BIODIVERSITAS
Volume 25, Number 5, May 2024
Pages: 1955-1964

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250512

Methyl jasmonate stimulates growth and upregulates the expression of Phenylalanine Ammonia-Lyase (PAL) gene in *Gynura pseudochina* in vitro micropropagation

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Manuscript received: 25 February 2024. Revision accepted: 7 May 2024.

Abstract. *Anjalani TR, Rasmi SA, Rahayu AE, Ramadhani MRN, Sholihah MF, Puspaningtyas I, Soleha ID, Sari SA, Rahmawati M, Nasori N, Jadid N. 2024. Methyl jasmonate stimulates growth and upregulates the expression of Phenylalanine Ammonia-Lyase (PAL) gene in* Gynura pseudochina *in vitro micropropagation. Biodiversitas 25: 1955-1964.* The use of medicinal plants as primary resources for traditional medicine is rising. *Gynura pseudochina* (L) DC. is a well-recognized traditional medicinal plant from Southeast Asia. It is rich in bioactive compounds like flavonoids, alkaloids, saponins, and tannins. Various techniques and methods have been developed to ensure plant quality and enhance phytomedicines' production, one of which involves plant in vitro culture. Elicitor-based in vitro culture, particularly using Methyl Jasmonate (MeJA), has been shown to boost the production of secondary metabolites in plants through the overexpression of Phenylalanine Ammonia-Lyase (PAL) genes, known to play a role in the excessive production of flavonoid compounds. This research aims to investigate the impact of MeJA on the growth and PAL gene expression in *G. pseudochina* in vitro culture. Several MeJA concentrations of MeJA (0, 75, 150, and 300 µM) were tested. Our findings revealed that the application of MeJA to *G. pseudochina* in vitro had a noticeable impact on callogenesis and organogenesis. The optimal condition for callogenesis was achieved with MeJA at a concentration of 150 µM. On the other hand, the highest frequency of root induction was observed at 75 µM of MeJA treatment, while the highest shoot frequency occurred at 150 µM of MeJA. Interestingly, the plant height, fresh weight, leaf, and shoot numbers were significantly affected after treatment with 150 µM of MeJA. Additionally, 75 µM of MeJA was found to promote optimal root growth compared to other MeJA treatments. Finally, 150 µM of MeJA treatment resulted in higher expression of the *PAL* gene (1.29 folds) than the non-treated plants. Our findings suggested that MeJA influenced the *G. pseudochina* growth and potentially increased the production of flavonoid compounds in vitro.

Keywords: Growth, *Gynura pseudochina*, in vitro culture, methyl jasmonate, phenylalanine ammonia-lyase

INTRODUCTION

Gynura pseudochina, locally named "*daun dewa*" is a part of around 46 Gynura species. It is widely distributed in Africa, China, and Southeast Asia. This species is part of an Asteraceae plant. *Gynura pseudochina*, among around 30,000 medicinal plants in Indonesia, is recognized for its healing properties. Its vegetative organs, including leaves, rhizome, and root, possess inflammatory-reducing activity, beneficial for treating herpes diseases and pain relievers as well as for fever ailment treatment, respectively (Saralamp et al. 2000; Lemmen and Bunyapraphatsara 2003; Siriwatanametanon and Heinrich 2011). In addition, combined *G. pseudochina* leaves and *Curcuma aeruginosa* rhizome are also used for dengue fever treatment (Moektiwardoyo et al. 2014). Moreover, it has been stated that G. pseudochina possesses anti-cancer and anti-allergenic activities and is used for treating ulcers, bleeding, rashes, and diabetes mellitus ailments (Meng et al. 2021). All these pharmaceutical activities are tightly correlated with their phytochemical content. Many reports showed that *G. pseudochina* contains alkaloids, flavonoids, saponins, and tannins. This contributes to Indonesia's wide range of phytochemical-based medicinal plant resources that require further development (Jadid et al. 2018). In recent years, there has been an increase in the market demand for medicinal herbs (Picking 2024). Therefore, preserving natural germplasm is paramount (Bisht et al. 2023). On the other hand, an effective method for accelerating medicinal plant propagation is necessarily required. However, the quantity of medicinal plants is not the only factor that should be considered. The quality of the plant, including the species traits, plant performance, and phytochemical content, should also be considered (Luo et al. 2023).

A traditional method of propagation through stem cutting faces several limitations, including longer establishment time, high risk of disease transmission, and

rooting difficulties (Qin et al. 2023). Thus, some techniques including genetic engineering and marker-assisted selection could help facing these fundamental problematiques in agriculture. However, the previously mentioned techniques, in some cases, have limitations. It includes food safety, toxicology, allergenic issues, and time consuming (Hasan et al. 2021). Other biotechnology-based technique such as in vitro plant cell culture offers an alternative method that can be used for accelerating plant propagation (Jadid et al. 2024) and phytochemical isolation (Abbasi et al. 2019). A few attempts have been made to boost the *G. pseudochina* in vitro multiplication. The use of Murashige and Skoog (MS) medium combined with 3 ppm of 6-benzylaminopurine (BAP) has successfully produced 34.8 shoots and 57.5 leaves (Nirwan and Aziz 2006). In addition, a combination of BAP (3 ppm) and Indole Acetic Acid (IAA) (0.5 ppm) illuminated with 1,156 lux of light intensity was also reported to increase the number of *G. pseudochina* shoots, height, and leaves (Ghulamahdi et al. 2008). However, no report has been made for *G. pseudochina* in vitro culture using an elicitor, a chemical compound that stimulates the plant secondary metabolites (Malik et al. 2020). Some elicitors include light (Murthy et al. 2024), nano-elicitors (Rahmawati et al. 2022), salicylic acid and Methyl Jasmonate (MeJA) (Mahendran and Rahman 2024). MeJA is a common abiotic elicitor widely applied through in vitro culture (Jeyasri et al. 2023). MeJA, a jasmonic acid derivative, is a plant protective mechanism that triggers a defense response. It promotes the control of gene expression, resulting in the accumulation of biologically active compounds like terpenoids, flavonoids, and alkaloids (Ho et al. 2020; Pisitpaibool et al. 2021). In addition, MeJA was also reported to affect the formation of organs in plants by stimulating an increase in metabolic-related gene expression levels, including *MsPAL*, *MsC4H*, and *Ms4CL*. They are recognized as pivotal genes participating in the phenylpropanoid pathway in *Mentha x piperita* (Krzyzanowska et al. 2012). In line with this research, Liu et al. (2022) also proved that the expression of the PAL (Phenylalanine Ammonia-Lyase) gene in *Isatis indigotica* increased under MeJA treatment. Nonetheless, the regulation of these genes is generally specific or species-dependent. No prior studies have documented the impact of MeJA treatment on the expression of genes associated with the biosynthesis of phenol compounds in *G. pseudochina*. Therefore, this work aims to investigate the effect of exogenous MeJA treatment in *G. pseudochina* in vitro culture.

MATERIALS AND METHODS

Plant materials, medium preparation, and inoculation

G. pseudochina plantlet was obtained from the collection of the Laboratory of Bioscience and Plant Biotechnology, Biology Department, Institut Teknologi Sepuluh Nopember, Surabaya. In this in vitro culture study, we employed an axenic nodal segment of the *G. pseudochina* as the primary explant. The solid MS basal medium (PhytoTech Lab®) was prepared with 30 g/L of sucrose (Duchefa Biochemie, Netherland) and supplemented by 0.25 ppm IAA and 3 ppm BAP. A series of MeJA (Sigma-Aldrich) concentrations (0, 75, 150, and 300 µM) were also added. Finally, the culture media was solidified using 8.2 g/L gelrite powder (PhytoTech Lab®). The pH of the medium was also set from 5.7 to 5.8. The measurement used in autoclaving is at 121°C for 20 minutes (Huang et al. 2023). The inoculation stage is performed aseptic or sterile in Laminar Air Flow (LAF) by placing the axenic nodal segments of the *G. pseudochina* in the MS medium supplemented with MeJA. All the inoculated explants were placed in a grow room at 25 ± 2°C under 40W of cool white fluorescent, 16/8h (light/dark) photoperiod for 75 days. Each treatment consisted of eight replications.

Growth response measurement

We evaluated the frequency of callogenesis and organogenesis of the explants after 75 days of MeJA treatment in vitro according to the equation below:

$$\%\ Callogenesis\ Induction = \frac{the\ number\ of\ explants\ that\ formed\ the\ callus}{total\ number\ of\ explants} x\ 100\%$$

$$\%\ Organogenesis\ Induction = \frac{the\ number\ of\ explants\ that\ formed\ the\ organ}{total\ number\ of\ explants} x\ 100\%$$

Additionally, we also recorded the number of leaves per explant. Furthermore, root and shoot proliferation was also calculated. Finally, the productivity of the plants formed from the in vitro system was also evaluated based on their fresh weight.

RNA extraction and gene expression measurement

The *G. pseudochina* leaves were prepared for total RNA isolation using Total RNA Mini Kit Plant Geneaid), following the manufacturer's guidelines (Jadid et al. 2016). The total RNA extracted was then quantified using NanoDrop 2000 Spectrophotometers (Thermo Fisher Scientific™). For PAL gene expression analysis, we used 25 ng of the extracted RNA to be then subjected to RT-qPCR using SensiFAST SYBR No-ROX One Step Kit (Meridian Bioscience). The primers used in this study include PAL1F (5'-CGGAACAACACAACCAAGATG-3') and PAL2-R (5'-CTGATTGGCATAGAGCGACTAA-3') and the Actin gene as a reference gene with ACT1-F (5'-CCAAGGCTAACAGAGAGAAGATG-3') and ACT2-R (5'-CGACCACTGGCATATAGAGAAAG-3'). The RT-qPCR procedure began with a 10 min reverse transcription phase at 45°C, succeeded by a 2 min activation of the polymerase at 95°C. Then, the PCR cycling consisted of approximately 39 cycles at 95°C for 5 seconds (denaturation), 61.3°C for 10 seconds (annealing), and 72°C for 20 seconds (extension). Finally, the gene expression was calculated using the Livak 2 -ΔΔCT method.

Statistical analysis

This study was performed utilizing a completely randomized design. The *G. pseudochina* growth data and GpPAL gene expression were analyzed using one-way ANOVA followed by post-hoc Tukey test (Minitab 19).

RESULTS AND DISCUSSION

Impact of Methyl Jasmonate (MeJa) on morphogenic responses of *Gynura pseudochina*

The morphogenic responses of *G. pseudochina* after MeJA treatments were determined by the frequency of explant forming callus, shoots and roots. Variations of callus, shoot and root formationswere observed in all MeJA treatments of *G. pseudochina* in vitro culture after 75 days of culture. We observed a significant delay in callus formation in explants treated with 300 µM of MeJA (47.5 ± 6.8 days) compared to other MeJA treatments. Meanwhile, explants treated with 150 µM of MeJA demonstrated the lowest values (14.4 ± 1.1 days), followed by the control and 75 µM of MeJA, which took 13.8 ± 3.4 days and 22.3 ± 5.1 days, respectively. All explants treated with zero MeJA formed callus (100%). At the same time, treatment of MeJA in all concentrations tested seemed to lower the ability of the explant to form callus. Even though the highest frequency of explant forming callus was obtained in *G. pseudochina* treated with 150 µM of MeJA (87.5%). Meanwhile, the other MeJA treatment-induced callus is only 75% of the explants (Table 1).

The reduction of callus formation frequency as a result of the MeJA treatment, follows a previous study by Sun et al. (2017). Another report also demonstrated that MeJA lower callus formation and growth in *Taxus x media var. Hatfieldii* (Furmanowa et al. 1997). However, some different results were also observed in other plants in vitro. This suggests that MeJA effect was species-dependent and concentration-dependent (Rodríguez-Sánchez et al. 2020). Overall, our results suggested that the optimal concentration of MeJA in inducing callogenesis and shoot formation is at 150 µM. Even though further studies are still needed to obtain an optimal MeJA concentration for root induction.

We also observed that MeJA treatments also resulted in different characteristics of callus (Table 2). Callus texture varies at various levels of MeJA concentration. Callus derived from the MS medium's explants with no more than 75 µM and 150 µM MeJA showed a compact (non-friable) callus texture. Furthermore, the 300 µM of MeJA treatment produced a friable callus texture. These results are similar to those obtained in *Salacia chinensis* in vitro culture after treatment with 50 and 100 µM of MeJA; the latter produced a compact and hard callus texture (Chavan et al. 2021). In addition, the callus color was also different among MeJA treatments (Table 2).

The callus obtained from zero MeJA treatment possesses a green callus. Meanwhile, 75 µM induced the formation of a green-yellowish callus. In contrast, 150 µM and 300 µM of MeJA produced green-purplish callus (Figure 1). The alteration of callus color indicates callus cells are actively dividing, while darker or browner callus color generally indicates a decrease in cell growth (Pisitpaibool et al. 2021). The green color in the control callus can occur due to the influence of Plant Growth Regulators (PGRs) supplemented with the medium. In comparison, the purple color in the callus is probably due to increased anthocyanin production from the MeJA treatment.

In addition to the callogenesis responses, we observed different shoot and root formations responses of the explants planted in the MeJA-containing medium (Table 1). The percentage of shoot formation ranged from 0-100%. The maximum shoot formation percentage was attained with a MeJA treatment concentration of 150 µM (100%), followed by 0 µM (87.5%) and 75 µM (50%) (Table 1). Interestingly, we did not observe any shoot formation in the explants treated with the highest concentration of MeJA treatment (300 µM).

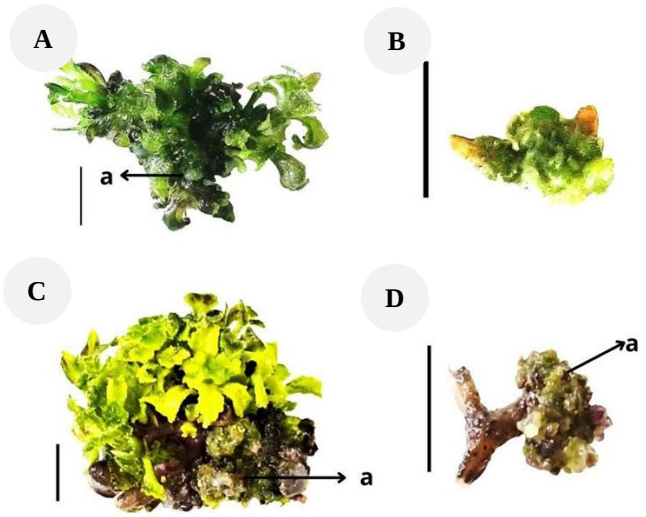

Figure 1. Callus formation in *Gynura pseudochina* in vitro culture after 75 days of culture. Note: A. Control, B. 75 µM, C. 150, D. 300 µM; (a) Callus (Scale bar: 1 cm)

Table 1. Morphogenic responses of *Gynura pseudochina* post-75 days of MeJA treatment

	Morphogenic responses (%)		
MeJA Treatment (µM)	**Frequency of explant forming callus**	**Frequency of shoot formation per explants**	**Frequency of root formation per explants**
0	100	87.5	62.5
75	75	50	75
150	87.5	100	0
300	75	0	0

Table 2. Texture and color of *Gynura pseudochina* callus after 75 days of MeJA treatment

MeJA treatments (µM)	Texture	Color
0	Compact	Green
75	Compact	Green yellowish
150	Compact	Green purplish
300	Friable	Green purplish

Some explants suffered mortality as a result of the elevated concentration of methyl jasmonate (Figure 2.D). We noticed that shoot formation appeared from the callus (Figure 1.C) and the nodal part (Figure 2.B). These data might suggest that MeJA treatment might induce the formation of embryogenic callus. Nevertheless, additional microscopic assessment should be carried to identify the presence of embryogenic callus (Zhang et al. 2021). Our results were similar to those observed in *Centella asiatica* in vitro culture, demonstrating that higher jasmonic acid concentrations might inhibit shoot formation (Krishnan et al. 2019). Machado et al. (2017) demonstrated that MeJA acts antagonistically with Gibberellic Acid (GA) to decrease shoot growth.

Another organogenesis response is the percentage of root formation. We noticed a variation in root formation frequency after MeJA treatments (Table 1, Figure 3). The percentage of root formation ranged from 0 to 75%. The highest percentage of root formation was obtained in 75 µM MeJA (75%), followed by 0 µM of MeJA (62.5%). Explants placed in the 150 and 300 µM of MeJA resulted in no root formation response (0%) (Table 1). This aligns with the results reported by Mangas et al. (2006), who observed that elevated concentrations of externally applied MeJA hinder root growth in the in vitro culture of *Ruscus aculeatus*.

Effect of MeJA on plant height of *Gynura pseudochina* in vitro culture

The increase in plant height resulted from the sequential progression involving cell division, elongation, and enlargement (Zhou et al. 2023). In this study, MeJA application in *G. pseudochina* in vitro culture significantly affects plant height ($P<0.05$). The *G. pseudochina* height ranged from 1.06 to 3.64 cm after MeJA treatment during 75 days of culture (Figure 4). The highest plant height was demonstrated by 150 µM of MeJA (3.64 cm), followed by the control plant (0 µM of MeJA). Meanwhile, we noted no significant difference between plant height after treatment 75 and 300 µM. They showed the lowest plant height of 1.32 and 1.06 cm, respectively. However, they significantly differ from the treatment at the control and 150 µM dose (Figure 4).

Gynura pseudochina plants cultured in vitro without MeJA (0 µM) exhibited greater growth than those subjected to 75 µM and 300 µM MeJA treatments. This could be attributed to the presence of IAA and BAP in the medium. The concurrent application of auxin and cytokinin can potentially enhance plant growth and development, influencing height, branching, and biomass (Talukdar et al. 2022).

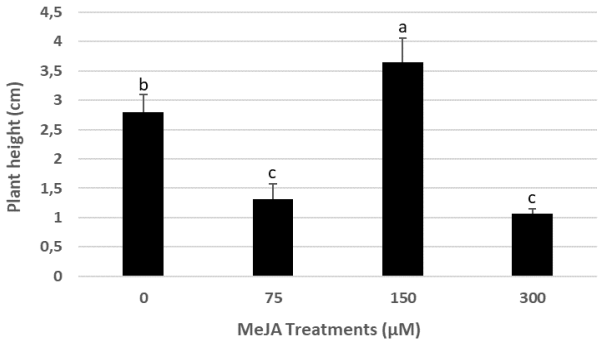

Figure 4. Effect of MeJA on plant height of *Gynura pseudochina* in vitro culture after 75 days of culture. Note: All data represented by mean ± standard deviation. Different letters represented a statistically significant effect by Tukey's test ($P<0.05$)

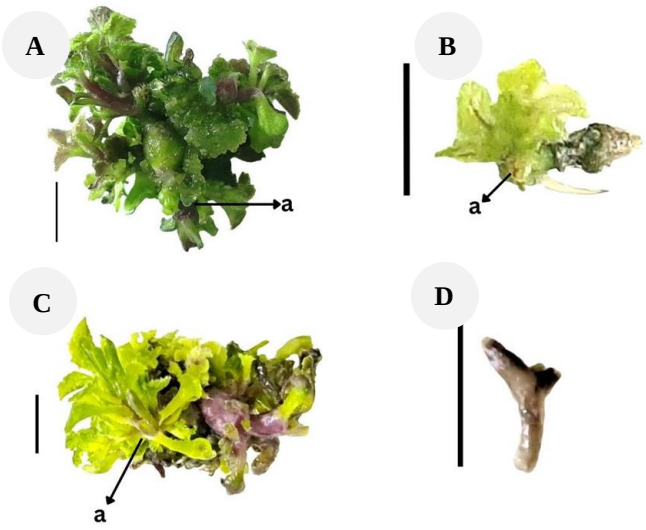

Figure 2. Shoot formation in *Gynura pseudochina* in vitro culture after 75 days of MeJA treatment. Note: A. Control, B. 75 µM, C. 150 µM, D. 300 µM (No shoot formation). A. Shoots (Scale bar: 1 cm)

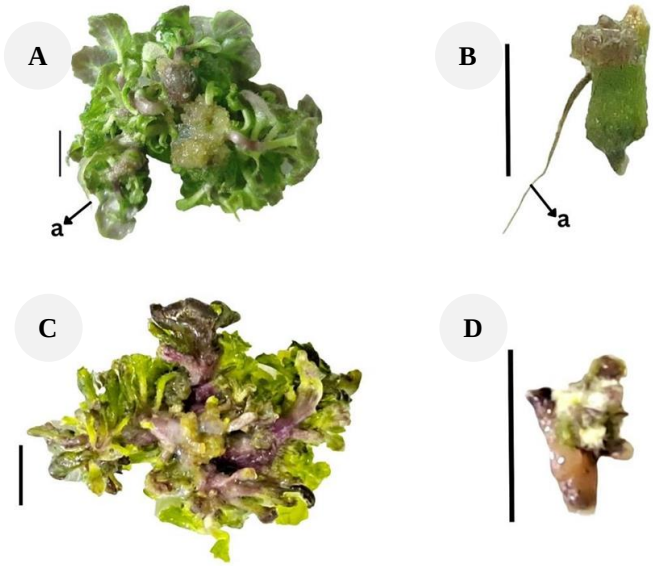

Figure 3. Root formation in *Gynura pseudochina* in vitro culture after 75 days of MeJA treatment. Note: A. Control, B. 75 µM, C. 150 µM (No roots detected), D. 300 µM (No roots formed); (a) Root (Scale bar: 1 cm)

Similar to the previous parameters observed, the effect of MeJA on plant height also depends on the concentration given. Alam and Albalawi (2020) demonstrated that 2 and 5 µM of MeJA treatment increased plant height of *Artemisia annua* in vitro culture. This effect is due to positive plant growth regulation due to exogenous MeJA application. In line with this study, Lalnunzawma et al. (2020) also showed that giving lower MeJA concentrations (0.3 and 0.6 mM) to strawberry plants (*Fragaria x ananassa*) increased plant height. However, it decreased plant growth at higher concentrations (0.9 mM). In contrast, other studies demonstrated different results, for instance, the application of MeJA (*Helianthus annuus*) and tomatoes (*Solanum lycopersicum*) (Li et al. 2018). It strongly supports the previous results, which explained that the effect of MeJA seems species-dependent. Moreover, plant height decreases because MeJA also plays an important role in plant senescence. Additionally, an unfavorable concentration of MeJA inhibits plant height by blocking the G1/S cell cycle transition, thereby reducing cell number and suppressing endoreduplication (which supports cell differentiation) (Heinrich et al. 2013). These consequently affect cell size and further generate a dwarf phenotype (Larrieu and Vernoux 2016).

Effect of MeJA on fresh weight of *Gynura pseudochina* in vitro culture

Fresh weight is a commonly employed quantitative measurement of plant growth and development. The augmentation of fresh weight primarily results from cell enlargement due to high water absorption rate and cell wall expansion regulated by turgor pressure within the cell (Dale 1988). In addition, fresh weight is also related to plant productivity, indicating good photosynthate transport (Mehmandar et al. 2023). In this investigation, the application of external MeJA had a notable impact on the fresh weight of the plants ($P<0.05$). The fresh weight ranged from 0.07-6.95 g per plant (Figure 5).

The highest average of *G. pseudochina* fresh weight was found at 150 µM of MeJA treatment (6.95 g), followed by control treatment (3.44 g). Meanwhile, the lowest fresh weight was at a MeJA concentration of 300 µM (0.072 g). However, the latter was not significantly different from those observed at 75 µM of MeJA treatment (0.354 gr). Our results suggested that 150 µM of MeJA was the optimal concentration; in contrast, the other concentration seemed to inhibit plant growth and development. This result was also supported by data obtained from the control plant. It indicates that PGRs added to the medium affected plant growth (Amoanimaa-Dede et al. 2022).

We noticed that the alteration of fresh weight of the *G. pseudochina* obtained from the MeJA treatment varies depending on the MeJA concentration. Other studies also exhibited similar results. For instance, administering exogenous MeJA at 50 and 200 µM to *Talinum paniculatum* callus increased biomass compared to control (Restiani et al. 2022). The application of MeJA at a concentration of 1 µM led to a 10.89% increase in the fresh weight of Brassica oleracea plants compared to those that did not undergo treatment (Sirhindi et al. 2020). The enhancement of the plant's fresh weight was also reported to be followed by an increase in the production of plant secondary metabolites. However, MeJA is also known to cause a decrease in biomass. MeJA treatment at 100 µM has been reported to decrease the biomass of *Polygonum multiflorum* and *Echinacea purpurea* by 5.8 and 22.97%, respectively. MeJA is proposed to potentially stimulate the production of Reactive Oxygen Species (ROS), subsequently negatively impacting plant growth (Ho et al. 2020). This is similar to those in other plants under environmental stress conditions. The plants tend to produce significant amounts of antioxidants to counter the existence of detrimental radicals (Jadid et al. 2017).

Impact of MeJA on the number of leaves of *Gynura pseudochina* in vitro culture

Leaves are plants' primary site for photosynthesis, making them vitally significant in plant development and biomass accumulation (Sari et al. 2018). In addition, leaves are considered the most frequently used plant organ for traditional ailment treatment. The leaves of the *G. pseudochina* plant are also used in traditional medicine. The leaf extract of this plant is utilized for treating inflammation and herpes infections (Siriwatanametanon and Heinrich 2011). Therefore, leave formation resulting from the MeJA treatment should be well-evaluated. Our study demonstrated that MeJA significantly affects the number of leaves in *G. pseudochina* treatment.

Our data showed that exogenous MeJA significantly affected the number of plant leaves with an average range of 0-80.6 ($P<0.05$) (Figure 6.A). The highest average number of leaves was found when MeJA was given at 150 µM (80.6). We also observed a purple leaf on explants treated with µM of MeJA. This might indicate the production of flavonoid-based compounds compared to plants possessing 40.75 leaves per explant (Figure 6).

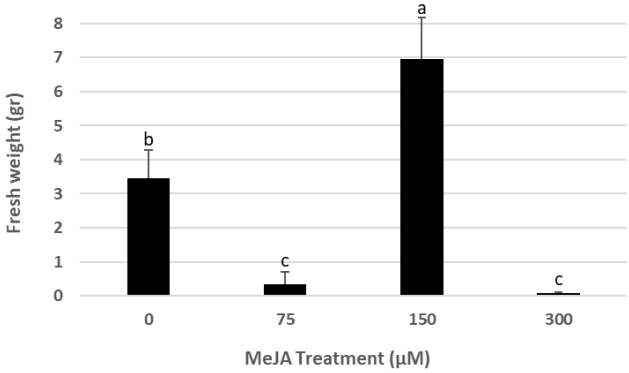

Figure 5. Effect of MeJA on fresh weight of *Gynura pseudochina* in vitro culture after 75 days of treatment. Note: All data represented by mean ± standard deviation. Different letters represented a statistically significant effect by Tukey's test ($P<0.05$)

The combination of IAA and BAP at the control medium successfully induced the formation of leaves. BAP is known as cytokinin, which contains nitrogen compounds, involved in protein synthesis; the latter is used for leaf growth (Pantin et al. 2011). Also, IAA increases the number of leaves and leaf area (Jin et al. 2023). No explants treated with 300 µM of MeJA exhibited zero leaf formation. This data suggests that MeJA application acts concentration-dependent, where the optimal concentration was obtained at 150 µM.

Previous studies demonstrated that leaves can generally induce callus (Rakesh et al. 2022). Another study explained that MeJA, in some cases, might increase leaf growth and senescence (Yamashita et al. 2021). In addition, 5 µM of MeJA has been reported to increase the number of leaves in wheat (*Triticum aestivum* L) (Islam et al. 2019). However, in other cases, MeJA can also cause a decrease in the number of leaves. For instance, adding 100 µM of MeJA decreased the number of *Celosia argentea* L. leaves (El-Sayed et al. 2023). The form of jasmonoyl-isoleucine (JA-Ile), which is catalyzed from the jasmonate compound, binds to the COI1 (coronatine insensitive-1) complex, which then recruits the transcription repressor JAZ (Jasmonate Zim-Domain) to degrade the COI-JAZ bond via the 26S proteasome. The JAZ repressor will ultimately inhibit the MYC transcription factor, increasing leaf growth. However, when the JA-Ile accumulation is low, the JAZ protein will be stably bound with MYC, which inhibits plant growth during defense responses (Kamińska 2021; Delgado et al. 2021). In addition, it is known that MeJA might delay the transition from the mitotic cell cycle to the endoreduplication cycle, which consequently reduces cell number and size (Noir et al. 2013).

Impact of MeJA on root number in *Gynura pseudochina* tissue culture

Plant roots absorb nutrients to support developmental processes (Lopez et al. 2023). A large number of roots can optimize the absorption of nutrients in the culture media (Sathyanarayan et al. 2023). In this study, the addition of MeJA significantly affected the number of roots of *G. pseudochina*, with an average range of root numbers of 1.25-1.60 ($P<0.05$) (Figure 7). Our data showed that the highest average number of roots was found when MeJA was added at 75 µM (1.6), followed by 0 µM of MeJA (1.25). We observed no root formation at 150 and 300 µM of MeJA treatment. Our results suggest that 75 µM of MeJA might help the plant to initiate root formation (Jásik and de Klerk 2006; Li et al. 2018).

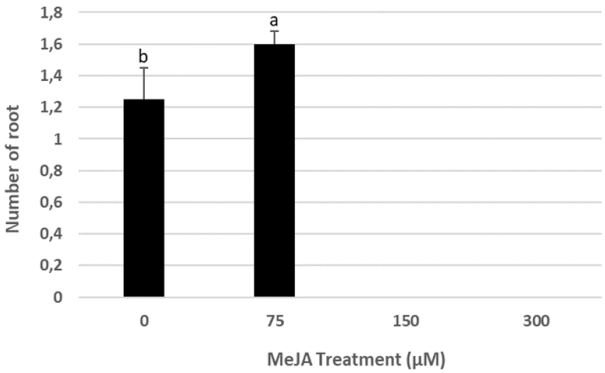

Figure 7. The Effect of MeJA on the root number of *Gynura pseudochina* in vitro culture after 75 days of culture. Note: All data represented by mean ± standard deviation. Different letters represent statistically significant differences by Tukey's test ($P<0.05$)

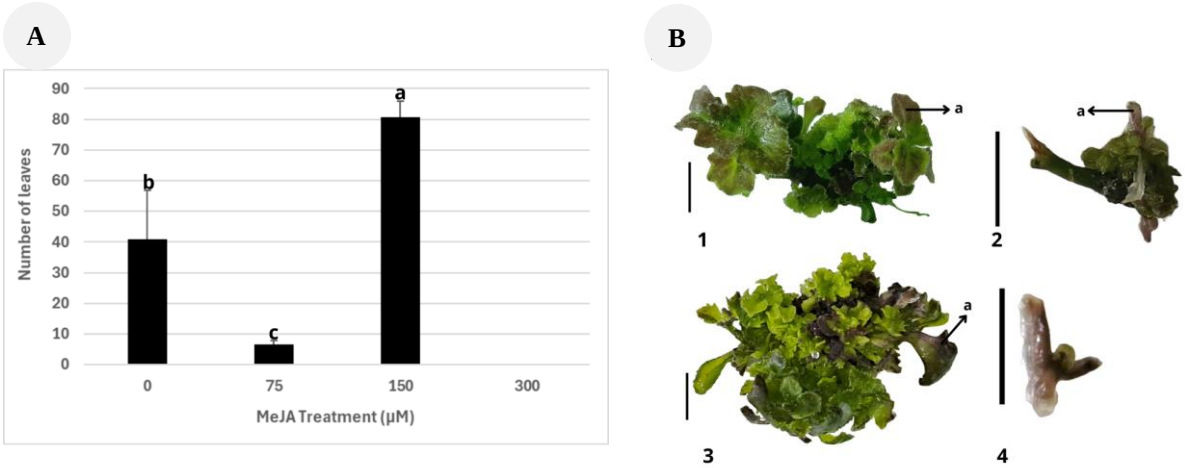

Figure 6. A. Effect of MeJA on the number of leaves of *Gynura pseudochina* in vitro culture after 75 days of treatment. Note: All data represented by mean ± standard deviation. Different letters represented a significant effect by Tukey's test ($P<0.05$). B. Morphological responses of G. pseudochina in vitro culture, showing leaf proliferation after MeJA treatment for 75 days. (1) Control (2) 75 µM (3) 150 µM (4) 300 µM (No shoot formed); (a) leaf (Scale bar: 1 cm)

MeJA application is known to modulate root and shoot growth (Yamashita et al. 2021). MeJA has been reported to increase the number of roots by 37.21% in *Cucumis sativus*. However, higher concentrations of MeJA (150 and 300 µM) inhibit root formation (Feng et al. 2023). On the other hand, 100 µM of MeJA also reduced the number of roots in Triticale (*Triticosecale wittmack*), as reported by Lahuta et al. (2017). In addition, root inhibition has also been reported in *Arabidopsis* after MeJA treatment. This might be due to the involvement of *COI1* (Kamińska 2021). Moreover, applying jasmonates (including MeJA) can inhibit root apical growth and produce a short root phenotype. The latter is due to Jasmonate-Auxin signaling (Jang et al. 2020).

Impact of MeJA on shoot multiplication of *Gynura pseudochina* in vitro culture

All MeJA treatment significantly affected shoot number generated from the nodal segment of *G. pseudochina* (p-value <0.05), except 300 µM of MeJA (Figure 8). Shoot proliferation is important in plant tissue culture (Wu et al. 2023); the number of shoots varied from 1.50 to 13.0 shoots per explant. The highest number of shoots was observed at 150 µM of MeJA. Stimulation of shoot proliferation was also increased when 100 µM of MeJA concentration was given to the medium in *Musa acuminata* in vitro culture (Mahmood et al. 2012). It is suggested that MeJA modulates the biosynthesis of cytokinin, which is important for shoot proliferation. Similarly, two-fold cytokinin accumulation after MeJA treatment has also been reported in *Triticum aestivum* (Avalbaev et al. 2016). Despite the findings of an adversarial relationship between jasmonic acid and cytokinin during Arabidopsis thaliana development, Jang et al. (2017) also observed that MeJA boosted cytokinin accumulation.

Moreover, adding MeJA at 75 µM resulted in the lowest shoot number (1.5 shoots per explant), which was not significantly different from those observed in the control plant. Interestingly, we did not detect any shoot formed in the explant treated with a higher amount of MeJA (300 µM). Rasouli et al. (2021) also reported a decrease in shoot multiplication rate when MeJA concentration exceeded 200 mg/L. Other studies also demonstrated similar results, indicating that a higher amount of MeJA negatively impacts shoot multiplications. MeJA-mediated growth suppression may result from reduced ATP production, proteins involved in energy metabolism, and alteration of the mitochondrial membrane (Patil et al. 2014).

Exogenous MeJA increases the expression of *GpPAL* in the in vitro culture of *Gynura pseudochina*

Gynura pseudochina contains bioactive compounds like flavonoids (Siriwatanametanon and Heinrich 2011). MeJA supplementation in this study increased Phenylalanine Ammonia-Lyase (*GpPAL*) gene expression. This gene is engaged in the initial committed step of phenylpropanoid synthesis (Yang et al. 2022). PAL functions to catalyze the formation of trans-cinnamic acid via phenylalanine deamination. In this present study, the change in expression of the *PAL* gene relative to the housekeeping gene (b-actin) was evaluated. We detected no significant difference in *GpPAL* expression of the explant after treatment with 75 and 300 µM of MeJA compared to the control (Figure 9). However, a significant expression of *GpPAL* was detected at MeJA 150 µM (1.29-fold compared to control) (Figure 9). *PAL*, *4CL*, *FLS*, and *F3'H* genes, and *DFR* gene gene are responsible for the biosynthesis of many secondary metabolites, including flavonoids (Olsen et al. 2008). Overexpression of the *PAL* gene is commonly correlated with the accumulation of flavonoid compounds (Xu et al. 2020). Kianersi et al. (2020) reported that adding MeJA at 150 µM increases the flavonoid production in *Capparis spinosa*. Similarly, the callus culture of *Givotia moluccana* treated with 150 µM of MeJA also accumulated a significant amount of total phenolic and flavonoid content compared to the control plant (Woch et al. 2023). Kianersi et al. (2020) also presented comparable findings concerning the application of MeJA to the PAL gene in *Salvia yangii* and *Salvia abrotanoides* plants. Their study indicated that adding MeJA at 150 µM yielded the highest PAL expression levels.

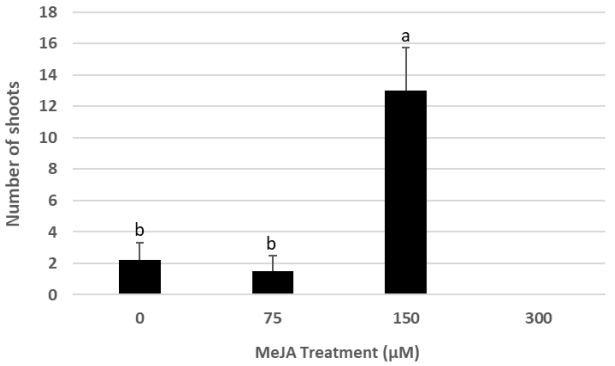

Figure 8. The Effect of MeJA on shoot proliferation of *Gynura pseudochina* in vitro culture after 75 days of culture. Note: Data shows mean ± standard deviation. Different letters represent a statistically significant effect by Tukey's test (P<0.05)

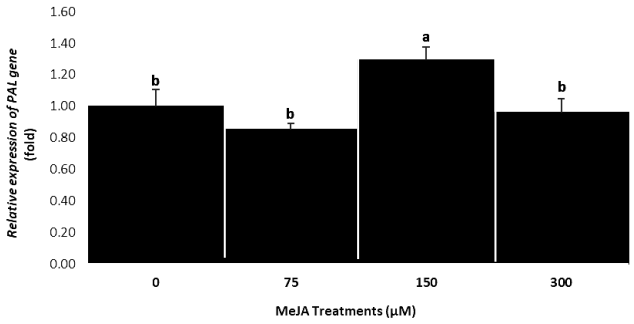

Figure 9. The expression of the *GpPAL* gene in *Gynura pseudochina* in vitro culture after 75 days of MeJA treatment

MeJA plays a pivotal role in plant defense mechanisms, safeguarding against both biotic and abiotic stressors (Li et al. 2018). This is supported by Liu et al. (2022), who stated that MeJA increases secondary metabolites under certain concentrations, including phenol-derived compounds, flavonoids, and lignin. These compounds contribute to seedling growth and increase plant resistance to adverse environments. Moreover, MeJA controls gene expression and promotes molecular signal transduction, leading to secondary metabolite build-up (Ho et al. 2020). The high affinity of MeJA and its receptor protein subsequently produces ROS. The latter causes an increase in enzymatic antioxidants, including superoxide dismutase, peroxidase, ascorbate peroxidase, and catalase. The ROS is also responsible for activating transcription factor proteins involved in the biosynthesis of phenol and flavonoid compounds (Ho et al. 2020).

Therefore, our results demonstrated that Methyl Jasmonate (MeJA) influenced the *G. pseudochina* growth responses in a concentration-dependent manner. All concentrations of MeJA also give a variation of callogenesis and organogenesis responses. The characteristics of callus obtained in this study also varied from compact, friable, green, green, yellowish, and green purplish. The 150 µM of MeJA treatment resulted in a significant response of the *G. pseudochina* on its plant height, fresh weight, number of leaves, and number of shoots. Furthermore, the latter concentration also promoted the over-expression of the *GpPAL* gene, which encodes key enzymes in accumulating phenol and flavonoid compounds. In addition, the concentration of MeJA at 75 µM was able to induce root formation. Briefly, our results offer a new insight into the application of exogenous MeJA on the in vitro culture and accumulation of economically important secondary metabolites in *G. pseudochina*. However, additional phytochemical validation and studies on the expression of flavonoid-related genes are necessary to confirm metabolite production. Micropropagation also grapples with challenges related to somaclonal variation in clonal plants, leading to genetic alteration (Espinosa-Leal et al. 2018). This phenomenon may result from prolonged exposure to elicitors and growth regulators or from abiotic stress during in vitro culture treatments. Hence, further research utilizing molecular markers such as Random Amplification (RAPD) (Andriyani and Jadid 2021), Amplified Fragment Length Polymorphisms (AFLP) (El-Naby 2022), and Inter-Simple Sequence Repeats (ISSR) (Alhasnawi 2023) can be conducted to assess the impact of MeJA on plant genetic stability. The genotoxicity of the plant also warrants thorough investigation (Kowalczyk et al. 2024).

ACKNOWLEDGEMENTS

The authors thank the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia for financially supporting this work through research grant number: 2004/PKS/ITS/2023.

REFERENCES

Abbasi BH, Zahir A, Ahmad W, Nadeem M, Giglioli-Guivarc'h N, Hano C. 2019. Biogenic zinc oxide nanoparticles-enhanced biosynthesis of lignans and neolignans in cell suspension cultures of *Linum usitatissimum* L. Artif Cells Nanomed Biotechnol 47 (1): 1367-1373. DOI: 10.1080/21691401.2019.1596942.

Alam P, Albalawi TH. 2020. In vitro alteration of artemisinin biosynthesis in *Artemisia annua* L. during treatment with methyl jasmonate. Trop J Pharm Res 19 (1): 33-37. DOI: 10.4314/tjpr.v19i1.5.

Alhasnawi AN. 2023. Genetic stability analysis associated with salt stress and salicylic acid in fenugreek (*Trigonella foenum-graecum*) plants. Biodiversitas 24 (6): 3601-3608. DOI: 10.13057/biodiv/d240660.

Andriyani A, Jadid N. 2021. Genetic diversity and morphological responses of *Capsicum annuum* varieties under aluminum stress. Biodiversitas 22 (5): 2576-2582. DOI: 10.13057/biodiv/d220516.

Amoanimaa-Dede H, Su C, Yeboah A, Zhou H, Zheng D, Zhu H. 2022. Growth regulators promote soybean productivity: A review. PeerJ 10: e12556. DOI: 10.7717/peerj.12556.

Avalbaev A, Yuldashev R, Fedorova K, Somov K, Vysotskaya L, Allagulova C, Shakirova F. 2016. Exogenous methyl jasmonate regulates cytokinin content by modulating cytokinin oxidase activity in wheat seedlings under salinity. J Plant Physiol 191: 101-110. DOI: 10.1016/j.jplph.2015.11.013.

Bisht A, Singh L, Pandey A, Pandey V, Dasila K, Bhatt ID, Pande V. 2023. Elicitor-induced phytochemicals production in *Berberis lycium* Royle. Ind Crops Prod 199: 116735. DOI: 10.1016/j.indcrop.2023.116735.

Chavan JJ, Kshirsagar PR, Jadhav SG, Nalavade VM, Gurme ST, Pai SR. 2021. Elicitor-mediated enhancement of biomass, polyphenols, mangiferin production and antioxidant activities in callus cultures of *Salacia chinensis* L. 3Biotech 11 (6): 285. DOI: 10.1007/s13205-021-02836-2.

Dale JE. 1988. The control of leaf expansion. Annu Rev Plant Physiol Plant Mol Biol 39 (1): 267-295. DOI: 10.1146/annurev.pp.39.060188.001411.

Delgado C, Mora-Poblete F, Ahmar S, Chen JT, Figueroa CR. 2021. Jasmonates and plant salt stress: Molecular players, physiological effects, and improving tolerance by using genome-associated tools. Intl J Mol Sci 22 (6): 3082. DOI: 10.3390/ijms22063082.

El-Naby ME. 2022. In vitro propagation for conservation and genetic fidelity of the near threatened *Dimocarpus longan* plant. J Genet Eng Biotechnol 20 (1): 130. DOI: 10.1186/s43141-022-00406-4.

El-Sayed SM, Mohamed AS, Abd Elhafez AE. 2023. Prospective effects of methyl jasmonate to ameliorate *Celosia argentea* L. growth under low light conditions. Middle East J Appl Sci 13 (2): 219-232. DOI: 10.0. 143.24/mejas/2023.13.2.18.

Espinosa-Leal CA, Puente-Garza CA, García-Lara S. 2018. In vitro plant tissue culture: Means for production of biological active compounds. Planta 248: 1-18. DOI: 10.1007/s00425-018-2910-1.

Feng L, Wei L, Liu Y, Hu D, Gong W, Liao W. 2023. Hydrogen peroxide is involved in methyl jasmonate-induced adventitious rooting in cucumber under cadmium stress. Sci Hortic 309: 111666. DOI: 10.1016/j.scienta.2022.111666.

Furmanowa M, Glowniak K, Syklowska-Baranek K, Zgórka G, Józefczyk A. 1997. Effect of picloram and methyl jasmonate on growth and taxane accumulation in callus culture of *Taxus x media var. Hatfieldii*. Plant Cell Tissue Organ Cult 49: 75-79. DOI: 10.1023/A:1005858329430.

Ghulamahdi MI, Aziz SA, Nirwan N. 2008. Effect of light on the growth quantity and content of anthocyanin of *daun dewa* (*Gynura pseudochina* (L.) DC) in vitro. Indones J Agron 36 (1): 8033. [Indonesian]

Heinrich M, Hettenhausen C, Lange T, Wünsche H, Fang J, Baldwin IT, Wu J. 2013. High levels of jasmonic acid antagonize the biosynthesis of gibberellins and inhibit the growth of *Nicotiana attenuate* stems. Plant J 73 (4): 591-606. DOI: 10.1111/tpj.12058.

Hasan N, Choudhary S, Naaz N, Sharma N, Laskar RA. 2021. Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. J Genet Eng Biotechnol 19 (1): 128. DOI: 10.1186/s43141-021-00231-1.

Ho T-T, Murthy HN, Park S-Y. 2020. Methyl jasmonate induced oxidative stress and accumulation of secondary metabolites in plant cell and organ cultures. Intl J Mol Sci 21 (3): 716. DOI: 10.3390/ijms21030716.

Huang T, Liu D, Cui X, Li M, Jin L, Paré PW, Li M, Wei J. 2023. In vitro bioactive metabolite production and plant regeneration of medicinal plant *Angelica sinensis*. Ind Crops Prod 194: 116276. DOI: 10.1016/j.indcrop.2023.116276.

Islam MM, Islam S, Parvin S, Haque MA. 2019. Mitigation of salt stress by foliar application of jasmonic acid in wheat. Am J Plant Biol 4 (1): 18-34.

Jadid N, Anggraeni S, Ramadani MR, Arieny M, Mas' ud F. 2024. In vitro propagation of Indonesian stevia (*Stevia rebaudiana*) genotype using axenic nodal segments. BMC Res Notes 17 (1): 45. DOI: 10.1186/s13104-024-06703-0.

Jadid N, Arraniry BA, Hidayati D, Purwani KI, Wikanta W, Hartanti SR, Rachman RY. 2018. Proximate composition, nutritional values and phytochemical screening of *Piper retrofractum* vahl. fruits. Asian Pac J Trop Biomed 8 (1): 37-43. DOI: 10.4103/2221-1691.221136.

Jadid N, Mardika RK, Nurhidayati T, Irawan MI. 2016. Reverse transcription-PCR analysis of geranylgeranyl diphosphate synthase (JcGGPPS) in *Jatropha curcas* L. and in silico analysis of Casbene Synthase (JcCS) among Euphorbiaceae. AIP Conf Proc 1744 (1): 020042. DOI: 10.1063/1.4953516.

Jadid N, Maziyah R, Nurcahyani DD, Mubarokah NR. 2017. Growth and physiological responses of some *Capsicum frutescens* varieties to copper stress. AIP Conf Proc 1854: 020018. DOI: 10.1063/1.4985409.

Jang G, Chang SH, Um TY, Lee S, Kim JK, Choi YD. 2017. Antagonistic interaction between jasmonic acid and cytokinin in xylem development. Sci Rep 7 (1): 10212. DOI: 10.1038/s41598-017-10634-1.

Jang G, Yoon Y, Choi YD. 2020. Crosstalk with jasmonic acid integrates multiple responses in plant development. Intl J Mol Sci 21 (1): 305. DOI: 10.3390/ijms21010305.

Jásik J, de Klerk GJ. 2006. Effect of methyl jasmonate on morphology and dormancy development in lily bulblets regenerated in vitro. J Plant Growth Regul 25 (1): 45-51. DOI: 10.1007/s00344-005-0048-4.

Jeyasri R, Muthuramalingam P, Karthick K, Shin H, Choi SH, Ramesh M. 2023. Methyl jasmonate and salicylic acid as powerful elicitors for enhancing the production of secondary metabolites in medicinal plants: an updated review. Plant Cell Tissue Organ Cult 153 (3): 447-458. DOI: 10.1007/s11240-023-02485-8.

Jin M, Liu Y, Shi B, Yuan H. 2023. Exogenous IAA improves the seedling growth of *Syringa villosa* via regulating the endogenous hormones and enhancing the photosynthesis. Sci Hortic 308: 111585. DOI: 10.1016/j.scienta.2022.111585.

Kamińska M. 2021. Role and activity of jasmonates in plants under in vitro conditions. Plant Cell Tissue Organ Cult 146 (3): 425-447. DOI: 10.1007/s11240-021-02091-6.

Kianersi F, Abdollahi MR, Mirzaie-Asl A, Dastan D, Rasheed F. 2020. Identification and tissue-specific expression of rutin biosynthetic pathway genes in *Capparis spinosa* elicited with salicylic acid and methyl jasmonate. Sci Rep 10 (1): 8884. DOI:10.1038/s41598-020-65815-2.

Kowalczyk T, Sikora J, Merecz-Sadowska A, Kukula-Koch W, Synowiec E, Majda A, Juda D, Śliwiński T, Sitarek P. 2024. Biological properties of extracts obtained from in vitro culture of *Plectranthus scutellarioides* in a cell model. Intl J Mol Sci 25 (2): 1043. DOI: 10.3390/ijms25021043.

Krishnan ML, Roy A, Bharadvaja N. 2019. Elicitation effect on the production of asiaticoside asiatic acid in shoot, callus, and cell suspension culture of *Centella asiatica*. J Appl Pharm Sci 9 (6): 67-74. DOI: 10.7324/JAPS.2019.90609.

Krzyzanowska J, Czubacka A, Pecio L, Przybys M, Doroszewska T, Stochmal A, Oleszek W. 2012. The effects of jasmonic acid and methyl jasmonate on rosmarinic acid production in *Mentha × piperita* cell suspension cultures. Plant Cell Tissue Organ Cult 108: 73-81. DOI: 10.1007/s11240-011-0014-8.

Lahuta LB, Zalewski K, Głowacka K, Nitkiewicz B, Amarowicz R. 2017. Effect of methyl jasmonate on carbohydrate composition, α-amylase activity and growth of triticale (*Triticosecale witmmack*) seedlings. J Agric Sci Technol 19 (5): 1008-0864.

Lalnunzawma, Karuna K, Rani R, Nahakpam S, Bahera SK, Sahay S. 2020. Effect of methyl jasmonate on growth and flowering behavior of strawberry cv. Nabila. Intl J Curr Microbiol Appl Sci 9 (9): 2690-2695. DOI: 10.20546/ijcmas.2020.909.335.

Larrieu A, Vernoux T. 2016. Q&A: How does jasmonate signaling enable plants to adaptand survive. BMC Biol 14: 79. DOI: 10.1186/s12915-016-0308-8.

Lemmen R, Bunyapraphatsara N. 2003. Plant resources of southeast Asia, Medicinal and Poisonous Plants 3. Backhuys Publishers, Leiden, The Netherlands.

Li C, Wang P, Menzies NW, Lombi E, Kopittke PM. 2018. Effects of methyl jasmonate on plant growth and leaf properties. J. Plant Nutr Soil Sci 181 (3): 409-418. DOI: 10.1002/jpln.201700373.

Liu R, Wang Z, Zheng J, Xu Z, Tang X, Huang Z, Zhang N, Dong Y, Li T. 2022. The effects of methyl on growth, gene expression and metabolite accumulation in *Isatis indigotica* Fort. Ind Crops Prod 177: 114482. DOI: 10.1016/j.indcrop.2021.114482.

Luo Y, Zheng K, Liu X, Tao J, Sun X, Deng Y, Deng X. 2023. In vitro propagation and genetic uniformity assessment of *Manglietiastrum sinicum*: A critically endangered Magnoliaceae species. Plants 12 (13): 2500. DOI: 10.3390/plants12132500.

Lopez G, Ahmadi SH, Amelung W, Athmann M, Ewert F, Gaiser T, Gocke MI, Kautz T, Postma J, Rachmilevitch S, Schaaf G. 2023. Nutrient deficiency effects on root architecture and root-to-shoot ratio in arable crops. Front Plant Sci 13: 1067498. DOI: 10.3389/fpls.2022.1067498.

Mahendran G, Rahman LU. 2024. Methyl jasmonate and salicylic acid as powerful elicitors for enhancing the production of secoiridoid glycoside from cell suspension cultures of *Enicostema axillare* (Poir. ex Lam.) A. Raynal. Plant Cell Tissue Organ Cult 156 (3): 68. DOI: 10.1007/s11240-023-02679-0.

Machado RA, Baldwin IT, Erb M. 2017. Herbivory-induced jasmonates constrain plant sugar accumulation and growth by antagonizing gibberellin signaling and not by promoting secondary metabolite production. New Phytol 215 (2): 803-812. DOI: 10.1111/nph.14597.

Mahmood M, Bidabadi SS, Ghobadi C, Gray DJ. 2012. Effect of methyl jasmonate treatments on alleviation of polyethylene glycol-mediated water stress in banana (*Musa acuminata* cv. 'Berangan', AAA) shoot tip cultures. Plant Growth Regul 68: 161-169. DOI: 10.1007/s10725-012-9702-6.

Malik NA, Kumar IS, Nadarajah K. 2020. Elicitor and receptor molecules: Orchestrators of plant defense and immunity. Intl J Mol Sci 21 (3): 963. DOI: 10.3390/ijms21030963.

Mangas S, Bonfill M, Osuna L, Moyano E, Tortoriello J, Cusido RM, Piñol MT, Palazón J. 2006. The efect of methyl jasmonate on triterpene and sterol metabolisms of *Centella asiatica*, *Ruscus aculeatus* and *Galphimia glauca* cultured plants. Phytochemistry 67 (18): 2041-2049. DOI: 10.1016/j.phytochem.2006.06.025.

Mehmandar MN, Rasouli F, Giglou MT, Zahedi SM, Hassanpouraghdam MB, Aazami MA, Tajaragh RP, Ryant P, Mlcek J. 2023. Polyethylene glycol and sorbitol-mediated in vitro screening for drought stress as an efficient and rapid tool to reach the tolerant *Cucumis melo* L. genotypes. Plants 12 (4): 870. DOI: 10.3390/plants12040870.

Meng X, Li J, Li M, Wang H, Ren B, Chen J, Li W. 2021. Traditional uses, phytochemistry, pharmacology and toxicology of the genus *Gynura* (Compositae): A comprehensive review. J Ethnopharmacol 276: 114145. DOI: 10.1016/j.jep.2021.114145.

Moektiwardoyo WM, Tjitraresmi A, Susilawati Y, Iskandar Y, Halimah E, Zahryanti D. 2014. The potential of dewa leaves (*Gynura pseudochina* (L) DC) and temu ireng rhizomes (*Curcuma aeruginosa* Roxb.) as medicinal herbs for dengue fever treatment. Procedia Chem 13: 134-141. DOI: 10.1016/j.proche.2014.12.017.

Murthy HN, Joseph KS, Paek KY, Park SY. 2024. Light as an elicitor for enhanced production of secondary metabolites in plant cell, tissue, and organ cultures. Plant Growth Regul 2024: 1-19. DOI: 10.1007/s10725-024-01139-9.

Nirwan N, Aziz SA. 2006. Multiplications and anthocyanins pigmentation of *Gynura pseudochina* (L.) DC in vitro. Indones J Agron 34 (2): 7930. DOI: 10.24831/jai.v34i2.1289.

Noir S, Bömer M, Takahashi N, Ishida T, Tsui TL, Balbi V, Shanahan H, Sugimoto K, Devoto A. 2013. Jasmonate controls leaf growth by repressing cell proliferation and the onset of endoreduplication while maintaining a potential stand-by mode. Plant Physiol 161 (4): 1930-1951. DOI: 10.1104/pp.113.214908.

Olsen KM, Lea US, Slimestad R, Verheul M, Lillo C. 2008. Differential expression of four *Arabidopsis PAL* genes; *PAL1* and *PAL2* have functional specialization in abiotic environmental-triggered flavonoiid synthesis. J Plant Physiol 165 (14): 1491-1499. DOI: 10.1016/j.jplph.2007.11.005.

Pantin F, Simonneau T, Rolland G, Dauzat M, Muller B. 2011. Control of leaf expansion: A developmental switch from metabolic to hydraulics. Plant Physiol 156 (2): 803-815. DOI: 10.1104/pp.111.176289.

Patil RA, Lenka SK, Normanly J, Walker EL, Roberts SC. 2014. Methyl jasmonate represses growth and affects cell cycle progression in cultured *Taxus* cells. Plant Cell Rep 33: 1479-1492. DOI: 10.1007/s00299-014-1632-5.

Picking D. 2024. The global regulatory framework for medicinal plants. In Pharmacognosy (pp. 769-782). Academic Press. DOI: 10.1016/B978-0-443-18657-8.00023-2.

Pisitpaibool S, Sukrong S, Kanjanaprapakul K, Phisalaphong M. 2021. Effects of preharvest methyl jasmonate elicitation and electrical stimulation on camptothecin production by in vitro plants *Ophiorrhiza ridleyana* Craib. Appl Sci 11 (10): 4555. DOI: 10.3390/app11104555.

Qin R, Zhao Q, Gu C, Wang C, Zhang L, Zhang H. 2023. Analysis of oxidase activity and transcriptomic changes related to cutting propagation of hybrid larch. Sci Rep 13 (1): 1354. DOI: 10.1038/s41598-023-27779-x.

Rahmawati M, Mahfud C, Risuleo G, Jadid N. 2022. Nanotechnology in plant metabolite improvement and in animal welfare. Appl Sci 12 (2): 838. DOI: 10.3390/app12020838.

Rakesh B, Srinatha N, KJ RK, Madhu A, MR SK, Praveen N. 2022. Antibacterial activity and spectroscopic characteristics of silver nanoparticles synthesized via plant and in vitro leaf-derived callus extracts of *Mucuna pruriens* (L.) DC. S Afr J Bot 148: 251-258. DOI: 10.1016/j.sajb.2022.04.047.

Rasouli D, Werbrouck S, Maleki B, Jafary H, Schurdi-Levraud V. 2021. Elicitor-induced in vitro shoot multiplication and steviol glycosides production in *Stevia rebaudiana*. S Afr J Bot 137: 265-271. DOI: 10.1016/j.sajb.2020.10.023.

Restiani R, Barlin N, Aditiyarini D. 2022. Effect of methyl jasmonate on biomass and saponin content in Javanese ginseng (*Talinum paniculatum* (Jacq.) Gaertn.). Scholars Acad J Biosci 10 (7): 154-158. DOI: 10.36347/sajb.2022.v10i07.003.

Rodríguez-Sánchez LK, Pérez-Bernal JE, Santamaría-Torres MA, Marquínez-Casas X, Cuca-Suárez LE, Prieto-Rodríguez JA, Patiño-Ladino OJ. 2020. Effect of methyl jasmonate and salicylic acid on the production of metabolites in cell suspensions cultures of *Piper cumanense* (Piperaceae). Biotechnol Rep 28: e00559. DOI: 10.1016/j.btre.2020.e00559.

Saralamp P, Chuakul W, Prathanturarug S. 2000. Medicinal plants in Thailand. Vol. I: Sirirukhachart Botanical Garden. 3rd ed., Amarin Printing & Publishing, Mahidol University, Bangkok, Thailand.

Sari YP, Kusumawati E, Saleh C, Kustiawan W, Sukartingsih S. 2018. Effect of sucrose and plant growth regulators on callogenesis and preliminary secondary metabolic of different explant *Myrmecodia tuberosa*. Nusantara Biosci 10 (3): 183-192. DOI: 10.13057/nusbiosci/n100309.

Sathyanarayan SR, Warke VG, Mahajan GB, Annapure US. 2023. Soil free nutrient availability to plants. J Plant Nutr 46 (5): 801-814. DOI: 10.1080/01904167.2022.2071736.

Sirhindi G, Mushtaq R, Gill SS, Sharma P, Abd_Allah EF, Ahmad P. 2020. Jasmonic acid and methyl jasmonate modulate growth, photosynthetic activity and expression of photosystem II subunit genes in *Brassica oleracea* L. Sci Rep 10 (1): 9322. DOI: 10.1038/s41598-020-65309-1.

Siriwatanametanon N, Heinrich M. 2011. The Thai medicinal plant *Gynura pseudochina* var. hispida: Chemical composition and in vitro NF-κB inhibitory activity. Nat Prod Commun 6 (5): 1934578X1100600512. DOI: 10.1177/1934578X1100600512.

Sun J, Wang Y, Chen X, Gong X, Wang N, Ma L, Qiu Y, Wang Y, Feng S. 2017. Effects of methyl jasmonate abscisic acid on anthocyanin biosynthesis in callus cultures of red-fleshed apple (*Malus sieversii* f. *niedzwetzkyana*). Plant Cell Tissue Organ Cult 130: 227-237. DOI: 10.1007/s11240-017-1217-4.

Talukdar M, Swain DK, Bhadoria PB. 2022. Effect of IAA and BAP application in varying concentration on seed yield and oil quality of *Guizotia abyssinica* (Lf) Cass. Ann Agric Sci 67 (1): 15-23. DOI: 10.1016/j.aoas.2022.02.002.

Wu K, Xiong Y, Wu Y, Da Silva JA, Zeng S, Ma G. 2023. Shoot proliferation and de novo shoot organogenesis induction in *Pandanus tectorius* Sol.—new insights for in vitro plant regeneration. In Vitro Cell Dev Biol-Plant 59: 354-364. DOI: 10.1007/s11627-023-10338-4.

Woch N, Laha S, Gudipalli P. 2023. Salicylic acid and jasmonic acid induced enhanced production of total phenolics, flavonoids, and antioxidant metabolism in callus cultures of *Givotia moluccana* (L.) Sreem. In Vitro Cell Dev Biol-Plant 59: 227-248. DOI: 10.1007/s11627-023-10335-7.

Xu N, Liu S, Lu Z, Pang S, Wang L, Wang L, Li W. 2020. Gene expression profiles and flavonoid accumulation during salt stress in *Ginkgo biloba* seedlings. Plants 9 (9): 1162. DOI: 10.3390/plants9091162.

Yang L, Li WC, Fu FL, Qu J, Sun F, Yu H, Zhang J. 2022. Characterization of phenylalanine ammonia-lyase genes facilitating flavonoid biosynthesis from two species of medicinal plant *Anoectochilus*. PeerJ 10: e13614. DOI: 10.7717/peerj.13614.

Yamashita F, Rodrigues AL, Rodrigues TM, Palermo FH, Baluška F, Almeida LF. 2021. Potential plant-plant communication induced by infochemical methyl jasmonate in sorghum (*Sorghum bicolor).* Plants 10 (3): 485. DOI: 10.3390/plants10030485.

Zhang S, Yan S, An P, Cao Q, Wang C, Wang J, Zhang H, Zhang L. 2021. Embryogenic callus induction from immature zygotic embryos and genetic transformation of *Larix kaempferi 3x Larix gmelinii*. PLoS One 16 (10): e0258654. DOI: 10.1371/journal.pone.0258654.

Zhou Y, Ju Y, Chi X, Shen P, Zhang Y, Cai M, Cheng T, Wang J, Zhang Q, Pan H. 2023. Three CYCDs positively regulate plant height of crape myrtle by increasing cell division. Sci Hortic 315: 111954. DOI: 10.1016/j.scienta.2023.111954.