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Effects of cadmium on meristem activity and nucleus ploidy in roots of *Pisum sativum* L. cv. Frisson seedlings

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Abstract

The effects of cadmium (Cd) administration on primary root growth, mitotic activity of apical meristems, mitotic aberrations and percentage of nucleus ploidy classes of differentiated roots were examined in *Pisum sativum* L. cv. Frisson. Cadmium caused a reduction of root length related to concentration, with an almost complete block of growth in plants treated with 250 μM Cd, from 24 h of treatment. Root lengthening is generally related to apical meristem activity, however, in the examined pea plants, mitotic activity was suppressed by 2.5 and 25 μM Cd treatment, while the highest Cd concentration, 250 μM, caused the occurrence of mitotic figures consisting almost exclusively of prophases. The lack of relation between root lengthening and mitotic activity was explained by the meristematic activity in the first period of treatment and by a different cell elongation. Lower (0.25, 0.5 and 1 μM), non-blocking Cd concentrations induced a number of mitotic aberrations, mainly consisting of sticky metaphases and anaphase bridges, whose frequency increased with Cd concentration. Besides, Cd induced variations of the percentages of nucleus populations in the differentiated roots, increasing the percentage of 4C nuclei and decreasing that of 2C. The mechanisms involved in the nuclear response to Cd, and the possible relations between Cd alteration of meristem cell activity and nuclear ploidy of differentiated cells are discussed.

Keywords: Cadmium; Root apical meristems; Mitotic index; Chromosome aberrations; Flow cytometry

1. Introduction

Cadmium is a widespread heavy metal and it is naturally present in the environment: in soils it occurs at trace concentrations; however, human activity, mainly some industrial processes and the use of phosphate fertilizers in agriculture, have increased its concentration (Prasad, 1995). Accumulation of Cd can be dangerous for all kinds of organisms, including plants. Although Cd is a non-essential element for plant growth, it is rapidly taken up by the roots, and interferes with the uptake, transport and use of several elements and water by plants (Das et al., 1997). It may induce symptoms of toxicity, which includes a general growth inhibition (Prasad, 1995) and numerous physiological and metabolic disturbances; among them, alterations in the photosynthetic process (Prasad, 1995; Di Cagno et al., 1999), alterations of enzyme activity (Sanitá di Toppi and Gabbrielli, 1999) and damage of cell membrane (Fodor et al., 1995; Hernánedez and Cooke, 1997; Llamas et al., 2000), related to oxidative damage to proteins, lipid and DNA (Valverde et al., 2001; Gichner et al., 2004; Sävenstrand and Strid, 2004), alteration of gene expression and inhibition of DNA repair processes (Waisberg et al., 2003).

Roots show visual symptoms of toxicity, as Cd causes root browning, twisting, reduction or disappearance of lateral roots (Wójcik and Tukendorf, 1999), and a progressive reduction of the growth rate. The latter mainly results from

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the reduced production of new cells due to mitotic activity in apical meristems (Zhang and Yang, 1994; Zhang and Xiao, 1998; Liu et al., 2003/4), and from the inhibition of cell elongation in the extension regions (Prasad, 1995; Voutsinas et al., 1997). Along with the decreased mitotic activity in the apical meristems, mitotic aberrations appear, consisting in C-mitoses, anaphase bridges, chromosome stickiness, vagrant chromosomes and micronuclei (Zhang and Yang, 1994; Fiskesjö, 1997; Panda and Panda, 2002), apoptosis and necrosis (Behboodi and Samadi, 2004). Most of chromosomal damages are related to clastogenesis and their evaluation may be used in short-term tests for genotoxicity. Genotoxicity assays based on plants have shown positive correlation with those based on animals. They are highly sensitive, relatively easy and inexpensive, hence, they are of particular relevance in developing countries (Fiskesjö, 1997; Panda and Panda, 2002).

The effects of Cd on meristem activity change in relation to species and genotype. A number of genotypes of pea plants have been utilized as an experimental system to investigate physiological, metabolic and molecular aspects of Cd administration (Metwally et al., 2005). However, despite the fact that the caryological characteristics of pea (low chromosome number, $2n = 14$; Bennett and Leitch, 1995) make the observations of chromosome damage easy, very few data (Von Rosen, 1954, in Das et al., 1997) exist about Cd impact on the meristem activity of root apices and their evaluation is a useful indication of plant sensitivity/tolerance towards the metal under examination.

Populations of cells within root meristems are distributed throughout pre-synthetic (G1), synthetic (S) and post-synthetic (G2) interphases and mitosis. During cell differentiation the cell cycle may arrest in the G1 and G2 phases or cells may undergo endoreduplication. Endoreduplication is the most common process of cell polyploidization in plants and occurs in over 90% of angiosperms, during differentiation and in tissues with high metabolic activity (D'Amato, 1998). Alteration of the meristem activity induced by Cd may then directly result in a different distribution of the nuclear class of ploidy in the differentiated roots. Besides, the metabolic alterations, which follow Cd application, may interfere with the process of endoreduplication, altering the proportion of nucleus populations. Up to now, the study of the proportion of nucleus populations, and the possible role of endoreduplication phenomena in response to Cd and, in general, to heavy metals toxicity has received little attention, although it has been shown that a clear relation exists between the physiological and metabolic status of the plant cells, and the regulation of the ploidy levels of the cells, in a number of experimental systems (Berta et al., 2000; Larkins et al., 2001).

In this paper, we investigated the short-term effects of increasing Cd concentrations on growth and nucleus populations of the primary roots of *Pisum sativum* L. cv. Frisson, a pea genotype widely utilized for physiological and molecular studies (Sgorbati et al., 1993; Morandi et al., 2000; Repetto et al., 2003; Atta et al., 2004). The mechanisms leading to alterations of these parameters were explored by analyzing mitotic activity and aberrations in the apical meristems and the length of cortex cells.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of *P. sativum* L. cv. Frisson (provided by V. Gianinazzi-Pearson, UMR PME, INRA-CMSE, Dijon, France) were surface-sterilized (10 min in 3.5% calcium hypochloride and 10 min in 96% ethanol) and germinated on moist sterile filter paper in the dark at 20–24 °C (16 h thermoperiod and 60% relative humidity). Three days after sowing seedlings were individually transferred into 1 L plastic pots containing 0.5 L of 0 (controls), 2.5, 25 and 250 μM $CdCl_2$ salt solution and grown in a growth chamber under controlled conditions (20–24 °C day/night, 16 h photoperiod, 175 $\mu mol\ m^{-2}\ s^{-1}$, 60% relative humidity). All solutions were replaced after 24 h. The seedlings were harvested after 24 or 48 h, and the following parameters were determined.

2.2. Root length

The primary roots of ten seedlings per treatment were measured and fixed in freshly prepared 3:1 (v/v) ethyl alcohol/acetic acid for 1 h at room temperature. They were rinsed with tap water and stored in 70% ethyl alcohol at 4 °C. Primary root growth, expressed as percentage, was calculated as: [(root length at harvest) − (root length at planting)] × (root length at planting)$^{-1}$ × 100.

2.3. Mitotic activity and aberrations

Mitotic activities were evaluated on squashes of root apices stained by Feulgen reaction, according to Hooker et al. (1998), using a hydrolysis of 9 min in 1N hydrochloric acid at 60 °C. Ten tips per treatment were evaluated and at least 1000 cells per tip were scored. The mitotic index (MI) and the mitotic phases distribution were calculated on the same slides. Because 2.5 and 25 μM Cd concentrations caused a block of the mitotic activity at 24 and 48 h of treatment, aberrant mitoses were studied on Feulgen squashes of 0.25, 0.5 and 1 μM root apices, after 24 and 48 h of treatment, and the percentage of anaphase chromosome bridges, considered as a firm evidence of clastogenesis (Panda and Panda, 2002), was calculated in each slide.

Feulgen-stained nuclei were observed using the Axioscope II optical microscope, connected to an AxioCam camera (Zeiss; Oberkochen, Germany), and digital images were acquired by using an AxioVision II 0.5 software.

Further analyses, related to root growth and meristem activity, were subsequently performed to explain some unexpected results, namely: