Elicitation of Canthin-6-one Alkaloid Accumulation in Cell Suspension Cultures of *Ailanthus altissima* (Mill.) Swingle

Gudrun Krauss*, Gerd-Joachim Krauss**, Renate Baumbach**, and Detlef Gröger*

* Institute of Plant Biochemistry, Academy of Sciences of the GDR, Weinberg 3, DDR-4050 Halle (Saale)

** Department of Biotechnology, Analytical Division, Martin-Luther-University Halle-Wittenberg, Weinbergweg, DDR-4050 Halle (Saale)

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Cell suspension cultures of *Ailanthus altissima* (Simaroubaceae) were cultivated under illumination and in the dark. Under influence of a yeast elicitor and cell wall preparations of various *Phytophthora* species, respectively, alkaloid formation was stimulated. Canthin-6-one and 1-methoxycanthin-6-one accumulation could be increased up to a 125-fold and 2.5-fold, respectively within 96 h by elicitors in dark grown cultures. In response to elicitors canthin-6-one is accumulated in the cells and the growth medium.

Introduction

Elicitor-induced formation and accumulation of secondary metabolites in higher plants is a well-known phenomenon. Especially the accumulation of phytoalexins in plants in response to the attack by pathogenic fungi is assumed to be an essential part in plant disease resistance [1–3]. The accumulation of phytoalexins may be also induced by biotic and abiotic elicitors in plant suspension cultures [4–6]. Recently it was shown that also alkaloid biosynthesis is triggered or stimulated by various elicitors ([7–11], for review see [12]).

Previously the occurrence of canthin-6-one derivatives in cell cultures of *Ailanthus altissima* was demonstrated [13–15] and their biosynthetic pathway has been studied using labeled precursors [16–19].

In this paper we report on the stimulation of canthine alkaloids synthesis under influence of various elicitors in cell suspension cultures of *Ailanthus altissima*.

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Experimental

Callus cultures were initiated in December 1987 from leaves, stems and roots of *Ailanthus altissima* seedlings. Suspension cultures were obtained from leaf-derived callus cultures. They were cultivated in 250 ml Erlenmeyer flasks containing 40 ml of a modified MS-medium [20] supplemented with 1 mg 2,4-dichlorophenoxyacetic acid/l and 1 mg kinetin/l [21] on gyratory shakers (80 rpm) at 27 °C either under illumination (2000 lux provided by white fluorescent tubes) with a daily photoperiod of 12 h or in the dark. Transfer of cells (10 ml) to fresh medium (30 ml) were performed every 10–12 days.

A yeast elicitor has been prepared according to [9]. Several *Phytophthora* species *viz. Ph. megasperma, Ph. palmivora* and *Ph. nicotianae* were grown in submerged culture according to [22]. The defatted cell walls of 4 days old *Phytophthora* mycelium were suspended in H_2O and autoclaved (120 °C, 3 h). After filtration the aqueous phase was evaporated, dialyzed and finally lyophilized [23] and used for elicitation.

For elicitor experiments the sterile preparations (Table) were added to 8 day old cultures and harvested after 4 days of incubation. Cells were harvested by suction filtration and lyophilized. Alkaloids from cells and culture medium both were separately extracted with chloroform. Alkaloid extracts were screened by TLC (silicia gel PF_{254} plates, Merck); solvent system: ethyl acetate. As main alkaloids of this particular cell line were canthin-6-one and 1-methoxycanthin-6-one identified by comparison with physical data of authentic samples [13, 24, 25].

Alkaloids were quantitatively analyzed by HPLC. A Lichrograph System (E. Merck, Darmstadt, F.R.G.) including a pump (model 655 A-11), a variable-wavelength UV detector (model 655 A-23) and an integrator (model D 2000) was used. The instrument was fitted with a valve-loop injector (Rheodyne 7125, 20 µl). Columns were a standard prepacked LiChrospher 100 NH_2 (5 µm) guard column and a LiChrospher 100 RP 8 (10 µm) column (250 mm × 4 mm) both from Merck. Samples were separated isocratically at ambient temperature, using methanol: water (70:30) as the mobile phase, at a flow rate of 1.5 ml/min. Alkaloids were detected at 260 nm and 320 nm with R_t values of canthin-6-one 5 min and 1-methoxycanthin-6-one 9.3 min. Quan-

tification was performed using the external standard method by measurement of peak areas. For peak identification, the absorbance ratios of two wavelengths (260 nm/320 nm) were measured and compared with reference substances [13, 25].

Results

Cell suspension cultures of *A. altissima* were grown either under illumination or in the dark. Light had no positive effect on the biosynthetic capacity of the cells. As main alkaloid 1-methoxycanthin-6-one besides the minor component canthin-6-one is accumulated in non-elicited cultures. A similar alkaloid profile has been described for *A. altissima* suspension cultures by Anderson *et al.* [13, 15]. However in *A. altissima* suspension cultures of Italian workers, who used a different cell line, canthin-6-one predominates clearly [14, 17].

In general alkaloid levels reach a maximum after 25 days of growth of *A. altissima* cell suspensions [14, 15].

We have added to cell suspension cultures of *Ailanthus* in the early growth phase some fungal elicitors and harvested the cells after 96 h of incubation. Both the yeast elicitor [9] and *Phytophthora* elicitors [23] gave similar results in stimulating the accumulation of canthin-6-one and 1-methoxycanthin-6-one under illumination and in the dark. Apparently 10 mg of yeast elicitor/40 ml culture represent the optimum for our cell line. In response to elicitor treatment canthine-6-one alkaloids were also found in the growth medium.

Under illumination the levels of canthin-6-one and 1-methoxycanthin-6-one were raised 10 times and 2 times respectively in cultures containing *Ph. megasperma* and yeast elicitor over the maximum found in controls. Canthin-6-one and 1-methoxycanthin-6-one accumulation could be increased up to a 125-fold and 2.5-fold respectively within 96 h by elicitors in dark grown cultures (Table). The elicitation of canthin-6-one accumulation is much more pronounced in dark grown cultures compared to illuminated ones. The reason is still unknown why

Table. Effect of fungal elicitors on production of canthine-6-one alkaloids by *Ailanthus altissima* cell suspension cultures.

Elicitor	Dry weight mg/ml medium	Canthin-6-one µg/g DW	Canthin-6-one µg/100 ml medium	1-Methoxycanthin-6-one µg/g DW	1-Methoxycanthin-6-one µg/100 ml medium
A					
Control	9.3	18	trace	509	trace
Ph. megasperma 10 mg	9.2	193	trace	1242	trace
Yeast 15 mg	9.5	222	trace	1185	trace
B					
Control	10.2	20	–	950	45
Ph. megasperma 5 mg	9.2	527	186	1697	220
10 mg	7.4	3326	920	2462	214
Ph. palmivora 10 mg	8.2	948	260	2131	162
Ph. nicotianae 10 mg	7.7	2616	506	2480	128
Yeast 5 mg	9.5	1016	274	2130	166
10 mg	7.5	2590	664	2360	190
15 mg	8.4	2401	618	2166	182

A, cultivated under illumination; B, cultivated in the dark; DW, dry weight; Ph., *Phytophthora*; the mg values of the first column refer to 40 ml culture medium.

light seems to inhibit stimulation of alkaloid formation or activate the degradation of canthin-6-ones in *A. altissima* suspension cultures under influence of elicitors. In general the response of cultured plant cells to elicitation is affected by various factors inter alia environmental conditions of a given cell line.

It is well documented [13, 25], that canthine alkaloids, especially canthin-6-one possess antibacterial, antifungal and cytotoxic activity. The latter has been tested by using guinea pig ear keratinocytes.

The elicitor-induced production of monoterpene indole alkaloids has been previously shown [26, 27]. This study demonstrates clearly, that also the accumulation of β-carbolines can be induced by elicitation.

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