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Effects of yeast extract and methyl jasmonate on the enhancement of solasodine biosynthesis in cell cultures of *Solanum hainanense* Hance

ABSTRACT

In this work, the effects of the elicitors methyl jasmonate (MeJA) and yeast extract (YE) on the growth and solasodine production of *Solanum hainanense* cells were investigated. The results showed that various concentrations of MeJA (50-250 µM) and YE (1-4 g/L) have different eliciting influences. The increase of solasodine content induced by the elicitation of 3 g/L of YE and 50 µM of MeJA at the beginning of cell culture was about 1.9- and 1.3-fold, respectively, as compared with that of the non-elicitated cells. In general, YE (biotic elicitor) was more effective in enhancing solasodine production than MeJA (abiotic elicitor).

Key words: Cell suspension, methyl jasmonate, solasodine, *Solanum hainanense*, yeast extract

Introduction

Different phytochemicals are produced by plants as secondary metabolites. These compounds can be classified into three main groups: the terpenes, phenolics and nitrogen-containing compounds. Secondary metabolites often play an important role in plant defense (Stamp, 2003). Humans used secondary metabolites as medicines, flavorings and recreational drugs (Davati & Najafi, 2013). According to DiCosmo & Misawa (1995), plant cell culture systems represent a potential renewable source of valuable medicinals, flavours, essences and colourants that cannot be produced by microbial cells or chemical syntheses.

Elicitation has been recognized as the efficient strategy to increase the biosynthesis of secondary compounds in plant cell cultures (Lu et al. 2001; Wang et al. 2004; Roat & Ramawat, 2009). An elicitor, in biology, is a molecule that enhances the production of another molecule. For example, jasmonic acid stimulates the biosynthesis of delta-viniferin in grapevine cell cultures (Santamaria et al. 2011). The effects of some elicitors (yeast extract, salicylic acid, methyl jasmonate, chitin) on the enhancement of secondary compounds production in the plant cell cultures has been studied.

Yan et al. (2006) reported accumulation of rosmarinic acid in hairy-root culture of *Salvia miltiorrhiza* elicited by yeast extract (YE). Some other authors as Goyal & Ramawat (2008), Turgut-Kara & Ari (2011), and Cai et al. (2012) reported elicitation efficiency of YE on the biosynthesis of isoflavonoids, cytochrome P450 and anthocyanin, and phenolic acid, respectively. Methyl jasmonate (MeJA) has been used as an elicitor for enhancing the production of secondary compounds in plant cell cultures such as ginsenoside in *Panax ginseng* (Thanh et al. 2005), anthocyanin in *Melastoma malabathricum* (See et al. 2011), or in hairy roots cultures such as glycyrrhizin in *Glycyrrhiza inflata* (Wongwicha et al. 2011), diterpenoid in *Salvia sclarea* (Kuźma et al. 2009).

Solanum hainanense, a member of Solanaceae family, accumulates mainly glycoalkaloid in roots (Hop & Phuong, 2003). Glycoalkaloids could inactivate human viruses such as *Herpes simplex*, *H. zoster* and *H. genitalis* (Stanker et al. 1996), prolong the duration of action of anestehetics (McGehee et al. 2000), reduce cholesterol content (Friedman et al. 2000a, 2000b), and also could be used as a malaria vaccine (Heal et al. 2001).

In an earlier report, we produced solasodine, a type of main glycoalkaloid in *Solanum* species, from cell culture of

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S. hainanense (Loc & Thanh, 2011). However, we have not found any reports on the effect of elicitors on production of solasodine from *S. hainanense* cell culture. Thus, the objective of this work was to investigate the effects of MeJA and YE on solasodine production of cell culture from this plant species.

Materials and Methods

Plant materials

In vitro stem segments (0.5 cm in length) of *S. hainanense* plants were cultured on the MS medium (Murashige & Skoog, 1962) supplemented with 3% (w/v) sucrose, 0.1 mg/L benzylaminopurine, 1 mg/L 2,4-dichlorophenoxyacetic acid, and 0.8% (w/v) agar for callus induction. Three-weeks old calli were subcultured and maintained on the same medium (Loc & Thanh, 2011). The medium was adjusted to pH 5.8, and then was sterilized at 121°C for 15 min. The cultures were incubated at 25±2°C under intensity of about 2,000 lux with a photoperiod of 10 h day light.

Cell suspension culture

Cell suspension cultures were established through the agitation of 3 g of callus in 250 mL Erlenmeyer flasks containing 50 mL of callus induction medium at a shaking speed of 150 rpm for 4 weeks under the same conditions as for the callus culture except the intensity of 500 lux until a suspension of free cells formed. In order to maintain the suspension culture, every 20 mL (approximately 1.65 g fresh cell biomass) of 3-weeks old culture was transferred to the same fresh medium.

Growth index was calculated as:

GI = Final fresh cell weight / Initial fresh cell weight

Elicitation

Elicitation effects of MeJA and YE were investigated by adding different concentrations to the medium at the beginning of culture with 3 g inoculum size. The cell biomass was harvested after 4 weeks, the fresh and dry cell weights were determined after they were harvested. The solasodine content was measured from the cell extract by high performance liquid chromatography (HPLC).

Optimal concentration of elicitors was added into the medium at days 7, 14 and 21 after inoculation (3 g). The addition of elicitor at the beginning of cell culture was used as control. The cells were harvested after 4 weeks of culture to determine the growth and solasodine content.

Quantification of solasodine

Solasodine content was determined by HPLC as our previous report (Loc & Thanh 2011) except a Hypersil MOS (C8) column (5 µm, 4.6×150 mm, Thermo Scientific) and run time of 5 min.

High performance liquid chromatography analysis was performed on a LC-20A Prominence system (Shimadzu, Japan) with LC-20AD pump, SPD-20A UV-VIS detector, SIL-20A HT autosampler and the LC-Solution software (ver. 1.22). All solvents were of analytical grade and were purchased from Sigma and Merck & Co., Inc.

A standard curve of solasodine (0.1-5 mg/mL in methanol, Santa Cruz) was used for determination of the solasodine content in the extracts.

Statistical analysis

Culture experiments were conducted with a minimum of ten replicates and all experiments were repeated 3 times. The data were analyzed as means ± standard error followed by comparisons of the mean by Duncan's test ($p<0.05$) using the SAS program.

Results and Discussion

Elicitation with methyl jasmonate

In present work, 50-250 µM MeJA was added to the medium on the beginning of culture and then 50 µM MeJA of optimal concentration on the days 7, 14 and 21 of culture. The results showed that the growth and solasodine production in cell cultures of *Solanum hainanense* were influenced by different concentrations of MeJA (Tables 1 and 2). The growth of the elicited cells was lower than that in the non-elicited cells (0.17-0.36 g vs. 0.45 g of dry weight with respective GIs of 0.76-1.19 and 1.66), while the solasodine production was increased and reached the highest value of 159 of mg/g of dry weight in the culture treated with 50 µM MeJA at the beginning of inoculation (Tables 3 and 4). The maximum content of solasodine was about 1.3 and 6.8 times higher in the elicited cells that recorded in non-elicited cells and *in planta* 1 year-old root, respectively. The most suitable elicitation day for adding 50 µM MeJA was the beginning of culture.

As shown in Figure 1, the HPLC chromatogram indicated that the solasodine standard had a retention time of 2.1 min. Solasodine was detected in *in planta* 1 year-old roots, non-elicited cells and MeJA elicited cells with retention time of 2.21 min, 2.19 and 2.1-2.19 min, respectively (Figures 2-4).

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Table 1. *Effect of MeJA concentration on Solanum hainanense cell growth.*

MeJA (μM)[1]	Fresh cell weight (g)	Dry cell weight (g)	Growth index
50	3.38^{ab}	0.35^{b}	1.13
100	3.57^{ab}	0.34^{bc}	1.19
150	2.96^{abc}	0.36^{b}	0.99
200	2.41^{bc}	0.34^{bc}	0.80
250	2.29^{bc}	0.35^{b}	0.76
Control[2]	4.98^{a}	0.45^{a}	1.66

[1] MeJA added to medium at the beginning of cell culture. Different letters in column indicate significantly different means using Duncan's test (p<0.05).
[2] Non-elicited cells.

Table 2. *Effect of elicitation day of MeJA (50 μM) on Solanum hainanense cell growth.*

Elicitation days	Fresh cell weight (g)	Dry cell weight (g)	Growth index
7	2.65^{c}	0.22^{bc}	0.88
14	2.98^{b}	0.28^{b}	0.99
21	2.51^{cd}	0.17^{d}	0.84
Control[1]	3.38^{ab}	0.35^{a}	1.13

[1] MeJA added to medium at the beginning of cell culture.

Table 3. *Effect of MeJA concentration on solasodine production of Solanum hainanense cells.*

MeJA[1] (μM)	Retention time (min)	Peak area (μV)[2]	Solasodine (mg/g)
50	2.12	2 743 907	159
100	2.12	2 376 911	138
150	2.11	1 689 790	98
200	2.10	653 237	38
250	2.10	626 007	36.5
Control[2]	2.19	2 128 800	123.5
Control[3]	2.21	157 473	23.5

[1] MeJA added to medium at the beginning of cell culture.
[2] Non-elicited cells.
[3] *in planta* 1 year-old root.

Table 4. *Effect of elicitation day of MeJA (50 μM) on solasodine production of Solanum hainanense cells.*

Elicitation days	Retention time (min)	Peak area (μV)[2]	Solasodine (mg/g)
7	2.10	1 369 289	79.5
14	2.19	1 283 280	74.5
21	2.15	1 872 148	108.5
Control[1]	2.12	2 743 907	159

[1] MeJA added to medium at the beginning of cell culture.

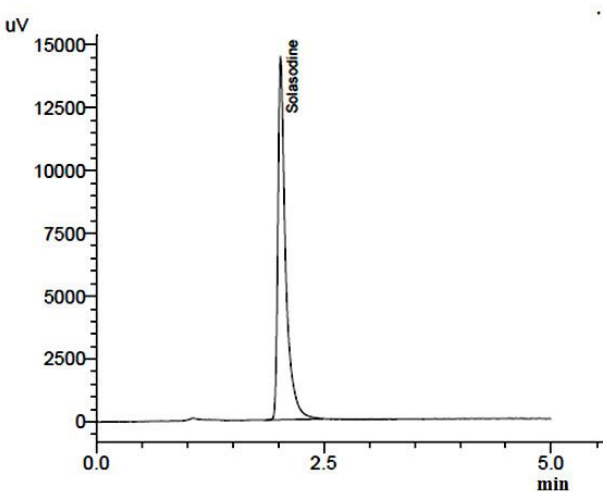

Figure 1. *HPLC chromatogram of the standard solasodine (0.5 mg/mL).*

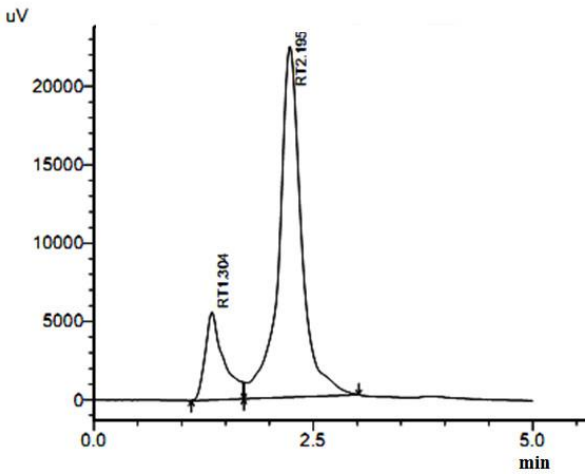

Figure 2. *HPLC chromatogram of the solasodine extract from in planta 1 year-old root.*

Elicitation with yeast extract

Table 5 showed YE at all concentrations (1-4 g/L) exhibited a stimulating effect on cell growth (0.43-0.54 g of dry weight with GIs of 2.69-3.1). YE also acts as a source of nitrogen, and this nutritional effect may also have played a role in the cell growth. However, while the GIs of elicitated cells increased from 1.89 to 2.93 in different elicitattion days with 3 g/L YE (non-elicitated cells: 1.66), the dry cell weight reduced from 0.35 to 0.45 g (non-elicitated cells: 0.48) (Table 6).

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As shown in Table 7, when 1-4 g of YE was supplemented in the medium, solasodine production increased from 125.5 to 220.5 mg/g dry weight. The days of elicitation with 3 g/L of YE for the induction of solasodine production are presented in Table 8. Solasodine accumulation reached a maximum value of 220.5 mg/g of dry weight when YE was added at the beginning of cell culture, and it was about 1.9 times higher than the non-elicited cells.

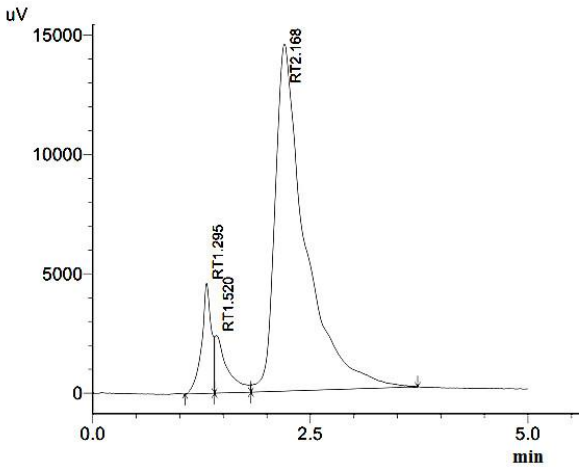

Figure 3. *HPLC chromatogram of the solasodine extract of 6 times dilution from non-elicited cells.*

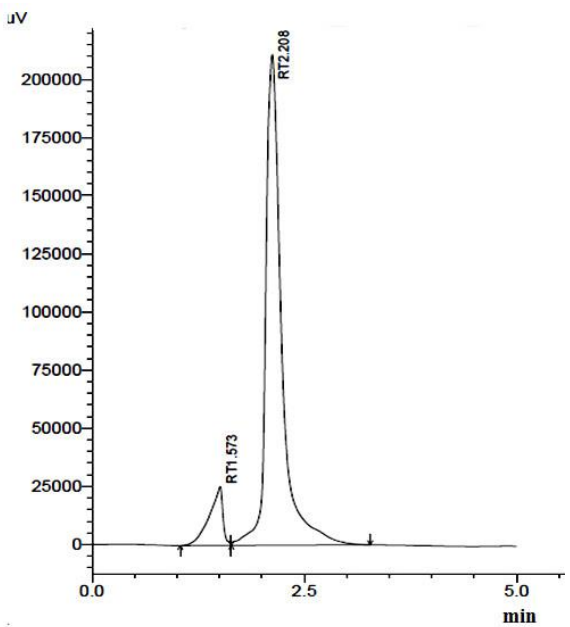

Figure 4. *HPLC chromatogram of the solasodine extract from cells elicitated by 50 µM MeJA at the beginning of culture.*

Table 5. *Effect of YE concentration on Solanum hainanense cell growth.*

YE (g/L)[1]	Fresh cell weight (g)	Dry cell weight (g)	Growth index
1	9.30[a]	0.54[a]	3.10
2	9.20[ab]	0.53[a]	3.07
3	8.80[b]	0.48[b]	2.93
4	8.08[c]	0.43[b]	2.69
Control[2]	4.98[d]	0.45[b]	1.66

[1] YE added to medium at the beginning of cell culture.
[2] Non-elicited cells.

Table 6. *Effect of elicitation day of YE (3 g/L) on Solanum hainanense cell growth.*

Elicitation days	Fresh cell weight (g)	Dry cell weight (g)	Growth index
7	8.79[a]	0.45[ab]	2.93
14	8.41[b]	0.36[c]	2.80
21	5.66[c]	0.35[c]	1.89
Control[1]	8.80[a]	0.48[a]	2.93

[1] YE added to medium at the beginning of cell culture.

Table 7. *Effect of YE concentration on solasodine production of Solanum hainanense cells.*

YE (g/L)[1]	Retention time (min)	Peak area (µV)[2]	Solasodine (mg/g)
1	2.19	2 161 702	125.5
2	2.18	2 841 851	165
3	2.15	3 804 932	220.5
4	2.14	3 413 115	198
Control[2]	2.19	2 128 800	123.5
Control[3]	2.21	157 473	23.5

[1] YE added to medium at the beginning of cell culture.
[2] Non-elicited cells.
[3] *in planta* 1 year-old root.

Table 8. *Effect of elicitation day of YE (3 g/L) on solasodine production of Solanum hainanense cells.*

Elicitation days	Retention time (min)	Peak area (µV)[2]	Solasodine (mg/g)
7	2.14	1 909 691	110.5
14	2.18	1 590 310	92
21	2.16	1 658 175	96
Control[1]	2.15	3 804 932	220.5

[1] YE added to medium at the beginning of cell culture.

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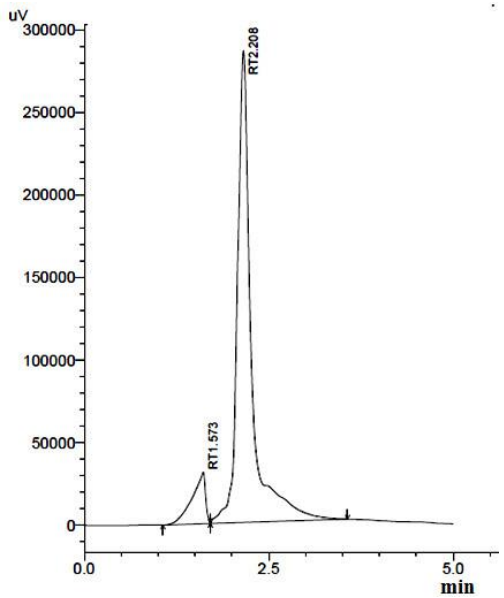

Figure 5. *HPLC chromatogram of the solasodine extract from cells elicitated by 3 g/L YE at the beginning of culture.*

As shown in Figure 5, solasodine was also detected at the retention times of 2.15 min when cells were grown in the medium with 3 g/L of YE. These results showed that elicitor treatments were suitable for enhancing solasodine production from cell culture of *S. hainanense*.

Conclusion

MeJA from 50-250 µM and YE from 1-4 g/L exhibited different eliciting effects on cell cultures of *S. hainanense*. The addition of suitable concentrations of MeJA and YE at the beginning of inoculation to the cultures significantly enhanced solasodine production in *S. hainanense* cells. The increase in solasodine content induced by MeJA and YE was about 1.3- and 1.9-fold, respectively, in comparison with the non-elicited cells. In conclusion, a biotic elicitor such as YE was more effective in enhancing solasodine biosynthesis than an abiotic elicitor such as MeJA.

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References

Cai Z, Kastell A, Mewis I, Knorr D, Smetanska I. 2012. Polysaccharide elicitors enhance anthocyanin and phenolic acid accumulation in cell suspension cultures of *Vitis vinifera*. Plant Cell Tiss. Organ Cult., 108(3): 401-409.

Davati N, Najafi MBH. 2013. Overproduction strategies for microbial secondary metabolites: A review. International Journal of Life science and Pharma Research, 3: L23-L37.

DiCosmo F, Misawa M. 1995. Plant cell and tissue culture: alternatives for metabolite production. Biotechnol. Adv., 13(3): 425-453.

Friedman M, Fitch TE, Levin CE, Yokoyama WH. 2000a. Feeding tomatoes to hamsters reduces their plasma low-density lipoprotein cholesterol and triglycerides. Journal of Food Science, 65(5): 897-900.

Friedman M, Fitch TE, Yokoyama WH. 2000b. Lowering of plasma LDL cholesterol in hamsters by the tomato glycoalkaloid tomatine. Food and Chemical Toxicology, 38(7): 549-553.

Goyal S, Ramawat KG. 2008. Increased isoflavonoids accumulation in cell suspension cultures of *Pueraria tuberosa* by elicitors. Indian J. Biotechnol., 7(3): 378-382.

Heal KG, Sheikh NA, Hollingdale MR, Morrow WJW, Taylor-Robinson AW. 2001. Potentiation by a novel alkaloid glycoside adjuvant of a protective cytotoxic T cell immune response specific for a preerythrocytic malaria vaccine candidate antigen. Vaccine, 19(30): 4153-4161.

Hop VV, Phuong VX. 2003. Alkaloid species belong to Solanaceae family in Vietnam. Vietnamese Journal of Biology, 25: 27-31.

Kuźma L, Bruchajzer E, Wysokińska H. 2009. Methyl jasmonate effect on diterpenoid accumulation in *Salvia sclarea* hairy root culture in shake flasks and sprinkle bioreactor. Enzyme and Microbial Technology, 44(6-7): 406-410.

Loc NH, Thanh LTH. 2011. Solasodine production from cell culture of *Solanum hainanense* Hance. Biotechnology and Bioprocess Engineering, 16(3): 581-586.

Lu MB, Wong HL, Teng WL. 2001. Effects of elicitation on the production of saponin in cell culture of *Panax ginseng*. Plant Cell Reports, 20: 674-677.

McGehee DS, Krasowski MD, Fung DL. Wilson B, Gronert GA, Moss J. 2000. Cholinesterase inhibition by potato glycoalkaloids slows mivacurium metabolism. Anesthesiology, 93(2): 510-519.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473-497.

Roat C, Ramawat KG. 2009. Elicitor-induced accumulation of stilbenes in cell suspension cultures of *Cayratia trifolia* (L.) Domin. Plant Biotechnology Reports, 3(2): 135-138.

Santamaria AR, Mulinacci N, Valletta A, Innocenti M, Pasqua G. 2011. Effects of elicitors on the production of resveratrol and viniferins in cell cultures of *Vitis vinifera* L. cv Italia. J. Agric. Food Chem., 59(17): 9094-9101.

See KS, Bhatt A, Keng CL. 2011. Effect of sucrose and methyl jasmonate on biomass and anthocyanin production in cell suspension culture of *Melastoma malabathricum* (Melastomaceae). Rev. Biol. Trop., 59(2): 597-606.

Stamp N. 2003. Out of the quagmire of plant defense hypotheses. Q. Rev. Biol., 78(1): 23-55.

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Stanker LH, Kampos-Holtzapple C, Beier RC, Levin CE, Friedman M. 1996. Detection and quantification of glycoalkaloids comparison of enzyme-linked immunosorbent assay and high performance liquid chromatography methods. ACS Symposium Series, 621: 243-251.

Thanh NT, Murthy HN, Yu KW, Hahn EJ, Peak KY. 2005. Methyl jasmonate elicitation enhanced synthesis of ginsenoside by cell suspension culture of *Panax ginseng* in 5L balloon type bubble bioreactor. Appl. Microbiol. Biotechnol., 67(2): 197-201.

Turgut-Kara N, Ari S. 2011. Analysis of elicitor inducible cytochrome P450 induction in *Astragalus chrysochlorus* cells. Plant Omics, 4(5): 264-269.

Wang HF, Provan GJ, Helliwell K. 2004. Determination of rosmarinic acid and caffeic acid in aromatic herbs by HPLC. Food Chemistry, 87(2): 307-311.

Wongwicha W, Tanaka H, Shoyama Y, Putalun W. 2011. Methyl jasmonate elicitation enhances glycyrrhizin production in *Glycyrrhiza inflata* hairy roots cultures. Z Naturforsch C, 66(7-8): 423-428.

Yan Q, Shi M, Ng J, Wu JY. 2006. Elicitor-induced rosmarinic acid accumulation and secondary metabolism enzyme activities in *Salvia miltiorrhiza* hairy roots. Plant Sci., 170(4): 853-858.

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