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Biomass and Flavonoid Production of *Talinum paniculatum* (Jacq.) Gaertn. Callus Culture Induced by Methyl Jasmonate

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Abstract **Original Research Article**

Talinum paniculatum (Jacq.) Gaertn has long been used as a medicinal plant in Indonesia. It has similar root morphology to *Panax ginseng*. Leaves of *T.paniculatum* is known as a source of flavonoids. It has antioxidant and antibacterial activities. In vitro culture is one of the tools in Biotechnology that can elevate secondary metabolites in cells by adding an elicitor. Methyl Jasmonate is a biotic elicitor that is naturally produced by plants as a defensive response against pathogens. Factors that affect the production of biomass and secondary metabolites are the concentration of elicitor and exposure (elicitation) duration. Therefore, this study aimed to evaluate the effect of methyl jasmonate concentration (0,50,100, and 150) µM and elicitation duration (0, 48, 96, 144) hours on callus biomass and flavonoid production of *T.paniculatum*. The results showed that the increase of methyl jasmonate concentration and duration of elicitation caused a decrease in callus biomass compared to the control. The highest callus (dry weight) biomass (0.062 g/g DW) was recorded in callus cultures elicited in 150 µM methyl jasmonate for 96 hours. Considering the flavonoid content, 150 µM methyl jasmonate with the duration of elicitation of 96 hours also produced the highest flavonoid content measured by TLC stained area (0.121 cm^2). It is concluded that the higher concentration of methyl jasmonate and the longer duration of elicitation influenced the inhibition of callus growth but increase the production of flavonoids.

Keywords: *Talinum paniculatum*, callus culture, elicitation, methyl jasmonate, flavonoid.

INTRODUCTION

The medicinal plant, *Talinum paniculatum* (Jacq.) Gaertn. belongs to the Talinaceae family and is widely distributed in Asia, especially in Indonesia [1] This plant is commonly known as "Som Jawa" [2]. This plant has long been used as a medicinal plant in Indonesia. It has similar root morphology to *Panax ginseng* [1]. It is well known for its antioxidant, antibacterial, and antifungal properties [3-6]. Its biological activities are based on the whole secondary metabolites, especially flavonoids.

Previous studies have reported that leaf extract of *T.paniculatum* predominantly contains flavonoids [2, 3, 7]. The extraction of *T.paniculatum* secondary metabolites as a bioactive compound in the pharmaceutical industry mainly by plant organs extraction. However, the quantity and quality of flavonoid extracts derived from field-grown plants may be affected by environmental conditions as well as the developmental stages of plants [8, 9] Furthermore, field cultivation of *T.paniculatum* requires a long growth period and plant management [10]. Therefore, an alternative method for reliable and controllable production of flavonoids from *T.paniculatum* is urgently required.

In vitro culture is a feasible technology that has been successfully applied to the production of various bioactive compounds from medicinal plants. For instance, cell suspension culture of *Hypericum perforatum* has been widely used for the production of flavonoids [11], *Panax ginseng* root culture has long been applied for the production of saponin [12] and cell suspension culture of *Thevetia peruviana* has been applied for the production of flavonoid and rosmarinic acid [13]. Many in vitro cultures have been established from plants, yet they frequently produce insufficient amounts of the targeted secondary metabolites. It might be because secondary metabolite production in the plant depends on environmental stress as well as pathogens infection [14, 15].

One of the strategies that can be used is by adding the elicitor to the culture media. Elicitor is

biological, chemical and physical factors that can induce enzymatic activity against stress [16]. Methyl Jasmonate (MeJa) is one of the biotic elicitors that is naturally produced by plants as a defensive response against environmental stress as well as pathogens. Previous studies reported that MeJa stimulates a signal transduction process that regulates defense response in plants and is effective to induce the production of secondary metabolites [11, 17-19]. Factors that affect the production of biomass and secondary metabolites are the concentration of elicitor and elicitation duration. Therefore, the present study aimed to evaluate the effect of MeJa concentration and elicitation duration on callus biomass and flavonoid biosynthesis of *T.paniculatum* callus culture. To the best of our knowledge, this is the first research on the production of flavonoids induced by MeJa in the callus culture of *Talinum paniculatum.*

MATERIAL AND METHODS

Research Materials

The plant material used in this study was the healthy young leaves of *Talinum paniculatum* obtained from CV Merapi Farma Herbal, Yogyakarta, Indonesia. The chemical materials were Murashige and Skoog (MS) medium, alcohol 50% (Onemed), tween 80 (Merck), Kinetin (Sigma Aldrich), 2,4-D (BDH), Methyl Jasmonate (Merck), filter paper, and silica gel plate GF254 (Merck).

Media Preparation

Medium Murashige and Skoog (MS) [20] was prepared by adding 5 mL macronutrient stock, 1 mL micronutrient stock, 5 mL iron stock, 4 mL vitamin, 100 mg myoinositol and 30 g of sucrose, 30 mL kinetin stock, 20 mL 2,4-D stock in 1 L distilled water then homogenized until all the materials have dissolved. Furthermore, the acidity of the medium solution was adjusted to the range 5.6-5.8 using a pH meter, then 8 g of agar powder was added to solidify the solution, then it was homogenized and placed into culture bottle. The medium was sterilized using an autoclave at a pressure of 1.2 atm and temperature of 121^0C for 15 minutes [19].

Explant Sterilization and Callus Initiation

The young leaves of *T.paniculatum* were washed with liquid detergent and 2 drops of Tween 80 for 5 minutes then rinsed using tap water until clean, then it was soaked in alcohol 50% for 3 minutes in LAF and rinsed using sterile distilled water three times. Then the leaves explant was cut (± 1 cm^2) and inoculated in MS medium for 8 weeks of culture at a temperature of 25 ^{0}C and light intensity 1000 lux for 24 h [19].

Callus Elicitation

The 58-day-old callus was elicited in an MS medium with various concentrations of MeJa (0, 50, 100, and 150) mM. The culture was incubated at 25°C, under light conditions for 24 h with a light intensity of 1000 lux. Each treatment was repeated 3 times. Callus was harvested after the elicitation process in 0, 48, 96, and 144 h.

Callus Extraction

The dried elicited callus was oven-dried until a constant weight was achieved. It was mashed into powder. Then 1 mL of methanol 96% was added to the vial containing the callus powder to be macerated for 24 h. Furthermore, the extract was filtered using filter paper and then was concentrated using waterbath at the temperature of 60 0C until the remaining volume of 0.1 mL [21].

Flavonoid Analysis by Thin Layer Chromatography (TLC)

A total volume of 3 µL extract was plotted in plat silica gel GF254 with a size of 10 cm x 8 cm, then they were diluted with eluent aquadest: methanol (3:7). Then, the plate was air-dried, sprayed with AlCl3. Furthermore, the plate was air-dried and observed under UV light at 366 nm. Identification of flavonoid content on the extract was measured by calculating the value of Retention Factor (Rf). Semi-quantitative analysis of flavonoids was done by calculating the stained area of the extract on the GF254 gel silica plate [21].

Data Analysis

The effect of elicitor concentration and elicitation duration on callus biomass was analyzed statistically using one-way Analysis of Variance (ANOVA) based on a Complete Randomized Block Design (CRBD), with a significance level of 0.05. The effect of elicitor concentration and elicitation duration on flavonoid biosynthesis of callus was analyzed descriptively.

RESULTS AND DISCUSSIONS

Based on the results in this study (Figure 1), callus can be induced from leaf explant in MS medium treatment with a combination of 3 mg/L kinetin and 2 mg/L 2,4-D. The initiation time of leaf-derived calluses was the 6th day. This combination and concentration of plant growth regulators was the optimum treatment to induce callus. The faster induction of callus from explant depends on the cell regeneration activity of the young explant tissue, the composition of medium nutrient, and the optimum concentration and combination of plant growth regulators, such as 2,4-D (auxin) and kinetin (cytokinin).

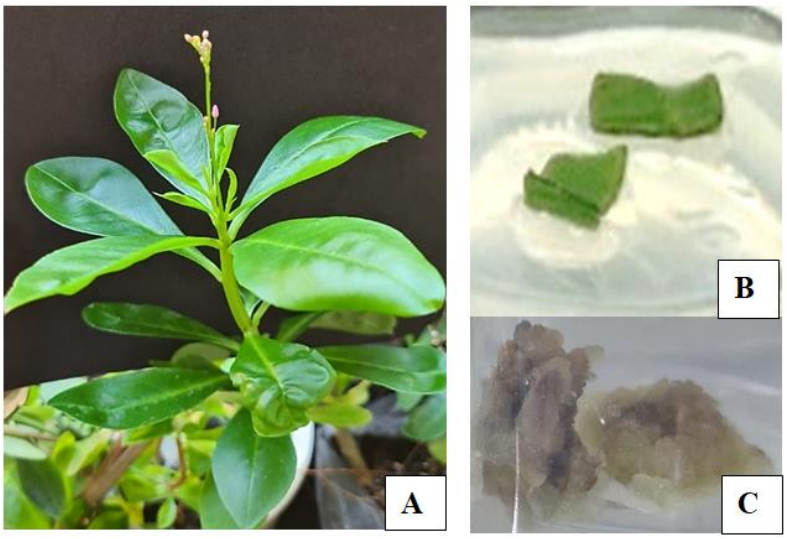

Figure 1: Steps of establishing callus culture (A) The plant of *Talinum paniculatum*, (B) Leaf explant and (C) Callus culture 58th days

The Effect of MeJa Concentration and Elicitation Duration on Callus Biomass

The effect of concentration of MeJa and elicitation duration on the growth of *T.paniculatum* callus was determined by callus dry weight. Based on the callus biomass in Figure 2, the growth of callus in the MeJa elicitation shows a decrease in biomass (0.055-0.062) g compared to the control (0.068 g). The highest biomass (0.062 g) was produced in MS medium elicited with 150 mM of MeJa for 96 h.

The higher concentration of MeJa and the longer elicitation duration affect the decrease of biomass of callus (0.055 g). The decrease of callus biomass in MeJa elicitation treatment was not significantly different based on ANOVA (P>0.05). However, the result in Fig 2 shows that there is a decreasing trendline amongst all elicitation treatments. This result was linear with the previous study [11] that reported the growth inhibitory effect of MeJa concentration (50-200) mmol/L on *Hypericum perforatum* cells after 15 days of elicitation compared to control. However [13], reported there is no significant effect on cell growth inhibitory of the *Thevetia peruviana* suspension cultures that were elicited with 3 µM of MeJa for 24 h.

These results indicated that the elicitor concentration and elicitation duration are factors that affect the cell growth and product yield of callus culture. The inhibitory effect was probably due to the higher concentration with long duration strongly stimulating the cell to produce secondary metabolite as a stress response thus switching the growth of cell (primary metabolism) to secondary metabolism. This metabolic shifting affects the growth of callus causing the cells to tend to use a nutrient in the medium to produce secondary metabolites rather than to grow [11, 14].

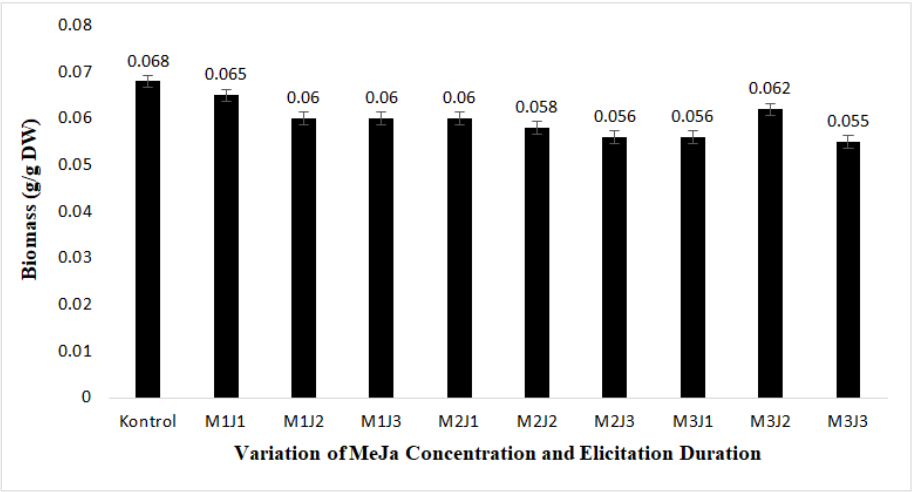

Figure 2: The effect of Meja concentration and elicitation duration on callus biomass Description: Control: without elicitation, M : Concentration of MeJa (0, 50, 100, and 150) µM, J : Elicitation duration (0, 48, 96, and 144) h

The Effect of MeJa Concentration and Elicitation Duration on Flavonoid Production

A suitable concentration of elicitor and elicitation duration are important factors that affect the A suitable concentration of elicitor and elicitation duration are important factors that affect the production of secondary metabolites in callus culture [11]. To determine the optimum concentration of elicitor as well as elicitation duration, callus culture of *T.paniculatum* was treated with different levels of MeJa concentration (0, 50, 100, and 150) mM and harvested after (0, 48, 96, and 144) hours of cultivation. The stained area resulting from TLC analysis can be used to measure the flavonoid content produced from callus extract semi-quantitatively [21].

Based on the result in Figure 3, shows that the increasing level of MeJa concentration and duration of elicitation tends to decrease flavonoid production up to the lowest level of flavonoid content (0.05 cm^2) compared to the control (0.071 cm^2). The longer elicitation duration influences the biosynthesis of flavonoids in callus culture rather than the higher elicitor concentration (0.05 – 0.067) cm^2. The optimum concentration of MeJa and elicitation duration was 150 mM of MeJa for 144 h resulting in the highest stained area (0.121 cm^2). It is indicated that this treatment was suitable to increase the production of flavonoids in callus culture hence increasing the stained area by TLC analysis. The negative effect of a high dose of MeJa was also reported in the cell suspension culture of *Hypericum perforatum* when treated with 150 - 200 mmol/L after 15 days of elicitation [11].

This is probably due to the concentration of MeJa at 150 mM for 144 hours of elicitation could stimulate the stress response for the cell. MeJa acts as an important signal that can be perceived by the membrane cell to initiate the signal transduction process that regulates defense response in plants and is effective to induce the production of secondary metabolites, especially flavonoids. MeJa could elicit flavonoids by activating the gene expression of PAL (Phenylalanine Ammonia Lyase). PAL is one of the key enzymes of phenylpropanoid biosynthesis in plants that plays an important role to produce flavonoid as well as phenolic compounds. This enzyme was related to plant stress response. The optimum level of MeJa concentration and elicitation duration could increase the activity of PAL resulting in enhanced flavonoid production in the cell. The increased level of MeJa concentration and elicitation duration led to a decrease in flavonoid production probably due to excessive production of ROS (Radical Oxygen Species) as a stress response of plant when treated with a high dose of MeJa for longer exposure. It can cause a toxic effect on the callus and then could inhibit cell growth and decrease the production of flavonoids as the yield of the cell.

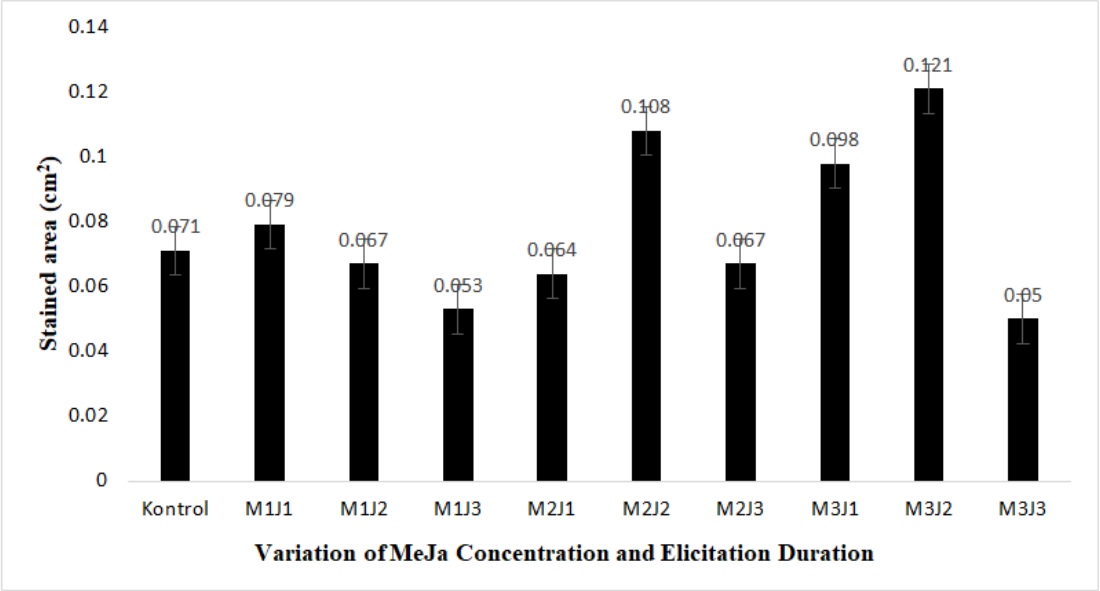

Figure 3: The effect of Meja Concentration and Elicitation Duration on Flavonoid Production Description: Control: without elicitation, M : Concentration of MeJa (0, 50, 100, and 150) µM, J: Elicitation duration (0, 48, 96, and 144) h

CONCLUSION

The higher concentration of MeJa and the longer elicitation duration decrease callus biomass and flavonoid production from *T.paniculatum* callus culture. The optimum concentration of MeJa and elicitation duration to increase callus biomass and flavonoid production was 150 µM of MeJa for 144 h resulting in callus biomass (0.062 g) and stained area (0.121 cm^2). These results suggest that optimum MeJa concentration and duration of elicitation could increase flavonoid production through *T. paniculatum* callus culture.

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