3. RESULTADOS

3.1. Artigo

Mercury toxicity induces oxidative stress in growing cucumber seedlings

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Mercury toxicity induces oxidative stress in growing cucumber seedlings

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Abstract

In this study, the effects of exogenous mercury ($HgCl_2$) on time-dependent changes in the activities of antioxidant enzymes (catalase and ascorbate peroxidase), lipid peroxidation, chlorophyll content and protein oxidation in cucumber seedlings (*Cucumis sativus* L.) were investigated. Cucumber seedlings were exposed to from 0 to 500 μM of $HgCl_2$ during 10 and 15 days. Hg was readily absorbed by growing seedlings, and its content was greater in the roots than the in shoot. Time and concentration-dependent reduction in root and shoot length was observed at all concentrations tested, equally in the roots and shoot, at both 10 and 15 days. At 50 μM $HgCl_2$, root fresh weight of 15-day-old seedlings increased, and at other concentrations, it reduced. For 10-day-old seedlings, reduction in root and shoot fresh biomass was observed. At 15 days, only at 50 μM $HgCl_2$ was there no observed reduction in shoot fresh biomass. Dry weight of roots increased at 500 μM both at 10 and 15 days, though at 250 μM $HgCl_2$ there was only an increase at 15 days. There was a significant effect on shoot dry weight at all concentrations tested. Hg-treated seedlings showed elevated levels of lipid peroxides with a concomitant increase in protein oxidation levels, and decreased chlorophyll content when exposed to between 250 and 500 μM of $HgCl_2$. At 10 days, catalase activity increased in seedlings at a moderately toxic level of Hg, whereas at the higher concentration (500 μM), there was a marked inhibition. Taken together, our results suggest that Hg induces oxidative stress in cucumber, resulting in plant injury.

Keywords: Catalase; Ascorbate peroxidase; Lipid peroxides; Cucumber; Chlorophyll; Protein oxidation

1. Introduction

The effects of certain heavy metals on cellular systems has received a great deal of attention in recent decades due to the increasing exposure of living organisms to these metals in the environment (Cavallini et al., 1999). Amongst heavy metals, mercury is one of the most hazardous pollutants of the environment and originates from various sources, such as gold and silver mining, copper and zinc mining and smelting areas, and in areas close to coal burning and other industrial activities (Du et al., 2005). It is known to accumulate in living organisms (Su et al., 2005), causing serious damage.

Its increasing levels in the soil exert a wide range of adverse effects on the growth and metabolism of plants (Verma and Dubey, 2003; Patra et al., 2004), such as reduced photosynthesis, transpiration, water uptake, chlorophyll synthesis (Godbold and Huttermann, 1986), and increased lipid peroxidation (Cho and Park, 2000).

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An important feature of mercury toxicity is the generation of free radicals. The generation of reactive oxygen species (ROS), such as the superoxide anion ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$) has been proven to be one of the underlying agents in the origin of tissue injury after the exposure of plants to a wide variety of stressful conditions, such as draught, heat, chilling, high light intensity, UV radiation, heavy metals, various organic chemicals and air pollutants (Cho and Park, 2000; Qureshi et al., 2005).

Complex antioxidant systems (Qureshi et al., 2005) such as catalase (E.C.1.11.1.6), ascorbate peroxidase (E.C.1.11.1.11), and superoxide dismutases (SOD, E.C.1.15.1.1) (Nakano and Asada, 1981; Cho and Park, 2000; Verma and Dubey, 2003), which neutralize and scavenge the ROS (Cho and Park, 2000; Mittler, 2002), are very important for plants in order to protect cellular membranes and organelles from the damaging effects of ROS, generated by various environmental stress, as heavy metals.

Cucumis sativus was selected as the test plant species, due to its sensitivity to a wide range of contaminants (Pereira et al., 2006) and also due to the insufficient information available on mercury toxicity in this species. Aiming to contribute to a better understanding of the toxicology of this metal, in this paper we present some data showing changes in antioxidative capacity, plant growth, chlorophyll content, protein oxidation and lipid peroxidation in seedlings of *C. sativus* exposed to mercury chloride.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of cucumber (*C. sativus* L.) obtained from Feltrin Ltd. (Santa Maria, RS) were germinated in glass recipients containing 20 mL of 10% of Murashige and Skoog (1962) medium, supplemented with 0.6% agar and various $HgCl_2$ levels. Seedlings were exposed to 0, 0.5, 50, 250 and 500 μM of $HgCl_2$. The medium pH was adjusted to 5.8. Each experimental unit consisted of six seeds, totalizing 15 replicates per treatment. After the radicle broke through, the seedlings were maintained in a growth chamber with controlled temperature (25 ± 1 °C) and photoperiod (16 h light; light intensity of 35 μmol m^{-2} s^{-1} at plant level) for 10 and 15 days. This time was selected to verify if there would be alterations in the biochemical parameters evaluated at a small interval of time.

2.2. Growth analysis

Cucumber growth was determined by measuring the length of the root system (Tennant, 1975) and of the shoot (measured with a ruler), both expressed in cm $plant^{-1}$. To obtain fresh weight, excess water from root washing was removed with a paper towel. To obtain dry weight, the plants were left at 65 °C to a constant weight. Fresh and dry weight was expressed as g $plant^{-1}$.

2.3. Metal determination

The Hg content was determined in the roots and cotyledons of 10 or 15 day-old cucumber seedlings. Between 20 and 300 mg of cotyledons and roots were digested with 5 mL HNO_3 and 0.2 mL H_2O in closed Teflon vessels, which were heated at 100 °C for 3 h in a digester block (Tecnal TE 007D). The samples were then diluted to 50 mL with high-purity water. Hg concentrations were determined using a Varian Atomic Absorption Spectrophotometer (Spectr AA 600, Australia) equipped with a vapor generative accessory (Varian VGA-76). The content absorbed was expressed as μg g^{-1} dry weight.

2.4. Protein oxidation

The reaction of carbonyls with 2,4-dinitrophenylhydrazine (DNPH) was used to determine the amount of protein oxidation, as described in Levine et al. (1990). Protein extract was obtained by the homogenization of cucumber seedlings (1 g) with 5 mL, 25 mM K_2HPO_4 (pH 7.0) which contained 10 mL L^{-1} Triton X-100. After the homogenate was centrifuged at 15.000 × *g* for 30 min at 4 °C, the supernatant was used for the immediate determination of protein oxidation. After the DNPH-reaction, the carbonyl content was calculated by absorbance at 370 nm, using the extinction coefficient for aliphatic hydrazones (22 l $mmol^{-1}$ cm^{-1}) and expressed as nmol carbonyl $(mg\ protein)^{-1}$.

2.5. Chlorophyll determination

Cotyledons were weighed and used for chlorophyll determination. Chlorophyll was extracted following the method of Hiscox and Israelstam (1979) and estimated with the help of Arnon's formulae (Arnon, 1949). 0.1 g chopped fresh cotyledons sample was incubated at 65 °C in dimethylsulfoxide (DMSO) until the pigments were completely bleached. Absorbance of the solution was then measured at 663 and 645 nm in a Spectrophotometer (Celm E-205D). Chlorophyll content was expressed as μg g^{-1} fresh weight.

2.6. Estimation of lipid peroxides

The level of lipid peroxidation products was estimated following the method of El-Moshaty et al. (1993) by measuring the concentration of malondialdehyde (MDA) as an end product of lipid peroxidation by reaction with thiobarbituric acid (TBA). Fresh whole plant samples (0.1 g fresh weight) were ground in 20 mL of 0.2 M citrate-phosphate buffer (pH 6.5) containing 0.5% Triton X-100, using mortar and pestle. The homogenate was filtered through two layers of paper and centrifuged for 15 min at 20.000 × *g*. One milliliter of the supernatant fraction was added to an equal volume of 20% (w/v) TCA containing 0.5% (w/v) TBA. The mixture was heated at 95 °C for 40 min and then