Effects of aluminium chloride on cell growth and production of coumarins in cell suspension cultures of *Angelica archangelica* L.

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SUMMARY

Effects of aluminium chloride on cell growth and production of coumarins in cell suspension cultures of *Angelica archangelica* L.
Elicitation of plant cells in culture represents a useful biotechnological tool to improve the production of secondary metabolites. In this study, aluminium chloride at various concentrations (1, 10, 50, 100, 500, and 1000 µM) was tested as a potential elicitor of the production of coumarins in angelica cell suspension cultures. In addition, the toxicity of aluminium ions for the culture was assessed by evaluating their effect on cell growth (characterized by fresh and dry biomass at the end of a two-week subculture). Cultures were cultured in the dark or in the light. Fresh biomass was not affected significantly in the presence of aluminium chloride at concentrations from 1 to 1000 µM. Dry biomass was reduced by about 10% at an aluminium concentration of 1000 µM. Production of coumarins was influenced by aluminium chloride depending on light conditions. In the dark-grown cultures, aluminium ions from a concentration of 10 and 50 µM enhanced accumulation of coumarins in the medium and cells, respectively. The contents of coumarins rose with an increasing aluminium level. The best results were achieved with 1000 µM aluminium chloride. The amounts of coumarins were increased by 33% in the medium and 24% in the cells as compared with control cultures. On the other hand, production of coumarins was not improved by aluminium chloride in the light-grown cultures. Moreover, higher aluminium concentrations lowered formation of coumarins in these cultures.

Key words: *Angelica archangelica* L. – cell suspension cultures – growth – coumarins – aluminium – elicitation – light conditions – sequential injection analysis *Má*

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SOUHRN

Vliv chloridu hlinitého na buněčný růst a produkci kumarinů v suspenzní kultuře *Angelica archangelica* L.
Elicitace rostlinných buněk v kultuře představuje užitečný biotechnologický nástroj pro zvýšení produkce sekundárních metabolitů. V této práci byl sledován vliv různých koncentrací chloridu hlinitého (1, 10, 50, 100, 500, 1000 µM) jako potenciálního elicitoru produkce kumarinů v suspenzní kultuře anděliky lékařské. Byla posuzována také toxicita hlinitých iontů pro kulturu hodnocením jejich účinku na buněčný růst (charakterizován čerstvou a suchou hmotností biomasy na konci čtrnáctidenní kultivace). Kultury byly kultivovány ve tmě a na světle. Čerstvá hmotnost

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nebyla chloridem hlinitým v koncentraci 1 až 1000 µM signifikantně ovlivněna. Suchá hmotnost byla snížena asi o 10 % při koncentraci chloridu hlinitého 1000 µM. Produkce kumarinů byla chloridem hlinitým ovlivněna v závislosti na světelných podmínkách. V kulturách kultivovaných ve tmě zvyšovaly hlinité ionty množství kumarinů v médiu od koncentrace 10 µM, v buňkách od koncentrace 50 µM. Obsah kumarinů rostl se zvyšující se hladinou hlinitých iontů. Nejlepší výsledky byly dosaženy s koncentrací 1000 µM chloridu hlinitého. Obsah kumarinů byl ve srovnání s kontrolní kulturou zvýšen o 33 % v médiu a o 24 % v buňkách. Naproti tomu v kulturách kultivovaných na světle chlorid hlinitý produkci kumarinů nezvýšil. Jeho vyšší koncentrace tvorbu kumarinů v těchto kulturách ještě snížily.

Klíčová slova: *Angelica archangelica* L. – suspenzní kultura – růst – kumariny – hliník – elicitace – světelné podmínky – sekvenční injekční analýza

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Introduction

Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavours, and other industrial materials. Plant cell culture technology has been developed as a promising alternative for producing metabolites that are difficult to be obtained by chemical synthesis or plant extraction. One of the major obstacles hindering its wider application is the low yield of plant secondary metabolites in plant cell cultures [1)]. Many approaches have been developed to overcome this problem [2, 3)]. Perhaps the most notable strategy for improving metabolite yields is elicitation [4)]. Elicitors are factors that can induce an up-regulation of genes. Some elicitors target secondary metabolic genes, which are often associated with defense responses to perceived environmental changes [1, 4)]. Elicitors may be biotic or abiotic. The biotic elicitors have biological origin (e.g. fungal homogenates, culture filtrates, and plant cell wall components). On the other hand, abiotic elicitors are not of a biological origin and are grouped in physical factors (such as thermal and osmotic stress, radiation, and wounding) and chemical compounds (e.g. heavy metal salts) [5)]. It is well known that treatment with elicitors causes an array of defence reactions, including the accumulation of secondary metabolites in intact plants [6–8)] as well as in cell cultures [9–12)].

We report here the effects of aluminium chloride as a potential elicitor on cell growth and production of coumarins in *Angelica archangelica* cell suspension cultures.

MATERIAL AND METHODS

Chemicals

2,4-dichlorophenoxyacetic acid and 6-benzylaminopurine (Sigma, Praha, Czech Republic); scopoletin (Fluka, Praha, Czech Republic); aluminium chloride, sodium phosphate dibasic, and potassium phosphate monobasic (Lachema, Brno, Czech Republic).

Instruments

A PS 20A autoclave (Chirana, Brno, Czech Republic); a roller (Vývojové dílny, Academy of Sciences of the Czech Republic, Praha, Czech Republic); a 200S analytical scale (Sartorius, Göttingen, Germany); a laboratory centrifuge MPW 342 (MPW Med. instruments, Warsaw, Poland); a laboratory shaker KS 501 (IKA Labortechnik, Staufen, Germany); a peristaltic pump (Alitea Instruments, Seattle, U.S.A); an eight position selection valve (Vici Valco Instruments, Brockville, Canada); and a FS 970 fluorescence detector (Schoeffel Instrument Corp., Westwood, U.S.A.).

Cell suspension cultures and culture conditions

Cell suspension cultures of *Angelica archangelica* were established as described previously [13)] and grown in liquid Murashige and Skoog medium [14)] supplemented with 2 mg l^{-1} 2,4-dichlorophenoxyacetic acid, 0.4 mg l^{-1} benzylaminopurine, and 30 g l^{-1} sucrose. The pH of all media was adjusted to 5.7 before autoclaving. The cultures were agitated in 250 ml flasks containing 30 ml of the medium on a roller apparatus at 8 rpm, incubated at 25 °C under a 16/8 light/dark photoperiod or in the dark, and subcultured every 14 days.

For testing the effects of aluminium chloride, the cultures were cultured in Murashige and Skoog media supplemented with an appropriate concentration of aluminium chloride (1, 10, 50, 100, 500, and 1000 µM). Control cultures were cultured in a medium without the addition of aluminium chloride. After 14 days, the cultures were harvested, and the cell growth and production of coumarins were evaluated.

All experiments were carried out in triplicate and were repeated at least twice. Student's t-test was used for statistical analysis of data, and differences with $P < 0.05$ were considered as statistically significant.

Analytical procedures

Cells were separated from the culture medium by vacuum filtration using a Buchner funnel with filter paper. For evaluation of the culture growth, filtered cells were washed with distilled water, weighed for fresh

Table 1. Effects of aluminium chloride on cell growth in Angelica archangelica cell suspension cultures
Values are means ± standard deviations (n = 3). Asterisks denote significant differences between Al-treated and control cultures, P < 0.05.

$AlCl_3$ concentration ($\mu mol\ l^{-1}$)	**Culture growth**			
	Cultures in the dark		**Cultures in the light**	
	Fresh weight (g)	**Dry weight (mg)**	**Fresh weight (g)**	**Dry weight (mg)**
0 (control)	5.76 ± 0.22	378 ± 5	4.34 ± 0.09	380 ± 10
1	6.15 ± 0.32	375 ± 14	4.35 ± 0.08	377 ± 8
10	5.61 ± 0.21	384 ± 2	4.34 ± 0.12	371 ± 4
50	5.68 ± 0.12	361 ± 12	4.42 ± 0.22	381 ± 2
100	5.91 ± 0.10	362 ± 10	4.22 ± 0.13	371 ± 3
500	5.48 ± 0.06	357 ± 17	4.29 ± 0.18	365 ± 5
1000	5.37 ± 0.12	336 ± 21*	3.98 ± 0.22	346 ± 8*

weight determination, and then dried to obtain dry weight.

Coumarins in cells and in the culture medium were quantified fluorometrically by sequential injection analysis (SIA) [15]. The powdered dry cells were extracted three times (always for 15 min) with a mixture of equal volumes of methanol and 0.066 M phosphate buffer (pH 6) by shaking at 150 rpm on an orbital shaker at laboratory temperature. The extracts were pooled, adjusted to 25 ml with the extraction mixture, centrifuged at 3,000 rpm for 10 min, and analysed. The culture media were analysed directly. The SIA conditions were as follows – a carrier stream: water; flow rate: 3 ml/min; sample volume: 40 µl; volume of 0.066 M phosphate buffer (pH 6): 100 µl; a 1.5 ml mixing coil; excitation wavelength: 345 nm; and emission wavelength: cut-off emission filter transparent at ≥ 390 nm. The contents of coumarins were expressed as scopoletin (mg l^{-1} in the medium and mg g^{-1} dry weight in the cells).

RESULTS AND DISCUSSION

Aluminium is not regarded as an essential nutrient in plants. Aluminium is phytotoxic, particularly at pH values below 5.0, but its low concentrations can sometimes increase plant growth or induce other desirable effects. Plant species and varieties vary widely in tolerance to aluminium [16]. Plants may adapt to higher levels of aluminium ions by various mechanisms. For example, aluminium can induce or stimulate biosynthesis of organic acids (such as citrate, malate and oxalate) [17, 18], callose [19] and pectin [20] formation, and synthesis of flavonoid type phenolics [18, 21, 22] and anthraquinones [23].

Aluminium ions in a wide range of concentrations were tested as a potential elicitor of production of coumarins in angelica cell suspension cultures. In addition, the toxicity of aluminium for the culture was assessed by evaluating its effect on cell growth, which was characterized by fresh and dry biomass at the end of a two-week subculture. Cultures were cultured in the dark or in the light because light conditions may influence both the growth and secondary metabolite formation in plant tissue cultures [24, 25].

As for culture growth (Table 1), fresh biomass was not influenced significantly in the presence of aluminium chloride at concentrations from 1 to 1000 µM. Dry biomass was not affected up to concentrations of 500 µM. Aluminium ions at 1000 µM reduced dry cell weight by about 12%, and by 9% in the dark-grown and light-grown cultures, respectively, in comparison with control cultures. An aluminium concentration of 5000 µM was lethal for angelica cell culture (data not shown).

Production of coumarins was influenced by aluminium chloride in dependence on light conditions (Fig. 1 and 2). Aluminium ions from a concentration of 10 and 50 µM enhanced accumulation of coumarins in the medium and cells, respectively, in the dark-grown cultures. The contents of coumarins rose with an increasing aluminium level. The best results were achieved with 1000 µM aluminium chloride. The amounts of coumarins were increased by 33% in the medium and 24% in the cells as compared with control cultures. On the other hand, production of coumarins was not improved by aluminium chloride in the light-

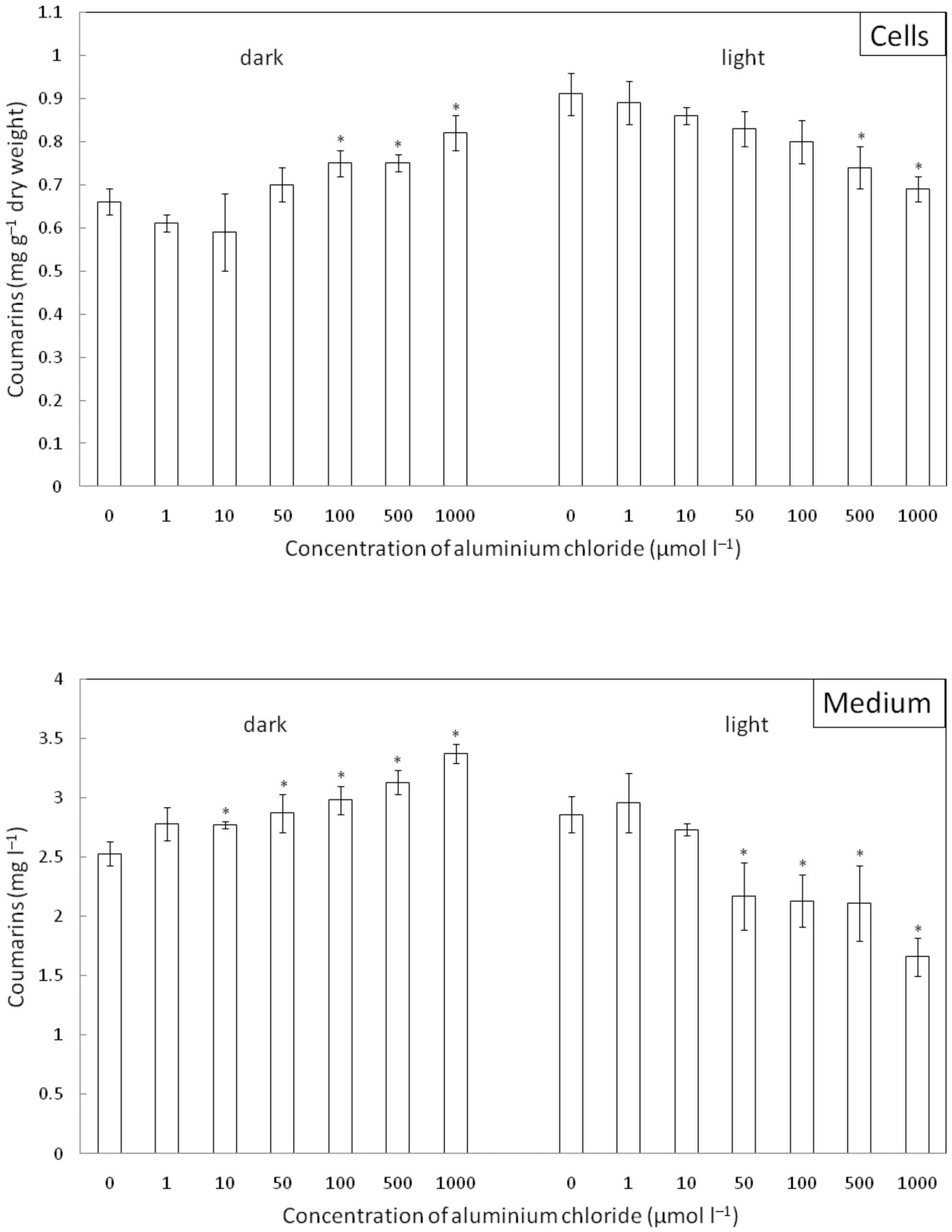

Fig. 1 and 2. Effect of aluminium chloride on production of coumarins in Angelica archangelica cell suspension cultures cultured in the dark or light
Values are means of three replicates. Vertical bars represent standard deviations. Asterisks denote significant differences between Al-treated and control cultures, P < 0.05.

-grown cultures. Moreover, formation of coumarins declined in angelica cell cultures cultured in the light at higher aluminium concentrations.

Stimulating effects of aluminium ions have been reported on the production of flavanols in *Vitis vinifera* callus cultures [26)] and diterpenoids in *Jatropha elliptica* root cultures [27)]. Similar findings regarding different response of a plant tissue culture to metal ions in dependence on light conditions have been observed, for instance, in cell suspension cultures of *Angelica archangelica* [28)] and *Digitalis lanata* [29)].

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