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Highlighted article

Effects of anthocyanin-rich extract from red cabbage leaves on meristematic cells of *Allium cepa* L. roots treated with heavy metals

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Abstract

The incubation of *Allium cepa* L. roots in $Pb(NO_3)_2$, $Cd(NO_3)_2 \times 4H_2O$ or $Cr(NO_3)_3 \times 9H_2O$ solution at the concentration of 100 μM lowered the mitotic index (MI) value in meristem by 58%, 39%, 48%, respectively. The proportion of mitotic phases (mainly prophases and telophases) in MI value was also changed. Moreover, mitotic disturbances such as: c-metaphases, sticky and lagging chromosomes, chromosome bridges, binucleate cells, micronuclei, "budding" nuclei and nucleoli partly outside nuclei were induced in the presence of the tested heavy metals, most frequently after Pb treatment. Pre-incubation in the ATH-rich extract from red cabbage leaves caused 2.5%, 1.8% or 1.6% increase in MI value as compared to the meristematic cells of *A. cepa* L. roots treated only with Cd, Pb, or Cr, respectively. Additionally, the ATH-rich extract was responsible for changing phase index values towards the control level in the material incubated in Pb or Cd. Moreover, the total number of mitotic abnormalities induced by the tested metals was reduced due to the preincubation in the ATH-rich extract, most effectively in the roots treated with Cd (by $\frac{2}{3}$) while by half in Cr presence. These data suggest a protective action of the ATH-rich extract from red cabbage leaves against heavy metal toxicity.

Keywords: Anthocyanins; Red cabbage; Heavy metals; *Allium cepa* L.; Root meristem

1. Introduction

Heavy metals introduced to environment both from natural and anthropogenic sources accumulate in soil and are taken up by plants inducing many toxic effects especially in roots. It was shown that heavy metals such as Pb and Cd inhibited root growth (Liu et al., 1994, 2003; Seregin et al., 2004) as a result of cell cycle disturbances (Wierzbicka, 1999) and decrease in mitotic index (MI) value accompanied by reduction of cell number in metaphase and anaphase (Wierzbicka, 1988, 1989; Samardakiewicz and Woźny, 2005; Fusconi et al., 2006). Additionally, both heavy metals induced c-mitoses, chromosome stickiness and chromosome bridges (Wierzbicka, 1988; Zhang and Xiao, 1998; Jiang and Liu, 2000; Samardakiewicz and Woźny, 2005). Pb also caused lagging chromosomes and nuclei with more condensed chromatin (Samardakiewicz and Woźny, 2005). Moreover, binucleate cells were found in root meristems after Pb treatment as a result of cytokinesis inhibition (Wierzbicka, 1989). It is also well documented that heavy metals such as Pb, Cd and Cr induced micronuclei (Knasmüller et al., 1998; Steinkellner et al., 1998; Samardakiewicz and Woźny, 2005; Qian et al., 2006). Apart from that, those metals disturbed RNA and DNA synthesis (Łbik-Nowak and Gabara, 1997) and induced changes in the root cell ultrastructure (Woźny et al., 1982).

Heavy metals incorporated *via* food chain are toxic to humans and animals (Fatur et al., 2002; Palus et al., 2003). Therefore, in numerous laboratories all over the world,

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scientists try to find agents reducing their toxicity. It is well documented that harmful effects of heavy metals can be minimized by Ca (Gabara and Gołaszewska, 1992; Gabara et al., 1992; Zhang and Xiao, 1998), Se (Landberg and Greger, 1994; Zhang and Xiao, 1998) or salicylic acid (Drazic and Mihailovic, 2005).

Moreover, in the recent years attention has been drawn to the "health-promoting" activity of phenolic compounds to which anthocyanins (ATH) also belong (Ferguson, 2001; Kowalczyk et al., 2003). ATH are plant pigments, present in numerous fruits and vegetables. These compounds possess pharmacological properties protecting against cardiovascular diseases and are postulated as anti-mutagenic and anti-tumor factors (Gąsiorowski et al., 1997; Kong et al., 2003). The above effects have been related mainly to the antioxidant properties of ATH, as it was demonstrated with experiments both *in vitro* and *in vivo* (Boveris et al., 2001; Kong et al., 2003; Lazzé et al., 2003). Although, knowledge about ATH actions in organisms exposed to heavy metals is fragmentary, experiments on rats showed that they reduced the harmful effects of Cd (Kowalczyk et al., 2003).

However, to the best of our knowledge, there is no information concerning the ATH role in plants treated with heavy metals. Therefore, the aim of the present work was to investigate the effect of the ATH-rich extract from red cabbage leaves on changes induced by these metals in the root meristem.

2. Materials and methods

2.1. Extraction procedure and ATH assay

Fresh leaves of red cabbage (*Brassica oleracea* var. *capitata rubra*) were extracted with a mixture of methanol/distilled water/0.01% HCl (MeOH/H_2O/HCl, 50/50/1, v/v/w) and then centrifugated. In the supernatant, ATH content was spectrophotometrically measured (Gitz et al., 1998). To obtain a more concentrated total ATH solution, the extract was condensed in a vacuum rotary evaporator in water bath at 40 °C.

Total ATH concentration [μM] was determined with cyanidin 3-glucoside as a standard and calculated using a molecular density coefficient $\varepsilon = 30\,\mathrm{mM\,cm^{-1}}$ at $\lambda = 525$ nm (Hodges and Nozzoillo, 1996).

2.2. HPLC analysis of phenolic compounds in extract

Phenolic profiles in the crude red cabbage extract were determined using HPLC Knauer system equipped with UV–Vis detector and a Eurospher-100 C-18 column (25 cm × 4.6 mm; 5 μm). The binary mobile phase according to Dyrby et al. (2001) consisted of water/formic acid (90:10, v/v) (solvent A) and water/acetonitrile/formic acid (40:50:10, v/v/v) (solvent B). The flow rate was 1 ml/min and a total run time was 50 min. The system was run with a gradient program: 0 min: 88% A + 12% B, 26 min: 70% A + 30% B, 40–43 min: 0% A + 100% B, 48–50 min: 88% A + 12% B. Phenolic compounds were divided into four subclasses and quantified on the basis of the maximum UV–Vis absorption of each group. The hydroxybenzoic acid derivatives were quantified at 280 nm and expressed as gallic acid equivalents, hydroxycinnamic acid derivatives at 320 nm as chlorogenic acid equivalents, flavonols at 360 nm as rutin equivalents, and ATH at 520 nm as cyanidin 3-glucoside equivalents.

2.3. Antioxidative activity test

Antioxidative activity of the extract was measured with ABTS (diammonium 2,2-anizobis) (3-ethylbenzothiazoline-6-sulfonate) as a free radical source (Bartosz, 2003). $ABTS^{\cdot}$ was generated by reacting ABTS (7 mM) with potassium peroxodisulfate (2.8 mM). The solution was diluted with Na-phosphate buffer pH 7.4 to obtain an absorbance of 0.900 at 732 nm. Two milliliters of the solution were added to 20 μl of the extract. The changes in absorbance at 732 nm were recorded during the first 30 s after mixing and until the absorbance reached a plateau. The obtained results were compared with the synthetic antioxidant—Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and presented as TAC (Total Antioxidant Capacity).

2.4. Plant material and treatment

Healthy and equally-sized bulbs of *Allium cepa* L. (cv. Polanowska), obtained from 'Polan' Company (Cracov, Poland), after scale removing were placed in containers filled with Hoagland solution containing: KNO_3 (0.51 g/l), $Ca(NO_3)_2 \times 4H_2O$ (1.18 g/l), $MgSO_4 \times 7H_2O$ (1.23 g/l), KH_2PO_4 (0.14 g/l), FeEDTA (5 mg/l) at pH 6.5 and cultured for 2 days at 21 °C in the darkness. The adventitious roots were treated with aqueous solutions of $Pb(NO_3)_2$, $Cd(NO_3)_2 \times 4H_2O$ or $Cr(NO_3)_3 \times 9H_2O$ at a concentration of 100 μM (each) for 2 h with or without preliminary incubation in 250 μM ATH extract for 3 h (such conditions were determined on the basis of preliminary experiments). Control roots were kept in ATH or in distilled water during all experimental time.

2.5. MI and disturbances of mitosis

Root meristems, 10 per each experimental series, after fixation in Carnoy, were stained with Schiff reagent and squashed specimens were made. Subsequently, MI was calculated according to equation:

$$\mathrm{MI}[\%] = \text{the number of scored cells (1000 per root)} / \text{the number of dividing cells} \times 100.$$

Moreover, the proportion of mitotic phases was determined (for example: Prophase index [%] = the number of cells in prophase/the number of dividing cells × 100).

The disturbances of mitosis such as: c-metaphases, chromosome bridges, sticky and lagging chromosomes, "budding nuclei", nucleoli partly outside nuclei, micronuclei and binucleate cells were also analyzed according to previously described methods (Fiskesjö, 1982; Ateeq et al., 2002; Gabara et al., 2006).

2.6. Electron microscopy

The Pb treated roots with or without pre-incubation in the ATH extract were fixed and embedded in Epon-Spurr's resin mixture as it was described earlier (Glińska and Gabara, 2000). Unstained ultrathin sections cut with Reichert ultramicrotome were examined in a Jeol 1010 transmission electron microscope at 80 kV. At least 20 microphotographs of nuclei from each treatment were viewed.

3. Results

3.1. Mitotic index

All the tested heavy metals diminished the number of dividing cells in *A. cepa* root meristem. The most pronounced effect was noticed in Pb presence, while the least after incubation in Cd. Additionally, proportion of mitotic phases (mainly prophases and telophases) in MI value changed in the presence of the tested metals. After Pb