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The Effect of Tomato (*Solanum lycopersicum* L) Filtrate and 6-Benzylaminopurine on the Growth of Moon Orchid (*Phalaenopsis* sp.) Plantlets In-vitro

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Abstract
Moon orchids *(Phalaenopsis* sp.*)* take a long time to propagate and produce limited results. Therefore, tissue culture is an alternative to overcome this problem. This study aims to investigate the effect of tomato filtrate and 6-benzylaminopurine (BAP) combinations on the growth of Moon Orchid plantlets in vitro Vacin and Went (VW) medium. The study employed a one-factor completely randomized design with five treatments: K(0% tomato filtrate + 0mg BAP/l BAP), A(5%tomato filtrate + 2mg BAP/l), B(10% tomato filtrate + 1.5mg BAP/l), C(15% tomato filtrate + 1mg BAP/l), and D(20% tomato filtrate + 0.5mg BAP/l). Plant height, number of leaves, and root length were analyzed using one-way ANOVA and Duncan's test at the 5% significance level. The combination of tomato filtrate and BAP was found to have a significant effect. The optimal treatments for each parameter were as follows: plantlet height treatments B and C (1.26 cm and 1.14 cm); number of leaves treatments A and B (21.4 and 20.6); and optimal root length treatments C and D (2.66 cm and 2.92 cm). The recommended combination for propagating moon orchid plantlets ranges from 10% tomato filtrate + 1.5mg BAP/l to 15% tomato filtrate + 1 mg BAP/l.

Keywords: growth regulator; in vitro; moon orchid tomato; biodiversity

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INTRODUCTION

Orchids are highly diverse and abundant plants. Orchids are well-known among the public due to their aesthetic value and have great potential for commercial development (Rusmaniah *et al.*, 2023). Despite considerable interest, orchid production in Indonesia remains slow (hardjo, 2023). Currently, efforts to improve the cultivation of ornamental orchids in Indonesia are suboptimal, leading to a decline in the quantity of orchids and placing them on the brink of extinction in the wild. The Central Statistics Agency (2022) reports that Indonesia produced 6.7 million stems of orchids, a 40.24% decrease from the previous year. Because of illicit logging and overexploitation for personal or commercial collections, often without consideration for conservation, orchid populations in the wild are declining.

The orchid was native to Indonesia and was highly sought after and very popular was the moon orchid (Phalaenopsis sp.). Through Presidential Decree No. 4/1993, the moon orchid was one of three national flowers designated by the government as Indonesia's national flower and has been recognized as a source of foreign exchange for the country (Widyastuti, 1993). The moon orchid has the highest sales value in the global market, reaching up to 80% market share. This orchid was loved by many people because of its long flowering period and high durability, so that the flowers can last for several months (Syamsiah *et al*., 2020).

Orchids can be propagated conventionally by separating mature orchid clumps or adventitious shoots and planting them in the same medium as their parent plants. However, such propagation methods are time-consuming and result in limited offspring, as producing a significant number of seedlings in a short time requires increased interest in orchids owing to the slow growth rate of seedlings until they reach maturity (Hardjo, 2018). Therefore, an effective method using in vitro culture technology must be applied to produce orchid plants up to the planlet stage, thereby rapidly increasing the number of orchid seedlings in a short time, producing disease-free plants that are consistent with their parent plants, and superior in their characteristics (Ashar *et al.*, 2023). Tissue culture, also known as in vitro propagation, produces new plants with improved quality and quantity and accelerates the growth and productivity of orchid plants. Shoot multiplication from plantlets is the stage following in

vitro culture that is used to propagate plant seedlings. Multiplication separates plant parts from newly planted tissue cultures or planting results (Nahlohy *et al.,* 2023).

Tissue culture methods involve isolating tissues, cells, or organs in optimized media under aseptic conditions in sterile containers. The advantage of the in vitro culture method is that it can produce offspring in large quantities with the same characteristics and form as the parent (Zulkarnain, 2024). Tissue culture techniques can reduce the risk of plants being susceptible to diseases and make their cultivation independent of seasonal conditions. The success of in vitro culture depends on the selection of explants, medium, aseptic environmental conditions, and the use of appropriate growth regulators (Smith and Emeritus, 1996).

The balance of growth regulators contributes to the success of in vitro cultures. In vitro cultivation involves adding a substance to the growth medium to influence the proliferation of growth regulators. Compounds known as growth regulators alter the physiological processes of plants by promoting or inhibiting them (Hardjo, 2018). The most commonly found growth regulators are auxins and cytokinins. Auxin was a growth regulator that plays an important role in the formation and development of roots and cell elongation in stems (Warisman *et al.,* 2024). This compound can be obtained from various organic sources, one of which was tomatoes. Meanwhile, 6-Benzylaminopurine (BAP) was a synthetic cytokinin commonly used in in vitro culture techniques to stimulate cell division and bud formation (Sualang *et al.,* 2023). The addition of organic compounds to the culture medium can help the growth of moon orchid *(Phalaenopsis sp.)* explants.

Dewi *et al.,* (2021) study on the effect of tomato filtrate on *Dendrobium* sp. Orchids showed that adding 10% tomato filtrate had a significant effect on the growth orchid plantlets. This significant effect was due to the complex plant hormones contained in tomato filtrate, which act as specific stimulants for plants at low concentrations, enabling optimal growth; however, these plant hormones inhibit growth at high concentrations. According to Ningsih *et al.* (2021), a concentration of 15% was the optimal concentration of tomato filtrate for increasing the number of leaves on Cattleya orchids. This optimal concentration of tomato filtrate was in line with the statement by Muharyati *et al.* (2015) that each species had a unique way of responding to the composition of the media in its environment. Conversely, the effect of different media compositions on different plant species has not been studied. Syamsiah *et al.* (2020) showed that the addition of BAP can prolong the emergence of shoots, leaves, and the number of leaves at a concentration of 2.00 mg/L. BAP belongs to the cytokinin group, which plays a crucial role in stimulating cell division, promoting shoot formation, and inducing the development of primary organs in plants so that it can trigger this growth. Based on the findings of the above study, research was conducted on the effects of tomato filtrate and BAP on the growth of moon orchid seedlings (*Phalaenopsis* sp.). This study was expected to produce superior plants that can quickly produce large numbers of healthy, high-quality seedlings.

MATERIALS AND METHODS

This study was conducted over a period of two months, from October to December 2024, at the Sememi Tissue Culture Laboratory in Surabaya. The objective of this study was to determine the effect of adding tomato filtrate and BAP to Vacin and Went medium in vitro on the growth of moon orchid seedlings (Phalaenopsis sp.). This study used an experimental method with a completely randomized design (CRD) with one factor, which tested combinations of growth regulators in the form of tomato filtrate and BAP added to the culture medium, consisted of 5 treatments: K (0% tomato filtrate + 0 mg BAP/l), A (5% tomato filtrate + 2 mg BAP/l), B (10% tomato filtrate + 1.5 mg BAP/l), C (15% tomato filtrate + 1 mg BAP/l), and D (20% tomato filtrate + 0.5 mg BAP/l). Each treatment was repeated five times, resulting in 25 experimental units. The procedures in this study included sterilization of tools, preparation of VW medium, medium sterilization, planlet inoculation, and observation.

Tomato filtrate was prepared by precisely weighing 100 g of ripe or red tomatoes using an analytical balance. The tomatoes were subsequently combined with distilled water at a volume/volume (v/v) ratio of 100 ml and blended using a blender. The tomato filtrate used in this study was diluted to 0%, 5%, 10%, 15%, and 20%. Dilution was performed by mixing the tomato filtrate into distilled water to achieve a total volume of 100 mL for each treatment sample.

Vacin and Went (VW) medium was prepared by adding 200 ml of distilled water to a 1000 ml beaker and turning on the stirrer at a speed of 6 rpm (revolutions per minute), followed by the addition of 10 ml of stock solution code (A–F) of VW medium, 10 ml of vitamins, BAP at the required concentration, and 20 g of sugar, and homogenized for 10 min. Tomato filtrate was then added at the desired concentration and homogenized for 5 min. Distilled water was added until the volume reached

1000 ml, followed by homogenization for 5 min, and the pH was regulated at 5.8–6.0. The solution was added with 7 grams of agar and homogenized for 2 minutes. The mixture was then heated on a stove in a pot until boiling (using medium heat) and poured into sterilized medium bottles, with the volume and concentration of the tomato filtrate and BAP adjusted according to the treatment. After that, the medium was sterilized using autoclave at a temperature of 121°C, pressure of 2 atm for 20 minutes

Plantlet inoculation was performed by placing orchid (*Phalaenopsis sp.)* explants in a bottle using tweezers and then placing them in a Petri dish. Moon orchid (*Phalaenopsis sp.)* plantlets in a Petri dish were sorted into good and poor quality plantlets. Three high-quality moon orchid plantlets were then planted in bottles containing Vacin and Went medium. The bottles containing the plantlets are labeled (with the orchid code and planting date) and stored in the tissue culture rack.

The height of the orchid planlets (*Phalaenopsis sp.)* and root length were observed at the beginning (0 WAT) and end of the study (8 WAT), while the number of leaves was observed once a week, starting from one week after planting (1 MST) to eight weeks after planting (8 WAT). The data were quantitatively analyzed, including plantlet height, number of leaves, and root length. The results were then analyzed using one-way analysis of variance (ANOVA) to determine the significant effects of the data, using the Statistical Package for the Social Sciences (SPSS) version 23. If this study had a significant effect, Duncan's Multiple Range Test (DMRT) was performed at the 5% level.

RESULTS

Based on the observed research on the addition of tomato filtrate and BAP to the growth of moon orchid *(Phalaenopsis sp.)* plantlets using VW medium in vitro, data were obtained in the form of three parameters, including plantlet height, number of leaves, and root length as presented in Table 1.

Table 1. Plant height, number of leaves, and root length of moon orchids (*Phalaenopsis sp.)* under various treatments at 8 WAT

Groups	Plantlet Height*	Number of Leaf*	Root Length*
K	0,39 ± 0,06^{a}	13,4 ± 1,67^{a}	0,93 ± 0,21^{a}
A	0,65 ± 0,20^{ab}	21,4 ± 2,61^{c}	1,58 ± 0,60^{b}
B	1,26 ± 0,18^{c}	20,6 ± 1,52^{c}	1,74 ± 0,53^{b}
C	1,14 ± 0,05^{c}	18 ± 1,30^{b}	2,66 ± 0,41^{c}
D	0,81 ± 0,20^{b}	17,6 ± 1,52^{b}	2,92 ± 0,53^{c}

Note: *) Different letter in the same column indicate a significant difference. K : VW, A : VW + 5% tomato filtrate + 2 mg BAP/l; B : VW + 10% tomato filtrate + 1,5 mg BAP/l; C : VW + 15% tomato filtrate + 1 mg BAP/l; D : VW + 20 % tomato filtrate + 0,5 mg BAP/l.

The height of moon orchid planlets (*Phalaenopsis sp*.) at 8 WAT shows that each treatment resulted in significant differences, where treatment K differed significantly from B, C, and D, but did not differ significantly from treatment A. Treatment A differred significantly from B and C, but not from treatments K and D. Treatment B differs significantly from treatments K, A, and D, but not significantly from treatment C. Additionally, treatment D significantly differed from treatments K, B, and C, but not from treatment A. Treatment B (10% tomato filtrate + 1.5 mg BAP/l) and C (15% tomato filtrate + 1 mg BAP/l) were found to be optimal for plantlet height.

Number of leaves also resulted in significant differences, where treatment K differs significantly from treatments A, B, C, and D (Figure 1). Treatment A differed significantly from K, C, and D, but did not differ significantly from treatment B. Treatment C differed significantly from K, A, and B, but not significantly from treatment D. Thus, the optimal treatment for leaves number was treatment A (5% tomato filtrate + 2 mg BAP/l) and B (10% tomato filtrate + 1.5 mg BAP/l).

Root length on the planlets over 8 MST also has significant differences, where treatment K differed significantly from treatments A, B, C, and D. Treatment A differed significantly from K, C, and D, but does not differ significantly from treatment B. Additionally, treatment C differed significantly from K, A, and B, but not significantly from D. The optimal treatment on root length was treatment D (20% tomato filtrate + 0.5 mg BAP/l) and C (15% tomato filtrate + 1 mg BAP/l).

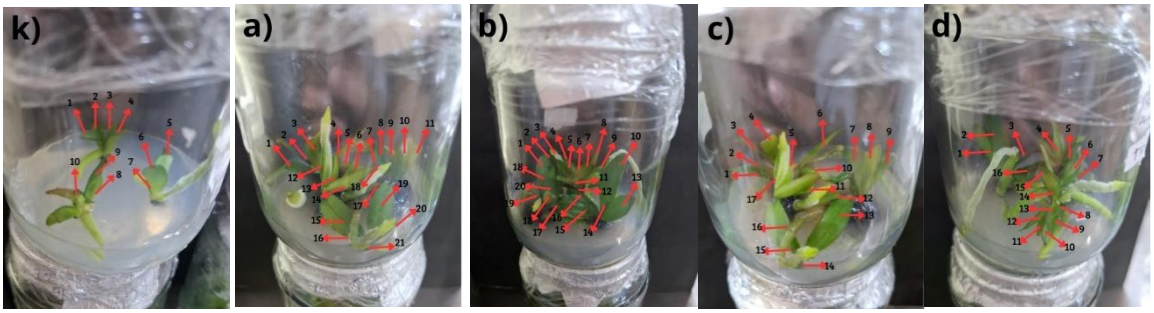

Figure 1. Number of leaves in the growth of moon orchid planlets. K : VW, A : VW + 5% tomato filtrate + 2 mg BAP/l; B : VW + 10% tomato filtrate + 1,5 mg BAP/l; C : VW + 15% tomato filtrate + 1 mg BAP/l; D : VW + 20 % tomato filtrate + 0,5 mg BAP/l.

DISCUSSION

Based on the research conducted, it was found that the combination of tomato filtrate and BAP (6-Benzylaminopurin) affects the growth of Moon Orchid *(Phalaenopsis* sp.*)* plantlets, with parameters including plantlet height, number of leaves, and root length being affected. Duncan's multiple range test statistical analysis showed that treatments B (10% tomato filtrate + 1.5 mg BAP/l) and C (15% tomato filtrate + 1 mg BAP/l) showed optimal results for plantlet height.

The presence of auxin hormone in tomato filtrate causes young moon orchid plants *(Phalaenopsis sp.)* to grow taller, as this hormone can help cell elongation through the formation of primordial cells, as well as combine with cytokinin to increase the growth of young plants (Wulandari et al., 2019). The increase in the height of orchid seedlings was also caused by variations in BAP concentrations of 1.5 and 1 mg. These Plant Growth Regulator (PGR) concentrations can act as stimulants for seedling growth, while excessive concentrations can inhibit seedling growth. The combination of tomato filtrate and BAP in the right proportions can effectively enhance orchid seedling growth, resulting in taller seedlings in treatments B and C compared to other treatments.

Endogenous auxin hormone activity originating from within the plant and exogenous auxin applied externally can trigger cell elongation in plant seedlings. Treatments B and C showed higher average heights compared to the other treatments. According to Ayuningtias *et al.* (2024), the application of auxin at the appropriate concentration in the medium allows for effective absorption by young plants, which in turn plays a role in stimulating cell division and accelerating elongation growth. Therefore, the combination of auxin and cytokinin hormones in the right amounts plays a crucial role in determining the height growth of you

Auxin, found in tomato filtrate, plays a role in stimulated organogenesis, somatic embryogenesis, and plant growth (Heriansyah *et al.*, 2020). Auxin enters the cytoplasm and stimulates specific proteins in the cell plasma membrane, which causes H^+ ions to be pumped into the cell wall. H^+ ions cause the pH to lower, activated enzymes that break the hydrogen bonds in the cellulose chains of the cell wall, allowing water to enter through osmosis and promoting cell growth (Illahi *et al.*, 2022).

The combination of tomato filtrate and BAP in the appropriate composition can also enhance leaf growth. High amounts of added cytokinin and low amounts of auxin can boost leaf formation. This is shown by the increase in leaf numbers in treatments A (5% tomato filtrate + 2 mg BAP/l) and B (10% tomato filtrate + 1.5 mg BAP/l). Treatments A and B had similar effects. Yulia et al. (2020) noted that adding extra cytokinin to the medium can promote leaf growth, with higher cytokinin levels leading to more leaves.

According to Saepudin et al. (2020), the number of leaves that grow on plant seedlings is caused by good growth during the bud phase. As the number of shoots increases, the number of leaves will also increase. Cytokinin that have been added to the media are translocated to the leaves; therefore, the main factor in leaf formation is influenced by the concentration of cytokinin added, namely BAP. BAP at this concentration can increase chlorophyll production, thereby increasing the photosynthetic efficiency of the plants. Rosniawaty *et al.* (2017) revealed that BAP is a growth regulator that contains nitrogen compounds. Cytokinin hormones also contain nitrogen compounds that increase amino acid and protein synthesis. Plants use these amino acids and proteins to support leaf growth.

BAP (6-Benzylaminopurine) is a cytokinin growth regulator that stimulates cell division and increases the number of leaves. Andany and Ratnasari (2023) stated that this happens when BAP moves into the plantlet cells through the area damaged by cutting. Cutting causes damage and partially breaks

down cells, disrupting the balance between the cell wall and protoplasm. This imbalance encourages plantlet activity, causing them to divide and promoting the formation of new shoots or leaves.

Root length is another factor that can be measured to assess the growth of plantlets. According to Duncan's test analysis, treatments C (15% tomato filtrate + 1 mg BAP/l) and D (20% tomato filtrate + 0.5 mg BAP/l) showed the best results for root length growth of Moon Orchid (Phalaenopsis sp.). There was no significant difference between the two treatments. The increase in auxin concentration in the tomato filtrate applied to the culture medium directly related to the number of roots formed. This effect is due to the stimulatory role of auxin in tomato filtrate, which encourages root formation in plants (Mubarak and Ratnasari, 2024).

Auxin in tomato filtrate increases root length. Research by Ambarwati *et al.* (2021) shows that providing the right amount of exogenous auxin with low cytokinin levels in VW medium can stimulate root growth. Exogenous auxins, like tomato filtrate, play an important role in cell differentiation and improve root development in plant seedlings. Lestari et al. (2018) noted that at certain concentrations, exogenous auxins can trigger root growth in plantlets, either alone or combined with cytokinin. The level of stem permeability has a strong effect on increased root growth to water. Here, the hormone auxin helps increase this permeability by breaking hydrogen bonds and flexing the stem's epidermal cell wall. Auxin can relax the epidermal cell walls, allowing the cells to expand. This expansion causes the epidermal cells to stretch quickly, followed by the dilation of nearby subepidermal cells. This process aids water penetration into the stem tissue, which accelerates root growth (Shofiana *et al.*, 2013).

Auxin content in tomato filtrate plays a role in elongated the root tip meristem by increased the sugar content in the cell vacuole, resulted in increased osmotic pressure. In addition, a decrease in pH causes the cell walls to become more organized and elastic. Auxin can also accelerate water diffusion into cells. Nutrients in the medium are absorbed along with water via diffusion (Malinda et al., 2022). This mechanism is based on the growth hypothesis, where auxin releases hydrogen ions that can reduce the pH, making cell walls more flexible and easier for plant roots to extend. Longer roots increase the efficiency of nutrient absorption, which, together with water, is transported into the xylem and phloem vessels and distributed to all parts of the plant. Additionally, the iron ion content in tomatoes plays a crucial role in chlorophyll synthesis, thereby supporting photosynthesis in plants (Zulkarnain, 2024).

The results of this study indicate that the optimal concentration of tomato filtrate and BAP for propagating moon orchid (Phalaenopsis sp.) seedlings on VW medium was in the range of (10% tomato filtrate + 1.5 mg BAP/l) to (15% tomato filtrate + 1 mg BAP/l). These results can be recommended as a solution for propagating *Phalaenopsis* orchid seedlings for public application in plant tissue culture science. These results are recommended as a solution because concentrations ranging from (10% tomato filtrate + 1.5 mg BAP/l) to 15% tomato filtrate + 1 mg BAP/l) In VW medium can optimize good growth in *Phalaenopsis* orchid seedlings (*Phalaenopsis* sp.).

CONCLUSION

The addition of tomato filtrate affected planlet height, number of leaves, and root length of moon orchid (*Phalaenopsis* sp.) plants grown on VW medium in-vitro. This growth effect can be seen from the optimal treatment for each parameter, including plant height, which was obtained optimally in the range of 10% tomato filtrate + 1.5 mg BAP/l to 15% tomato filtrate + 1 mg BAP/l.

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CONFLICT OF INTEREST

There is no conflict of interest

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