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Identification by NMR and accumulation of a neolignan, the dehydrodiconiferyl alcohol-4-β-D-glucoside, in *Linum usitatissimum* cell cultures

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Abstract

Phenylpropanoids represent a broad range of secondary metabolites in plants, in which they are involved in defense mechanisms. This study deals with the NMR identification of a phenylpropanoid, which belongs to the class of neolignans, dehydrodiconiferyl alcohol-4-β-d-glucoside (DCG), in in vitro cultures of Linum usitatissimum. The combination of 1- and 2-D NMR experiments such as COSY, HMBC and HMQC allowed the identification of this compound's structure unambiguously. In order to evaluate its implication in defense mechanism, the L. usitatissimum suspension cells were placed together with fungal extracts. Consequently, the DCG concentration decreased dramatically after 96 h of treatment. In correlation, the phenylcoumaran benzylic ether reductase (PCBER) expression increased rapidly and constantly immediately after elicitation until 96 h post elicitation, as shown by semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR). These two results are in agreement, since the aglycone form of DCG is one of the two substrates of PCBER, thus suggesting PCBER activation in plant defense mechanisms. ***To cite this article: J. Attoumbre et al., C. R. Chimie 9 (2006)***.

Résumé

Les phénylpropanoïdes représentent une large classe de métabolites secondaires chez les végétaux chez lesquels ils sont souvent impliqués dans des mécanismes de défense. Cette étude présente l'identification par RMN d'un phénylpropanoïde

Abbreviations: BAP, benzylaminopurine; DCG, dehydrodiconiferyl alcohol-4-β-D-glucoside; DW, dry weight; FW, fresh weight; HPLC, high pressure liquid chromatography; IFR, isoflavone reductase; NAA, naphthaleneacetic acid; NMR, nuclear magnetic resonance; PCBER, phenylcoumaran benzylic ether reductase; PLR, Pinoresinol lariciresinol reductase; RT-PCR, reverse transcriptase polymerase chain reaction.

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appartenant à la classe des néolignanes, l'alcool déhydrodiconiférylique-4-β-D-glucoside (DCG) dans des cultures in vitro de *Linum usitatissimum*. La combinaison d'expériences mono- et bidimensionnelles, telles que COSY, HMBC et HSQC a permis d'identifier la structure de ce composé sans ambiguité. Afin d'évaluer son implication dans les mécanismes de défense, les suspensions cellulaires de *L. usitatissimum* ont été mises en présence d'extraits fongiques. La concentration en DCG a alors chuté de manière importante, après 96 heures de traitement. Corrélativement, il a été remarqué par RT-PCR semi-quantitative que l'expression de la phénylcoumarane benzylique éther réductase (PCBER) avait augmenté rapidement dès les premières heures après l'élicitation et de manière stable jusqu'à 96 h post élicitation. Ces deux résultats sont en accord, puisque la forme aglycone du DCG est l'un des deux substrats de la PCBER. Ils suggèrent l'activation de la PCBER dans des situations de défense chez les végétaux. ***Pour citer cet article : J. Attoumbre et al., C. R. Chimie 9 (2006)***.

Keywords: Cell suspension cultures; Dehydrodiconiferyl alcohol-4-β-D-glucoside (DCG); Elicitation; HPLC; *Linum usitatissimum*; PCBER; RMN

Mots-clés : Alcool déhydrodiconiférylique-4-β-D-glucoside (DCG) ; Cultures in vitro ; Élicitation ; CLHP ; *Linum usitatissimum* ; PCBER ; RMN

1. Introduction

In plants, the oxidative dimerization of two phenylpropanoid units leads to a great variety of secondary metabolites. When the oxidative process implies the 8-8′ bonds, products are named lignans while the term neolignan is used to define all of the other type of linkage. Lignans and neolignans, found in a wide range of plant species, are responsible for their pharmacological activities and implied in their defense mechanisms [1,2]. Nevertheless, despite the importance of lignans in human health protection and in plant biology, systematic studies on the enzymes involved in their biosynthesis have only recently begun [3]. In this context it has been shown that phenylcoumaran benzylic ether reductases (PCBERs) that catalyze reductive process in 8-5′ linked lignans are ubiquitous vascular plant enzymes, which could have a role in plant defense. Flaxseed is known to be a rich source of lignans and *Linum usitatissimum* in vitro cultures have been established as a very useful tool to study the biosynthesis and the accumulation of metabolites [4–6].

In these cultures, a 8-5′ phenylpropanoid dimer (dehydrodiconiferyl alcohol-4-β-D-glucoside (DCG)) has been isolated and identified by nuclear magnetic resonance (NMR) spectroscopy. To evaluate the role of this compound in the cell cultures defense response, elicitation experiments by two fungal pathogens have been carried out. In these cultures DCG accumulation was quantified as a function of time and the gene expression of *Lu PCBER* was monitored by semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR).

2. Material and methods

2.1. Plant material

Cell suspension cultures of *L. usitatissimum* cv Barbara were established from hypocotyl-derived calli in MS-derived medium [7] supplemented with 3% sucrose, 8.88 μM benzylaminopurine (BAP) and 2.68 μM naphthaleneacetic acid (NAA). All suspension cultures were incubated on a gyratory shaker at 120 rpm in darkness at 25 °C and subcultured every 14 days.

2.2. DCG isolation and identification by NMR and mass spectrometry

A freeze-dried and ground sample of cells (5 g) cultured on MS-derived medium [7] was extracted with methanol/water (70/30, v/v) (200 ml) for 3 h at 60 °C. After filtration, the extract was concentrated to 15 ml. DCG was separated and purified by semi-preparative high pressure liquid chromatography (HPLC) using a μBondapack C18 prepacked column [10 μm, 25 × 100 mm], eluted with acetonitrile/H_2O 0.2% acetic acid (15/85, v/v), flow rate 8 ml min^{-1}, UV detection 280 nm, retention time 25 min. DCG was identified by ^{1}H, ^{13}C NMR and ESI-MS.

The 1 D and 2 D NMR spectra were recorded at 300 K on a Bruker Avance 300 spectrometer operating