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Original article

Induction of oxidative stress and antioxidative mechanisms in *Phaseolus vulgaris* after Cd application

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Abstract

Oxidative stress has been shown to be of great importance in the toxicity of several metals (copper, zinc, ...). In this study, the relationship of cadmium phytotoxicity and antioxidative reactions in bean (*Phaseolus vulgaris* L.) plants was investigated. Eleven-day-old seedlings were exposed to an environmentally realistic concentration of cadmium (2 μM $CdSO_4$). Several biochemical and physiological parameters were influenced even by these low concentrations. At the biochemical level, the antioxidative defence mechanism was significantly activated after 24 h of cadmium exposure. Some enzymes able of quenching reactive oxygen species (syringaldazine peroxidase, EC 1.11.1.7; guaiacol peroxidase, EC 1.11.1.7) as well as enzymes important in the reduction of $NAD(P)^+$ (isocitrate dehydrogenase, EC 1.1.1.42; malic enzyme, EC 1.1.1.40) were significantly elevated by cadmium exposure. Furthermore, the ascorbate–glutathione cycle appeared to be a very important mechanism against cadmium-induced oxidative stress. In leaves, significant increases of ascorbate peroxidase (EC 1.11.1.11) and glutathione reductase (EC 1.6.4.2) and significant changes in the ascorbate and glutathione pool were observed. Morphological and other biochemical parameters (lipid peroxidation) were significantly enhanced 48 h after the start of the cadmium exposure. At the end of the experiment (72 h after the start of the metal treatment), even visual effects, such as chlorosis, were observed. The present data indicate that cadmium, like other metals, induces cellular redox disequilibrium suggesting that an environmentally realistic concentration of cadmium can cause oxidative stress.

Keywords: Antioxidative defence; Cadmium toxicity; Environmentally realistic metal-concentrations; Oxidative stress; *Phaseolus vulgaris*

1. Introduction

Due to anthropogenic activity, mainly in the 20th century, the Kempen region (Belgium), as many other regions worldwide, suffers from significant soil cadmium pollution [35]. In this area the cadmium pollution is of growing concern because the metal easily leaches from the sandy soil into the groundwater and is therefore a hazard to the environment and to human health.

In plants, exposure to cadmium causes a reduction of several physiological processes including photosynthesis, respiration and transpiration [6,32]. As a consequence, cadmium-exposed plants show various symptoms of injury such as stunted growth, leaf epinasty and chlorosis. At lower concentrations, these visible symptoms are less pronounced or even absent, but various cellular processes can already be affected.

To control the metal homeostasis and the redox status, plants dispose of several cellular defence mechanisms against elevated concentrations of heavy metals, such as complexation and transport processes. Important metal-binding pep-

Abbreviations: AsA, ascorbic acid; APOD, ascorbate peroxidase; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; EDTA, ethylenediaminetetraacetic acid; GPOD, guaiacol peroxidase; GSH, glutathione; GSSG, glutathione disulphide; GR, glutathione reductase; ICDH, isocitrate dehydrogenase; MDA, malondialdehyde; ME, malic enzyme; ROS, reactive oxygen species; SPOD, syringaldazine peroxidase; TBA, thiobarbituric acid.

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tides are metallothioneins and phytochelatins [19,27], which reduce the free metal concentration in the cytosol by binding the metals and transporting them to specific compartments. Phytochelatins have a very high antioxidant capacity [8] and their synthesis can be induced by cadmium [39].

Cadmium is known to affect cellular processes such as membrane damage (alteration in permeability due to changes in lipid composition and/or lipid peroxidation [3,17]), disruption of the electron transport, inhibition/activation of enzymes and interaction with nucleic acids [7,32,37]. Possible mechanisms by which these disorders are generated are induction of oxidative stress and replacement of elements such as Zn, Fe, Mn, ...which are essential cofactors of many enzymes.

Cadmium almost always adopts only one oxidation state, which is as a bivalent cation. Unlike redox-active metals (Cu, Fe, ...), cadmium is not able to induce production of reactive oxygen species (ROS) through a Fenton-like reaction. Cadmium causes oxidative stress probably through indirect mechanisms such as interaction with the antioxidative defence, disruption of the electron transport chain or induction of lipid peroxidation [4,5,25]. Activation of lipoxygenase, an enzyme that stimulates lipid peroxidation, has been reported after cadmium exposure [34].

Plants possess several protective mechanisms to cope with ROS in which enzymes as well as reducing metabolites, such as ascorbate, glutathione and α-tocopherol, are involved [14]. The enzymatic antioxidant system operates with the sequential and contemporaneous action of enzymes such as peroxidases, catalases and superoxide dismutases. Furthermore, the ascorbate–glutathione cycle appears to be an important defence mechanism against oxidative stress caused by metals [10,11]. The reduction of H_2O_2 by ascorbate can occur directly or it can be catalysed by ascorbate peroxidase. Then, the oxidised form of ascorbate can be reduced enzymatically by dehydroascorbate reductase using glutathione as a substrate, which in turn is reduced by glutathione reductase in the presence of NAD(P)H [15].

In summary, high concentrations of cadmium often irreversibly disturb several cellular (membrane damage, disruption of the electron transport, ...) and physiological (growth, photosynthesis, ...) processes, probably because of a severe induction of oxidative stress. The purpose of this project was to identify the importance and a possible role for oxidative stress in plants only shortly exposed to environmentally realistic, relatively low, cadmium concentrations. This is an important improvement compared to most of the previous studies where longer exposure times and/or higher cadmium concentrations were used. In our study, bean (*Phaseolus vulgaris*) seedlings were exposed to low concentrations of cadmium (2 μM) in the culture medium. Several biochemical and physiological parameters were measured to reveal the capacity of the organisms to cope with stress induced by this realistic cadmium concentration.

2. Results

2.1. Cadmium uptake and growth responses

In bean seedlings exposed to 2 μM $CdSO_4$ in a hydroponic system (Hoagland nutrient solution), increasing cadmium concentrations were detected in the primary leaves as a function of time after onset of exposure (Fig. 1). Increased values were found from the first experimental point at 24 h.

Growth parameters were reduced 48 h after the start of the treatment. Significant decreases in leaf area (results not shown) and leaf weight (Fig. 2) were observed as compared to the control-grown seedlings.

2.2. Biochemical effects

The level of lipid peroxidation was determined in terms of the thiobarbituric acid (TBA)-reactive compounds, such as malondialdehyde (MDA). The kinetic responses of the MDA content to 2 μM $CdSO_4$ are summarised in Fig. 3. A significant increase (approximately doubling) in lipid peroxidation

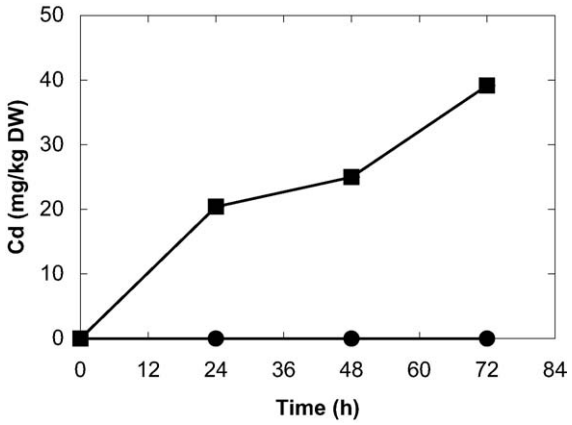

Fig. 1. Cadmium accumulation (μg g^{-1} DW) in the primary leaves of *P. vulgaris* treated with 2 μM $CdSO_4$ (■) compared to the control (•) after different periods of treatment.

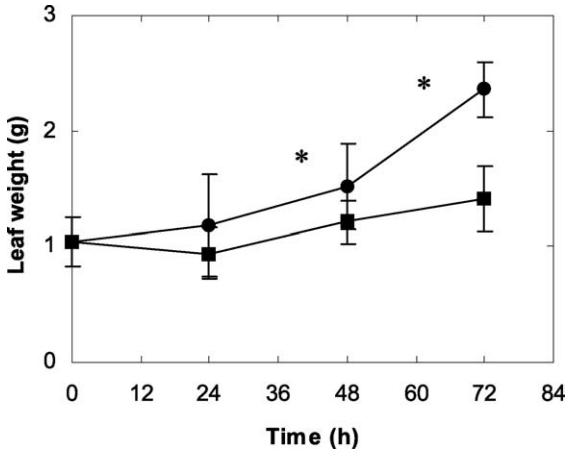

Fig. 2. Fresh weight of two primary leaves of *P. vulgaris* treated with 2 μM $CdSO_4$ (■) compared to the control (•) after different periods of treatment. Each point represents the mean ± S.D. of seven determinations. Significance level: * $P < 0.05$.