsabila *et al*. (2022), protein added to the culture medium can increase cell division activity and cell elongation. Zahra *et al*. (2023) stated that thiamine can stimulate the formation of auxin which plays a role in orchid growth and morphogenesis.

4. CONCLUSIONS

Treatment of NPK foliar fertilizer (21:21:21) at a concentration of 2.5 g/L(K4) can accelerate the formation of shoots and roots without the addition of organic materials. Treatment of a combination of NPK leaf fertilizer concentration (21:21:21) and potato organic materials can replace VW media as a medium for protocorm regeneration to become plantlets in *Vanda tricolor* orchids at an affordable cost. The best and most efficient treatment as a regeneration medium for *Vanda tricolor* orchids to support growth and development replacing VW media is NPK foliar fertilizer (21:21:21) with a concentration of 2 $g.L^{-1}$ with the addition of 50 $g.L^{-1}$ organic potato material. Further research is

needed including the use of foliar fertilizer + potato media for the stage of plantlets to become seedlings. For orchid protocorm regeneration, it is recommended to use foliar fertilizer with a higher N content than P and K. For subsequent growth and development, the concentration of activated charcoal should be increased to stimulate root growth. For *Vanda tricolor* orchid protocorm regeneration, the concentration of bananas should be increased.

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