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Cycloheximide-Induced Phenolic Burst in Roots of *Pisum sativum* L.

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Abstract—Chromatography and histochemical analysis of soluble phenolic compounds demonstrated their higher content in the roots of cycloheximide-treated pea plants. These substances accumulated together with lignin in the endodermis and xylem cells of conducting bundles. This finding confirms the antipathogenic cycloheximide effect based on the previous results of proteome analysis.

Keywords: phytoimmunity, cycloheximide, phytoalexins, lignin
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INTRODUCTION

Cycloheximide (CH) is an antibiotic that is known to suppress protein synthesis via the inhibition of 80S ribosomes. This compound is frequently used to estimate the degree to which protein synthesis and proteolysis contribute to changes in the protein content. Proteome analysis demonstrated earlier that CH completely suppressed salicylate-inducible proteins in pea roots, which was not observed in the control [1]. Also, the content of approximately 30 proteins, which largely participated in protein metabolism, was decreased in the presence of CH [2].

The higher level of chalcone reductase, chalcone isomerase, chalcone–flavone isomerase, sophorol reductase, and kofeil-CoA-methyltransferase in the presence of CH was unexpected. These enzymes participate in the synthesis of antipathogenic products of phytoalexins and lignin, which are constituents of phenylpropanoid metabolism. Moreover, the content of isopentenyl-pyrophosphate isomerase, which is a catalyst of the synthesis of antipathogenic terpenes, was elevated [2]. The higher level of these defense proteins suggested that CH has an antipathogenic effect that, on one hand, is manifested by the inhibition of protein synthesis in response to CH and, on the other hand, may be a signal of pathogen attack [1].

The goal of this study is to demonstrate whether CH-induced increasing of enzyme level, one which catalyzes the synthesis of phytoalexins and lignin, is realized in the intensification of their formation.

METHODS

Seedlings of *Pisum sativum* L. were grown in 1/4 Hoagland-Arnon medium at 25°C, 250 μM/(m^2 s) light intensity, and a 16-h light period. Three-day-old seedlings were placed in a CH solution (10 μM) for 48 h to study the CH effect on pea roots. This CH concentration was chosen because the total protein, in this case, remained constant, while the protein ratio changed [1, 2]. The roots of the treated plants were frozen in liquid nitrogen and lyophilized.

To extract phenolic compounds (PCs), 10 mg of ground roots was mixed with 0.2 mL 80% methanol and incubated at 80°C for 30 min with subsequent centrifugation at 12000 *g*. The supernatant was used to study the soluble PC content and composition by high performance liquid chromatography (HPLC). The total PC content was determined with Folin-Ciacolteu's reagent. The optical density of solutions was determined at 765 nm with an Infinite 200 PRO plate reader (Tecan, Switzerland).

Liquid chromatography was used to resolve the cycloheximide-dependent PCs [3] on a Zorbax SB C 18 reversed-phase column (4.6 × 150 mm, 5-Micron) (Agilent Technologies, United States) with a Dionex Ultimate 3000 HPLC system (Thermo Scientific, United States). The following eluents were used: mQ H_2O with 5% acetonitrile and 0.5% acetic acid (eluent A) and acetonitrile with 5% acetic acid (eluent B). The gradient elution was as follows: 100% A for 0–1 min, to 98% A for 1–2 min, to 60% A for 2–10 min, to 50% A for 10–25 min, 50% A for 25–30 min, to 98% A for 30–32 min, to 100% A for 32–33 min, and 100% A for 33–34 min. The flow constituted 0.5 mL/min. A UV detector was used to monitor the process at 285 and 360 nm.

A Calcofluor White fluorochrome (Megazyme, Ireland) was used for lignin and PC detection (yellow-green and red-brown fluorescence, respectively). Root