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Production of solasodine by calli from different parts of *Solanum eleagnifolium* Cav. plants

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ABSTRACT

Callus tissues from different explants (hypocotyl, cotyledon, root, leaf and fruit) of Solanum eleagnifolium Cav. were cultured on a modified Murashige-Skoog medium, with 1 $mg.l^{-1}$ 2,4-D as the sole growth regulator. The presence of the alkaloid solasodine was determined by spectrophotometric and TLC methods. Its concentration ranged from 1.00 to 2.15 $mg.g^{-1}$ DW. The calli from different explants showed a direct association between the solasodine production and their growth, although they have a different production rate. It was also observed that about the seventh week of culture the metabolite concentration decreased in all cases.

ABBREVIATIONS: 2,4-D (2,4-dichlorophenoxyacetic acid)
DW (dry weight)
TLC (thin layer chromatography)

INTRODUCTION

Diosgenin is the most important raw material for steroidal drugs. The difficulties in providing plant raw materials for diosgenin production has led to the selection of alternative sources, such as solasodine, tomatidine, solanidine, hecogenine, etc. Of these, solasodine has advantages over the others and it seems to be in a strong competitive position with diosgenin (Mann, 1978).

In spite of the fact that a hundred species of the genus Solanum contain solasodine, only a few of them are considered to be important for commercial production. In fact, in the USSR, solasodine obtained from S. laciniatum has been used to produce cortisone and progesterone (Alekseenko et al., 1976) and in India solasodine extracted from S. khasianum and from other cultivated species have been used in the pharmaceutical industry (Mann, 1978).

The presence of steroidal glycoalkaloids in Solanum eleagnifolium Cav., native to Argentina, has been reported. According to this study solamargine, glycoside of solasodine is present in the whole plant with the highest concentration in the fruits (Rodriguez, 1984). The "in vitro" production of solasodine for several species of Solanum has been reported (Khanna et al., 1976; Khanna and Manot, 1976; Hosoda and Yatazawa, 1979; Hosoda et al., 1979; Chandler and Dodds, 1983; Emke and Eilert, 1986). The only reference of S. eleagnifolium is one reported by Khanna et al., (1976) where there is no specifications of the explant origin. The aim of this paper was to study the solasodine production from plant cell cultures of S. eleagnifolium initiated from different explant sources.

MATERIALS AND METHODS

Explants were obtained from seedlings and fruits of S. eleagnifolium Cav. Seeds were sterilized for 15 min in sodium hypochlorite (4% Cl_2 active) with 0.1% Triton X-100, washed thoroughly with sterile distilled water and germinated on 0.8% agar medium containing Murashige-Skoog (MS) mineral nutrients (Murashige and Skoog, 1962) and 5% sucrose at 25 ± 2°C and exposed to 16 h photoperiods by fluorescent lamps at approximately 1.8 $W.m^{-2}$.

Fruits were collected from wild plants; they were sterilized for 2-3 min in 70% ethanol and then for 10 min in sodium hypochlorite (1% Cl_2 active) with 0.1% Triton X-100 and washed with sterile water.

Explants from seedling (cotyledons, hypocotyls, roots and leaves) as well as from fruits were cultured in flasks on 10 ml of modified MS nutrient medium with the vitamin complex described by Khanna and Staba (1968), myo-inositol (100 $mg.l^{-1}$), 2,4-D (1 $mg.l^{-1}$) and solidified with 0,8% agar. The incubation conditions were the same as mentioned above. Calli from different tissue sources were subcultured in the same medium every 15-20 days for 5 months before starting the growth and solasodine determinations.

In order to determine growth and solasodine production of the calli, small pieces of about 50 mg fresh weight were placed in flasks, with 10 ml of solid nutrient medium and incubated for 7-9 weeks. Growth was determined as fresh and dry weight each week. For dry weight determination, callus pieces were dried to constant weight at 45°C.

The solasodine content was measured using 15-20 mg dry powdered callus pieces that were extracted and hydrolysed with 4 ml of 1 N HCl by refluxing at 100°C for 2 h.

For TLC, the hydrolysed samples were extracted three times with 1 ml of Cl_3CH, and concentrated to about 0.5 ml. The concentrated extracts as well as solasodine (Sigma Chem. Co.) and solasodiene standards were applied on silica-gel (Kieselgel G. type 60) plates. The solvent system was hexane:acetone (4:1). The spots were detected by H_2SO_4:CH_3-COOH:

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anisaldehyde (1:50:1) reagent, followed by heating at 100°C for 10 min. Solasodiene was obtained from Dr. J. Rodríguez (Facultad de Química, Bioquímica y Farmacia, Universidad de San Luis, Argentina).

The spectrophotometric assay was based on the procedure described by Lancaster (1975). Firstly, the hydrolysed samples were alkalinized with 60% NaOH. For the colorimetric determination, solasodine was extracted from those samples with chloroform, and 2 ml aliquots were made up to 5 ml with chloroform. Then 3 ml of 2 x 10^{-4} M bromothymol blue in borax buffer (pH 8) was added and mixed with a Vortex stirrer. The aqueous layer was taken out and 1 ml of methanolic 0.01 N NaOH was added to 4 ml of the chloroform layer. The optical density was determined at 610 nm. Standard curves were obtained using standard solutions of solasodine. For determining the solasodine in the culture medium, the same method was used. Controls (4 ml 1 N HCl) and standards were included with each batch of callus analysed. The spectrophotometry technique employed is rather specific, because other compounds of related chemical structure are not capable of binding bromothymol blue (Chandler and Dodds, 1983) except solasodiene. This compound is formed during the hydrolysis, so all the values observed are referred to solasodine. In all determinations 9 to 15 replicates were employed.

RESULTS AND DISCUSSION

Calli of the different plant parts (cotyledons, hypocotyls, roots, leaves, fruits) showed a DW increase between 13-15 times the initial weight in seven weeks (Fig. 1). Although the growth curves showed similar growth rates (μ = 0.080-0.086 day^{-1}), callus formed from fruit explants showed a longer lag period. Callus initiation from fruit explants was more difficult to obtain than those from seedling sources. It is possible to relate the longer induction period to a different growth potential (Aitchison et al., 1977).

TLC examination revealed the presence of solasodine. Its spot was present at the same Rf, 0.30, as the standard; solasodiene was also detected with Rf, 0.38 with no other spots being detectable.

Total solasodine production per flask was associated with the callus growth (Fig. 1). The correlations between growth (mg DW/flask) and solasodine production (g/flask) in calli from different explants were significant in all cases ($P < 0.001$). The values obtained were R^2 = 0.73 for cotyledon calli, R^2 = 0.78 for root-calli, R^2 = 0.89 for leaf-calli, R^2 = 0.89 for fruit-calli and R^2 = 0.92 for hypocotyl-calli. These results are different to those reported by Emke and Eilert (1986), who working with Solanum dulcamara suspension cultures, found that the steroidal alkaloids production was not restricted to a particular growth period.

In cotyledon-calli, hypocotyl-calli and leaf-calli the highest solasodine concentration was reached between the fourth and the fifth weeks of culture. The fruit-calli showed the highest concentration at the seventh week and the root-calli at the third one. Solasodine concentration decreased beyond its maximum in all calli (Fig. 1). However the total solasodine per flask increased until attaining the stationary growth phase.

The decrease in the solasodine concentration after the fourth and fifth week of culture could be explained either by a low production rate or by the initiation of a degradation process. On the other hand, the total amount of solasodine per flask decreased during the stationary phase. This may be due to the degradation of solasodine or changes in the chemical structure, leading to other compounds non-detectable by the technique used, since the presence of solasodine in the culture medium was not detected.

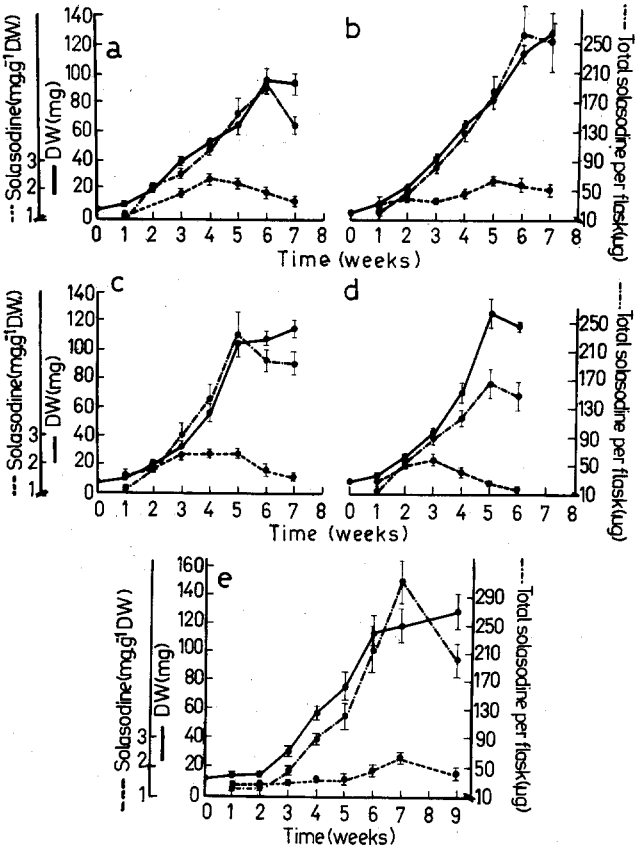

Fig. 1: Time course of growth and solasodine accumulation in S. eleagnifolium calli. a. cotyledon-calli. b. leaf-calli. c. hypocotyl-calli. d. root-calli. e. fruit-calli. Mean ± SE from 9 to 15 replicates.

The values of solasodine obtained in this study (1.0-2.15 $mg.g^{-1}$ DW) are higher than those reported by Khanna et al. (1976) who found values of 0.32 $mg.g^{-1}$ DW for the same species. However, they are comparable to those obtained by Chandler and Dodds (1983) who reported values of 1.24 $mg.g^{-1}$ DW in calli cultures of S. laciniatum using 2-chloroetyl trimethyl ammonium chloride as a growth regulator. Comparing with whole plant, the values of solasodine concentration informed in this paper for calli are higher than those determined for stems and leaves (0.3-0.6 $mg.g^{-1}$ DW) but lower than those found in fruits (30-50 $mg.g^{-1}$ DW) (Nigra et al., 1985).

In conclusion the reported experiments have revealed that all S. eleagnifolium calli have the ability to produce solasodine, despite their different explant origin. The highest solasodine concentration reached during the experiment by the different cultures, was similar for all although they showed a different production rate. These data

are similar to those found by Hosoda et al. (1979) who did not find any differences between the alkaloid concentration in root and hypocotyl calli of Solanum laciniatum. Also Sasse et al. (1982), investigating the accumulation of β-carboline alkaloids by cell cultures of Peganum harmala, reported similar results.

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