EFFECTS OF METHYL JASMONATE AND SALICYLIC ACID ON *IN VITRO* ALKALOID PRODUCTION OF *Stemona kerrii*

Jessadaporn Ruangsak,* Srisulak Dheeranupattana
Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
*e-mail: nazumi_jung@hotmail.com

Abstract: Effects of methyl jasmonate and salicylic acid on *in vitro* alkaloid production of *Stemona kerrii* were investigated. The 8-week old plantlets of *S. kerrii* were cultured in liquid MS medium containing 1 mg/l NAA with various concentrations of methyl jasmonate; 0, 100 and 200 µM and salicylic acid; 0, 100 and 200 µM for 7 and 14 days. Root and medium extracts were analyzed quantitatively by HPLC and the total oxyprotostemonine, stemocurtisine and stemocurtisinol in both root and medium extracts were determined. It was found that at 7 days when cultured in liquid MS medium supplemented with 200 µM methyl jasmonate. *S. kerrii* produced the highest total oxyprotostemonine (318.4638 µg/g DW), stemocurtisine (4.3397 µg/g DW) and stemocurtisinol (379.9040 µg/g DW) at 7 days when cultured in liquid MS medium supplemented with 200 µM methyl jasmonate.

Introduction: Non Tai Yak (*Stemona* sp.) is a monocotyledonous flowering plant in the Family Stemonaceae. It is a herbaceous plant with more than 80 different *Stemona* alkaloids. e.g. stemonal, stemofoline, 16,17-didehydro-16(E)-stemofoline and rotenone) (Shiengthong *et al*., 1974; Sakata *et al*., 1978; Lin *et al*., 1994 ; Jiwajinda *et al*., 2001). The pure alkaloids derived from the extracts of leaves and roots of *Stemona* species have insect toxicity and antiparasitic activity against maggot, common cutworm, Aphid, *Rhizoctonia solani* an *Erwinia caratovora* (Brem *et al*., 2002; Pacher *et al*., 2002). Useful secondary metabolites from plants have been of interest in recent years for their flavors, fragrances, dyes, pharmaceuticals and pesticide properties. The plant is gaining popularity and is widely used as insecticides and pesticides including *Derris elliptica* (Roxb.) Benth., *Azadirachta indica* A. Juss. , *Gloriosa superba* L., *Heliotropium indicum* L., and *Stemona* spp. Valuable secondary metabolites from plants under cultivation or grown in nature are not always satisfactory. It is often restricted to species or genus and might be activated only during a particular growth and developmental stage or under specific season, stress or nutrient availability. For these reasons in the past several decades, a lot of effort has been put into plant cell cultures as a possible production method for plant secondary metabolites. The promotion of secondary metabolism in plant tissue cultures by adding elicitor or precursor into medium culture to enhance productivity in a short period of time is successful in many plants i.e. *Rubia tinctorum, Morinda citrifolia* and *Taxus* sp. (Jian *et al*., 2005; Smetanska, 2008). Moreover, it depends on the type of secondary metabolites, type and concentration of elicitor and precursors. In this work, the effects of methyl jasmonate (MeJa) and salicylic acid (SA) on alkaloids production from root cultures of *S. kerrii* were investigated.

Methodology: *Stemona kerrii* (Fig. 1) was collected from Hariphunchai Education Center, Chiang Mai University, Lamphun Province, Thailand. Shoot tips and axillary buds were surface sterilized with 0.9 % active Cl for 15 min followed by washing 3 times with sterile distilled water. Each single shoot was cultured on Murashige and Skoog (MS) medium supplemented with 2 mg/l of benzyladenine (BA), 3% (w/v) sucrose and 0.2% (w/v) gelrite. The pH of the medium was adjusted to 5.8 before autoclaving at 121ºC for 15 min. Cultures were incubated at 25±2ºC under 16 h/d photoperiods. Multiple shoots were induced from the buds explants after 4 weeks of culturing which showed extensive proliferation. Shoot

explants were placed on MS medium supplemented with 1.0 mg/l of naphthalene acetic acid (NAA) and solidified with 0.2% (w/v) gelrite at 25±2ºC, under 16 h/d photoperiod. Roots were generated after culturing for 8 weeks. The 8-week old plantlets of *S. kerrii* were cultured in liquid MS medium containing 1 mg/l NAA with various concentrations of 0, 100 and 200 µM MeJa and 0, 100 and 200 µM SA for 7 and 14 days. Dry roots of *Stemona kerrii*. from each period were ground and extracted 3 times with methanol (Merck, HPLC grade, Germany). The solution was filtered and evaporated to get crude extract which was extracted again with dichloromethane (DCM) (Merck, HPLC grade Germany). The extract was concentrated to get crude DCM extracts and its weight was also recorded. The crude DCM extract was dissolved in methanol and filtered with 0.45 µM membrane filter (Filtrex syringe membrane filtration). Finally, Root and medium extracts were analyzed quantitatively by HPLC (Agilent 1200 series) equipped with UV detector at wavelength of 297 nm (Agilent Technologies, Palo Atto, CA, USA) and eluted with methanol (Merck, HPLC grade, Germany)-Milli-Q water (70:30, v/v) at flow rate 1.0mL/min and the retention times of oxyprotostemonine, stemocurtisine and stemocurtisinol were 2.975, 3.594 and 4.858 min, respectively (Figure 2). The total oxyprotostemonine, stemocurtisine and stemocurtisinol in both root and medium extracts were determined. All experiments were repeated at least thrice with 30 replicates per treatment. Significance of treatment effects was determined by using one-way analysis of variance (ANOVA) followed by Turkey's test and $P<0.05$ was considered statistically significant.

Figure 1. *Stemona kerrii*

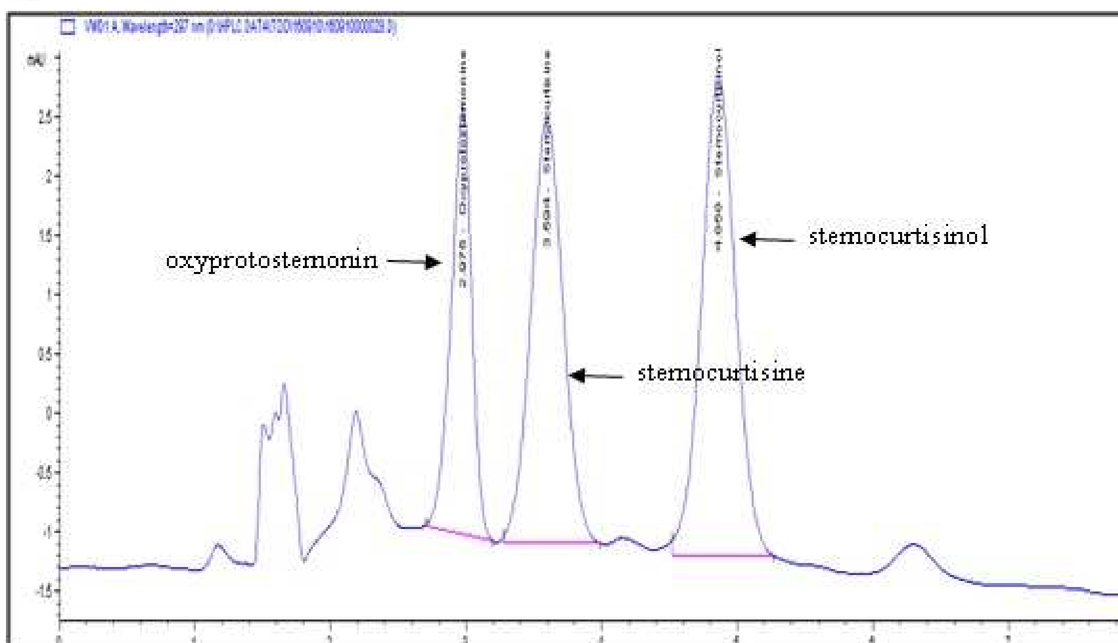

Figure 2. Chromatogram of a standard mixtures of oxyprotostemonine, stemocurtisine and stemocurtisinol.

Results, Discussion and Conclusion: The effects of MeJa on the production of oxyprotostemonine, stemocurtisine and stemocurtisinol are shown in Figure 3. A comparison was made between cultures treated with MeJa and controlled cultures for alkaloid production. The results suggested that *S. kerrii* produced the highest total oxyprotostemonine (318.4638±91.2901 µg/g DW) at 7 days when cultured in liquid MS medium supplemented with 200 µM MeJa. It was increased 3.96-folds over control (80.4748±9.8497 µg/g DW). The maximum total stemocurtisine production (4.0309±0.3076 µg/g DW) was observed in the treatment with 200 µM MeJa at 7 days. It was increased 7.29-folds over control (0.5952±0.1170 µg/g DW). Moreover, the 200 µM MeJa increased the stemocurtisinol content (379.9040±58.0920 µg/g DW) up to 4.04-folds compared with the control (93.9531±17.3097 µg/g DW) at 7 days. Increasing MeJa concentrations increased oxyprotostemonine, stemocurtisine and stemocurtisinol accumulation in the medium. This was in contrast with Chotikadachanarong (2011) who reported that oxyprotostemonine production in tissue culture of *S. curtisii* Hook f. in liquid MS medium was inhibited by 200µM MJ treatment but stemocurtisinol was increased. This result indicates that the optimal concentration of elicitor to elicit metabolite production varies depending on the metabolite. MeJa 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 MeJa is associated with the accumulation of some secondary metabolites (van der Fits and Memelink, 2000). Moreover, Cosio *et al*. (1990) reported the inhibitory effects of MeJa on the growth and many other metabolic activities in plants. There is a report that MeJa concentration above 0.01mM inhibited root growth in some plant species (Lois *et al*., 1989).

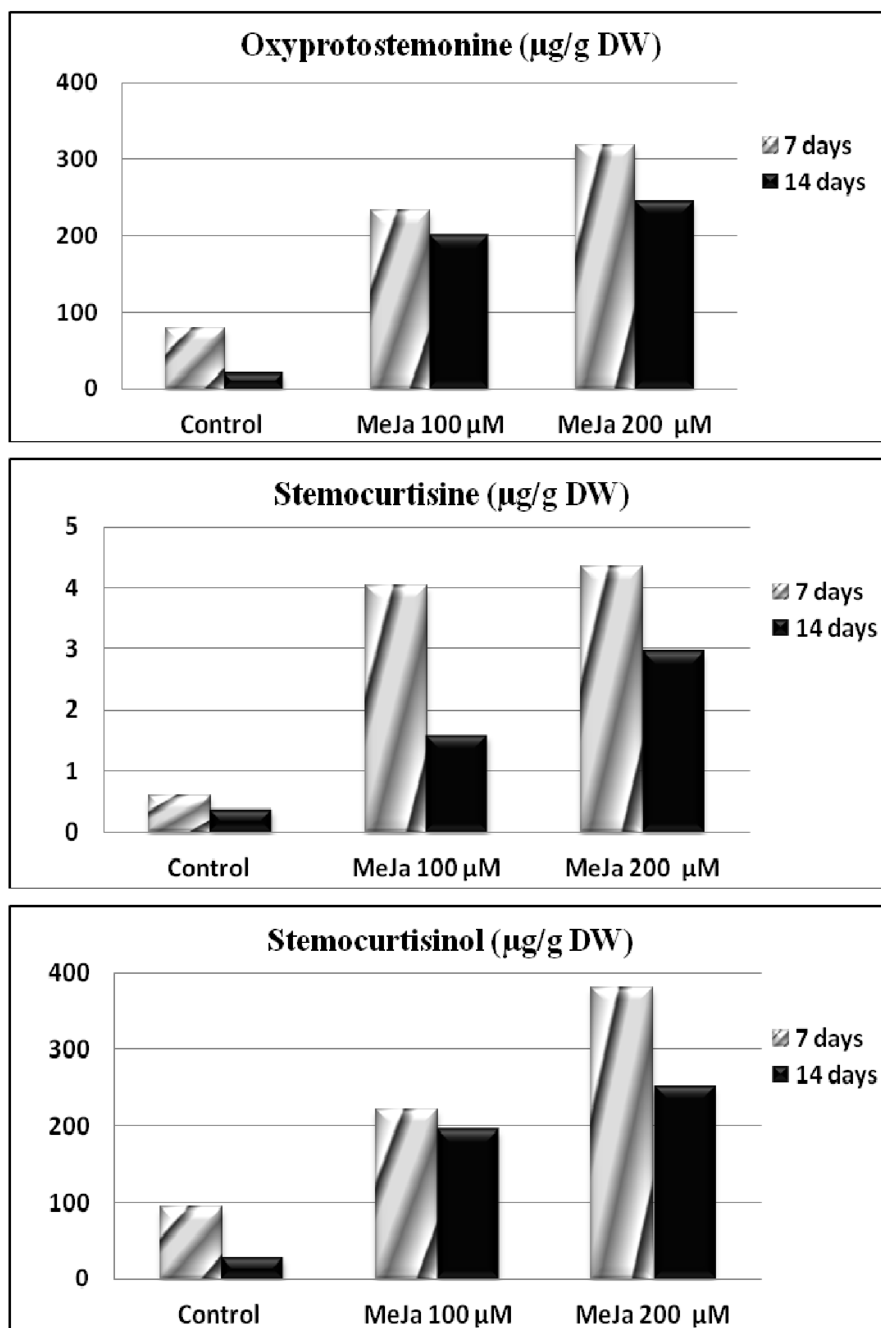

Figure 3. Effect of methyl jasmonate (MeJa) on oxyprotostemonine, stemocurtisine and stemocurtisinol production of *S. kerrii* after 7 and 14 days.

The effects of salicylic acid (SA) on alkaloid production are shown in Figure 4. *S. kerrii* produced the highest total oxyprotostemonine (91.4633±14.7998 µg/g DW) when cultured in liquid MS medium supplemented with 100 µM SA at 7 days. It was increased 1.14-folds over control (80.4748±9.8497 µg/g DW). Increasing SA concentrations decreased oxyprotostemonine production significantly at 14 days. The maximum total stemocurtisine production (1.4668±0.7480 µg/g DW) was observed in the treatment of 100 µM SA at 7 days. It was increased 2.47-folds over control (0.5952±0.1170 µg/g DW). Similarly, Chaichana *et al*. (2010) reported that stemocurtisine production in tissue culture of *S. curtisii* in liquid MS medium supplemented with 1.0 mg/l NAA and 100 µM SA at 4 weeks had the respective amount of stemocurtisine 7.42 -folds over that found in the natural root. For stemocurtisinol production, it was found that 200 µM SA at 7 days increased the

stemocurtisinol content (111.5499±7.6741 µg/g DW) up to 1.19-folds compared with the control (093.9531±17.3097 µg/g DW) and decreased when cultured for 14 days. SA is one of the key endogenous signals involved in the activation of numerous plant defense responses (Shah *et al*., 1999), As previously reported, an elicitor could be highly selective in stimulating the metabolite synthesis, for example, causing the enhancement of monomeric over than dimeric alkaloids in *Catharanthus roseus* (Aerts *et al*., 1996). Moreover, SA also increased the expression of putrescine N-methyltransferase and hyoscyamine 6 ß-hydroxylase enzyme which enhanced tropane alkaloid production in adventitious root cultures of *Scopolia parviflora* (Kang *et al*. 2004)

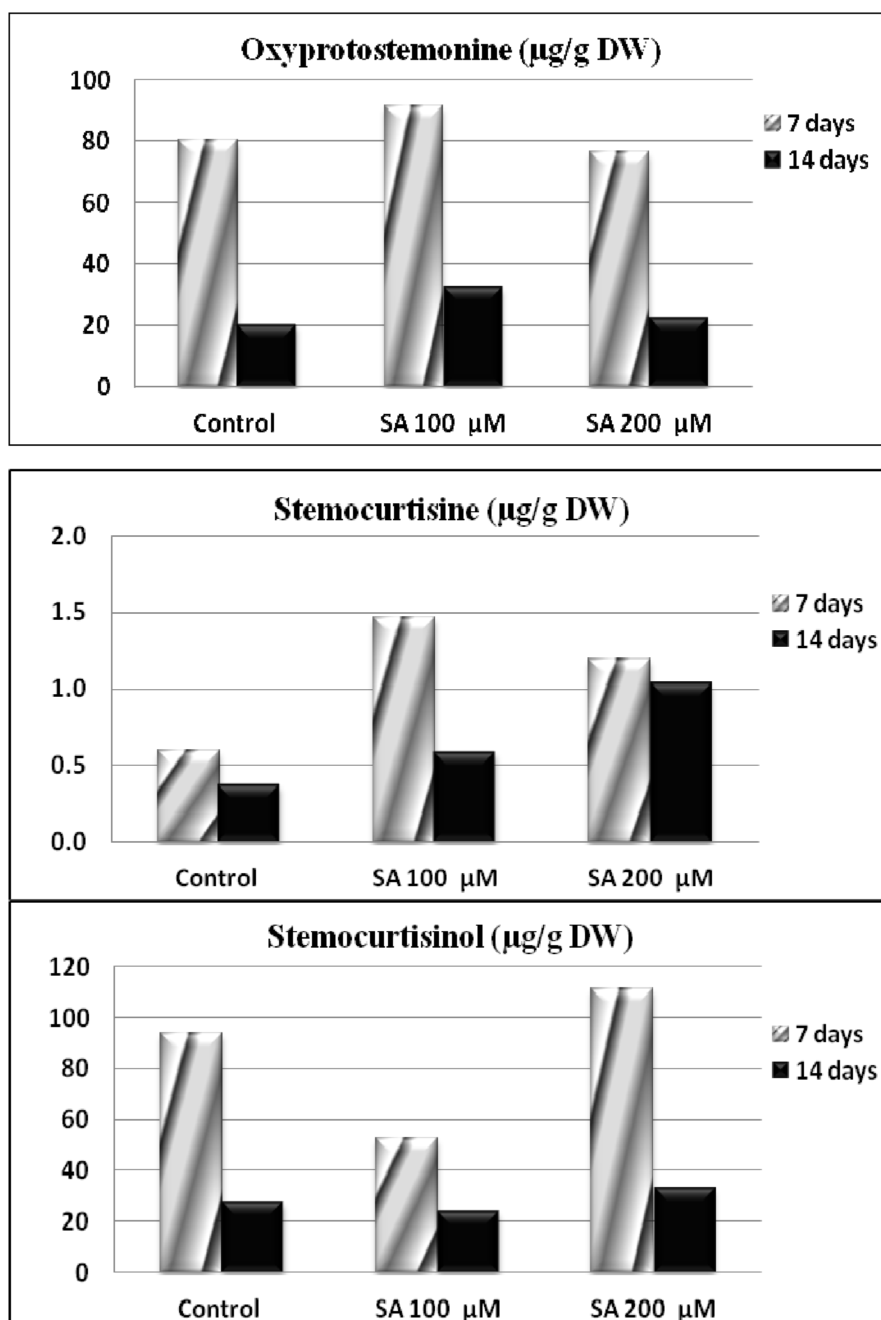

Figure 4. Effect of salicylic acid (SA) on oxyprotostemonine, stemocurtisine and stemocurtisinol production of *S. kerrii* after 7 and 14 days.

It was also found that MeJa can enhance the production of oxyprotostemonine, stemocurtisine and stemocurtisinol better than SA. These methods could be potentially developed for the future large scale production of *Stemona* alkaloids. However, it is possible

to increase the alkaloid production *in vitro* root cultures by adding 200 µM MeJa for 7 days in order to produce 3 types of alkaloids at high yield within a short time.

References:

1. Aert R, Gisi D, De Carolis E, De Luca V, Baumann TW.Plant J 1994;5:635-643.
2. Brem B, Seger C, Pacher T, Hofer O, Vajrodaya S, Gregor H. Agric Food Chem 2002;50:6383-6388.
3. Chaichana N, Dheeranupattana S, Jatisatienr A.STT36 Congress 2010. 26-28 October, 2010. Bangkok, Thailand.
4. Chotikadachanarong K. Ph.D. Thesis, Chiang Mai University 2011:98.
5. Cosio EG, Frey T, Verduyn R, van Boom J, Ebel J.FEBS Lett 1990;271:223-226.
6. Jian Z, Lawrence C D, Verpoorte R.Biotechnol Adv 2005; 23:283-333.
7. Jiwajinda S, Hirai N, Watanabe K, Santisopasri V, Chuengsamarnyart N, Koshimizu K, Ohigashi H.Phytochem 2001;56:693-695.
8. Kang SM, Jung HY, Kang YM, Yun DJ, Bahk JD, Yang JK, Choi MS. Plant Sci 2004;166 : 745-751.
9. Lin WH, Ma L, Cai MS, Barnes RA. Phytochem 1994; 36:1333-1335.
10. Lois R, Dietrich A, Hahlbrock K, Schulz W.EMBO J 1989; 8:1641–1648.
11. Pacher T, Seger C, Engelmeier D, Vajrodaya S, Hofer O, Gregor H. J Nat Prod 2002; 65:820-827.
12. Sakata K, Aoki K, Chang CF, Sakurai A, Tamura S, Murakoshi S. Agri Biol Chem 1978;42:457-463.
13. Shah J, Kachroo P, Klessig DF. Plant Cell 1999; 11:191-206.
14. Shiengthong D, Donavanik T, Uaprasert V, Roengsumran S, Massy-Westropp RA.Tetrahedron Lett 1974; 23:2015-2018.
15. Smetanska I.Adv Biochem Eng Biotechnol 2008; 111:187-228.
16. Song SH, Byun SY.Biotechnol Bioprocess Eng 1998; 3:91-95.
17. van der Fits L, Memelink J.Sci 2000; 289:295-297.
18. Wang C, Wu J, Mei X.Appl Microbiol Biotechnol 2001; 55:404-410.

Acknowledgements: We would like to thank the National Research Council of Thailand, Chiang Mai University, Graduate School, Department of Biology, Faculty of Science for financial support. This research is a part of the Plant Genetic Conservation Project Under the Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn.

Keywords: *Stemona kerrii*, methyl jasmonate, salicylic acid, *Stemona* alkaloids

© 38th Congress on Science and Technology of Thailand (Full paper)