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Effect of Moringa Leaf and Shallot Filtrate in MS Media on the Growth of *Sambung Nyawa* (*Gynura procumbens*) Plantlets

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Abstract
Sambung nyawa (*Gynura procumbens*)is a local plant that sees a lot of uses, however its cultivation is still limited. Thus, tissue culture can be examined as potential propagation methods. This study determined the effect of moringa leaves and shallots filtrate to Murashige and Skoog (MS) media on *sambung nyawa* plantlets's growth. This study was an experimental with 2 combined factors; moringa leaves and shallots filtrate at various concentration and BAP and IAA control treatment. The growth parameters observed were plantlet height, leaves's number, root length and roots's number. The data obtained were then tested for normality, Friedman Test, and Mann-Whitney Test. This study found that there was effect on varying concentration of moringa leaf filtrate, concentration of shallot filtrate, and the interaction of both on the growth of *sambung nyawa* plantlets with 37.5 g/L moringa leaf filtrate resulted in best plantlet height, 37.5 g/L shallot filtrate had the greatest number of leaves, 50 g/L shallot filtrate resulted in highest root length and combination of 25 g/L moringa leaf and 37.5 g/L shallot filtrated resulted in the highest number of roots.

Keywords: moringa; natural growth regulator; planlet; *sambung nyawa*; shallots; tissue culture

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INTRODUCTION

Sambung nyawa plant (*Gynura procumbens*) is a wild plant in the Asteraceae family often used for consumption and medicinal purposes (Bakhtra *et al*., 2018). Cultivation of the *sambung nyawa* plant is still limited due to various challenge to conventional propagation, such as the lack of seeds for generative propagation, long cultivation time, low biomass yields, limited agricultural land, and lower priority compared to other commodities such as food and plantations (Sakinah *et al*., 2018; Manuhara *et al*., 2019). One of the methods can be applied to increase *sambung nyawa* production is by propagating seeds using tissue culture.

Tissue culture is a cultivation method using adult plant tissue to produce new plants with the same characteristics as parent plant (Apriliyani and Wahidah, 2021). In-vitro plant propagation is affected by the composition in the growth medium and growth regulators from the auxin and cytokinin groups (Nurcahyani *et al*., 2021). Plant growth regulators (PGRs) are divided into two types; natural and synthetic regulators. Natural growth regulators are environmentally friendly, safe to use, readily available, and less expensive (Kaffi, 2018). Moringa leaves and shallots are readily available potential sources of natural growth regulators.

Moringa leaves contain significant amounts of ascorbate and phytohormones, such as cytokinin (zeatin), auxin, and gibberellin (Nasir *et al*., 2016; Howladar, 2014). A 50% moringa leaf extract was found to significantly increased plant height, fresh and dry weight, flower diameter, and leaf area in sunflowers (Iqbal *et al*., 2020). Shallots can be used as an alternative growth regulator due to their auxin and thiamine content, which can stimulate plant growth and development (Tarigan and Nurbaiti, 2017). Application of shallot extract has been shown to affect corn plant growth by increasing root length and number (Gresiyanti *et al*., 2021).

In vitro propagation of *sambung nyawa* plant cultures have been carried out using MS media with the addition of BAP, NAA, and IBA, resulting in higher number of shoot induction, elongation of shoot buds, number of roots and root length (Majumder *et al*., 2016). Research on the growth of *sambung nyawa* plantlets on MS media supplemented with natural growth regulators has not been conducted.

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Therefore, the aim of this study was to determine the effect of adding natural growth regulators, from moringa leaf and shallots filtrate, to MS media on the growth of *sambung nyawa* plantlets in vitro.

MATERIALS AND METHODS

This research was conducted in March-June 2025 at the laboratory of PT Pawitra Jaya Sakti Biotek Sidoarjo. This experimental study used a factorial Completely Randomized Design (CRD) with two combined factors; moringa leaf (M) and shallots (S) filtrate at various concentration (0 g/L, 12.5 g/L, 25 g/L, 37.5 g/L, and 50 g/L), and one control treatment of 1 mg/L BAP and 0.5 mg/L IAA. This study had 26 treatment media with two replications.

The preparation of study began with sterilization of the equipment using an autoclave, then filtrate of moringa leaf and shallot was produced by weighing 0 g, 12.5 g, 25 g, 37.5 g, and 50 g of moringa leaves and shallots which then pureed using a blender in a 1 L of aquades and filtered. BAP hormone was prepared at 1 mg/L while IAA at 0.5 mg/L. Medium was made by dissolving MS with filtrate and synthetic hormones using a magnetic stirrer and hotplate then pH was adjusted to 5.6 – 5.8. Medium adjusted for its pH were the sterilized using an autoclave.

Explants were taken from *sambung nyawa* plant at age 2–3 months old, cut to a size of 0.5 cm. The subculture process began with sterilization of equipment using autoclave and 96% alcohol, sterilization of plantlet bottles and media bottles with 96% alcohol, sterilization of the Biological Safety Cabinet (BSC) with 96% alcohol and exposure to UV light for 30 minutes. Then, plantlets were inoculated into medium in BSC by placing two explants in each bottle, capped with PP plastic, and labeled accordingly. The inoculated bottles were then stored in a sterile room with 14-watt LED lights and a temperature of 25°C under controlled conditions. Growth parameters were observed after 7 weeks of incubation period.

The growth parameters observed including plantlet height increase, number of leaves, root length, and number of roots. The data obtained were then analyzed for normality, followed by a Friedman test at 5% significance level to determine significant differences between treatments. Then, a Mann-Whitney test at 5% significance level was performed to compare two treatments and determine any significant differences between samples.

RESULTS

Growth parameters observed in *sambung nyawa* planlets are presented in Table 1 and Table 2. Based on Table 1, it can be seen that the MS media with the addition of 37.5 g/L moringa leaf filtrate without shallot filtrate resulted in the highest increase in plantlet height and MS media with the addition of 37.5 g/L shallot filtrate and without the addition of moringa leaf filtrate treatment produced the highest average number of leaves. Meanwhile in Table 2 showed that MS media with the addition of 50 g/L shallot filtrate and without the addition of moringa leaf filtrate treatment produced the highest average root length, MS media with the addition of 25 g/L moringa leaf filtrate and 37.5 g/L shallots treatment produced the highest average root number.

The Friedman Test results for all growth variables showed a significance value of $p<0.05$, indicating a significant difference in growth outcomes across all sambung nyawa plantlet growth variables (Table 1 and Table 2).

Table 1. Effect of various concentration of Moringa leaf and shallot filtrate to the growth of *Sambung nyawa* planlet above the ground

Treatments*	Height (cm)**	Number of Leaves (pc)**
M_0S_0	$1.500 \pm 0.354^{acdehiklm}$	$7.750 \pm 1.299^{adfgijkmo}$
$M_0S_{12.5}$	$2.150 \pm 0.384^{abcefhjk}$	$8.500 \pm 1.118^{adfghijkmo}$
M_0S_{25}	$2.150 \pm 0.377^{abcdefhjk}$	$9.250 \pm 1.920^{acdefghijklmo}$
$M_0S_{37.5}$	$2.325 \pm 0.370^{bcfghjk}$	16.750 ± 1.479^{bl}
M_0S_{50}	$2.275 \pm 0.444^{abcefhjk}$	$11.750 \pm 1.299^{cdehjl}$
$M_{12.5}S_0$	2.500 ± 0.339^{bfghj}	12.750 ± 1.479^{cdehl}
$M_{12.5}S_{12.5}$	$1.600 \pm 0.400^{cdefhiklm}$	$8.000 \pm 0.707^{dfgijkmo}$
$M_{12.5}S_{25}$	$1.675 \pm 0.342^{cdefhiklm}$	$7.750 \pm 0.829^{dfgijkmo}$
$M_{12.5}S_{37.5}$	$1.850 \pm 0.477^{abcdefhijkl}$	$9.750 \pm 1.479^{defhijlmo}$
$M_{12.5}S_{50}$	1.325 ± 0.205^{acdilm}	12.000 ± 0.707^{cehl}
$M_{25}S_0$	$1.500 \pm 0.367^{dehiklm}$	$8.000 \pm 0.707^{dfgijkmo}$
$M_{25}S_{12.5}$	1.325 ± 0.327^{deiklm}	$5.750 \pm 1.479^{dgikmno}$
$M_{25}S_{25}$	1.725 ± 0.192^{ehik}	$6.500 \pm 1.118^{dgikmno}$
$M_{25}S_{37.5}$	2.450 ± 0.364^{bfghj}	10.250 ± 1.090^{hjl}
$M_{25}S_{50}$	1.775 ± 0.178^{ehik}	$8.250 \pm 0.829^{dfgijkmo}$
$M_{37.5}S_0$	2.950 ± 0.150^{gh}	9.500 ± 1.118^{cjlmo}

$M_{37.5}S_{12.5}$	2.000 ± 0.616^{abcdefghijk}	6.750 ± 1.479^{dfgijkmno}
$M_{37.5}S_{25}$	2.050 ± 0.415^{efhijk}	6.750 ± 0.829^{dgikmo}
$M_{37.5}S_{37.5}$	2.125 ± 0.507^{efhijk}	7.000 ± 1.225^{dfgijkmo}
$M_{37.5}S_{50}$	2.25 ± 0.482^{hjk}	6.750 ± 0.829^{dgikmo}
$M_{50}S_{0}$	1.450 ± 0.229^{iklm}	7.000 ± 0.707^{dgikmo}
$M_{50}S_{12.5}$	2.200 ± 0.234^{jk}	12.250 ± 2.278^{l}
$M_{50}S_{25}$	1.775 ± 0.268^{k}	7.750 ± 0.829^{dfgijkmo}
$M_{50}S_{37.5}$	1.350 ± 0.050^{l}	4.500 ± 1.118^{n}
$M_{50}S_{50}$	1.150 ± 0.112^{m}	8.000 ± 0.707^{dfgijkmo}
Control (BAP + IAA)	1.450 ± 0.364	8.250 ± 2.165

Note: *) Number typed in subscript is the concentration of the filtrate.
**) The same superscript notation indicates no significant difference, while different superscript indicates a significant different at 5% level (Mann-Whitney test).

Table 2. Effect of various concentration of Moringa leaf and shallot filtrate to the growth of *Sambung nyawa* planlet under the ground

Treatments*	Root Length (cm)**	Number of Roots (pc)**
$M_{0}S_{0}$	15.250 ± 1.639^{afhjklp}	2.250 ± 1.090^{acdehkl}
$M_{0}S_{12.5}$	4.625 ± 2.902^{abilmopq}	2.500 ± 1.500^{abcdefhjkl}
$M_{0}S_{25}$	20.125 ± 2.301^{abckn}	3.000 ± 1.225^{acdehjkl}
$M_{0}S_{37.5}$	21.750 ± 3.897^{abcdk}	4.750 ± 0.433^{befhj}
$M_{0}S_{50}$	24.500 ± 2.958^{abck}	1.500 ± 0.500^{achkl}
$M_{12.5}S_{0}$	22.525 ± 4.132^{abck}	2.750 ± 0.433^{acdehkl}
$M_{12.5}S_{12.5}$	10.000 ± 2.449^{efhijklmp}	3.250 ± 1.090^{aefhjkl}
$M_{12.5}S_{25}$	12.250 ± 3.491^{fhijklmp}	7.250 ± 3.112^{bfhij}
$M_{12.5}S_{37.5}$	13.375 ± 3.990^{fhijklp}	6.000 ± 4.243^{ahijkl}
$M_{12.5}S_{50}$	0 ± 0^{efginr}	0 ± 0^{gkm}
$M_{25}S_{0}$	12.500 ± 2.179^{fhijklp}	3.250 ± 1.090^{ahjkl}
$M_{25}S_{12.5}$	6.900 ± 5.112^{iklmnopq}	6.000 ± 4.301^{ahijkl}
$M_{25}S_{25}$	5.375 ± 5.447^{iklmnopqr}	3.500 ± 3.571^{aijklm}
$M_{25}S_{37.5}$	15.000 ± 2.622^{fhijklp}	7.750 ± 1.479^{gij}
$M_{25}S_{50}$	18.625 ± 9.296^{klmp}	4.750 ± 1.299^{j}
$M_{37.5}S_{0}$	10.250 ± 3.491^{lmop}	4.750 ± 1.785^{ahjkl}
$M_{37.5}S_{12.5}$	5.075 ± 3.208^{fmnopq}	7.000 ± 3.937^{ahijkl}
$M_{37.5}S_{25}$	1.875 ± 3.248^{noqr}	1.750 ± 3.031^{aklm}
$M_{37.5}S_{37.5}$	1.000 ± 1.225^{noqr}	3.500 ± 5.500^{aklm}
$M_{37.5}S_{50}$	3.625 ± 3.664^{nopq}	4.000 ± 2.915^{ahijkl}
$M_{50}S_{0}$	1.500 ± 1.500^{nqr}	1.500 ± 1.500^{acklm}
$M_{50}S_{12.5}$	9.750 ± 3.345l^{mop}	2.250 ± 0.829^{ackl}
$M_{50}S_{25}$	2.925 ± 1.618^{q}	5.750 ± 3.112^{ah}
$M_{50}S_{37.5}$	0 ± 0^{efgr}	0 ± 0^{gm}
$M_{50}S_{50}$	0 ± 0^{efgr}	0 ± 0^{gm}
Control (BAP + IAA)	0 ± 0	0 ± 0

Note: *) Number typed in subscript is the concentration of the filtrate.
**) The same superscript notation indicates no significant difference, while different superscript indicates a significant different at 5% level (Mann-Whitney test).

According to the Mann-Whitney test results, the dominant treatments significantly different from the other treatments were $M_{37.5}S_0$, which was significantly different from 19 other treatments; $M_{50}S_{50}$, which was significantly different from 17 other treatments; $M_{50}S_{37.5}$, which was significantly different from 16 other treatments; $M_{12.5}S_{50}$, which was significantly different from 14 other treatments; $M_{12.5}S_0$, which was significantly different from 12 other treatments; and $M_{50}S_{12.5}$, which was significantly different from 12 other treatments. Each dominant treatment significantly different from the other treatments was then compared on average to determine the best treatment. The $M_{37.5}S_0$ treatment, a treatment with MS media supplemented with 37.5 g/L of moringa leaf filtrate and without the addition of shallot filtrate, was the treatment with the most significant differences compared to the other treatments and had the highest average growth rate in plantlet height.

Based on the Mann-Whitney test results, the dominant treatments significantly different from the other treatments were $M_0S_{37.5}$, which was significantly different from 24 other treatments; $M_{50}S_{37.5}$, which was significantly different from 21 other treatments; $M_{12.5}S_{50}$, which was significantly different from 18 other treatments; M_0S_{50}, which was significantly different from 17 other treatments; $M_{12.5}S_0$, which was significantly different from 17 other treatments; $M_{50}S_{12.5}$, which was significantly different from 17 other treatments; and $M_{25}S_{37.5}$, which was significantly different from 16 other treatments. Each dominant treatment significantly different from the other treatments was then compared on average to

determine the best treatment. The $M_0S_{37.5}$ treatment, the MS media treatment with the addition of 37.5 g/L shallot filtrate and without the addition of moringa leaf filtrate, was the treatment with the most significant differences compared to the other treatments and had the highest average leaf growth rate.

The dominant treatment significantly differed from the other treatments: the M_0S_{25} treatment was significantly different from 21 other treatments, the $M_0S_{37.5}$ treatment was significantly different from 21 other treatments, the M_0S_{50} treatment was significantly different from 19 other treatments, and the $M_{12.5}S_0$ treatment was significantly different from 20 other treatments. Each dominant treatment significantly differed from the other treatments and was then compared to determine the best treatment. The M_0S_{50} treatment, a MS medium treatment with 50 g/L of shallot filtrate and without the addition of moringa leaf filtrate, had the highest average root length growth rate.

According to the test results, the dominant treatments significantly differed from the others: the $M_{12.5}S_{50}$ treatment, which was significantly different from 18 other treatments; the $M_{50}S_{37.5}$ treatment, which was significantly different from 18 other treatments; the $M_{50}S_{50}$ treatment, which was significantly different from 18 other treatments; the $M_{25}S_{37.5}$ treatment, which was significantly different from 14 other treatments; and the M_0S_{50} treatment, which was significantly different from 11 other treatments. Each treatment that was significantly different from the others was then compared on average to determine which treatment was superior. Treatment $M_{25}S_{37.5}$, a treatment using MS media supplemented with 25 g/L moringa leaf filtrate and 37.5 g/L shallot filtrate, had the highest average root growth rate.

The growth of sambung nyawa plantlets in MS media supplemented with 1 mg/L BAP and 0.5 mg/L IAA only resulted in plantlet height and leaf number. Furthermore, the lower part of the plantlets did not grow roots but rather callus, making it impossible to calculate the growth parameters of root. The result of number of leaves from each treatment can be viewed in the following Figure 1.

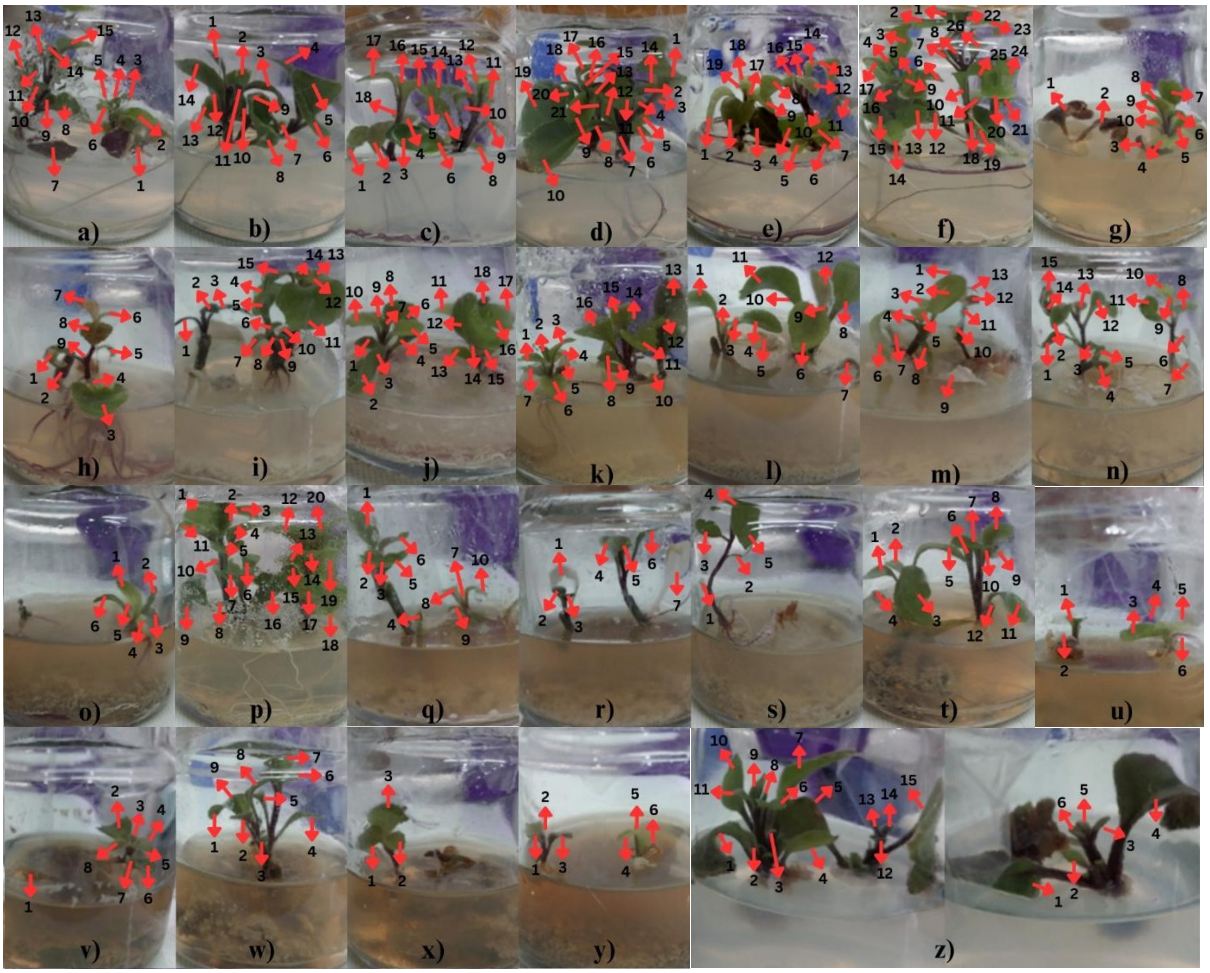

Figure 1. Observation results of the number of leaves based on treatment. a) **M_0S_0**; b) **$M_0S_{12.5}$**; c) **M_0S_{25}**; d) **$M_0S_{37.5}$**; e) **M_0S_{50}**; f) **$M_{12.5}S_0$**; g) **$M_{12.5}S_{12.5}$**; h) **$M_{12.5}S_{25}$**: i) **$M_{12.5}S_{37.5}$**; j) **$M_{12.5}S_{50}$**; k) **$M_{25}S_0$**; l) **$M_{25}S_{12.5}$**; m) **$M_{25}S_{25}$**; n) **$M_{25}S_{37.5}$**; o) **$M_{25}S_{50}$**; p) **$M_{37.5}S_0$**; q) **$M_{37.5}S_{12.5}$**; r) **$M_{37.5}S_{25}$**; s) **$M_{37.5}S_{37.5}$**; t) **$M_{37.5}S_{50}$**; u) **$M_{50}S_0$**; v) **$M_{50}S_{12.5}$**; w) **$M_{50}S_{25}$**; x) **$M_{50}S_{37.5}$**; y) **$M_{50}S_{50}$**; z) Control (MS + 0.5 mg/L IAA and 1 mg/L BAP)

DISCUSSION

Plantlet height increases due to the division, elongation, and enlargement of new cells in the apical meristem and stem internodes, which causes the plantlets to grow taller (Widiastoety, 2014). Plant growth requires growth regulators from the auxin and cytokinin groups (Nurcahyani *et al*., 2021). Moringa and shallot leaves contain endogenous hormones from the auxin and cytokinin groups (Tini *et al*., 2022). The administration of growth regulators increases protein synthesis in plant cells. Proteins are used as a building block for plant organs such as roots, stems, and leaves (Salisbury and Ross, 1992).

Moringa leaf filtrate is known to be rich in natural phytohormones such as auxins, cytokinins, and gibberellins, which can stimulate root growth, shoot growth, and cell elongation (Saini *et al*., 2016). Moringa leaf extract contains natural cytokinin hormones such as zeatin, dihydrozeatin, and isopentyladenine. Furthermore, moringa leaves contain protein, minerals, essential amino acids, glucosinolates, isothiocyanates, and phenolics, which can stimulate plant growth (Culver *et al*., 2012). However, excessively high concentrations can cause inhibitory effects due to hormonal imbalance or phytotoxicity. Plant growth regulators, such as auxin, can act as growth stimulants and inhibitors depending on the concentration used (Farman *et al*., 2019). This is reflected in the decrease in average plantlet height and leaf number at a concentration of 50 g/L, indicating possible toxic effects or physiological stress.

Shallots contain compounds such as flavonoids, such as quercetin, organic sulfur, and phenolic compounds, which have antioxidant activity and potentially support root and shoot cell elongation (Hasibuan *et al*., 2020; Sagar *et al*., 2022). Other studies have also shown that several shallot concentration treatments, such as 7.5 mL/L – 22.5 mL/L, showed no significant differences in plant height parameters (Mutryarny *et al*., 2022b). This may be due to the higher auxin content in shallots than cytokinins (Tini *et al*., 2022). The auxin hormone is known to play a vital role in almost every aspect of root development, from the cellular level to the overall root system level (Roychoudhry and Kepinski, 2022). Active sulfur compounds at high concentrations can also trigger oxidative stress or antimicrobial reactions that can inhibit the process of early root differentiation (Benkeblia, 2004). Therefore, plantlet growth tends to decrease in almost all growth variables at a concentration of 50 g/L.

Based on the Mann-Whitney test, it was found that there was an interaction between the concentration of moringa leaf filtrate and the concentration of shallot filtrate on plantlet height. This can be seen from the treatment media where the combination of moringa leaf filtrate and shallot filtrate can also differ significantly from other treatments, although this does not necessarily result in better growth. This finding indicates that the response of sambung nyawa plantlets to one treatment factor may depend on the concentration of the other factor. The effect on sambung nyawa plantlet growth of one concentration of moringa leaf filtrate may differ depending on the concentration of shallot filtrate used simultaneously, and vice versa.

Biologically, the interaction between the concentration of moringa leaf filtrate and shallot filtrate may occur due to the possible synergy or interaction between the bioactive compounds contained in the two types of filtrate. The hormones auxin and cytokinin in moringa leaves are known to play a role in stimulating root and shoot growth (Saini *et al*., 2016). Meanwhile, shallots also contain sulfur compounds and flavonoids such as quercetin, which act as antioxidants and promote tissue elongation (Hasibuan *et al*., 2020; Sagar *et al*., 2022).

Moringa leaves have a higher auxin content than shallots (Tini *et al*., 2022). However, the treatment medium containing 50 g/L shallots resulted in the highest root growth. This may be due to the endogenous hormones contained in the grafted explants. The endogenous hormones in the grafted explants are thought to be sufficient to meet the explant's growth hormone requirements. Therefore, the addition of exogenous hormones from shallots at lower auxin levels than those from moringa leaves is thought to meet the requirements for optimal root growth. Thus, a synergistic reaction occurs between the endogenous hormones in the grafted explants and the exogenous hormones in shallots. Meanwhile, exogenous hormones from moringa leaves, with higher auxin levels than shallots, are thought to exceed the requirements for root growth, resulting in an antagonistic reaction between the endogenous hormones of the grafted explants and the exogenous hormones of the moringa leaves. Treatment media containing 25 g/L of moringa leaves and 37.5 g/L of shallots also resulted in the highest root growth. This may be due to the presence of endogenous hormones contained in the grafted explants. The endogenous hormones of the grafted explants are thought to be sufficient to meet the explant's growth hormone needs, so adding exogenous hormones from shallots at a higher concentration than those from moringa leaves is thought to meet the requirements for optimal root growth. This creates a synergistic reaction between the endogenous hormones of the grafted explants and the exogenous hormones of the

shallots and moringa leaves. This combination of compounds has the potential to create synergistic or antagonistic interactions, depending on their concentration.

Several media treatments combining moringa leaf and shallot filtrates have resulted in lower growth of grafted explants compared to media treatments using only one filtrate. This can occur due to the interaction between exogenous and endogenous hormones in plants. Explant growth and morphogenesis are regulated by the interaction and balance of growth regulators in the media with endogenous hormones present in the explants (Rainiyati and Kristiana, 2009). The interaction between endogenous hormones and the administered growth regulators results in a balanced ratio of their action to cytokinins (Aziz *et al.*, 2014).

Root length growth in media supplemented with 1 mg/L BAP and 0.5 mg/L IAA did not show good results. Plantlets did not produce root growth, but instead produced callus growth. Higher concentration of cytokinin in undifferentiated plant cells can encourage explants to form shoots (Svolacchia and Sabatini, 2023). However, callus formation is also influenced by the interaction of exogenous hormones added to the media and endogenous hormones contained in the explants (Wahyuni *et al.*, 2020).

This indicates that moringa leaf filtrate and shallot filtrate are capable of promoting stable root differentiation, unlike synthetic hormones, which tend to trigger the proliferation of non-differentiated cells into callus. Therefore, moringa leaf filtrate and shallot filtrate can be considered effective natural plant growth regulators, as they can simultaneously support vegetative and root growth without producing callus that could interfere with acclimatization.

According to research that has been carried out, the best treatment for increasing plantlet height is $M_{37.5}S_0$ treatment (MS media with the addition of 37.5 g/L of Moringa leaf filtrate), the best treatment for the number of leaves is $M_0S_{37.5}$ treatment (MS media with the addition of 37.5 g/L of shallot filtrate), the best treatment for root length is the M_0S_{50} treatment (MS medium with the addition of 50 g/L of shallot filtrate), and the best treatment for root number is the $M_{25}S_{37.5}$ (MS media with the addition of 25 g/L Moringa leaf filtrate and 37.5 g/L shallot filtrate).

Furthermore, control media using synthetic hormones resulted in callus formation but not root formation, indicating that hormonal balance significantly determines growth direction. The use of filtrates from natural sources, such as moringa leaves and shallots, provides a more stable approach and is closer to natural conditions, as the compounds work more slowly and support tissue differentiation rather than triggering excessive proliferation. Utilizing filtrates from natural sources offers the opportunity to develop cheaper, more sustainable growing media that are free from the risk of synthetic hormonal imbalances. This makes the filtrate a relevant and strategic biological additive option in tissue culture-based propagation.

CONCLUSION

Based on the research that has been done and the research results obtained, it can be concluded that there is an effect of the concentration of moringa leaf filtrate, the concentration of shallot filtrate, and the interaction between the concentration of moringa leaf filtrate and the concentration of shallot filtrate on the growth of sambung nyawa (*Gynura procumbens*) plantlets with 37.5 g/L moringa leaf filtrate increasing the best plantlet height, 37.5 g/L shallot filtrate increasing the best number of leaves, 50 g/L shallot filtrate increasing the best root length and 25 g/L moringa leaf filtrate and 37.5 g/L shallot increasing the best number of roots.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

REFERENCES

Apriliyani R, and Wahidah BF, 2021. Perbanyakan anggrek *Dendrobium* sp. secara *in vitro*: Faktor-faktor keberhasilannya. *Filogeni: Jurnal Mahasiswa Biologi 1*(2): 33–46.

Aziz MM, Ratnasari E, and Rahayu YS, 2014. Induksi Kalus Umbi Iles-Iles (*Amorphophallus muelleri*) dengan Kombinasi Konsentrasi 2, 4-D dan BAP Secara *In Vitro*. *LenteraBio 3*(2): 109–114.

Bakhtra DD, Jubahar J, and Yusdi E, 2018. Uji Aktivitas Fraksi Dari Ekstrak Daun Sambung Nyawa (*Gynura procumbens* (Lour) Merr.) Terhadap Bakteri *Shigella dysenteriae*. *Jurnal Farmasi Higea 10*(1): 10–18.

Benkeblia N, 2004. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *LWT-Food Science and Technology 37*(2): 263–268.

Culver M, Fanuel T, and Chiteka AZ, 2012. Effect of moringa extract on growth and yield of tomato. *Greener Journal of Agricultural Sciences 2*(5): 207–211.

Farman S, Mushtaq A, and Azeem MW, 2019. Plant growth regulators (PGRs) and their applications: A review. *International Journal of Chemical and Biochemical Sciences 15*: 94-103.

Gresiyanti DM, Anissa RK, Setyawati FD, Susanto AD, and Ratnasari E, 2021. Perbandingan Efektivitas Ekstrak Bawang Merah dan Auksin Sintetik Terhadap Pertumbuhan Akar Jagung (*Zea mays* L.). *Prosiding Seminar Nasional Biologi 1*: 715–724.

Hasibuan AS, Edrianto V, and Purba N, 2020. Skrining fitokimia ekstrak etanol umbi bawang merah (*Allium cepa* L.). *Jurnal Farmasimed (JFM) 2*(2): 45–49.

Howladar SM, 2014. A novel *Moringa oleifera* leaf extract can mitigate the stress effects of salinity and cadmium in bean (*Phaseolus vulgaris* L.) plants. *Ecotoxicology and Environmental Safety 100*: 69–75.

Iqbal J, Irshad J, Bashir S, Khan S, Yousaf M, and Shah AN, 2020. Comparative study of water extracts of Moringa leaves and roots to improve 52 the growth and yield of sunflower. *South African Journal of Botany*, *129*: 221-224.

Kaffi U, 2018. Uji Efektifitas Pertumbuhan Vegetatif Bunga Nusa Indah (*Mussaenda Pubescens*) Terhadap Pemberian ZPT Organik Jagung Muda Pada Berbagai Sumber Setek. *AGROVITAL: Jurnal Ilmu Pertanian 2*(2): 62–66.

Manuhara YSW, Noviyanti R, Hardjo PH, and Tanjung M, 2019. Enhancement of flavonoid production of *Gynura procumbens* (Lour.) Merr adventitious roots in baloon-type bubble bioreactor influenced by phenylalanine and tyrosine. *Ecology Environment dan Conservation Journal 25*: S61–S66.

Majumder S, Biswas A, and Rahman MM, 2016. In vitro mass propagation of *Gynura procumbens* (Lour.) Merr.-an important medicinal plant. *Asian Journal of Natural & Applied Sciences 5*(3): 71-79.

Mutryarny E, Endriani E, and Purnama I, 2022. Efektivitas Zat Pengatur Tumbuh dari Ekstrak Bawang Merah pada Budidaya Bawang Daun (*Allium porum* L). *Jurnal Pertanian 13*(1): 33–39.

Nasir M, Khan AS, Basra SMA, and Malik AU, 2016. Foliar application of moringa leaf extract, potassium and zinc influence yield and fruit quality of 'Kinnow' mandarin. *Scientia Horticulturae 210*: 227–235.

Nurcahyani E, Zulkifli Z, and Kanedi M, 2021. Pengenalan dan Pelatihan Teknik Kultur Jaringan Tumbuhan Bagi Guru Biologi SMA Se-Kabupaten Tanggamus Provinsi Lampung. *Jurnal Pengabdian Kepada Masyarakat (JPKM) TABIKPUN 2*(1): 39–46.

Rainiyati L, and Kristiana M, 2009. Peranan IAA dan BAP terhadap perkembangan nodul pisang (Musa AAB) raja nangka secara *in vitro*. *Jurnal Agronomi 13*(1): 51–57.

Roychoudhry S, and Kepinski S, 2022. Auxin in root development. *Cold Spring Harbor Perspectives in Biology 14*(4): a039933.

Sagar NA, Pareek S, Benkeblia N, and Xiao J, 2022. Onion (*Allium cepa* L.) bioactives: Chemistry, pharmacotherapeutic functions, and industrial applications. *Food Frontiers 3*(3): 380–412.

Saini RK, Sivanesan I, and Keum YS, 2016. Phytochemicals of *Moringa oleifera*: a review of their nutritional, therapeutic and industrial significance. *3 Biotech 6*: 1–14.

Sakinah DG, Putra ETS, and Rogomulyo R, 2018. Produksi dan kadar flavonoid daun sambung nyawa (*Gynura procumbens* (lour.) merr.) pada tiga fase agroforestri. *Vegetalika 7*(3): 1-15.

Salisbury FB, and Ross CW, 1992. *Plant physiology*. Belmont: Wadsworth Publishing.

Svolacchia, N, and Sabatini, S, 2023. Cytokinins. *Current Biology 33*(1): R10-R13.

Tarigan PL, and Nurbaiti SY, 2017. Pemberian ekstrak bawang merah sebagai zat pengatur tumbuh alami pada pertumbuhan setek lada (*Piper nigrum* L.). *Jurnal Online Mahasiswa (JOM) FAPERTA 1*: 1-11.

Tini EW, Sakhidin S, Saparso S, and Haryanto TAD, 2022. Perbandingan Hormon Endogenous pada Beberapa Tanaman. *Jurnal Galung Tropika 11*(2): 132–142.

Wahyuni A, Satria B, and Zainal A, 2020. Induksi kalus gaharu dengan NAA dan BAP secara *in vitro*. *Agrosains: Jurnal Penelitian Agronomi 22*(1): 39 44.

Widiastoety D, 2014. Pengaruh auksin dan sitokinin terhadap pertumbuhan planlet anggrek Mokara (Effect of auxin and cytokinin on the growth of Mokara orchid plantlets). *Journal of Tropical Horticulture 24*(3): 230–238.