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How cobalt facilitates cadmium- and ethylene precursor-induced growth inhibition and radial cell expansion in barley root tips

Research Article

Katarína Valentovičová, Igor Mistrík, Veronika Zelinová, Ladislav Tamás*

Institute of Botany, Slovak Academy of Sciences,
SK-84523 Bratislava, Slovak Republic

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Abstract: Significant root growth inhibition was observed during the very short 5 minute exposure time of barley roots to the low 10 μM concentration of cadmium. In addition to the cadmium-induced root growth inhibition, considerable radial expansion of roots was observed as a characteristic symptom of transient short-term exposure of roots to cadmium. The cadmium-induced radial expansion of roots was observed mainly the cortical cells of elongation zone that were twice as large as in control roots. Similarly as in cadmium-treated roots, short-term treatment with ACC significantly inhibited root growth and caused a marked radial expansion of cortical cells. The ethylene synthesis inhibitor cobalt significantly alleviated both the cadmium- and ethylene precursor-induced root growth inhibition and radial root expansion. The results indicate that ethylene probably plays a crucial role in the short-term cadmium-induced inhibition of root growth and radial cell expansion of barley root tips, which are the very early symptoms of cadmium toxicity.

Keywords: *Cadmium • Cobalt • Root growth inhibition • Swollen roots*

Abbreviations

ACC - 1-aminocyclopropane-1-carboxylic acid

1. Introduction

Environmental pollution by metals is an increasingly difficult problem over the world affecting crop production, breeding, in addition to human health [1]. Cadmium (Cd) is one of the most toxic heavy metals due to its great solubility in water and high toxicity at very low concentration. The most general visible symptoms of cadmium toxicity are stunting and chlorosis of leaves due to the inhibition of several physiological processes [2]. However, the earliest symptom is the inhibition of root growth, which may appear within few minutes after Cd treatment [3]. Various physiological and metabolic processes affected by Cd have been suggested as a crucial factor for the inhibition of root growth including inhibition of water and mineral uptake [4], alteration of membrane composition and function [5], retardation of enzymes activities [6], the inhibition of cell division [7], production of reactive oxygen species (ROS) or disturbances in the antioxidative systems [8,9].

Apart from the Cd-induced inhibition of root growth in certain cases, also a radial expansion of root cells has been reported [10,11]. The radial expansion of root cortex cells resulting in the swelling of roots is symptomatic for a number of stress conditions such as iron or copper deficiency [12], progressive drought [13],excess salinity [14], UV light [15], mechanical inhibition [16], chemicals [17] and hormones such as auxins and chemicals like ethylene [18]. In most cases the swollen roots are covered with well-developed root hairs in comparison to non-swollen root sections [12,17,18].

Plant hormones play a crucial role in the regulation of root growth and probably are involved in both Cd-induced root growth inhibition and radial expansion of root cells. It is well known that roots grow away from Cd suggesting cadmium inhibits basipetal auxin flow [19]. In contrast to auxin flow inhibition, ethylene production was stimulated by Cd treatment in suspended carrot cell cultures [20]. With suspended tomato cells, ethylene

* E-mail: Ladislav.Tamas@savba.sk

signalling was involved in the Cd-induced programmed cell death [21]. In addition, enhanced generation of ethylene has been reported in both roots and leaves after cadmium treatment in wheat and barley seedlings [22,23]. Alterations in the level of hormones and reactive oxygen species in roots, are the key events during different stress conditions, leading to the morphological stress responses as part of general acclimation to stress conditions [24].

Numerous studies showed that application of various mild stress conditions or biologically active molecules such as salicylic acid or nitric oxide, may alleviate heavy metal induced toxicity symptoms, specifically by the modulation of antioxidative systems [25-29]. It has also been reported that some elements at low doses have beneficial effect on growth or curb toxic symptoms induced by other elements. It is well known that low doses of aluminium, cobalt, sodium or selenium have beneficial effects, while at higher concentrations, toxic effects on plant growth have been observed [30]. Aravind and Prasad [31] have reported the protective role of zinc, by modulating the redox status of the plant system combating Cd-induced oxidative stress.

In the present study, we investigated the short-term cadmium- and ethylene precursor aminocyclopropane-1-carboxylic acid (ACC)-induced changes such as root growth inhibition and radial root expansion in barley root tips and their modification by employing cobalt (Co), an ethylene synthesis inhibitor. The results indicated the crucial role of ethylene in the short-term Cd-induced inhibition of root growth and the radial swelling of barley root tips.

2. Experimental Procedures

2.1 Plant material and growth conditions

Barley seeds (*Hordeum vulgare* L.) cv. Jubilant (Plant Breeding Station – Hordeum Ltd Sládkovičovo-Nový Dvor) were soaked in distilled water for 6 h followed by their germination between two sheets of filter paper (density 110 g/m^2, Papírna Perštejn, Czech Republic) moistened with distilled water in Petri dishes (5 ml per sheet of filter paper of 18 cm in diameter) at 25°C in darkness. The uniformly germinating seeds 24 h after the their soaking, were then placed into the row between two sheets of filter paper moistened with distilled water, into rectangle trays. The trays were placed into a nearly vertical position in order to allow downward radical growth The filter papers were irrigated with distilled water, from the reservoir through a filter paper wick. Seedlings with roots approximately 4 cm long, 72 h after the onset of seed inhibition, were used for treatments. The roots of seedlings were immersed in distilled water (control) or into 10 mM $CdCl_2$, for 5, 10, 15, 20, 25 or 30 min, followed by washing with distilled water up to 35 min. The ethylene precursor, ACC, and the ethylene synthesis inhibitor $CoCl_2$ were applied at 10 and 40 mM concentrations for 30 min respectively. After these short-term treatments, the seedlings were incubated as described above between two sheets of filter paper moistened with distilled water. Analyses were performed 24 h after the short-term treatments.

2.2 Cd content determination

Immediately after short-term treatments (11 mm long), root tips were oven-dried at 60°C for 2 days and after digestion and extraction, they were analysed by using the atomic absorption spectrometer (Perkin Elmer 4100, Norwalk, CT, USA) [32].

2.3 Root length measurement

For determination of root length growth, the position of root tips following the treatments were marked on the filter paper. After 24 hours, roots were excised at the position of the marks and growth of length was measured with a ruler.

2.4 Root cross-section and cortex-layer area measurement

Measurements were performed with the Cell* - Imaging Software for Life Sciences Microscopy (Olympus). For the cortex-layers, ten adjacent cells from individual cortex layers were measured on handmade cross sections of 0.5-1 mm in thickness. Cells under the rhizodermis were marked as the first cortex layer, while the cells adjacent to the endodermis were labelled as the fifth cortex layer.

2.5 Cell length determination

Rhizodermal and cortical cell lengths (20 adjacent cells), were measured on the handmade longitudinal sections obtained from the swollen part of roots, employing the Cell* - Imaging Software for Life Sciences Microscopy (Olympus).

2.6 Statistical analyses

The experiments were conducted in five independent trials, with and repeated three times. Data was analysed by a one-way analysis of variance (ANOVA), and the means were determined by using Tukey's test.

3. Results

Significant root growth inhibition was observed during a very short (5 min long) exposure time of barley roots to the low 10 mM concentrations of cadmium, and inhibition further increased with greater exposure time (Figure 1A). The time-dependent Cd-induced inhibition of root growth correlates with the time-dependent increase of Cd uptake in barley root tips (Figure 1B). In addition to Cd-induced root growth inhibition, radial expansion of roots was observed as a characteristic symptom of transient (short-term) exposure of roots to Cd (Figure 1C). Despite the significant inhibition of root growth induced by the immersing of roots into distilled water for 35 min compared to untreated seedlings (Figure 1A), swelling of root tips was not detected (Figure 1C) in this instance. No correlation was observed between the time-dependent Cd-induced root growth inhibition and radial root expansion (Figure 1C). While the Cd-induced inhibition of root growth increased linearly with increasing exposure-time, radial expansion of roots reached nearly the maximum level after 10 min of Cd treatment. The length of the rhizodermal and cortical cells in part of swollen roots showed a significant decrease with regard to cadmium exposure-time (Figure 2).

Figure 3A. shows that cadmium-induced radial expansion of root cells occurred in root tissues from 0 to 3 mm behind the root tip, representing meristem and elongation zone during the short-term Cd treatment. Root cross-section analysis revealed that the Cd-induced radial expansion of roots occurred mainly in cortical cells (Figure 3B) that were twice as large as in the control roots (Figure 4). Apart from the radial swelling of cortical cells, a significant increase in the number of cortex cells representing 130.5±14.8 in control or 151.1±11.1 in swollen roots was also delected. Similarly, as in Cd-treated roots, short-term treatment with ACC significantly inhibited root growth and caused a marked radial expansion of cortical cells in barley root tips (Figure 5). The use of cobalt (Co) as an ethylene synthesis inhibitor at 40 mM concentration was found to significantly alleviate both cadmium- and ethylene precursor (ACC)-induced root growth inhibition and radial root swelling (Figure 5).

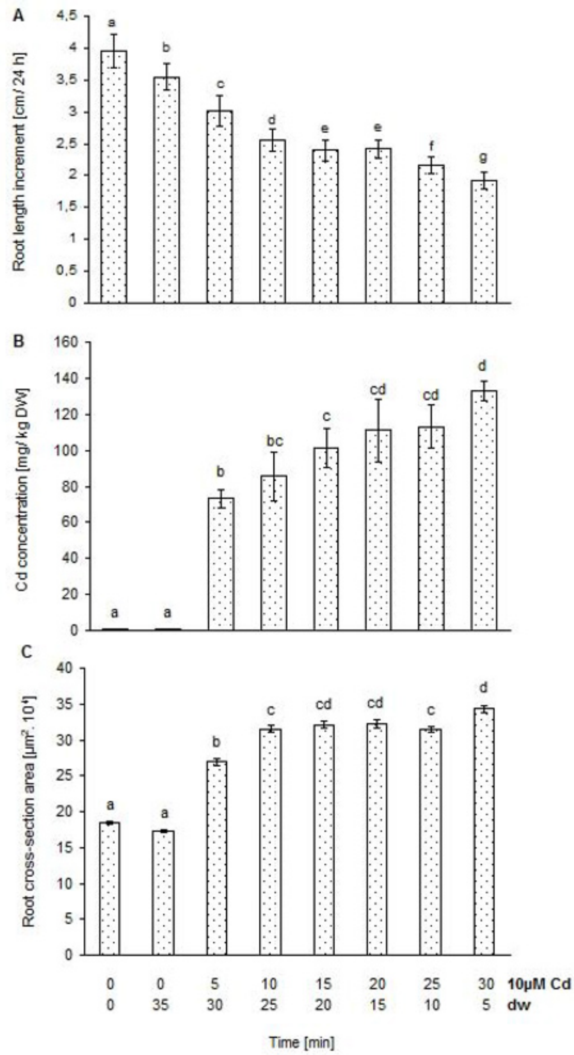

Figure 1. Root length increments (A), Cd contents (B) and root cross-section areas of control and swollen root zones (C) 24 h after short-term treatment with 10 µM Cd for 0, 5, 10, 15, 20, 25 or 30 min and following washing with distilled water (dw) up to 35 min of incubation. (The first column – untreated seedlings, the second column - control roots treated with dw). Mean values ± SD (n=5). Different letters indicate statistical significance according to Tukey's test ($P<0.05$).

4. Discussion

The observed results demonstrate that only a few minute long exposure of roots to Cd was sufficient

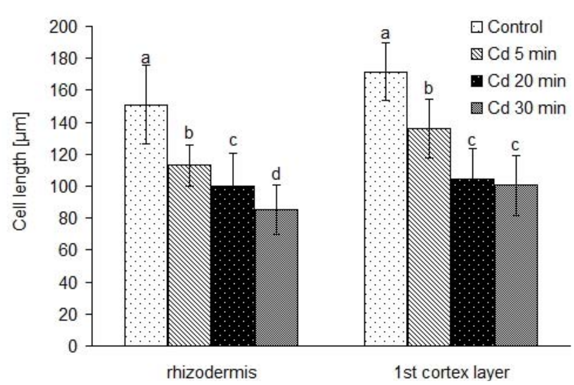

Figure 2. Cell lengths in control and swollen root zones 24 h after the short-term treatment with 10 µM Cd for 0, 5, 20 or 30 min and following washing with distilled water (dw) up to 35 min of incubation. Mean values ± SD (n=5). Different letters indicate statistical significance according to Tukey's test ($P<0.05$)

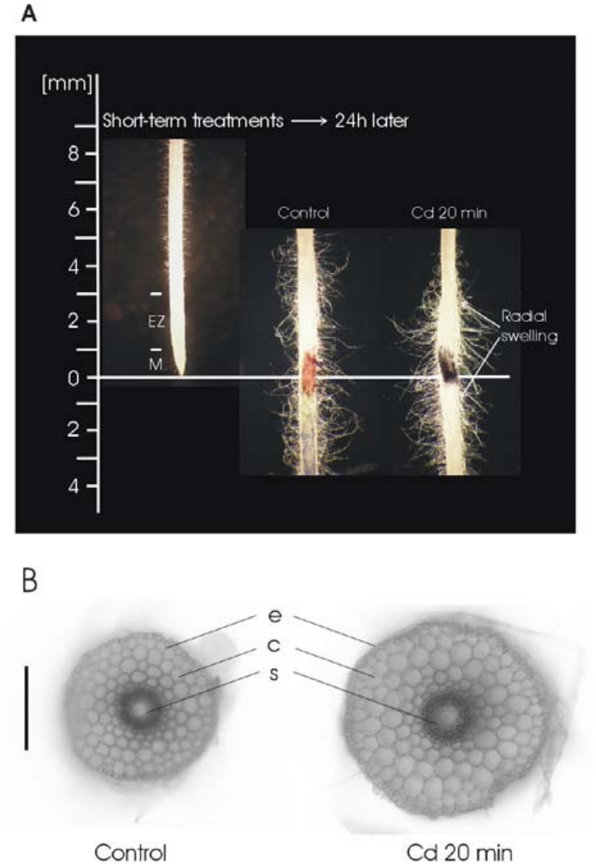

Figure 3. Localization of radial root swelling (A) and cross sections (B) 24 h after the short-term treatment with 10 μM Cd for 0 or 20 min and following washing with distilled water (dw) up to 35 min of incubation. After these short-term treatments roots were arranged into the row that root tips were on the line marked on the tray. After 24 h root part on this line was marked with black stain. (e – epidermis, c – cortex, s – stele).

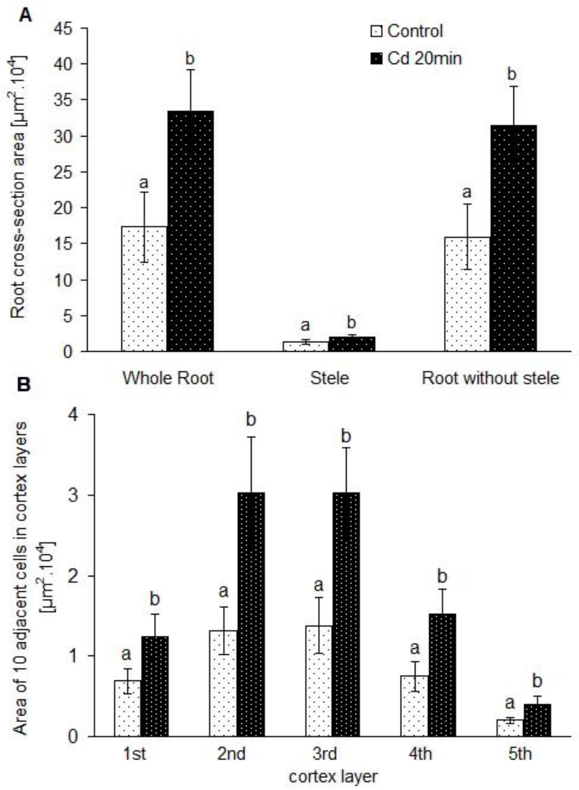

Figure 4. Root-cross section areas of swollen root zones (A) and areas of individual cortex layers (B) 24 h after short-term treatment with 10 μM Cd for 0 or 20 min and following washing with distilled water (dw) up to 35 min of incubation. Mean values ± SD (n=5). Different letters indicate statistical significance according to Tukey's test ($P<0.05$).

to induce the inhibition of root growth, an example of typical Cd-induced toxicity in barley root tips. It has been previously reported that Cd accumulation - including intracellular entry - is very rapid and detectable after few minutes [33]. This study demonstrated that transient short-term Cd treatment induced root growth inhibition and is correlated with the elevated accumulation of Cd in root tips in a time-dependent manner. It has been previously demonstrated that Cd uptake by roots is a metabolically dependent process even during short-term exposure [34].

Apart from cadmium-induced root growth inhibition, it was shown that the radial swelling of root tips is characteristic of short-term Cd-treatment in barley seedlings. However, prolonged low cadmium concentration treatment induced roots with a larger cortex to reductions in the numbers of cells, indicating that the whole roots did not show swelling [35]. In contrast to these results, Rodríguez-Serano *et al.* [36] have observed thickening of lateral roots due to an increase in the number of cortical cells in Cd-treated pea plants. The results in this study showed that radial swelling of root tips induced by short-term cadmium treatment was the consequence of the marked enlargement of cortical cells and partly due to increase of their number. Similar results were reported in studies where there was radial enlargement of roots behind the root tip in pea plants [10]. Ci *et al.* [37] found that Cd-tolerant wheat lines have smaller root diameter than sensitive ones after long-term exposure to Cd. Enlargement of epidermal and cortical cell layers in the apical region has been reported in Cd-treated roots of rice where also the formation of electro-dense vacuolar inclusion appeared, suggesting the sequestration of cadmium into the vacuole [11]. Similar to analysis of cadmium treatment, examination of Cu-induced root swellings revealed destroyed epidermis and cortex of large cells with folded walls [38].

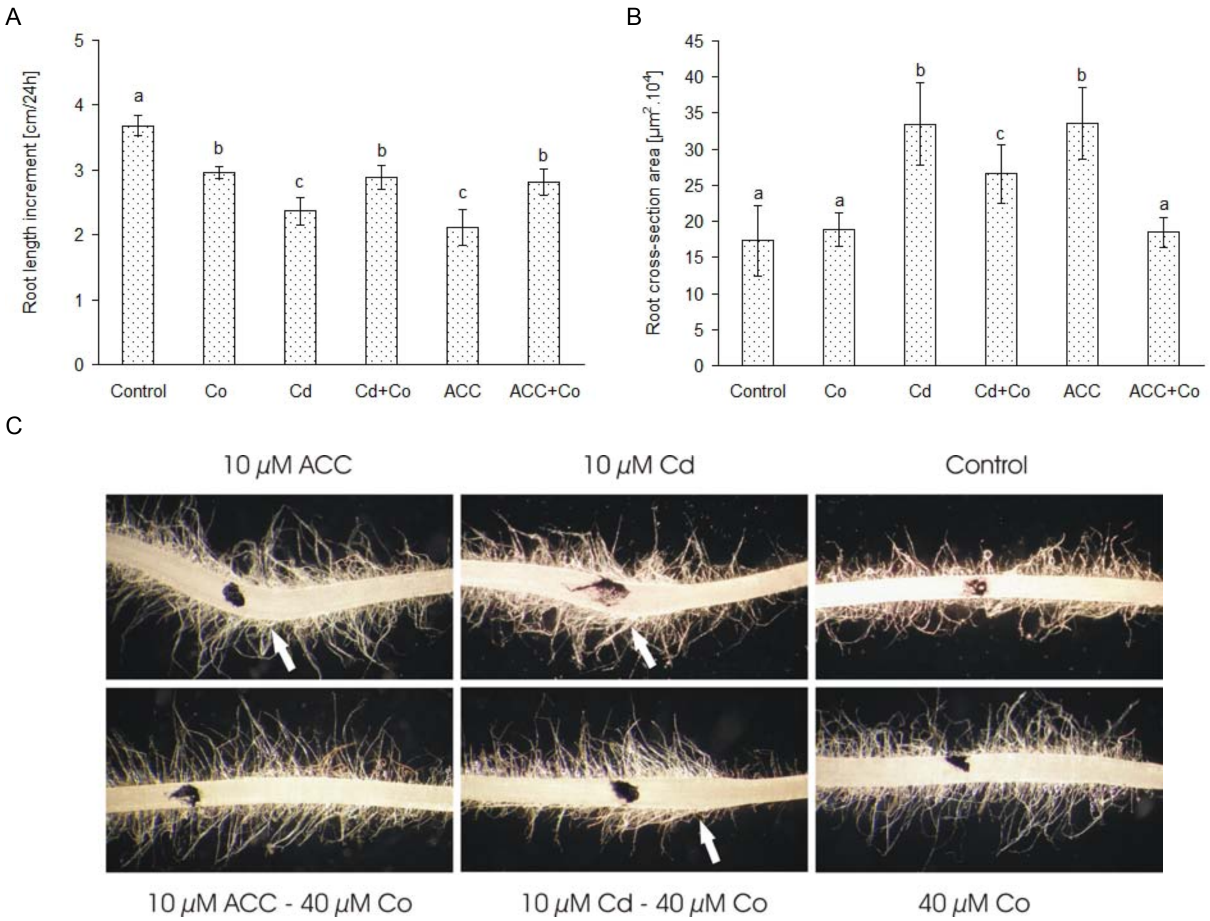

Figure 5. Root length increments (A) root-cross section areas (B) and root swellings (C) 24 h after short-term treatment with dw (control), 10 μM Cd, 40 μM Co, 10 μM Cd and 40 μM Co, 10 μM ACC or 10 μM ACC and 40 μM Co for 0 or 30 min and following washing with distilled water for 5 min. Mean values ± SD (n=5). Different letters indicate statistical significance according to Tukey's test ($P<0.05$). Black stain – see Figure 3.

The observations indicate that cadmium-induced inhibition of root growth is positively correlated with cadmium uptake in an exposure-time dependent manner. On the other hand, radial expansion of roots reached nearly its maximum level 10 min after Cd treatment. These results suggest that apart from the changes in the direction of cell expansion, additional mechanisms negatively influencing the root structure and function are involved in the Cd-induced inhibition of root growth.

Morphogenic analysis of the maize root apex has revealed a distinct transitional region between the meristem and elongation zone where the isodiametric growth of cells occurred [39]. This study reveals that rapid changes in cell growth direction may occur also in the zone characterized by the rapid elongation of cells (1-3 mm behind the root apex during short-term Cd treatment) that is probably associated with alterations in hormone levels in root tips. It is known that both enhanced ethylene production and exogenously applied ethylene caused thick roots as a consequence of ethylene-induced growth inhibition and radial swelling of root cells [40]. Likewise, it is commonly recognized that heavy metals, including Cd are effective inductors of ethylene synthesis in plants [41]. In carrot cell suspension cultures, ethylene production was highly stimulated by Cd treatment [20]. On the other hand, in tomato mutants that showed the loss of sensitivity to ethylene, cadmium caused the development of thinner roots in comparison to control groups [42]. The study demonstrated that the short-term exposure of barley roots to cadmium, besides root growth inhibition, induces similar root swelling as an ethylene precursor (ACC), thus suggesting enhanced production of ethylene in barley root tips. In accordance with these results, it has been shown that ethylene production from excised root tips doubled after 15 min of aluminium treatment [43].

It is well known that cobalt inhibits ethylene production in root tissues, both during non stressful or stressful conditions [44]. The present study demonstrated that cobalt effectively alleviated both ACC- and Cd-induced root growth inhibition and root swelling, further suggesting a crucial role of enhanced ethylene synthesis in the response of roots to Cd. Cobalt substantially ameliorated aluminium-induced root growth inhibition and ethylene production in *Lotus japonicus* [45]. In addition, lower concentrations of cobalt stimulated root growth of germinating barley seedlings [46].

Other results as well as this study suggests that ethylene plays a crucial role in the short-term cadmium-induced inhibition of root growth and radial swelling of barley root tips, which are the very early morphological symptoms of cadmium toxicity.

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References

[1] Pan J., Plant J.A., Voulvoulis N., Oates C.J., Ihlenfeld C., Cadmium levels in Europe: implications for human health, Environ. Geochem. Health, 2010, 32, 1-12
[2] Das P., Samantaray S., Rout G.R., Studies on cadmium toxicity in plants: review, Environ. Pollut., 1997, 98, 29-36
[3] Ortega-Villasante C., Hernández L.E., Rellán-Álvarez R., Del Campo F.F., Carpena-Ruiz R.O., Rapid alteration of cellular redox homeostasis upon exposure to cadmium and mercury in alfalfa seedlings, New Phytol., 2007, 176, 96-107
[4] Perfus-Barbeoch L., Leonhardt N., Vavasseur A., Forestier C., Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status, Plant J., 2002, 32, 539-548
[5] Hernández L.E., Cooke D.T., Modification of the root plasma membrane lipid composition of cadmium-treated Pisum sativum, J. Exp. Bot., 1997, 48, 1375-1381
[6] Tamás L., Bočová B., Huttová J., Mistrík I., Ollé M., Cadmium-induced inhibition of apoplastic ascorbate oxidase in barley roots, Plant Growth Regul., 2006, 48, 41-49
[7] Fusconi A., Repetto O., Bona E., Massa N., Gallo C., Dumas-Gaudot E., et al., Effects of cadmium on meristem activity and nucleus ploidy in roots of Pisum sativum L. cv. Frisson seedlings, Env. Exp. Bot., 2006, 58, 253-260
[8] Romero-Puertas M.C., Rodríguez-Serrano M., Corpas F.J., Gómez M., Del Río L.A., Sandalio L.M., Cadmium-induced subcellular accumulation of O2.- and H2O2 in pea leaves, Plant Cell Environ., 2004, 27, 1122-1134
[9] Tamás L., Valentovičová K., Halušková Ľ., Huttová J., Mistrík I., Effect of cadmium on the distribution of hydroxyl radical, superoxide and hydrogen peroxide in barley root tip, Protoplasma, 2009, 236, 67-72
[10] Fusconi A., Gallo C., Camusso W., Effects of cadmium on root apical meristems of Pisum sativum L.: Cell viability, cell proliferation and microtubule pattern as suitable markers for assessment of stress pollution, Mutat. Res., 2007, 632, 9-19
[11] Rascio N., Vecchia F.D., La Rocca N., Barbato R., Pagliano C., Raviolo M., et al., Metal accumulation and damage in rice (cv.Vialone nano) seedlings exposed to cadmium, Env. Exp. Bot., 2008, 62, 267-278
[12] Romera F.J., Frejo V.M., Alcántara E., Simultaneous Fe- and Cu-deficiency synergically accelerates the induction of several Fe-deficiency stress responses in Strategy I plants, Plant Physiol. Biochem., 2003, 41, 821-827
[13] Couot-Gastelier J., Vartanian N., Drought-induced short roots in Arabidopsis thaliana: structural characteristics, Bot. Acta., 1995, 108, 407-413
[14] Burssens S., Himanen K., van de Cotte B., Beeckman T., Van Montagu M., Inzé D., et al., Expression of cell cycle regulatory genes and morphological alterations in response to salt stress in Arabidopsis thaliana, Planta, 2000, 211, 632-640
[15] Ktitorova I.N., Demchenko N.P., Kalimova I.B., Demchenko K.N., Skobeleva O.V., Cellular analysis of UV-B-induced barley root subapical swelling, Russ. J. Plant Physiol., 2006, 53, 824-836
[16] Wilson A.J., Robards A.W., Goss M.J., Effects of mechanical impedance on root growth in barley Hordeum vulgare L. II. Effects on cell development in seminal roots, J. Exp. Bot., 1977, 28, 1216-1227
[17] Svensson S.B., The effect of coumarin on root growth and root histology, Physiol. Plant., 1971, 24, 446-470
[18] Pulgarín A., Casero P.J., Casimiro I., Lloret P.G., Polar transverse expansion of pericycle cells in

auxin-treated adventitious roots of onion (Allium cepa), Can. J. Bot., 1996, 74, 544-552
[19] Hasenstein K.H., Evans M.L., Stinemetz C.L., Moore R., Fondren W.M., Koon E.C., et al., Comparative effectiveness of metal ions in inducing curvature of primary roots of Zea mays, Plant Physiol., 1988, 86, 885-889
[20] Sanita di Toppi L., Lambardi M., Pazzagli L., Cappugi G., Durante M., Gabbrielli R., Response to cadmium in carrot in vitro plants and cell suspension cultures, Plant Sci., 1998, 137, 119-129
[21] Yakimova E.T., Kapchina-Toteva V.M., Laarhoven L.-J., Harren F.M., Woltering E.J., Involvement of ethylene and lipid signalling in cadmium-induced programmed cell death in tomato suspension cells, Plant Physiol. Biochem., 2006, 44, 581-589
[22] Milone M.T., Sgerri C., Clijsters H., Navari-Izzo F., Antioxidative responses of wheat treated with realistic concentration of cadmium, Env. Exp. Bot., 2003, 50, 265-276
[23] Vassilev A., Lidon F., Scotti P., Da Graca M., Yordanov I., Cadmium-induced changes in chloroplast lipids and photosystem activities in barley plant, Biol. Plant., 2004, 48, 153-156
[24] Potters G., Pasternak T.P., Guisez Y., Palme K.J., Jansen M.A.K., Stress-induced morphogenic responses: growing out of trouble?, Tr. Plant Sci., 2007, 12, 98-105
[25] Hsu Y.T., Kao C.H., Cadmium toxicity is reduced by nitric oxide in rice leaves, Plant Growth Regul. 2004, 42, 227-238
[26] Hu Y., Ge Y., Zhang C., Ju T., Cheng W., Cadmium toxicity and translocation in rice seedlings are reduced by hydrogen peroxide pre-treatment, Plant Growth Regul., 2009, 59, 51-61
[27] Popova L.P., Maslenkova L.T., Yordanova R.Y., Ivanova A.P., Krantev A.P., Szalai G., et al., Exogenous treatment with salicylic acid attenuates cadmium toxicity in pea seedlings, Plant Physiol. Biochem., 2009, 47, 224-231
[28] Chao Y.-Y., Kao C.H., Heat shock-induced ascorbic acid accumulation in leaves increases cadmium tolerance of rice (Oryza sativa L.) seedlings, Plant Soil, 2010, 336, 39-48
[29] Xu J., Yin H., Liu X., Li X., Salt affects plant Cd-stress responses by modulating growth and Cd accumulation, Planta, 2010, 231, 449-459
[30] Pilon-Smits E.A.H., Quinn C.F., Tapken W., Malagoli M., Schiavon M., Physiological function of beneficial elements, Curr. Opin. Plant Biol., 2009, 12, 267-274
[31] Aravind P., Prasad M.N.V., Modulation of cadmium-induced oxidative stress in Ceratophyllum demersum by zinc involves ascorbate-glutathione cycle and glutathione metabolism, Plant Physiol. Biochem., 2005, 43, 107-116
[32] Koreňovská M., Poláčeková O., Trace elements content in virgin sunflower oil production, Czech J. Food Sci., 2000, 18, 61-65
[33] Kevrešan S., Kiršek S., Kandrač J., Petrovič N., Kelemen D.J., Dynamics of cadmium distribution in the intercellular space and inside cells in soybean roots, stems and leaves, Biol. Plant., 2003, 46, 85-88
[34] Uraguchi S., Mori S., Kuramata M., Kawasaki A., Arao T., Ishikawa S., Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice, J. Exp. Bot., 2009, 60, 2677-2688
[35] Vázquez M.D., Poschenrieder Ch., Barceló J., Ultrastructural effects and localization of low cadmium concentration in bean roots, New Phytol., 1992, 120, 215-226
[36] Rodríguez-Serano M., Romero-Puertas M.C., Zabalza A., Corpas F.J., Gómez M., Del Río L.A., et al., Cadmium effect on oxidative metabolism of pea (Pisum sativum L.) roots. Imaging of reactive oxygen species and nitric oxide accumulation in vivo, Plant Cell Environ., 2006, 29, 1532-1544
[37] Ci D., Jiang D., Dai T., Jing Q., Cao W., Effects of cadmium on plant growth and physiological traits in contrast wheat recombinant inbred lines differing in cadmium tolerance, Chemosphere, 2009, 77, 1620-1625
[38] Panou-Filotheou H., Bosabalidis A.M., Root structural aspects associated with copper toxicity in oregano (Origanum vulgare subsp. hirtum), Plant Sci., 2004, 166, 1497-1504
[39] Baluška F., Barlow P.W., Kubica Š., Importance of the post-mitotic isodiametric growth (PIG) region for growth and development of roots, Plant Soil, 1994, 167, 31-41
[40] Pierik R., Verkerke W., Voesenek R.A.C.J., Blom K.W.P.M., Visser E.J.W., Thick root syndrome in cucumber (Cucumis sativus L.): A description of the phenomenon and an investigation of the role of ethylene, Ann. Bot., 1999, 84, 755-762
[41] Maksymiec W., Signaling responses in plants to heavy metal stress, Acta Physiol. Plant., 2007, 29, 177-187
[42] Gratão P.L., Monteiro C.C., Rossi M.L., Martinelli A.P., Peres L.E.P., Medici L.O., et al., Differential ultrastructural changes in tomato hormonal mutants exposed to cadmium, Env. Exp. Bot., 2009, 67, 387-394

[43] Massot N., Nicander B., Barceló J., Poschenrieder Ch., Tillberg E., A rapid increase in cytokinin levels and enhanced ethylene evolution precede Al3+-induced inhibition of root growth in bean seedlings (Phaseolus vulgaris L.), Plant Growth Regul., 2002, 37, 105-112

[44] Lau O.-L., Yang S.F., Inhibition of ethylene production by cobaltous ion, Plant Physiol., 1976, 58, 114-117

[45] Sun P., Tian Q.-Y., Zhao M.-G., Dai X.-Y., Huang J.-H., Li L.-H., et al., Aluminum-induced ethylene production is associated with inhibition of root elongation in Lotus japonicus L, Plant Cell Physiol., 2007, 48, 1229-1235

[46] Locke J.M., Bryce J.H., Morris P.C., Contrasting effects of ethylene perception and biosynthesis inhibitors on germination and seedling growth of barley (Hordeum vulgare L.), J. Exp. Bot., 2000, 51, 1843-1849