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COMPARISON OF ROOT GROWTH AND MORPHOLOGICAL RESPONSES TO CADMIUM AND NICKEL IN TWO MAIZE CULTIVARS

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Abstract. Two maize cultivars *'Premjia 190 MB'* and *'Blitz 160 MB'*, in their seedling stage were exposed for 3 days to Cd^{2+} and Ni^{2+}, separately and also jointly, in concentrations that caused significant inhibition but not cessation of root growth. Primary root growth, cell viability, tissue organization and root system morphology of both cultivars were similar under control conditions. Length of primary roots of both cultivars was significantly reduced by Cd and Ni ions and by the joint treatment (Cd+Ni), under which also early reduction of cell viability occurred. Comparing the two metals, Cd and Cd+Ni proved to be more toxic than Ni alone, with the exception of the effects on *'Blitz 160 MB'* lateral root formation. Comparing the cultivars, *'Premija190 MB'* seemed to be more sensitive to Cd and Cd+Ni than to Ni in all studied parameters, while the early reduction of cell viability, activity of lateral root formation and root thickness in *'Blitz 160 MB'* were more sensitive to Cd+Ni and Ni. The studied growth and morphological characteristics of roots suggest that, at least in the stage of 5 to 6-day-old seedlings, the cultivar *'Blitz 160 MB'* responded more sensitively to experimentally induced heavy metal stress conditions than did the cultivar *'Premjia 190 MB'*.

Key words: maize cultivars, cadmium, nickel, root growth, root system, cell viability, quantitative anatomy

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Introduction

Heavy metals (Ni and Cd) uptake, distribution and accumulation in plants as well as their effects on physiological and morphological traits have been summarized in several recent reviews (Seregin *et al.* 2008; Hasan *et al.* 2009; Lux *et al.* 2011; Gallegoa *et al.* 2012). There is no evidence for Cd acting as a mineral nutrient, whereas Ni stimulates seed germination and plant growth (Bollard 1983), and also plays an important role in nitrogen metabolism. Therefore it is considered to be an essential nutrient for plants (Marschner 1995; Gerendás 1999). Though beneficial (Ni) or harmless (Cd) at low concentrations, both elements induce phytotoxic effects at concentrations higher than the tolerance threshold of the different plant species, since they are taken up through metal transporters with low specificity (Rogers *et al.* 2000; Clemens *et al.* 2002; Sanz *et al.* 2009). Many physiological processes are impaired by Cd and by excess Ni resulting not only in common but also in specific symptoms of metal toxicity (Foy *et al.* 1978; Sun & Wu 1998; Sanità di Toppi & Gabbrielli 1999; Seregin & Kozhevnikova 2006; Chen *et al.* 2009). Maize plants although belonging to excluders, are able to take up and accumulate Cd and Ni. Their response to elevated concentrations of these metals results among others in growth reduction and modification of tissue organization and differentiation in maize roots (Lux *et al.* 2011).

The aim of this work was to demonstrate the responses of primary root growth, cell viability, tissue organization and root system morphology of two commercially available maize cultivars and to compare their sensitivity to increased concentrations of cadmium and nickel acting separately as well as together.

Material and methods

Two maize cultivars, *'Premija 190 MB'* and 'Blitz 160 MB' differing in environmental stress

tolerance, yield and productivity were used (http://maize.com.ua/catalog). Seedlings (2 to 3-day-old) with primary root length from 1 to 2 cm were exposed to aerated hydroponic treatments of distilled water (control), or 2μM concentrations of either Cd^{2+}, or Ni^{2+} sulphate salts or their combination (hereinafter referred as Cd, Ni, or Cd+Ni) for 3 days at laboratory conditions. Control and heavy metal solutions pH was adjusted to 5.8.

Primary and lateral root lengths as well as cross section areas of root tissues were assessed using the imaging software Olympus CellF. Hand made cross sections of primary roots taken at 5 mm distance from the root tip were investigated microscopically (bright field, Axioskop 2 plus, Carl Zeiss) and photographed with digital camera Olympus DP72. Viability of peripheral root cells was assessed following 24h treatments by staining 5 mm apical root segments with the fluorescent dye propidium iodide (PI) excited at 488 nm and detected at 560-660 in confocal laser-scanning microscope Olympus FV1000 (Oh *et al.* 2010). Data were statistically evaluated with the program Excel software.

Results and discussion

Under control conditions the studied root growth and morphological parameters of both cultivars were similar: no significant differences were found. Increases in the root lengths revealed that the relatively low concentrations of metal ions caused significant reduction but not cessation of primary root growth in both cultivars (Fig. 1). This agrees with the finding that already 1μM Cd concentration reduced maize seedling root growth by 30% (Široká *et al.* 2004). The inhibitory effect of Cd was stronger than that of Ni and the growth was most reduced by Cd+Ni in both cultivars. Comparing the response of the two cultivars, primary root growth of *'Blitz 160 MB'* was more reduced than that of *'Premija190 MB'*. Expressed as the percentage of the control roots, length the roots of *'Blitz 160 MB'* reached 17.7%, 27.5% and 22.2% following 3 day-exposition to Cd, Ni and Cd+Ni respectively, while the roots of *'Premija190 MB'* reached 48.8%, 81.0%, and 44.4%.

The capability of primary roots to produce lateral roots was higher in the cultivar *'Blitz 160 MB'* than in *'Premija190 MB'*. After the 3 days of cultivation almost all primary roots of control seedlings (81.5% and 96.7% in *'Premija190 MB'* and *'Blitz 160 MB'* respectively) developed at least one lateral root. This percentage decreased under Cd (81.4%), Ni (53.3%) and was the lowest under Cd+Ni treatment (46.7%) in *'Blitz 160 MB'*. In *'Premija190MB'* the percentages of primary roots forming lateral ones were significantly lower: 3.4% (Cd), 33.4% (Ni) and 22.8% (Cd+Ni). Also, the lateral root density (expressed as the number of laterals per 1 cm of the primary root length) in *'Blitz 160 MB'* was higher under heavy metal treatments, particularly Cd (Fig. 2). In contrast, the development of lateral roots in *'Premija190 MB'* was completely inhibited by Cd and strongly inhibited by Ni or Cd+Ni. However, after 3-day-exposition lateral roots were short and did not contribute substantially to the total root length of the root system that was greater in *'Premija190 MB'* (data not shown).

In the propidium iodide cell viability test, the intact cells of the peripheral root tissues in control plants were characteristic with stained cell walls only (Fig. 3 A), while in non-viable cells with damaged cytoplasmic membrane, also nuclei and cytoplasm were stained. After 24 h treatments almost all cells of the peripheral root tissues revealed drastic effect of Cd+Ni in both cultivars (Fig. 3 B). Interestingly, early damage was not evident in the roots of either of the cultivars exposed to Cd, and the staining was similar to the control. Under Ni treatment the root cell viability was not disturbed in *'Premija190 MB'* (Fig. 3 C) but a substantial number of cells lost their viability in *'Blitz 160 MB'* (Fig. 3 D). This would correspond to the higher primary root growth sensitivity in the cultivar *'Blitz 160 MB'* (Fig. 1).

The areas of the primary root cross sections in both cultivars were similar under control conditions (Fig. 4 A). The significant increase of the root area was observed in the Cd-treated

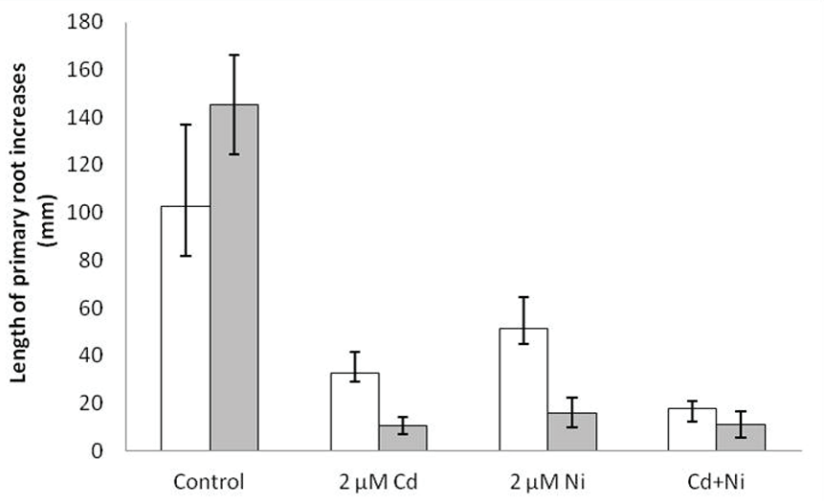

Fig. 1. Primary root length increases (in mm, average±sd) of seedlings of the cultivars *'Premjia 190 MB'* (white columns) and *'Blitz 160 MB'* (gray columns). In Cd+Ni treatment the same 2µM concentrations of Cd^{2+} and $Ni2^{+}$ were applied.

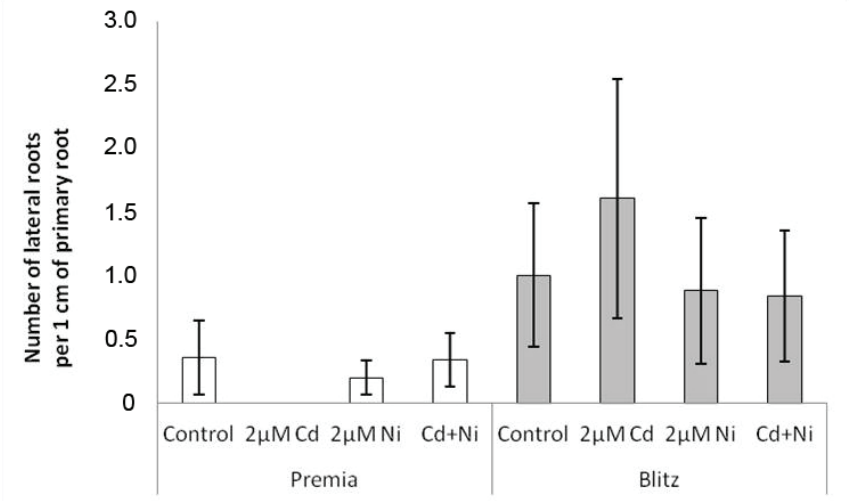

Fig. 2. Lateral root density expressed as number of lateral roots per 1 cm (average±sd) of primary root length in *'Premjia 190 MB'* and *'Blitz 160 MB'* seedlings after 3-day cultivation in distilled water (control) and metal solutions. In Cd+Ni treatment the same 2µM concentrations of Cd^{2+} and Ni^{2+} were applied.

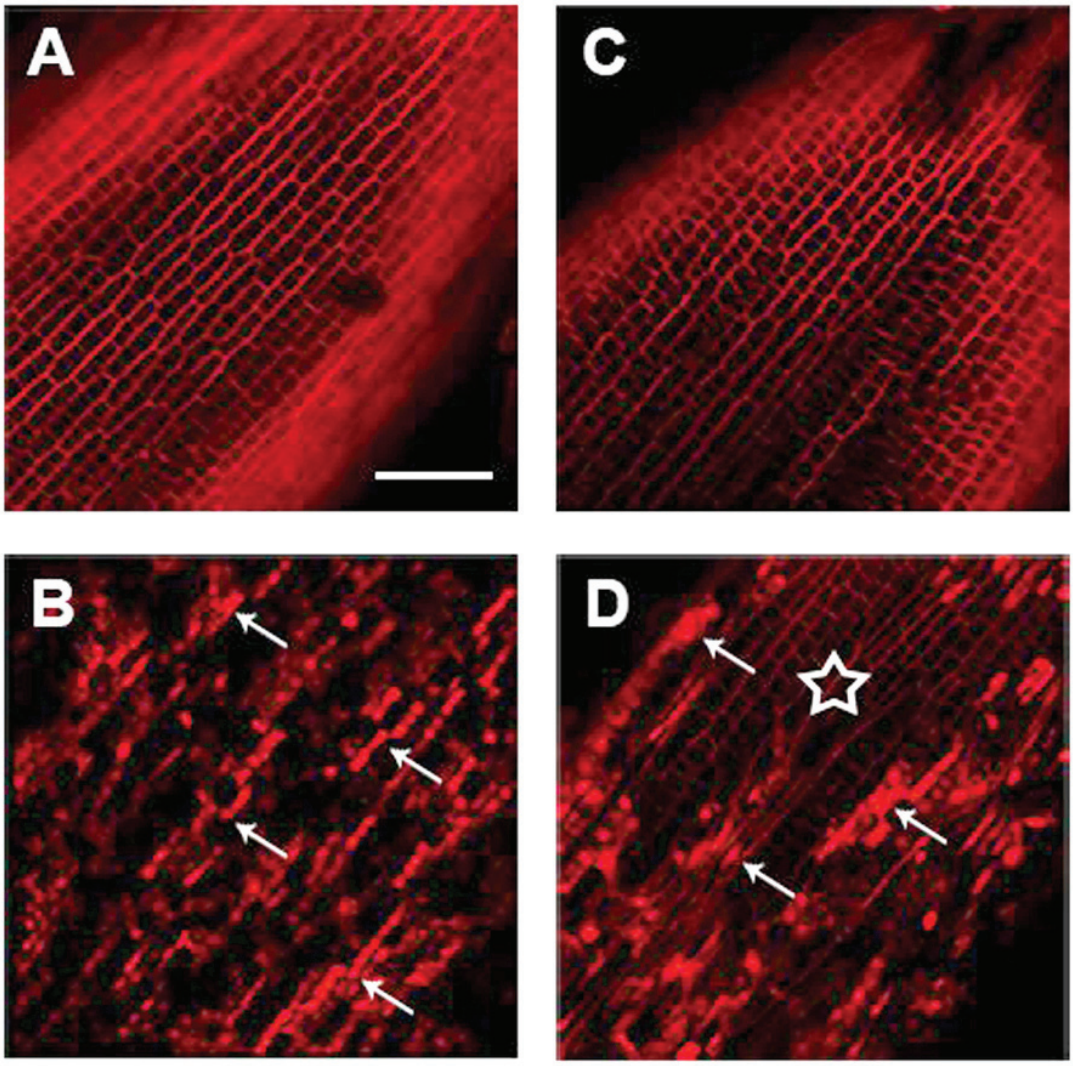

Fig. 3. Detection of viable (with cell wall stained only) and dead cells (with nuclei and cytoplasm stained) in the peripheral tissues of the root tip following 24 h of exposition to heavy metals, with propidium iodide using confocal microscope. **A** – *'Premjia 190 MB'*, control with all cells viable; **B** – *'Blitz 160 MB'*, Cd+Ni, with majority of cells damaged (e.g. arrows); **C** – *'Premjia 190 MB'*, Ni with viable, undamaged cells; **D** – *'Blitz 160 MB'*, Ni with viable (asterisk) and damaged (arrows) cells. Bar represents 30 μm in all figures.

cultivar *'Premija190 MB'* (Fig. 4 B) while in *'Blitz 160 MB'* such increase was induced by Ni and Cd+Ni (Fig. 4 A). The increases of root thickness were due to the adequate enlargements of the areas of root cortex. The areas of other tissues (rhizodermis, central cylinder) remained unchanged under all the heavy metal treatments. Likewise, there was no difference in the number of large metaxylem elements either between the cultivars or between the treatments. Comparing to the control the total area of all large metaxylem elements in the stele was greater in Cd-treated cultivar *'Premija190 MB'* while in the cultivar *'Blitz 160 MB'* the area enlargement was induced by Ni (Fig. 5).

Based on the recorded increases of root lengths under control and all three metal stress variants we can assume that the cross sections of the primary roots were taken from the newly formed root tissues that had developed under

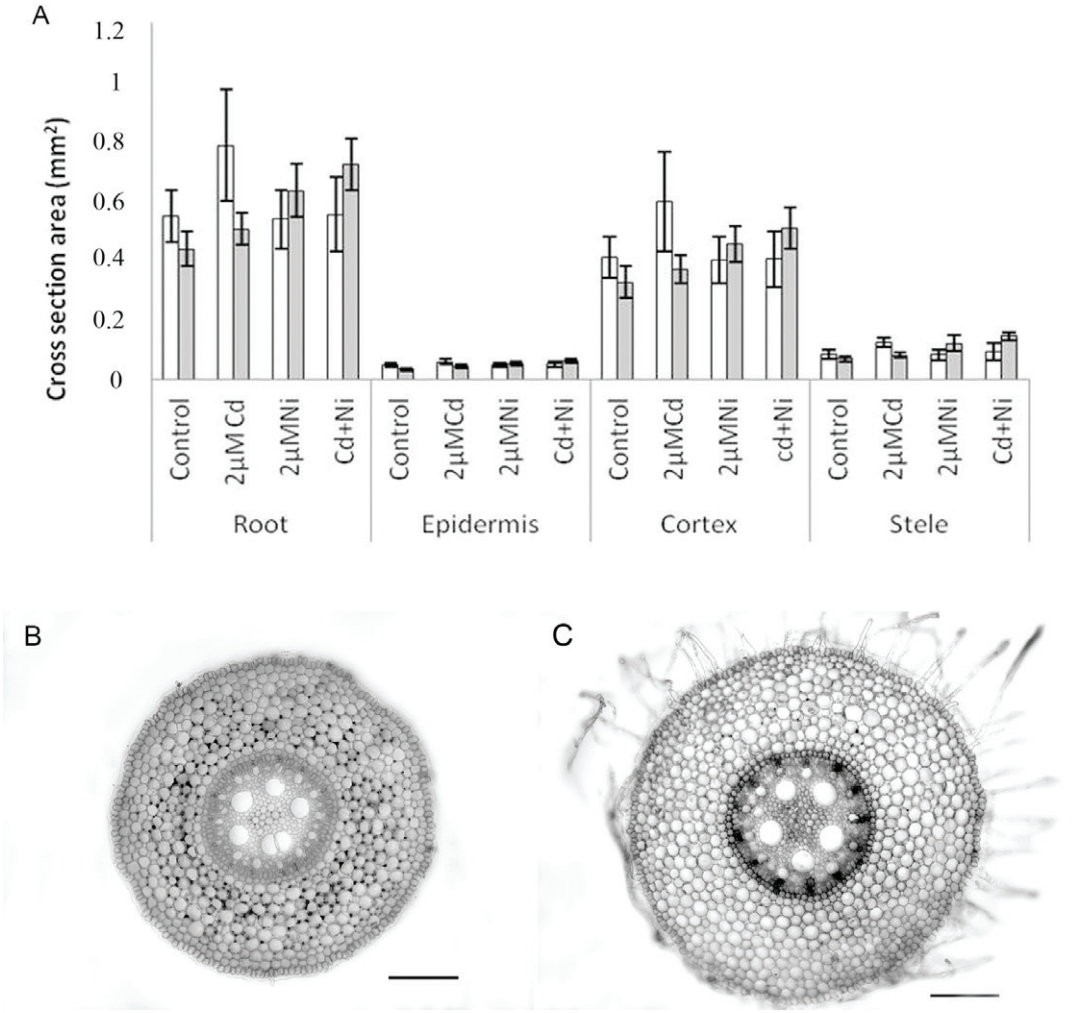

Fig. 4. Quantitative anatomy of primary roots. **A** – cross section area (in mm², average±sd) of the whole root and root tissues in the seedlings of the cultivar *'Premjia 190 MB'* (white columns) and *'Blitz 160 MB'* (gray columns). In Cd+Ni treatment the same 2µM concentrations of Cd^{2+} and Ni^{2+} were applied. **B, C** – cross sections taken at 5 mm distance from the root tip of the cultivar *'Premjia 190 MB'* after 3 days of treatment. **B** – control. **C** – increased root thickness and cortex area induced by Cd treatment. Bars represent 200 µm.

experimental conditions. Thus the differences in the whole root thickness and cortex area between control and Cd, Ni, or Cd+Ni treatments may be considered as the effects of the metals. Elevated Cd concentrations that do not cause significant necrosis induced several modifications of root anatomy and increased root diameter (Lux *et al.* 2011). In maize cultivar used by Maksimović *et al.* (2007) similar effects of Cd on root anatomical structure were more pronounced than the effect of Ni. With this, only results concerning the cultivar *'Premija190 MB'* were in accordance, while the root anatomy was more sensitive to Ni and Cd+Ni in the cultivar *'Blitz 160 MB'*, indicating differences between maize genotypes. Generally, the enlargement of cortical tissues is considered to play a role in resistance to radial flows of water and solutes (Maksimović *et al.* 2007).

In conclusion, the maize cultivars

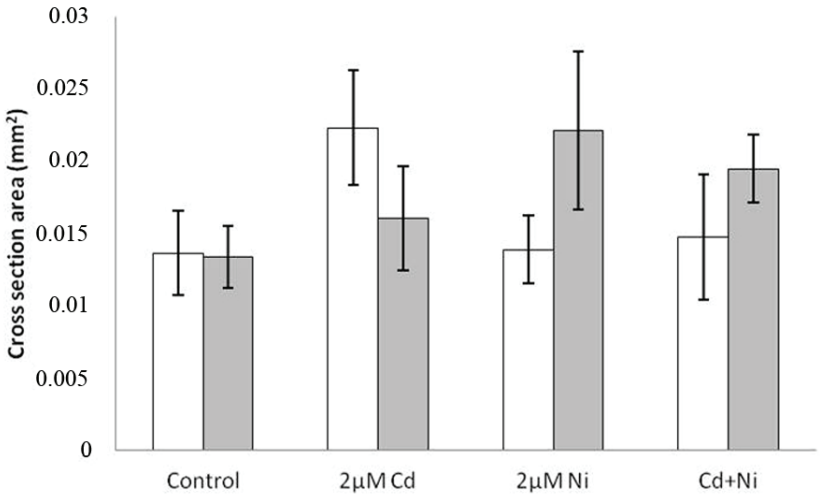

Fig. 5. The sum of cross section areas (average±sd) of the large metaxylem elements in the central cylinder of the cultivar *'Premjia 190 MB'* (white columns) and *'Blitz 160 MB'* (gray columns). In Cd+Ni treatment the same 2µM concentrations of Cd^{2+} and Ni^{2+} were applied.

'Premija190 MB' and *'Blitz 160 MB'* did not differ in the studied characteristics under control conditions. The Cd and Ni ions and their joint action significantly reduced the elongation growth of primary roots of both cultivars. They had different effects on early responses of the root cell viability, lateral root formation and quantitative proportions of root tissues in the two cultivars. *'Premija190 B'* seemed to be more sensitive to Cd or Cd+Ni than to Ni in all studied parameters. In comparison with *'Premija190 MB', 'Blitz 160 MB'* responded more sensitively to Cd only in primary root growth, while cell viability, lateral root development and root thickness were more sensitive to Cd+Ni or Ni. The studied growth and morphological characteristics of roots confirmed intraspecific differences in the responses to heavy metal stress conditions. Our data suggest that at least in the stage of 5 to 6-day-old seedlings the cultivar *'Blitz 160 MB'* responded more sensitively to experimentally induced heavy metal stress conditions than the cultivar *'Premija190 MB'* did.

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