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DIFFERENTIAL SOLASODINE ACCUMULATION IN PHOTOAUTOTROPHIC AND HETEROTROPHIC TISSUE CULTURES OF *SOLANUM LACINIATUM*

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Key Word Index—*Solanum laciniatum*; Solanaceae; plant tissue culture; glycoalkaloid; solasodine.

Abstract—Foliage from a *Solanum laciniatum* plant contained 7.64 mg solasodine per g dry weight. In contrast, leaf-derived callus cultures incubated under light yielded only 0.09 mg/g solasodine. A similar low level was recovered from shoots regenerated from this callus and cultured under heterotrophic conditions. However, shoots cultured photoheterotrophically or photoautotrophically yielded solasodine concentrations approaching those of field grown plants. Solasodine biosynthesis in *S. laciniatum* is therefore promoted by actively photosynthesising chloroplasts, and cell cultures yield only low solasodine levels as a consequence of their heterotrophic mode of nutrition.

INTRODUCTION

The biosynthesis of secondary metabolites in plant cell cultures has been frequently reported; however yields are often very low compared to the plants from which the cultures were derived [1]. A good example is *Solanum laciniatum* Ait., a native shrub of New Zealand cultivated for the extraction of solasodine, a glycoalkaloid used as a raw material for the synthesis of steroid drugs [2]. Whole plants of *S. laciniatum* typically contain up to 3% solasodine (on a dry weight basis) [2], whereas callus cultures have been reported to contain either no or very low levels of solasodine [3–6]. Manipulating various cell culture parameters such as growth regulators, sucrose concentration, nutrient stress, temperature and light versus dark can alter the level of solasodine synthesis in *S. laciniatum* cell cultures [6–9], but the maximum concentrations still remain well below those of whole plants. The screening of numerous *S. laciniatum* cell lines has revealed considerable variation between clones for glycoalkaloid production, with accumulation of up to 3% dry weight in a few rare variants [7, 10]. Unfortunately these variants were unstable for glycoalkaloid accumulation [7, 11]. The aim of this study was to examine reason for the low level of solasodine synthesis in tissue cultures of *S. laciniatum*.

RESULTS AND DISCUSSION

The solasodine content of the field grown *S. laciniatum* plant used in this study was 7.64 ± 0.65 mg/g dry weight in leaves, 0.93 ± 0.12 in stems and 3.22 ± 0.09 in roots. These values are typical for this species [12], although plants with higher levels have been reported [2]. Rapidly proliferating callus cultures originating from leaf tissue contained approximately 100-fold less solasodine (Table 1). Since these callus cultures were off-white in colour and devoid of any chlorophyll pigmentation, their growth can be considered solely heterotrophic. Shoots regenerated from this callus and cultured under heterotrophic conditions (in darkness with sucrose in the medium) yielded a solasodine concentration similar to the callus cultures (Table 1). However, when regenerated shoots were cultured under photoheterotrophic conditions (in light with sucrose in the medium) or photoautotrophic conditions (in light without sucrose in the medium), the solasodine yields were considerably greater than heterotrophic shoot or callus cultures (Table 1), and approached those of field grown plants (see above).

The higher dry weight of photoheterotrophically cultured shoots compared to those cultured photoautotrophically (Table 1) is indicative of the extent to which exogenous sucrose contributes to *in vitro* shoot growth. This is also reflected in the lower solasodine yield in photoheterotrophic cultures compared to photoautotropic cultures (Table 1). The difference in solasodine content between the photoautotrophic shoot cultures (Table 1) and the field grown plant (see above) is probably a result of the low light intensity in the growth cabinet compared to sunlight. Shading of field grown *S. laciniatum* plants is known to reduce solasodine yields [13].

The results of this investigation illustrate several important points. The low solasodine yield in *S. laciniatum* cell cultures results from a change in gene expression rather than a loss in genetic potential, since shoots regenerated from callus cultures remain capable of normal solasodine synthesis. Furthermore, this change in gene expression is associated with the heterotrophic mode of nutrition in cell culture. This supports several previous studies which report the differential biosynthesis of harman alkaloids [14], quinolizidine alkaloids [15], various lipids [14, 16], quinones [17] and betalain [18] in

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Table 1. Growth and solasodine content of *S. laciniatum* tissue cultures (Mean ± s.d, $n = 4$)

Plant culture	Sucrose concentration (g/l)	Light or dark	Dry weight (g/flask)	Solasodine content (mg/g dry wt)
Callus	30	light	0.31 ± 0.07	0.09 ± 0.03
Shoots	30	dark	0.13 ± 0.01	0.04 ± 0.02
Shoots	30	light	0.58 ± 0.30	1.09 ± 0.13
Shoots	0	light	0.29 ± 0.01	2.23 ± 0.06

cell suspension cultures grown under heterotrophic, photoheterotrophic and photoautotrophic conditions. Since this study employed differentiated shoots, it can be also concluded that solasodine biosynthesis in leaves of *S. laciniatum* is either greatly promoted or dependent upon actively photosynthesizing chloroplasts.

EXPERIMENTAL

Plant material originated from a single adult plant of *S. laciniatum* growing on the Lincoln College campus. All tissue culture procedures were performed as previously described [19]. Callus cultures originating from leaf explants were grown on MS salts, vitamins and sucrose [20], plus 1 μM 6-benzylaminopurine and 1 μM β-naphthoxyacetic acid. Shoots were regenerated from this callus (see [19]) and cultured on MS salts with and without 30 g/l sucrose.

Cultures were incubated at 26° under cool white fluorescent tubes providing continuous light at 72 μmol/m^2/sec. Culture vessels were wrapped in aluminium foil for dark treatments.

After 5 weeks the plant material was removed from culture vessels and oven dried overnight at 75°. Leaves, stems and roots of the field grown parent plant from which the cultures were established were simultaneously dried. Solasodine determinations were performed as previously described [12], except that the temperature during the precipitation of glycoalkaloids from the crude extracts was lowered to 60°.

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