Effects of Methyl Jasmonate on Alkaloid Production by Root Cultures of *Stemona curtisii* Hook. f.

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**Abstract**  
The roots of *Stemona curtisii* Hook. f. (Thai vernacular, Non Tai Yak, Family Stemonaceae) containing oxyprotostemonine, stemocurtisine and stemocurtisinol (the important insecticidal alkaloids). To enhance the production of these alkaloids, The roots were cultured on semi-solid MS medium containing 1 mg/l NAA with different concentrations of methyl jasmonate for 16 weeks. The amounts of the individual alkaloids were determined by HPLC. The results suggested that root cultures medium without methyl jasm onate (control) produced the highest oxyprotostemonine (2.927 mg/g dw). But the highest total stemocurtisine (0.025 mg/g dw) and stemocurtisinol (0.385 mg/g dw) occurred when the roots were stimulated with 200 and 100 µM methyl jasmonate, respectively.

**Key Words:** *Stemona* alkaloid, elicitor, oxyprotostemonine, stemocurtisine, stemocurtisinol

**INTRODUCTION**

The *Stemona* plant is known in the Thai vernacular as “Non Tai Yak”. The extracts of *Stemona* roots have been used in traditional medicine in South East Asia, China and Japan to treat the symptoms of bronchitis, pertussis and tuberculosis and have been used as antiparasitic agent on humans and animals (Greger, 2006.). In 2003-2004, Mongkornasawakul et al. reported the isolation of two new *Stemona* alkaloids, stemocurtisine and stemocurtisinol along with oxyprotostemonine from the root extracts of *Stemona curtisii* with the investigation of the larvicidal activity on mosquito larvae (*Anopheles minimus*).

However, propagation of this plant through seeds is unreliable due to poor germination. On other hand, harvesting the plant on a mass scale from natural habitats (southern region of Thailand) leads to depletion of natural propagation and they need 3-5 years for growth prior to harvesting and the alkaloid contents are low depending on various conditions such as genetics and environmental factors. Therefore, The production of important alkaloids through different biotechnological means is an interesting alternative, since it would guarantee a stable and uniform year-round supply, independent of seasonal variations of field-grown plants. Furthermore, the use of elicitor such as methyl jasmonate (MJ) is one of the effective strategies employed to increase the production of important alkaloids in cell and organ cultures.
OBJECTIVE

To study the effects of methyl jasmonate on root growth and alkaloids production from root cultures of S. curtisii.

MATERIAL AND METHODS

Plant materials and elicitation of methyl jasmonate: In vitro roots of S. curtisii (1 g fresh weight) were inoculated into Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 1 mg/L naphthalene acetic acid (NAA), solidified with 0.2% (w/v) gelrite (control medium) or addition with 100, 200, 300 and 500 µM of filter-sterilized MJ (elicitation medium) for 16 weeks. After 16 weeks of elicitation roots were collected and freshed weight was determined. The roots were dried with ventilat at 35 ºC. After total elimination of water was achieved, the dried roots were weighed and alkaloid content was analyzed by HPLC.

Root extract: Dried root powder (1 g) was macerated sequentially with 3 x 50 ml methanol at room temperature over 3 days before filtered and evaporated at 35 ºC. Then the crude methanol extract was dissolved in 1 ml methanol and 1 ml water before extraction with dichloromethane (3 x 5 ml) to give the partially purified extract. The weight was recorded. The extract was analyzed by HPLC.

Medium extract: The medium was extracted with dichloromethane at a ratio of medium: dichloromethane of 1:1 (3 times). The dichloromethane fraction was filtered before evaporated at 35 ºC to give the partially purified extract. The weight was recorded. The extract was analyzed by HPLC.

High-Performance Liquid Chromatography (HPLC) Analysis of Alkaloids from the Roots and the Exudates: Quantification was based on the external standard method using calibration curve. The analysis of these compounds were performed using an Agilent 1100 HPLC system equipped with UV detector at wavelength of 297 nm (Agilent Technologies, Palo Alto, CA, USA). 20 µL of solution was injected onto reversed phased (Inertsil ODS-3, 5 µm, 4.6 I.D. x 150 mm, GL sciences Inc., Japan). HPLC column and eluted at flow rate 1.0ml/min with methanol (Merck, HPLC grade, Germany)-Milli-Q water (60:40, v/v). Prior to the next run, the HPLC column was equilibrated further for 30 min. Data acquisition and analysis were performed by the Agilent ChemStation software. The retention times of oxyprotostemonine, stemocurtisine and stemocurtisinol were 2.37, 4.15 and 7.67 min, respectively (Figure 1).

Statistical analysis: All experiments were repeated at least thrice with 15 replicates per treatment. Significance of treatment effects was determined by using one-way analysis of variance (ANOVA) followed by Turkey’s test. P<0.05 was considered statistically significant.
RESULTS

The MJ treatment led to a repression of root growth in cultures with no significant difference with control (0.363±0.028 g dw) and induced root browning evidently (Figure 2). MJ has been studied for its inhibitory effects on growth and many other metabolic activities in plants (Cosio et al., 1990). One previous study reported that MJ concentrations above 0.01mM inhibited root growth in some species (Lois et al., 1989).

In Figure 3, we analysed the accumulation of three alkaloids already identified in *S. curtisii* roots: oxyprotostemonine, stemocurtisine and stemocurtisinol. The amount of oxyprotostemonine decreased significantly in all concentrations of MJ treatments. The highest level of total oxyprotostemonine accumulation was observed with control at 2.927±0.019 mg/g dw. Moreover oxyprotostemonine production was inhibited with 200µM MJ treatment. The maximum total stemocurtisine production (0.025±0.0002 mg/g dw) was observed in treatment of 200 µM MJ. It was increased 25-fold over control. The 100 µM MJ increased the stemocurtisinol content up to 10.19-fold compared with the control (0.385±0.0051 mg/g dw).

![Figure 1. Chromatogram of a standard mixtures of oxyprotostemonine, stemocurtisine and stemocurtisinol.](image1.png)

![Figure 2. Effects of methyl jasmonate on cultured roots.](image2.png)
Figure 3. The effects of methyl jasmonate on alkaloids production.
DISCUSSIONS

In this study, the effect of MJ on the production of stemocurtisine was maximal at 200 µM, whereas the effect of MJ on the production of stemocurtisinol was maximal at 100 µM. This result indicates that the optimal concentration to elicit metabolite production varies depending on the metabolite. This conclusion agrees with those reached for several other metabolite and plant cell systems; indole alkaloid is produced by *Catharanthus roseus* (Sim *et al*., 1994). MJ has proved to be an effective signaling molecule that can strongly stimulate taxane biosynthesis in cultured Taxus cells (Wang *et al*., 2001) and camptothecin in *Camptotheca acuminata* (Song and Byun, 1998).

It is known that methyl jasmonate have been associated with the accumulation of some secondary metabolites (van der Fits and Memelink, 2000). Our experiments showed that MJ increased the content of stemocurtisinol, which corresponds with previous results that MJ addition can elicit the accumulation of alkaloids (Aerts *et al*., 1994) in plants. The effects of MJ on promoting plant secondary metabolism has been reported to be elicitor signal transducers for the production of plant secondary metabolites (Gundlach *et al*., 1992). They induce an accumulation of compounds belonging to different structural classes, including phenolics, terpenoids, alkaloids and others. (van der Fits and Memelink, 2000).

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