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ACCUMULATION OF CAPSIDIOL IN TOBACCO CELL CULTURES TREATED WITH FUNGAL ELICITOR*

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Key Word Index—*Nicotiana tabacum*: Solanaceae; cell cultures; fungal elicitor; sesquiterpenoid accumulation; capsidiol.

Abstract—Addition of fungal elicitor to tobacco cell suspension cultures induced extracellular accumulation of capsidiol.

INTRODUCTION

The accumulation of stress-associated sesquiterpenoids in pathogen challenged plants [1] and tissue cultures [2–6] of the Solanaceae has been well documented. The bicyclic sesquiterpenoids of tobacco identified include capsidiol, lubimin, phytuberin, phytuberol, rishitin and solavetivone, and all increase within a 24 hr period in tobacco leaves challenged with *Pseudomonas lachrymons* [7]. Helgeson [8] has reviewed the work on the accumulation of four stress-associated sesquiterpenoids, including rishitin and capsidiol, in *N. tabacum* callus cultures infected with *Phytophthora parasitica*. Recently, Watson *et al.* [9] documented the production of capsidiol and three other stress metabolites in tobacco suspended callus cultures treated with cellulase. We now report the induced accumulation of capsidiol in tobacco cell suspension cultures upon addition of fungal elicitor prepared from *Phytophthora megasperma* f. sp. *glycinea* [10].

RESULTS AND DISCUSSION

Capsidiol accumulation in the culture media was significant six hr after addition of elicitor to the cell cultures, reached a maximum by 18–24 hr, and declined thereafter (Fig. 1). Less than 10% of the total capsidiol was found in cellular extracts, and intracellular capsidiol accumulated with a similar time course as that observed in the media. Very little if any capsidiol (1/100–1/1000 of maximum elicitor induced levels) was normally detected in control cultures. None of the other previously identified sesquiterpenoids were consistently observed, but, if measurable, were present in very low quantities (less than 0.1 μg/g fr. wt). Large amounts of capsidiol (greater than 10 μg/g fr. wt) were uniformly produced in elicitor treated cultures.

Production of capsidiol by suspension cultures was dependent on the amount of elicitor added (Fig. 2).

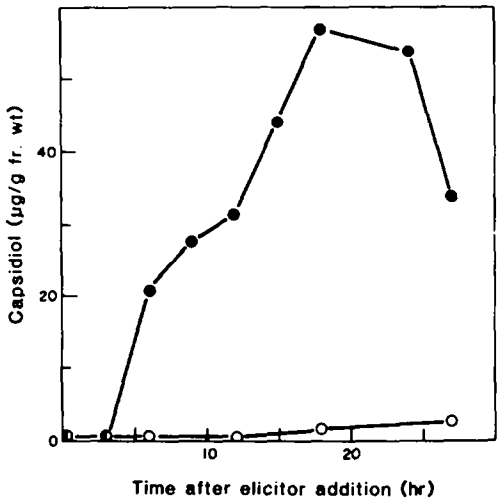

Fig. 1. The time course of capsidiol accumulation in the media of tobacco suspension cultures in the absence (O) or presence of fungal elicitor (●). Fifty μg glucose equivalents of fungal elicitor per ml of culture media were added to the latter cell cultures.

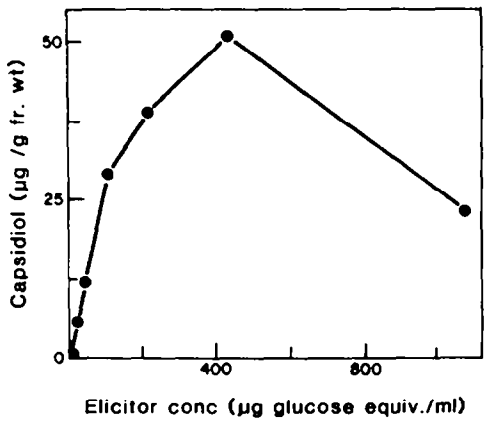

Fig. 2. Dose response of tobacco suspension cultures to fungal elicitor. Capsidiol accumulation in the media was determined 11 hr after addition of the indicated amount of elicitor.

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Maximum capsidiol accumulation occurred with 200–400 μg glucose equivalents of elicitor per ml of culture. Optimal elicitor-induced capsidiol accumulation was also dependent on the number of cells present as well as their relative growth rate. Cells in the rapid growth phase (fr. wt doubling every 2–4 days) at a density of 0.5–1 g fr. wt per 10 ml media gave the greatest capsidiol production in response to elicitor.

Elicitor-induced accumulation of secondary metabolites has previously been observed in plant cell suspension cultures of parsley [11] and soybean [12]. Although the metabolites in those systems are phenylpropanoid derivatives, the induction time courses of their accumulation are similar to that observed for capsidiol, a sesquiterpenoid, in tobacco suspension cultures. It should be noted however, that the amount of elicitor required for maximum secondary metabolite accumulation in tobacco was much greater than that of parsley or soybean.

In the presence of elicitor, tobacco cells readily took up ^{14}C-acetate from the media and incorporated a significant proportion (~1%) into the extracellular capsidiol, indicating *de novo* synthesis of the capsidiol. Our present work is concentrating on elucidating mechanisms responsible for elicitor induced changes in the sesquiterpenoid biosynthetic pathway, and especially the enzyme 3-hydroxy-3-methylglutaryl CoA reductase.

EXPERIMENTAL

Cell suspension cultures of *Nicotiana tabacum* Ky 14, obtained from Dr Glenn Collins, University of Kentucky, were grown in modified Murishige-Skoog media [13]. The cultures were originally derived from callusing cotyledons and have been in continuous suspension culture for more than two yr. The cultures consisted mostly of cell aggregates (2–20 cells per aggregate) and appeared creamy to light brown in colour. Three days after subculturing cells (20% v/v inoculum), experimental manipulation with fungal elicitor (50–400 μg glucose equivalents per ml of cell suspension) was initiated. Fungal elicitor was prepared from *Phytophthora megasperma* f. sp. *glycinea*, a non-pathogen of tobacco, according to ref. [10]. Cells and media were collected by filtration at specified times and frozen at −80° until utilized. Both cells and media turned distinctly brown 2–3 hr after elicitor addition to the cultures. Sesquiterpenoids were extracted from the media using $CHCl_3$ and from cells using MeOH [14]. Continuous subculturing of the suspension cultures for more than one yr has not diminished their ability to synthesize sesquiterpenoids in response to elicitor treatment.

Chromatographic and spectral analyses. Organic extracts were evaluated qualitatively by TLC using silica G plates developed with cyclohexane–EtOAc (1:1) and stained with vanillin–H_2SO_4 (2.8 g vanillin in 100 ml MeOH containing 0.5 ml conc H_2SO_4) [7]. Sesquiterpenoids were quantitated by GC (30 M × 0.25 mm SE-54, WCOT capillary column programmed 60°–180° at 12°/min, then to 220° by 2°/min; injector and detector temperatures 240° and 300°, respectively; He carrier gas at 3 ml/min) according ref. [14] except hexadecane was used as the int. standard.

Capsidiol was identified by comparison to authentic standards kindly provided by Dr J. Kuć. Capsidiol identification was verified by capillary GC-MS using conditions similar to those employed for quantitation. A Finnigan Ion Trap Detector interfaced to a Varian 3700 GC was used. Mass spectra for authentic capsidiol and the experimental component were identical ie *m/z* (rel. int.): 236[M]$^+$ (0.5), 201 (10), 119 (10), 107 (23), 105 (19), 69 (19), 67 (17), 57 (54), 55 (100), 53 (16).

All experiments were repeated a minimum of three times with similar results.

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