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Heavy metal-induced reactive oxygen species and cell death in barley root tip

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ABSTRACT

Transient exposure of roots to Cd, Pb, Hg or Cu for 30 min reduced root growth in a concentration-dependent manner. While the lower concentrations of metals evoked a marked root growth inhibition accompanied by a visible radial root expansion, the higher concentrations caused root growth arrest without the radial expansion of root cells. At lower metal concentrations, evoking mild stress, the amount of reactive oxygen species (ROS) increased linearly after the treatments. They probably function in both stress signalization and metabolic adaptation processes without damaging the root cells. In turn, at high metal concentrations, causing moderate or severe stress, their production has a biphasic character. The amount of ROS, mainly superoxide, increased rapidly in a metal dose-dependent manner, causing a marked cell death at the site of their generation in the root tips. After this transient burst, ROS generation increased again, together with highly ROS, during the destructive processes associated with cell death in the root tips. Our results show that despite the different origin of ROS induced by these heavy metals, the rate of their generation, depending on the metal concentration, determines the development of typical mild stress-activated morphogenic changes or severe stress-caused cell death within a few minutes after the exposure of roots to toxic level of metals.

1. Introduction

Increased reactive oxygen species (ROS) production is an early and general response of living organisms to both abiotic and biotic stresses. Although first studies described ROS mainly as the inevitable toxic by-products of aerobic metabolism and focused mostly on their destructive effect on cells, it is now clear that ROS are key components of aerobic organisms with a wide range of regulatory and metabolic functions (Foyer and Allen, 2003). ROS include various chemical species that are formed upon incomplete reduction of oxygen (Halliwell, 2006). In plants, the most frequently studied ROS are the singlet oxygen, superoxide anion, hydrogen peroxide and hydroxyl radical with a plethora of functions in cell metabolism and signalling (Das et al., 2015; Mignolet-Spruyt et al., 2016). While under moderate stress conditions ROS function as both local or systemic signals and reactants of various metabolic pathways involved in the adaptive responses required for acclimation and survival of organisms, under severe stresses, a high rate of their production causes irreversible damages, which may be detrimental, leading even to cell death (Mullineaux and Baker, 2010). Consequently, the rapid activation of the antioxidant defence mechanisms is crucial for the elimination of the severe stress-induced toxic ROS in plants. Therefore, both production and elimination of ROS in cells are highly regulated to maintain their steady-state level in a non-toxic concentration (Mittler et al., 2004).

Although the anthropogenic release of heavy metals into the environment had started with the advancement of human civilization, it rose enormously mainly during the industrial revolution and further increased exponentially during the 20th century (Han et al., 2002). Recently, the heavy metal pollution of soils is a serious environmental problem all over the world, affecting not only crop production, but also human health. Many physiological, biochemical and molecular mechanisms have been described as a potential target site of excess metal concentrations. However, the disturbance of cell redox homeostasis, resulting in oxidative stress, is the most common and probably the very early symptom of metal toxicity (Sharma and Dietz, 2009). Consequently, the activation of various antioxidant mechanisms is a key and general component of plant defence response and tolerance to metal toxicity (Sytar et al., 2013; Anjum et al., 2014). Redox-active metals, such as copper (Cu) or iron, can undergo redox cycling reaction and directly generate ROS. On the other hand, these redox-active metals are

Abbreviations: DCF, 2,7-dichlorodihydrofluorescein; DPI, diphenyleneiodonium; hROS, highly ROS; mROS, mitochondrial ROS; NBT, nitro-blue tetrazolium chloride; NOX, NADPH oxidase; ROS, reactive oxygen species

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the key components of various proteins with broad range of biological functions in cells. Therefore, the uptake, transport and storage of these metals are strictly regulated to avoid their participation in the uncontrolled ROS generation (Jomova et al., 2012). Mercury (Hg) is also a redox-active metal but without any biological function in plant cells, and in comparison to Cu it is highly toxic to living organisms even at very low concentrations (Chen and Yang, 2012). In spite of the fact that cadmium (Cd) and lead (Pb) are non-redox-active heavy metals without a known biological function in higher plants, numerous studies have shown that ROS generation is a main component of their toxicity in plant cells (Sharma and Dubey, 2005; Gallego et al., 2012). These heavy metals may activate ROS generation either through the activation of ROS-generating enzymes, such as NADPH oxidase (NOX) and peroxidase, or as a consequence of exhaustion or inhibition of antioxidative systems in plants (Sharma and Dietz, 2009). In addition, recently increasing evidence indicates the mitochondrial origin of ROS generation induced by toxic metal concentrations in plants (Keunen et al., 2011).

The aim of the present study was to analyze the role of ROS generation in the responses of barley root tip exposed to Cd, Pb, Hg and Cu stress. Previous studies based on the root growth inhibition test analysis have revealed that these metals had similar degree of toxicity to plants, causing a marked root growth inhibition even at micromolar concentrations (Naumann et al., 2007; Zelinová et al., 2014). Our results show that despite the different origin of ROS induced by these heavy metals, the rate of their generation, depending on the metal concentration, determines the development of typical mild (low metal concentrations) stress-activated morphogenic changes or severe (high metal concentrations) stress-caused cell death within a few minutes after the exposure of roots to metals. While under mild stress ROS generation has a linear, under severe stress a biphasic character.

2. Materials and methods

2.1. Plant material and growth conditions

Barley seeds (*Hordeum vulgare* L.) cv. Slaven (Plant Breeding Station, Hordeum Ltd, Sládkovičovo-Nový Dvor, Slovakia) were imbibed in distilled water for 15 min followed by germination between two sheets of filter paper (density 110 g/m^2) moistened with distilled water in Petri dishes at 25 °C in darkness. The uniformly germinating seeds, 20 h after the onset of seed imbibition, were arranged into rows between two sheets of filter paper moistened with distilled water in rectangle trays. The trays were placed in a vertical position and moistened through the filter paper wick from the reservoir with distilled water. For short- term treatment, seedlings with approximately 4 cm long roots were used 60 h after the onset of seed imbibition.

2.2. Short-term treatments

During the short-term treatments, roots were immersed into distilled water (dw; control) or into appropriate test solutions, such as 10, 20, 30 or 60 μM $CdCl_2$; 5, 10, 15 or 20 μM $CuCl_2$; 25, 50, 100 or 200 μM $PbCl_2$; 10, 25, 50 or 100 μM $HgCl_2$; or in combination with 0.5 μM diphenyleneiodonium – DPI (10 mM stock in DMSO, the final concentration of DMSO was 0.1%), or 5 μM rotenone (4 mM stock in methanol, the final concentration of methanol was 0.25%) for 30 min. KCN at 1 mM concentration was used for the pre-treatment of roots for 10 min before the different metal treatments. Following the rinse in dw for 5 min, the seedlings were immediately used for analysis (0 h – without incubation after the short-term treatments; see Fig. 2) or were incubated between two sheets of filter paper moistened with distilled water in the vertically oriented trays as described above for 1, 2, 3, 6 or 9 h. For the analysis the longest root for root length measurement and the two longest roots for ROS and cell death measurement and localization of each seedling were used.

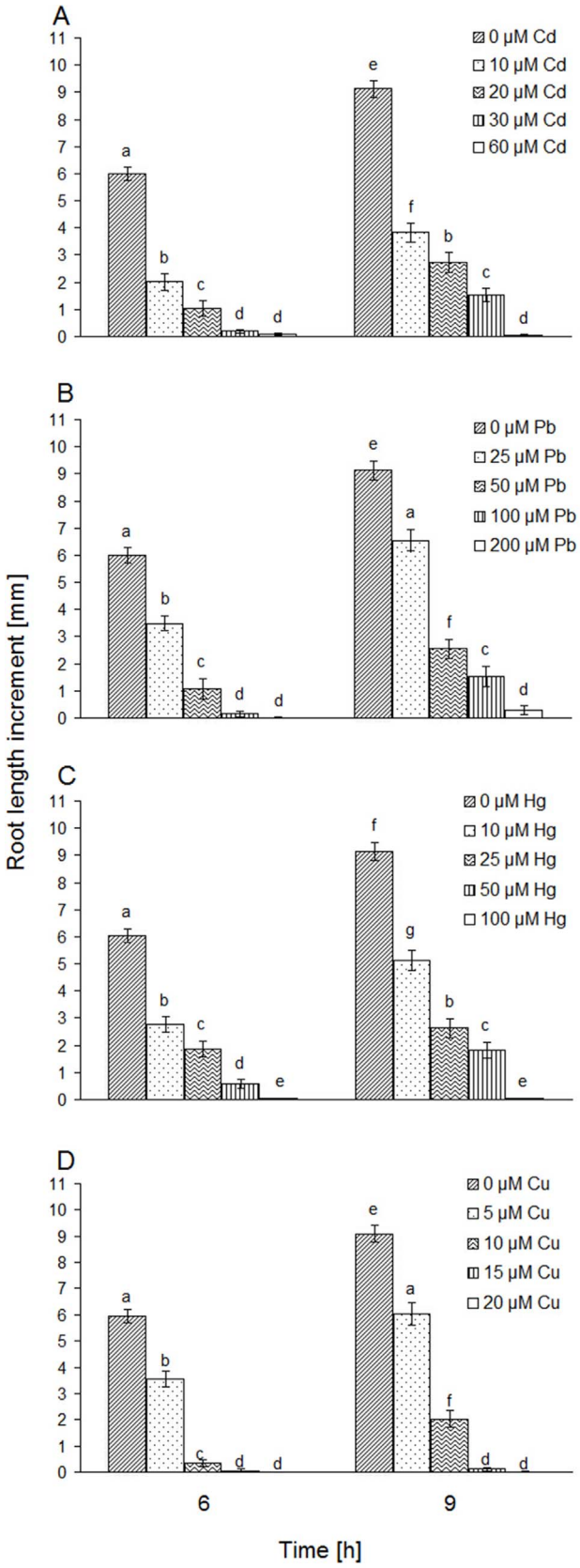

Fig. 1. Root length increments 6 and 9 h after the short-term treatment of roots with 0, 10, 20, 30 or 60 μM Cd (**A**); 0, 25, 50, 100 or 200 μM Pb (**B**); 0, 10, 25, 50 or 100 μM Hg (**C**); and 0, 5, 10, 15 or 20 μM Cu (**D**); for 30 min. Mean values ± SD (n = 5). Different letters indicate statistical significance according to Tukey's test (P < 0.05).