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Effect of L-arginine, casein hydrolysate, banana powder and sucrose on growth and solasodine production in shoot cultures of *Solanum laciniatum*

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Abstract

The addition of L-arginine, casein hydrolysate, banana powder and the reduction of the concentration of sucrose in the media could increase the solasodine content in the shoot cultures of *Solanum laciniatum*. No linear correlations between growth index, chlorophyll content and solasodine content were observed, however a positive linear correlation between solasodine productivity and chlorophyll content occurred in these shoot cultures.

Abbreviations: Ch – chlorophyll content; DW – dry weight; fl – flask; FW – fresh weight; GI – growth index; LOD – limit of detection; LOQ – limit of quantitation; SDc – solasodine content; SDp – solasodine productivity; w – week

Introduction

Solasodine, a steroidal alkaloid which can be produced commercially from *Solanum laciniatum* and *Solanum khasianum* plants, is one of the most important raw materials for steroidal drug production (Macek, 1989; Nigra *et al.*, 1990). Several papers have described solasodine production in tissue cultures of *S. laciniatum*, and this has been reviewed by Macek (1989). Indrayanto (1983) reported that no solasodine was detectable in callus and suspension cultures of *S. laciniatum*. Chandler & Dodds (1983a) described the effect of phosphate, nitrogen and sucrose concentration variation on solasodine content in callus cultures of *S. laciniatum*. It was also reported that the solasodine concentration in these calluses was enhanced by organogenesis and by incubation in darkness (Chandler & Dodds, 1983b). Conner (1987) reported that callus cultures of *S. lacinatum* contained approximately 100-fold less solasodine compared to the concentration in plants, however, shoot cultures cultivated photoheterotrophically or photoautotrophically yielded solasodine concentrations approaching those of field grown plants. To the best of our knowledge, there are no reports concerning the effect of variation in the concentration of the media components on solasodine production in shoot cultures of *S. laciniatum*.

In this paper, the effect of L-arginine, casein hydrolysate, banana powder and sucrose on growth and solasodine production in the shoot cultures of *S. laciniatum* are presented.

Materials and methods

Shoot cultures of *Solanum laciniatum* Ait were provided by Dr. Ika Mariska, Research Institute for Spice, Medicinal Crops & Industry, Department of Agriculture, Bogor, Indonesia. The shoot cultures were cultivated on modified MS medium (Murashige & Skoog, 1962) with benzyladenine (4 mg l^{-1}), sucrose (30 g l^{-1}) and solidified with agar (7 g l^{-1}). The shoot cultures were subcultured every four weeks by inoculated ca. 1.0 g shoots on fresh medium in 250 ml glass-bottles closed with aluminium foil. The incubation conditions were 25 ± 1 °C under continuous light, using fluorescent lamps (Phillips TL 40W/54) at a light intensity of approximately 60 μmol m^{-2} s^{-1}.

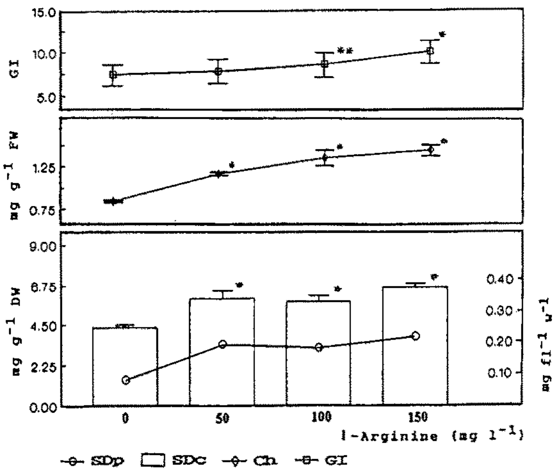

Fig. 1. Effect of the addition of L-arginine in media on solasodine content (SDc), chloropphyll content (Ch), solasodine productivity (SDp) and growth index (GI) in shoot cultures of *Solanum laciniatum*. Values represent the mean ± SD; Significantly different (ANOVA-HSD test) from medium without L-arginine, * (p<0.01), ** (p<0. 05).

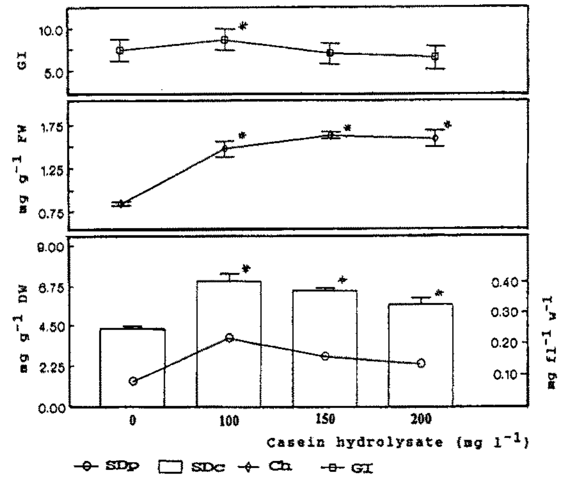

Fig. 2. Effect of the addition of casein hydrolysate in media on solasodine content (SDc), chlorophyll content (Ch), solasodine productivity (SDp) and growth index (GI) in shoot cultures of *Solanum laciniatum*. Values represent the mean ± SD; *Significantly different (p<0.01;ANOVA-HSD test) from medium without casein hydrolysate.

In order to study the influence of some media components on growth and solasodine production in these shoot cultures, the cultures were transferred to a series of treatment media and incubated as described above for 4 weeks. In the first, second and third series of the treatment media, various concentration of L-arginine (Sigma), casein hydrolysate (Sigma) and banana powder (Sigma) were added into the media (see Fig. 1–3). All other components were the same as the modified MS medium as described above. In the fourth series of treatment media, the concentration of sucrose (E. Merck) was reduced from 30 to 0 g l^{-1} (see Fig. 4). The growth rate of the shoot cultures was expressed as GI (growth index) defined as the ratio of the final fresh weight and the initial fresh weight of the shoot cultures.

The total chlorophyll content was estimated according to the method of Harborne (1973).

In order to determine the solasodine content in these shoot cultures, 100.0 mg of the oven dried (50 °C) powdered biomass was extracted three times with 5.0 ml $CHCl_3$ on a vortex mixer to remove the non polar components, after filtration the residue was hydrolyzed with 2 N HCl in methanol (2 h; 75 °C), then neutralized with 10 N NaOH, solasodine was extracted three times with 7.5 ml $CHCl_3$. The $CHCl_3$ phase was collected and evaporated to dryness under N_2. The hydrolysate extracts were dissolved with 2.0 ml (accurately, measured with a Socorex adjustable pipette) $CHCl_3$ and 4

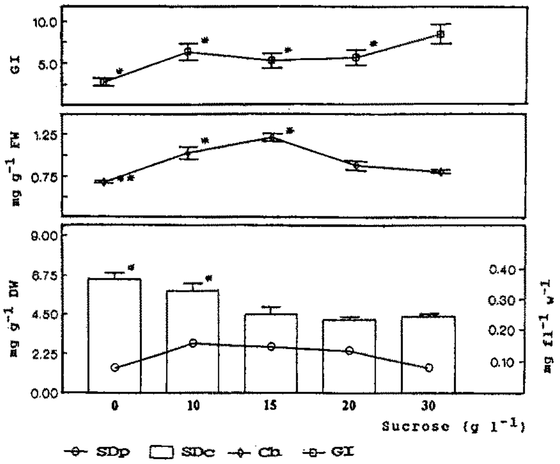

Fig. 3. Effect of decreasing of sucrose in media on solasodine content (SDc), chlorophyll content (Ch), solasodine productivity (SDp) and growth index (GI) in shoot culture of *Solanum laciniatum*. Values represent the mean ± SD; Significantly different (ANOVA-HSD test) from medium with sucrose 30 g l^{-1}, * (p<0.01), ** (p<0.05).

μl of this solution was spotted on precoated Kieselgel G 60 F254 TLC plates (E. Merck), and developed using a mixture of chloroform:methanol:diethylamine (20:2:0.5) as eluent. The solasodine spots (R_f = 0.68) were detected by anisealdehyde-H_2SO_4 reagent (100 °C, 10 min). Quantitation was performed by measuring the absorbance reflectance (at λ_{max}, 385 nm) of the solasodine spots, using a Shimadzu GS-930 TLC-Scanner. The determination of solasodine was made

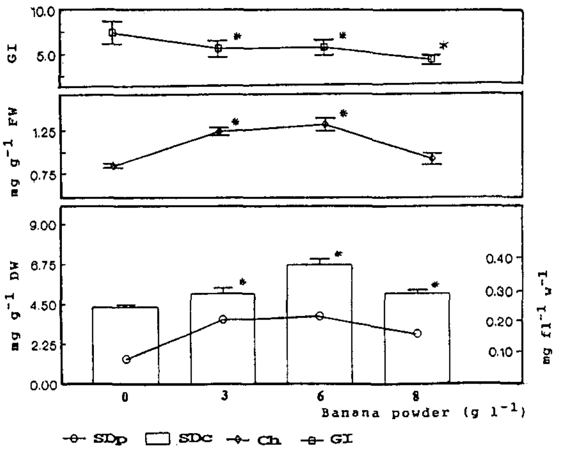

Fig. 4. Effect of the addition of banana powder in media on solasodine content (SDc), chlorophyll content (Ch), solasodine productivity (SDp), and growth index (GI) in shoot cultures of *Solanum laciniatum*. Values represent the mean ± SD; *Significantly different (p<0.01; ANOVA-HSD test) from medium without banana powder.

by calculation with a calibration graph obtained using solasodine (Sigma) as external standard on the same TLC plate. With this method linearity of solasodine was achieved from 0.3 to 1.6 μg/spot (r = 0.998, n = 7, V_{xo} = 2.1%); LOD = 0.11 μg/spot; LOQ = 0.33 μg/spot; accuracy (mean ± SD) by the standard addition method was 98.92 ± 2.35%, n = 14; RSD of precision determination was 1.87% (n = 10). The presence of solasodine in the hydrolysate extracts was confirmed by GC-MS (Jeol DX 303 GC-MS system) analysis (according to Carle, 1979) by comparison with authentic solasodine standard (Sigma).

Solasodine productivity was expressed as the average of solasodine formation in one flask for 1 week, defined as mean of solasodine content (mg g^{-1} DW) mean of biomass formation in one flask for 1 week (g DW fl^{-1} w^{-1}).

Results and discussion

Figure 1 shows that addition of L-arginine (50–150 mg l^{-1}) to the media significantly increased the solasodine and chlorophyll content in these shoot cultures, however the GI was significantly increased only by the addition of 100 mg l^{-1} and 150 mg l^{-1} L-arginine. Solasodine productivity was also increased (2.4–2. 7 times) by the addition of L-arginine into media. This result shows that L-arginine might be the donor molecule for the nitrogen atom in solasodine, as in the case of solanidine as previously reported by Kaneko *et al.* (1976).

The addition of casein hydrolysate (Fig. 2) to media (100–200 mg l^{-1}) increased the solasodine and chlorophyll content significantly but the GI was increased only by the addition of 100 mg l^{-1} casein hydrolysate. This may explain the decreasing solasodine productivity by the addition of 150 and 200 mg l^{-1} casein hydrolysate. The increase of solasodine content in shoot cultures cultivated on media with the addition of casein hydrolysate, might be due to its sterols (Heble *et al.*, 1976), or amino acids content. Atipayakul & Jatisatienr (1989) also reported that the solasodine content in callus cultures of *S. laciniatum* increased by the addition of cholesterol.

Decreasing the sucrose concentration in the media from 30 g l^{-1} to 10 and 0 g l^{-1} (Fig. 3) increased the solasodine content significantly. The increase of the solasodine content in shoot cultures of *S. laciniatum* cultivated on media without sucrose has been reported previously by Conner (1987). This effect is opposite to the one published by Schripsema (1991), which reported that the production of the alkaloid O-acetylvallesamine in the *Tabernaemontana divaricata* cell suspension cultures was decreased when the sucrose concentration in the media reduced to 5 g l^{-1}. Although the solasodine content was increased in cultures cultivated on media without sucrose, its productivity (0.07 mg fl^{-1} w^{-1}) was the same as cultures cultivated on media with the original sucrose concentration (30 g l^{-1}). This was due to the very low GI and chlorophyll content of the cultures cultivated on this medium.

Figure 4 shows that the solasodine content in these shoot cultures increased significantly by the addition of 3–8 g l^{-1} banana powder in the media, Chlorophyll content also increased in media with the addition of 3 and 6 g l^{-1} banana powder. The increase of chlorophyll content in callus cultures of *S. wrightii* cultivated on media with the addition of banana powder has been reported previously (Handayani, 1991). Although banana homogenate or powder usually used to promote growth in plant tissue cultures (Hartojo, 1986; Pierik, 1987), addition of banana powder in the media decreased the GI of these shoot cultures significantly. This might be due to the toxic effect of certain components of banana powder.

The solasodine content in these shoot cultures (4.1–7.0 mg g^{-1} DW) was higher than the previously reported in the shoot cultures of *S. laciniatum* by Macek, 1989 (1.1–3.9 mg g $^{-1}$ DW) and Conner (1987) (0.04–2.23 mg g $^{-1}$ DW) or in shoot cultures of *S. dulcamara* (1.05 mg g^{-1} DW) by Ehmke & Eilert (1986). Ehmke & Eilert (1993) reported that a positive correlation occurred between solasodine level and chlorophyll content and aggregate size in suspension cultures of *S. dulcamara*, however in this study no linear correlation between solasodine and chlorophyll content was found ($r_{calc.}$ = 0.479; $r_{tab.}$ = 0.532; n = 14; p>0.05), but a positive linear correlation was observed between solasodine productivity and chlorophyll content ($r_{calc.}$ = 0.679; $r_{tab.}$ = 0.661; n = 14; p<0.01). Nigra *et al.* (1987) reported that a correlation occurred between solasodine production (g fl^{-1}) and growth in calli of *S. eleagnifolium* but in this study no correlation was observed between solasodine productivity (mg fl^{-1} w^{-1}) and GI ($r_{calc.}$ = 0.449; $r_{tab.}$ = 0.532; n = 14; p>0.05). This result confirms the previous report by Mak & Doran (1993) which stated that solasodine biosynthesis and growth could be uncoupled.

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