

*Full Length Research Paper*

# Enhancement of saponin accumulation in adventitious root culture of Javanese ginseng (*Talinum paniculatum* Gaertn.) through methyl jasmonate and salicylic acid elicitation

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Javanese ginseng (*Talinum paniculatum*) is an herb and one of the traditional medicinal plants in Indonesia which accumulate saponin in the root organ. However, slow root growth hampers the accumulation of the compound in this plant. Therefore, *in vitro* culture of adventitious roots offers an alternative way to increase the production of saponin. Furthermore, this study was performed to investigate the effect of methyl jasmonate (MeJA) and salicylic acid (SA) on the growth and saponin content in adventitious root of Javanese ginseng. Adventitious root culture was induced on solid Murashige and Skoog (MS) medium supplemented by 10  $\mu$ M IBA (indole-3-butyric acid). Subsequently, adventitious roots were subcultured into a half strength MS liquid medium with the addition of different concentrations of MeJA or SA and incubated for 5, 10 and 15 days. Despite inhibition on the root growth, saponin production increased by 1.5 and 1.3 fold upon elicitation with 0.2 mM MeJA and SA for 15 days, respectively. Thus, MeJA and SA elicitation regulate saponin biosynthesis in adventitious root culture of Javanese ginseng as a time and dose-dependent manner.

**Key words:** Adventitious root, elicitation, Javanese ginseng, saponin, *Talinum paniculatum*.

## INTRODUCTION

*Talinum paniculatum* (family Talinaceae, is a succulent plant that grows up to 100-120 cm tall and characterized by a large and many-flowered terminal panicle with pink color. In Indonesia, this plant is also called Javanese ginseng as it has swelling roots similar to *Panax ginseng*

root and its extract is used as a traditional medicine for multiple diseases (Manuhara et al., 2015). Investigation of root extract from Javanese ginseng resulted in the identification of triterpenoid saponin mixtures. Further characterization from the same genus resulted in the

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identification of oleanane-type saponins which is responsible for different pharmacological activities such as estrogenic, antifertility, antibacterial, antifungal, antioxidant, and cytotoxic activities (Kohda et al., 1992; Reis et al., 2015; Thanamool et al., 2013).

In the natural habitat, the root of Javanese ginseng has a slow growth rate. Generally, it takes about 2-3 years to get more than 100 g of root per plant (Manuhara et al., 2015). Therefore, *in vitro* root culture is essential for preservation of this plant, especially to support sustainable saponin production. Adventitious root culture has been used to produce wide range of secondary metabolites which have been naturally accumulated in root organ. The advantages of adventitious root culture are the root, not influenced by geotropism, fast growth of root branch and genetically stable (Murthy et al., 2016; Inabuy et al., 2017).

Methyl jasmonate (MeJA) is a widely used elicitor to induce secondary metabolite compounds in plant cell culture. MeJA and its derivatives have been proposed to be the key signaling compounds in the process of elicitation leading to the accumulation of secondary metabolites (Ramirez-Estrada et al., 2016). Salicylic acid (SA) is another elicitor that is widely studied as a stressed molecule signal to respond to the pathogen in plants (Hernández et al., 2017). Furthermore, SA was reported to enhance phytoalexin production in cell culture and root culture of several medicinal plants such as *Withania somnifera*, *Anisodus luridus* and *Lepechinia caulescens* (Sivanandhan et al., 2013; Qin et al., 2014; Vergara Martínez et al., 2017). Among all biotic and abiotic elicitors, MeJA and SA are the most important elicitors used as inducers of triterpene saponin production (Moses et al., 2013). Therefore, this experiment was established to compare the effect of different elicitors (MeJA and SA) in various concentrations (0.05, 0.1 and 0.2 mM) and duration of elicitation (5, 10 and 15 days) on saponin production from adventitious roots culture of Javanese ginseng.

## MATERIALS AND METHODS

### Adventitious root culture

Adventitious roots were induced from the shoot culture of Javanese ginseng grown on a phytohormone-free Murashige and Skoog (MS) solid medium (Murashige and Skoog, 1962) containing 30 g/L sucrose and 8 g/L agar. 20 mg of adventitious roots were then isolated and placed onto a half-strength MS solid medium supplemented with 1  $\mu$ M and 10  $\mu$ M of IBA (indole-3-butyric acid) (Duchefa Biochemie, the Netherlands) or NAA (1-naphthaleneacetic acid) (Duchefa Biochemie, the Netherlands). Root cultures were incubated at 25 $\pm$ 1°C in the dark. After 28 days, adventitious roots were harvested and the respective number of lateral roots that appear as well as final biomass were measured. Subsequently, the adventitious roots were subcultured in a 100 ml Erlenmeyer flask containing 15 ml of half-strength MS liquid medium for treatment with elicitors.

### Effect of MeJA and SA on adventitious root growth and saponin production

Treatments of adventitious roots were performed with an inoculum of about 0.7 g fresh weight root segment in 20 ml of half-strength MS liquid medium supplemented with 10  $\mu$ M IBA and 15 g/L sucrose. Different concentrations of MeJA (0, 0.05, 0.1, and 0.2 mM) and SA (0, 0.05, 0.1, and 0.2 mM) were added in the medium. The root was harvested after elicitation for 5, 10, and 15 days. After that, dry weight and saponin content were measured.

### Extraction and determination of total saponin content

Roots were collected from MeJA and SA treated and non-treated samples at different incubation time. Subsequently, the dry weight was measured after drying the roots in the oven at 50°C for 24 h. All samples were stored on the desiccator until constant weight was reached.

Thirty milligram of dried sample was ground to a fine powder and dissolved in 1.5 ml of 96% methanol. The mixture was sonicated for saponin extraction using sonicator (Branson sonicator 3510) for 1 h and then centrifuged at 10,000 rpm for 10 min. Collected supernatant was used for total ginseng saponin analysis.

Total saponins were measured through spectrophotometer (Optima Japan SP3000 nano) as described by Fiallos-Jurado et al. (2016) with minor modification. The Liebermann-Burchard (LB) reagent was used for saponin quantification as it is capable of producing a light brown staining if this compound is present in a sample. The LB reagent consists of a 1:5 mixture of acetic acid and sulfuric acid, respectively. After mixing 1 ml sample solution with 3.5 ml of LB reagent, absorbance at 540 nm was measured in all samples after 20 min. A calibration curve based on oleanolic acid was used to determine the final concentration of saponin (mg/ml) in each solution. The total saponin content was calculated on the basis of dry weight.

### Statistical analysis

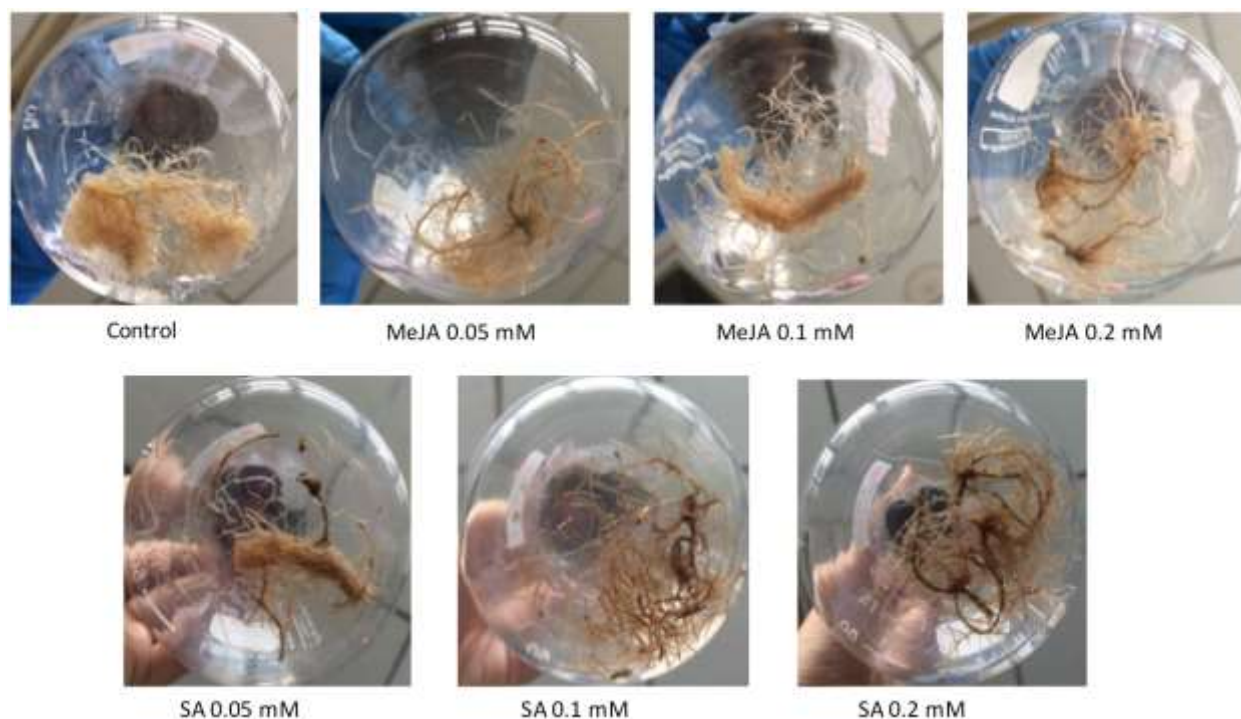
Data were analyzed using one-way ANOVA followed by Duncan's multiple range tests. All statistical analyses were performed at the level of P value less than 0.05 using SPSS 23.0 (SPSS Inc. USA).

## RESULTS

### Adventitious root cultivation

The number of lateral roots and biomass was affected by auxin type and respective concentration. In general, roots treated by IBA form whitish yellow lateral roots with numerous root hairs while those treated by NAA form brown yellowish lateral roots and also callus in their main roots. The roots formed in a medium containing NAA were also shorter and thicker than roots grown in a medium containing IBA.

The number of lateral roots that emerged from adventitious root explant varied in all treatments (Figure 1). The result showed that IBA induced the development of more lateral roots compared with control, while NAA showed a contrasting result. Furthermore, there was no significant difference between the roots induced by 1  $\mu$ M



**Figure 1.** Morphology of adventitious roots of Javanese ginseng after treatment with different elicitors for 15 days.

**Table 1.** Effect of the type and auxin concentrations on the induction of lateral root and root biomass of Javanese ginseng after 4 weeks of culture.

| Treatments | Concentration ( $\mu\text{M}$ ) | Number of lateral root | Root biomass (mg)       |
|------------|---------------------------------|------------------------|-------------------------|
| Control    | 0                               | $15.67 \pm 0.58^b$     | $30.069 \pm 0.002^{bc}$ |
| IBA        | 1                               | $23.00 \pm 3.00^a$     | $30.072 \pm 0.001^b$    |
|            | 10                              | $24.33 \pm 4.16^a$     | $30.075 \pm 0.001^a$    |
| NAA        | 1                               | $9.00 \pm 4.36^c$      | $20.068 \pm 0.001^c$    |
|            | 10                              | $4.00 \pm 1.00^c$      | $20.067 \pm 0.001^c$    |

Values were presented as mean  $\pm$  standard deviation using five replicates. Values with same alphabets are significant at  $p < 0.05$  (one-way ANOVA) according to Duncan Multiple Range test.

and 10  $\mu\text{M}$  IBA in terms of the number of lateral roots, yet 10  $\mu\text{M}$  IBA resulted in the highest root biomass (Table 1). The adventitious roots incubated in 10  $\mu\text{M}$  IBA has growth rate of 0.035 g/day with doubling time for 27 days. Therefore, IBA 10  $\mu\text{M}$  was further added to the medium for adventitious root cultivation.

#### Effect of MeJA and SA on adventitious root growth

Both treated and non-treated roots were growing on the liquid MS medium. However, the treated roots showed a slower growth rate compared to non-treated control. Furthermore, the color of treated roots changed and

tends to be darker compared to that in control (Figure 1). Dry weight of adventitious root culture during the 5, 10, and 15 days after treatment with various concentrations of MeJA and SA are shown in Figure 2. In each incubation time, root biomass decreased from 32-37% when treated with MeJA. Similar result was also observed on SA treated samples in which root biomass decreased from 23 to 35%.

#### Effect of MeJA and SA elicitation on saponin production

Increased concentration of MeJA and SA resulted in the

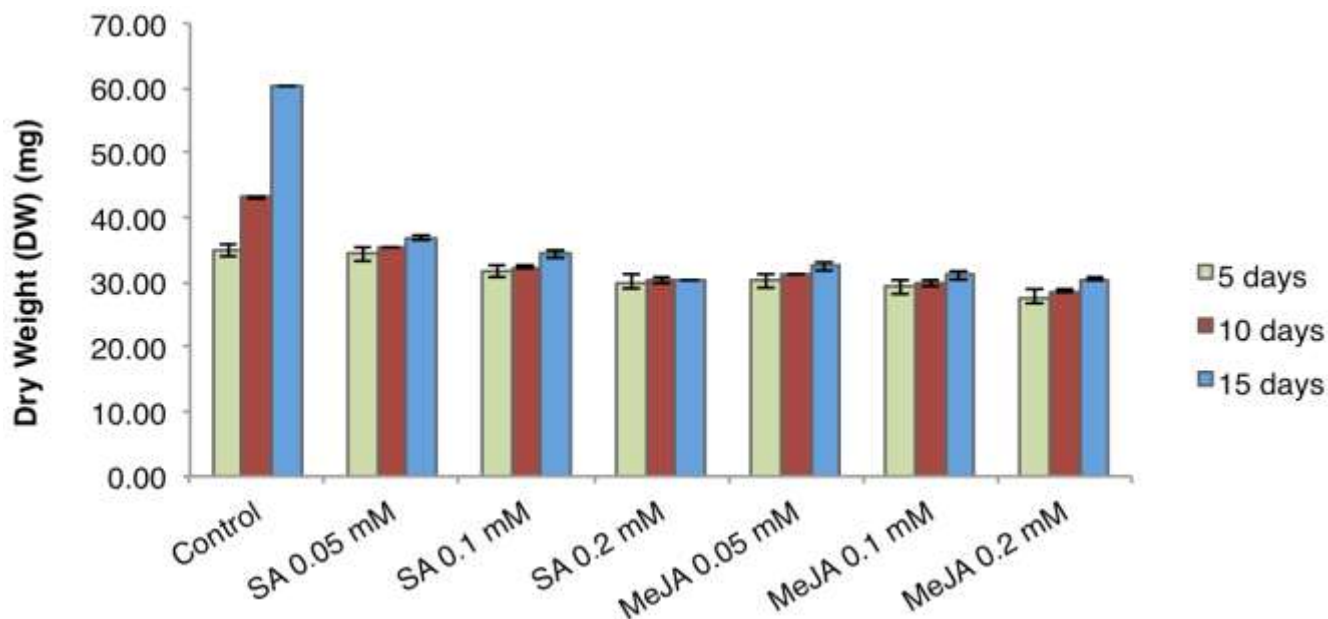


Figure 2. The effect of various concentrations and durations of MeJA and SA on adventitious root growth.

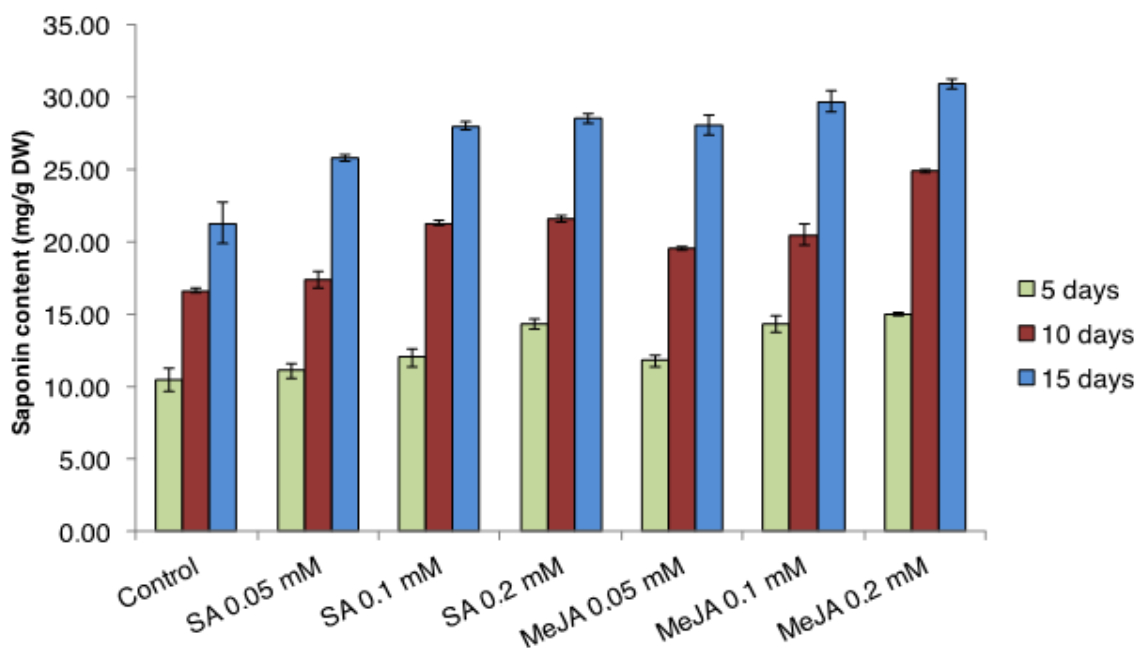


Figure 3. The effect of various concentrations and durations of MeJA and SA on saponin content in adventitious roots.

accumulation of saponin content in the adventitious roots (Figure 3). The longer the incubation period which allow contact with elicitors, the more there is saponin accumulation in the root culture. At the 5-day treatment,

saponin content increased as long as there is increase in elicitor concentration; however, the results slightly increase. This is because during the 5 days incubation, the roots are still in the adaptation stage with the new

medium. At the 10-day treatment, saponin content at the root also increase with increasing elicitor concentration of 0.2 mM of MeJA and SA increased saponin content of about 1.4 and 1.2 times respectively compared to control.

Amount of total saponin increased with increasing MeJA and SA concentrations, and reached a maximum accumulation up to 30 mg/g dry weight (DW) upon treatment with 0.2 mM MeJA for 15 days. In every treatment compared to control, MeJA with concentration of 0.05, 0.1 and 0.2 mM increased saponin content by 1.2, 1.3 and 1.5 fold, respectively, whereas SA with concentration of 0.05, 0.1 and 0.2 mM increased saponin content by 1.1, 1.26 and 1.3 fold, respectively.

## DISCUSSION

*In vitro* tissue cultures have been selected as alternatives for the production of desired saponin compounds in many plants (Espinosa-Leal et al., 2018; Leonard et al., 2018). These techniques could solve different problems associated with saponin extraction from natural-grown plants as well as offer a more sustainable mean for the production of this secondary compound. Furthermore, some plant secondary compounds are controlled in a tissue-specific manner, thus root or shoot culture are more preferred as the production vehicles (Faizal et al., 2013). Taken together, plant tissue culture could help to facilitate the improvement of saponin production through biotechnological approaches.

In this study, adventitious root culture was induced from Javanese ginseng by employing different concentrations of IBA and NAA. IBA was found to be more superior over NAA which may be due to the effects of IBA on the induction of lateral root. In morphological and histological aspects, the roots formed under IBA treatment developed normally compared to NAA. Based on the study conducted by Pacurar et al. (2014), growth regulators such as IBA have a higher potential to induce roots than other auxin hormones. The study also reported that IBA has a prominent effect on the stimulation of *in vitro* lateral roots from a mature root tissue. Similarly, adventitious roots of *P. ginseng* with IBA-containing medium were more effective in inducing lateral root and root growth compared to NAA (Murthy and Paek, 2016).

Many important plant secondary metabolites are hindered by low production titer which could prevent their commercial application. One of the technical approaches used to overcome this problem is application of elicitor to trigger a physiological response in plants and results in the accumulation of secondary metabolites. Moreover, elicitation depends on many factors such as elicitor type and concentration, the growth stage of the culture at the time of elicitor addition and incubation period with elicitor (Ramirez-Estrada et al., 2016). In this context, we used different concentrations of MeJA and SA together

with different incubation periods to improve saponin production in adventitious roots of Javanese ginseng.

Both treatment of MeJA and SA induced a negative growth effect on adventitious roots of Javanese ginseng, which is a common phenomenon observed in plants. MeJA is suggested to inhibit root growth by interfering with transmembrane auxin flux pathway (Yan et al., 2016). Likewise, it has been reported that root growth in Arabidopsis was decreased upon modulation with SA signaling pathway (Rivas-San Vicente and Plasencia, 2011). Similarly, the elicitation using MeJA and SA on *P. ginseng* adventitious root culture showed inhibition of the root growth up to 25% (Ali et al., 2006) as reported in other plants (Saini et al., 2013; Eichmann and Schäfer, 2015).

The different growth shown by the treated roots indicate a stress condition. SA as a phenolic compound was produced naturally on every plant, and accumulation of phenolic compound on roots might occur as indicated by browning in response to additional SA put into the medium. In view of the inverse relationship between the production of biomass and the accumulation of the secondary metabolite, the root growth depression arising from elicitation may favor the synthesis of the secondary metabolite.

Amount of total saponin in the adventitious root cultures of Javanese ginseng increased with increasing MeJA or SA concentrations, and reached a maximum following the addition of 0.2 mM MeJA for 15 days to the cultivation medium. Previous study in whole root culture of *G. glabra* showed that 2 mM MeJA and 1 mM SA increased respective glycyrrhizin production by 3.8 and 4.5 fold after elicitation for 24 h (Shabani et al., 2009); whereas, adventitious root culture of *P. ginseng* required 7 days incubation with 0.2 mM of MeJA or SA for increasing the production of ginsenoside to 4 and 3 fold, respectively (Ali et al., 2006). The prolonged elicitation for 35 days in root culture of *Centella asiatica* significantly increased the accumulation of saponin by 4 to 6 fold (Mangas et al., 2008). The result indicated that the optimum concentration of signaling compounds varies according to the species. Also, contact time with elicitor was an important factor for enhanced production of secondary metabolite. In conclusion, MeJA and SA are potentially used to improve the yield of saponins in adventitious root cultures in Javanese ginseng.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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