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Research article

Impact of cadmium stress on two maize hybrids

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ABSTRACT

Some physiological parameters and composition of the root cell walls of two maize hybrids (monocots), the sensitive Novania and the tolerant Almansa were studied after treatment with cadmium cations. After 10 days of Cd^{2+} treatment (1×10^{-5} M and 5×10^{-5} M), plant growth inhibition, in the sensitive hybrid in particular, as well as a certain alteration in root structure and pigment content were observed. The Cd^{2+} accumulation was ten times higher in the roots than in the shoots. Chemical analyses and atomic absorption spectroscopy proved that Cd^{2+} modified the composition of the root cell walls by a significant increase in the content of alkali-soluble polysaccharide fractions, particularly in the tolerant hybrid. An increase in the content of phenolic compounds, mainly in the tolerant hybrid, and a decrease in protein content were observed in the presence of Cd^{2+} in the alkali fractions. The results indicate that the changes in the cell wall polysaccharide fractions and their proportion to lignin and cellulose are obviously involved in the tolerance and/or defence against Cd^{2+} of the maize hybrids studied.

1. Introduction

Plants, as non-moving organisms, have to resolve unfavourable changes (e.g. drought, soil salinity, organic pollutants, and toxic metals) in their imminent environment in specific ways. In the case of toxic metal stress plants defend by sequestering heavy metals in specific organelles segregated from vital cellular parts, or by the synthesis of enzymes involved in detoxification (Parrotta et al., 2015). One of the protective mechanisms against toxic metals is thought to be related to the plant cell wall. There is a wide diversity in species specific responses, e.g. between non-hyperaccumulating ecotype and hyperaccumulator in which the hyperaccumulation is partly regulated by cell-wall polysaccharide modification in roots (Li et al., 2015). To understand how different taxons are responding to toxic metals, it is crucial to compare the chemical composition and structure of cell walls from different species, hybrids, clones. This information might shed light on alternative resistance mechanisms and on how to improve the tolerance to Cd (Parrotta et al., 2015).

The cell wall of terrestrial plants is a structural complex, extensively cross-linked and metabolically dynamic that contains heteromolecular assemblies composed of polysaccharides, phenolic compounds, and proteins. Its functions include the provision of mechanical support to individual cells and to the plant as a whole, control of cell growth, and regulation of cell and tissue differentiation. As the first barrier, the plant cell wall protects the cells and plant organs against environmental impact, e.g. invading pathogens or toxic metals. The structure of primary cell walls in grasses, including maize, varies in comparison with all other plants in the content of non-cellulosic polysaccharides which interlace and cross-link the cellulose microfibrils to form a strong framework (Carpita, 1996). Differences also pertain in the amount of phenolic compounds and structural proteins that covalently cross-link the primary and secondary walls and lock cells into shape.

Cadmium, a dangerous pollutant, is a divalent cation with a high affinity to cell walls. Cations binding to the apoplast provide protection against Cd-induced oxidative damage to cells. It has been confirmed that most cells accumulate divalent cations mainly in the apoplast (Redjala et al., 2009; Xu et al., 2011). However, the cell walls function not only as a sink for toxic metal accumulation, they are also modified under metal stress (Krzeslowska, 2011). Cd cations induce alterations in cell wall compartments. Little information is currently available on the composition of cell wall polysaccharides after abiotic stress. Studies have focused on the isolation of cell wall polysaccharides such as pectin, hemicelluloses and cellulose. Dicots (cell walls type I) with a high proportion of pectin (around 20–35%) often serve as model systems (O'Neill and York, 2003). Pectin, an anionic plant cell wall polysaccharide composed of D-galacturonic acid, is the main binding site of divalent and trivalent cations with very good cation exchange capacity

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(Schiewer and Patil, 2008). By contrast, in grasses the amount of pectin is low (around 5%) (O'Neill and York, 2003) and its function is partially replaced with another acidic polysaccharide - glucuronoarabinoxylan (GAX) (Kim and Carpita, 1992). GAX consists of a D-xylose backbone with L-arabinose and D-glucuronic acid as side-chains. It has been estimated that 70–90% of the total charge of cells is provided by pectin or GAX. The sorption of cations by the cell walls depends on several factors, e.g. the cations' exchange capacity of the cell wall, the affinity of the binding cations to the cell wall, ionic strength, and pH of the cell wall (Wehr et al., 2010).

Maize (*Zea mays* L.), a member of monocots of the order Poales, is a fast-growing plant with a rich root system, hence a good model for studying grass cell walls. As a food crop, information on cations binding to cell walls can be useful not only for the phytoremediation of soils, but also for the fortification of plants with various essential divalent cations, for example, selenium. The present study sought to link the physiological state and root structure of the plant with the root cell wall composition under Cd^{2+} stress. Two maize hybrids, Novania and Almansa, characterised by Lukačová-Kuliková and Lux (2010) as hybrids sensitive and tolerant to toxic metals, were studied to obtain more information about the distribution and effects of Cd^{2+} and to better understand the changes which could be crucial for the defence mechanism and plant tolerance in this monocot.

2. Materials and methods

2.1. Plant material, sterilisation and germination

Maize (*Zea mays* L.) seeds, Novania and Almansa (sensitive and tolerant hybrids to toxic metals) (Lukačová-Kuliková and Lux, 2010) were obtained from Sempol, Bratislava- Podunajské Biskupice, Slovakia. Prior to germination, the seeds were immersed in a 10% solution of JAR, a detergent with anion-active components' content of 5–15% (10 min), rinsed under running tap water (30 min), the surface decontaminated in 10% NaClO (10 min), and rinsed under running tap water. After 3 h of imbibition, the seeds were germinated on wet Perlite for 3 days in the dark at 25 °C and 70% ambient humidity.

2.2. Plant cultivation

After germination, the plants were cultivated for 10 days in Hoagland solution (Hoagland and Arnin, 1950) at pH 6.8 (control), or in the same solution supplemented with 1×10^{-5} M and 5×10^{-5} M $Cd(NO_3)_2$, under photosynthetic photon flux of 130–140 μmol m^{-2} s^{-1}, at an ambient humidity of 70%, and a 16-h photoperiod. The Cd^{2+} concentrations were chosen on the basis of previous experiment where the lower concentrations didn't show unambiguous impact on cells, mainly on cell walls (unpublished results). The length of the primary roots and shoots was measured. Part of the material was used for determination of the photosynthetic pigments and for detection of apoplasmic barriers. The remaining material was dried at 40 °C for 10 days for cell wall analyses.

2.3. Plant growth parameters and pigments analysis

After 10 days of cultivation, the plant material was harvested, washed 3 times with distilled water and the following growth parameters: root length and shoot length, root and shoot fresh and dry mass and content of photosynthetic pigments were determined.

The Cd tolerance indices expressed as the stress index of R_{LMAX} (relative maximum of root length), R_{RDM} (relative root dry mass), R_{SL} (relative shoot length), R_{SDM} (relative shoot dry mass), R_{CHLA} (relative content of chlorophyll *a*), R_{CHLB} (relative content of chlorophyll *b*), R_{CAR} (relative content of carotenoids) were calculated as a ratio of a respective parameter obtained under Cd treatment and without Cd treatment (control).

The photosynthetic pigments were extracted using 80% (v/v) acetone and their concentrations were determined spectrophotometrically (Chl *a* at 663 nm, Chl *b* at 646 nm, and carotenoids at 470 nm) following the method detailed by Lichtenthaler (1987).

2.4. Apoplasmic barriers visualisation

Cross-sections of the primary roots, from the apex to the base in equal distances (each 2.5% of the whole root length), were prepared for microscopic observations. A solution of 0.2% berberine hemisulphate was used for visualisation of the Casparian strips, and a solution of 0.2% Fluoride Yellow 088 for visualisation of suberin lamellae (Zelko et al., 2012). Samples were examined using a Leica DMI3000 B inverted microscope fitted with an exciting filter BP 450–490 nm, dichromatic mirror 510 nm and emission filter LP 515 nm. The distance from the root apex to the point with the first appearance of the Casparian strips/suberin lamellae, expressed as a percentage of the root length (Redjala et al., 2011) was examined in five samples of each hybrid in each experimental group.

2.5. Isolation of cell walls

10 g of dry root material for cell walls isolation was homogenised using X-Press (−25 °C, 100 kPa). The disintegrated cells were filtered through Miracloth. The cell wall fraction was subsequently lyophilised.

2.6. Polysaccharides isolation

For isolation of the polysaccharides, a method proposed by Capek et al. (2000) with modifications was used. The lyophilised cell walls were pre-extracted for 6 h in a Soxhlet apparatus using chloroform: methanol (2:1). From the resulting cell walls and 50 mM phosphate buffer with α-amylase, a 0.5% suspension was prepared under stirring at ambient temperature for 24 h. Subsequently, the suspension was filtered through an S4 frit, dialysed and lyophilised. The extractives-free and de-starched root cell walls were subjected to a three-step extraction procedure using hot water, 1 M NaOH and 4.2 M KOH as extracting agents (Fig. 1). All the polysaccharide fractions (PS I - PS IV) were characterised by yield, monosaccharides composition, the content of proteins, phenolic compounds, and Cd^{2+}. PS V denotes the residue from the PS IV fraction.

2.7. Analytical methods

The nitrogen content (% N) in the cell walls and in the PS V fraction was determined by elementary analysis using EA 1108 apparatus (FISONS Instruments, UK). The protein content was calculated as % N × 6.25.

The content of proteins in the PS I - PS IV fractions was determined using the method by Bradford (1976).

Klason lignin was determined by the method devised by Schwanninger and Hinterstoisser (2002).

Total phenolic compounds content was determined using a Folin-Ciocalteu agent and gallic acid as standard (Thaipong et al., 2006).

The cadmium and calcium cations contents in the cell walls and in PSs were determined by atomic absorption spectrometry (Perkin Elmer 1100 spectrometer, USA) at the Geological Institute, Faculty