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Selenium affects physiological parameters and phytochelatins accumulation in cucumber (*Cucumis sativus* L.) plants grown under cadmium exposure

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ABSTRACT

The aim of the present study was to examine the effects of exogenous selenium (Se) on the tolerance of cucumber plants to cadmium (Cd) stress. The cucumber plants grown in a nutrient solution were supplied with Cd (0, 25 or 50 μM) and Se (0, 5 or 10 μM), individually or simultaneously. Under Cd-stress, there were no significant differences in the shoot biomass of the Se-supplied plants as compared to the Cd-exposed plants. However, the fresh weight (FW) of roots increased significantly after supplementation of a 50 μM Cd-polluted nutrient solution with 10 μM Se. The accumulation of phytochelatins (PCs) was detected in the plants treated with 25 or 50 μM Cd, individually or simultaneously with Se, with a higher level noted in the roots. When the individual PCs isoforms were considered, the predominance of PC4 in the Cd-exposed roots and of PC2 in the Cd-exposed leaves was evident. An enrichment of Cd-treated plants with Se caused a reduction of PCs accumulation in roots, but did not change its concentration in leaves. The addition of Se to the Cd-containing growth media did not affect GSH accumulation in roots and leaves, as compared to Cd-alone treatment. Moreover, the supplementation of Cd-exposed plants with Se provoked a significant decrease in the level of lipid peroxides as well as a reduction of Cd content in the root tissues. Our results imply that the degree of disturbances provoked by Cd could be partially ameliorated by Se supplementation, probably due to the inhibition of Cd accumulation and a reduced lipid peroxidation in the root tissues as well as the improvement of cell membranes stability in the leaf tissues. However, the effect of Se on the Cd-stressed plants greatly depended on the proportion of these two elements in the nutrient solution.

1. Introduction

Besides the ability to take up essential elements, plants are able to absorb and accumulate other metals, even those of toxic or with unidentified metabolic functions. The occurrence of heavy metals in excess poses a global problem, threatening the health of vegetation, wildlife and humans (Nikolić et al., 2008). Cadmium (Cd) is one of the most dangerous heavy metals due to its high toxicity, mobility and availability for all living organisms. Cadmium is released into the environment mainly by power stations, heating systems, metalworking industries, waste incinerators, urban traffic, cement factories, and as a by-product of phosphate fertilisers. In areas with low anthropogenic pressure, it can be released as a result of rock mineralisation processes (Das et al., 1997; Sanitá di Toppi and Gabbrielli, 1999). Although plants have no metabolic requirement for Cd, it is easy taken up by the roots, revealing a serious health risk. The content of Cd in crop plants varies in a wide range (from 5 up to 400 μg kg^{-1} DW) (Kabata-Pendias and Mukherjee, 2007). The toxic effect of Cd on plant growth and development is widespread and was broadly reviewed over the last 30 years (Das et al., 1997; Sanitá di Toppi and Gabbrielli, 1999; Deckert, 2005; Dong et al., 2007). The main and most visible symptoms of Cd toxicity are: growth reduction, chlorosis and necrosis of leaves, red-brown colouration of leaf margins or veins. Moreover, Cd can disturb the water balance, mineral nutrition, photosynthesis, respiration as well as general plant development (Wójcik and Tukiendorf, 2005).

To protect themselves from the toxicity of heavy metals, plants have developed several mechanisms to inactivate metal ions thus protecting metabolically and structurally important proteins. One of these mechanisms consists of the biosynthesis of phytochelatins

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(PCs), which are linear iso-peptides with varying chain lengths as $(\gamma\text{-Glu-Cys})_n$-Gly, where $n = 2–11$. Numerous studies have confirmed that a reduced form of glutathione (GSH) is the direct substrate for PCs biosynthesis (Cobbett, 2000; Inouhe, 2005). The chelation of Cd with PCs in the cytoplasm and compartmentalisation of the PC-Cd complexes in the vacuole are generally considered as a "first line" of defence mechanism against Cd phytotoxicity (Inouhe, 2005). The PC-Cd complexes are up to 1000 times less toxic to many enzymatic proteins than the free Cd ions (Solt et al., 2003).

The biological activity of metal ions can be markedly affected by the presence of other ions in the root zone. Although selenium (Se) has not been confirmed to be an essential micronutrient in higher plants, several studies have demonstrated that at relatively low concentrations Se can exert beneficial effects on the growth of plants under both normal and stress conditions. Selenium acts probably as an antioxidant agent and it can increase the tolerance of plants to several abiotic stresses (Hartikainen, 2005). Recent research has identified the protective effect of Se against salt stress (Kong et al., 2005; Hawrylak-Nowak, 2009), drought (Germ et al., 2007; Proietti et al., 2013), and UV-irradiation (Hartikainen and Xue, 2000). Moreover, many studies have shown that Se has a detoxifying effect on animals poisoned by heavy metals (Newairy et al., 2007; Talas et al., 2008; Lazarus et al., 2009). In the recent years, increased attention has been paid to the potential role of Se in the alleviation of heavy metal toxicity, including Cd, also in plants (Filek et al., 2008; Pedrero et al., 2008; Cartes et al., 2011; Mroczek-Zdyrska and Wójcik, 2012; Feng et al., 2013; Saidi et al., 2014). Several previous studies have shown that Se can increase a plant's tolerance to heavy metals mainly through: (1) alleviation of oxidative stress, (2) inhibition of the uptake of heavy metals, (3) rebuilding chloroplasts and increasing the contents of chlorophyll, and (4) maintaining the integrity of cell membranes (Feng et al., 2013 and references therein). However, to our knowledge, the influence of the applied exogenous Se on the accumulation of PCs in tissues of Cd-exposed higher plants has not been studied earlier.

Thus, the aim of the present study was to investigate the influence of Se supplementation on the tolerance of cucumber (*Cucumis sativus* L.) plants to the Cd stress. Particularly, the biomass and some physiological and biochemical parameters of stress such as content of photosynthetic pigments, level of lipid peroxidation, electrolyte leakage, root viability, and GSH and PCs accumulation were determined in plants exposed to Se and Cd, supplied individually or simultaneously. The accumulation and translocation of Se and Cd in the plants were also determined.

2. Materials and methods

2.1. Plant material and growth conditions

Experiments were carried out on cucumber (*Cucumis sativus* L.) plants cv. Polan F1 grown in a controlled-climate chamber (Sanyo, model MRL 350HT). Plants were cultivated under the following conditions: photosynthetic photo flux density (PPFD) of 230 $\mu mol\ m^{-2}\ s^{-1}$, 14-h day photoperiod, temperature 25/20 °C (day/night) and relative humidity of 65–70%. Seeds of cucumber germinated in wet quartz sand for seven days at 25 °C. After germination, the best-developed seedlings of uniform size were transferred to 1 L glass jars (2 per jar) filled with 1.5-times strength Hoagland's II nutrient solution supplemented with ferric citrate and an A–Z solution containing the essential microelements (Hoagland and Arnon, 1950). The pH of the nutrient solution was adjusted to 5.5. Three Cd treatments (0, 25 or 50 μM, as $CdCl_2 \cdot 2.5\ H_2O$) were applied with combinations of three Se levels (0, 5 or 10 μM as Na_2SeO_4). Detailed treatments were as follows: control (0 Cd/Se), 5 μM Se, 10 μM Se, 25 μM Cd, 25 μM Cd + 5 μM Se, 25 μM Cd + 10 μM Se, 50 μM Cd, 50 μM Cd + 5 μM Se, 50 μM Cd + 10 μM Se. After 14 days of growth in the presence of Se and/or Cd, the plant samples were examined for photosynthetic pigments concentrations, relative electrolyte leakage (REL), the level of lipid peroxidation, root viability, GSH and PCs accumulation, as described below. Moreover, the fresh weight (FW) and Se and Cd accumulation were determined. We presented the results of the root and shoot FW, because all physiological and biochemical parameters were measured in the fresh biomass. We have also determined the dry weight (DW) of plant's organs, but the tendencies were similar as for FW.

2.2. Determination of photosynthetic pigments

The chlorophyll *a* and *b* as well as total carotenoid (xanthophyll + carotene) concentrations were determined spectrophotometrically following the procedures of Lichtenthaler and Wellburn (1983). The samples were collected from the second true leaves and pigments were extracted from the fresh leaf discs by homogenising in 80% (*v*/*v*) acetone. The absorbance of the resulting extracts was measured at 663, 646 and 470 nm.

2.3. Lipid peroxidation assay

The level of a membrane lipid peroxidation in the root tissue extracts was estimated by measuring the malondialdehyde (MDA) content, as a by-product of lipid peroxidation. MDA content was assayed following the method of Heath and Packer (1968) with minor modifications. In order to determine the MDA content 500 mg of fresh tissues were ground in 4.5 mL of 0.1% (*w*/*v*) trichloroacetic acid (TCA), and centrifuged at 10,000 rpm for 10 min. Then, 4 mL of 20% TCA containing 0.5% of thiobarbituric acid (TBA) (*w*/*v*) were added into 1 mL of the obtained supernatant. The solution of TCA + TBA was enriched with butylated hydroxytoluene (BHT) to prevent MDA formation during the assay, which could result in falsely elevated TBA reactivity. The reaction mixture was heated at 95 °C for 30 min, quickly cooled on ice and re-centrifuged at 10,000 rpm for 10 min. The absorbance was measured at 532 and 600 nm. The amount of MDA–TBA red complexes was calculated from the extinction coefficient of 155 $mM^{-1}\ cm^{-1}$.

2.4. Analysis of relative electrolyte leakage

The relative electrolyte leakage (REL) was measured by the electrical conductivity method as described by Luo et al. (2005) with slight modifications. Ten freshly cut leaf discs (0.5 cm^2 each), originating from the same leaf, were rinsed with deionised water and put into 10 mL of deionised water in a test tube. The initial electrolyte leakage (EL0) was measured after 24 h of floating of the leaf discs at room temperature using a conductimeter. The tubes were subsequently boiled at 100 °C for 30 min to release all the electrolytes into the solution, cooled to room temperature and the final electrolytic leakage (EL1) was measured. REL was calculated according to the following formula: (EL0/EL1) × 100 and expressed as a percentage of total conductivity.

2.5. Determination of root viability

The root viability was determined using the fluorescein diacetate/propidium iodide (FDA/PI) staining mixture at final concentrations of: 12.5 (FDA) and 5 (PI) $\mu g\ mL^{-1}$ (Pan et al., 2001). The apical fragments of primary roots (about 2 cm length) were gently incubated in FDA/PI mixture for 10 min and then rinsed with distilled water. Observations were carried out in a drop of distilled