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Galactoglucomannan oligosaccharides alleviate cadmium stress in *Arabidopsis*

Danica Kučerová[a,b], Karin Kollárová[a,*], Ivan Zelko[a], Zuzana Vatehová[a], Desana Lišková[a]

[a] Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia
[b] Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 9, 845 23 Bratislava, Slovakia

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SUMMARY

Our study focused on the mediatory role of galactoglucomannan oligosaccharides (GGMOs) in plant protection against cadmium stress, examined mainly on the primary root growth of *Arabidopsis thaliana*. The application of GGMOs diminished the negative effect of cadmium on root length, root growth dynamics and also on photosynthetic pigment content. We tested the hypothesis that the effect of GGMOs is associated with decreased cadmium accumulation or its modified distribution.

Cadmium distribution was observed chronologically from the first day of plant culture and depended on the duration of cadmium treatment. First, cadmium was stored in the root and hypocotyl and later transported by xylem to the leaves and stored there in trichomes. The protective effect of GGMOs was not based on modified cadmium distribution or its decreased accumulation. In cadmium and GGMOs + cadmium-treated plants, the formation of suberin lamellae was shifted closer to the root apex compared to the control and GGMOs. No significant changes between cadmium and GGMOs + cadmium variants in suberin lamellae development corresponded with any differences in cadmium uptake.

GGMOs also stimulated *Arabidopsis* root growth under non-stress conditions. In this case, suberin lamellae were developed more distantly from the root apex in comparison with the control. Faster solute and water transport could explain the faster plant growth induced by GGMOs.

Our results suggest that, in cadmium-stressed plants, GGMOs' protective action is associated with the response at the metabolic level.

Introduction

Heavy metal pollution is a serious and complex problem linking different environmental components. Soil and water contamination lead to plants growing in stressful conditions and accumulating toxic metals in their tissues (Clemens, 2006). This may cause human health problems (Järup and Åkesson, 2009), mostly as a consequence of the heavy metals entering the food chain. Among heavy metals, cadmium is the element of major concern, not only because of its widespread occurrence, but also because of its high toxicity and bioavailability for plants (Prokop et al., 2003). Considering the fact that phosphate fertilizers constitute a significant source of cadmium pollution (Chen et al., 2007), the level of cadmium is likely to be elevated in agricultural soils. Cadmium from soil is readily absorbed by roots, where it may cause growth inhibition and then be transported from roots to aerial plant parts (Clemens, 2006). Studies on cadmium uptake and distribution are essential to understanding the mechanisms involved in cadmium tolerance, transportation and accumulation. When cadmium is absorbed by plants, it affects the water balance, photosynthetic apparatus and causes leaf chlorosis, oxidative stress, and the inhibition of stomata opening (Hasan et al., 2009). Root growth inhibition is one of the most visible and common symptoms of its phytotoxicity. This characteristic has been observed in almost all cadmium reports; however, more detailed studies in this field are rare.

With regard to heavy metal toxicity, the development of approaches to clean up the soil, as well as strategies to decrease the accumulation and alleviate the damaging effects on plants, has become the object of interest (Sarwar et al., 2010). In addition to well-known, useful, but time-consuming methods such as breeding new and more resistant cultivars to be grown in contaminated soils, other approaches have also appeared. Preliminary studies are focused on the application of substances able to alleviate heavy metal stress. Among them, in the case of cadmium stress, are signaling molecules – salicylic acid (Belkadhi et al., 2008), polyamines (Hsu and Kao, 2007), plant nutrients (Sarwar et al., 2010), antioxidants – *N*-acetyl-L-cysteine (Deng et al., 2010), inorganic compounds – silicon (Vaculík et al., 2012), plant hormones (Munzuroglu and Zengin, 2006), and also biologically active oligosaccharides derived from bacteria (Ma et al., 2010).

* Corresponding author. Tel.: +421 2 59410265; fax: +421 2 59410222.
E-mail addresses: kollarova.sav@gmail.com, Karin.Kollarova@savba.sk (K. Kollárová).

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Biologically active oligosaccharides represent a group of regulatory molecules serving as signaling molecules in various processes of plant growth and development (Aldington et al., 1991; Ochoa-Villarreal et al., 2012) and playing a role in plant-adaptive responses to abiotic and biotic stress (Van den Ende and Valluru, 2009; Zabotin et al., 2009). Our research is focused on a specific class of oligosaccharide-signaling molecules, galactoglucomannan oligosaccharides (GGMOs), derived from spruce galactoglucomannan (Karácsonyi et al., 1996; Capek et al., 2000). Galactoglucomannans are structural constituents of both the primary and secondary cell walls of higher plants (Kubačková et al., 1992; Lundqvist et al., 2002). Galactoglucomannan polysaccharides may fulfill a role comparable to that of xyloglucans, impacting flexibility and forming growth-constraining networks with cellulose (Schröder et al., 2004). Galactoglucomannans have attracted a great deal of interest because of their possible applications as an oxygen barrier film in packaging materials, as a hydrogel in biomedical products and as an emulsion stabilizer in food and feed (Krawczyk and Jönsson, 2011). Zhao et al. (2013) found that a *Populus* endo-1,4-β-mannanase gene, *PtrMAN6*, which suppresses cell wall thickening during xylem differentiation, catalyzes the hydrolysis of mannan-type wall polysaccharides to produce GGMOs. GGMOs in turn serve as signaling molecules to regulate the transcriptional program of cell wall thickening. Exogenously added GGMOs regulate xylem cell differentiation (Beňová-Kákošová et al., 2006; Richterová-Kučerová et al., 2012; Kákošová et al., 2013). GGMOs influence several other processes, such as elongation growth in various plants (Auxtová et al., 1995; Lišková et al., 1999; Kollárová et al., 2007, 2009; Richterová-Kučerová et al., 2012), as well as cell division in *Zinnia* xylogenic culture (Beňová-Kákošová et al., 2006), and regeneration of isolated protoplasts (Kákoniová et al., 2010). Experimental evidence indicates that their action could be based on the interaction with auxin (Auxtová-Šamajová et al., 1996). Exogenously applied GGMOs behave as auxin antagonists in both IBA (indole-3-butyric acid)-stimulated and -inhibited elongation growth (Kollárová et al., 2010). Their action is connected with changes in peroxidase activity (Kollárová et al., 2009, 2010). GGMOs are known as inhibitors of TNV virus infection (Slováková et al., 2000). They induce resistance manifested by the decrease of local lesions. Moreover, with the exception of plant growth and resistance, GGMOs are assumed to have a positive effect on human health because of their potential prebiotic activity (Willför et al., 2008; Polari et al., 2012).

In this study, we hypothesized the potential of GGMOs to protect *Arabidopsis* plants against cadmium stress. The possible effects of GGMOs on root growth, root growth dynamics, and photosynthetic pigments content, as cadmium toxicity markers, were investigated. The second hypothesis was that GGMOs' action in the presence of cadmium is connected with changes in cadmium accumulation, uptake or distribution in *Arabidopsis* plants.

Methods

Plant material and growth conditions

Surface decontaminated *Arabidopsis thaliana* (*Ler*) seeds were placed in Petri dishes on MS medium (Murashige and Skoog, 1962) supplemented with or without $Cd(NO_3)_2$ (10^{-4} M), GGMOs (10^{-9} M), or GGMOs (10^{-10} M) + $Cd(NO_3)_2$ (10^{-4} M). The most effective concentrations of GGMOs (chosen on the basis of previous experiments – data not shown) were used. Two days after stratification at 4 °C in the dark, the dishes were transferred to a growth chamber at 24 ± 1 °C, 60% relative humidity, under a 16 h photoperiod, at irradiance of 50–60 μmol m^{-2} s^{-1}, in sterile conditions for 7/21 days. The length of the primary roots was determined after 7/21 days of cultivation.

Preparation of galactoglucomannan oligosaccharides (GGMOs)

GGMOs were obtained from spruce galactoglucomannan (composed of galactose, glucose and mannose in a 1:8:33 mol proportion) by partial acid hydrolysis, as described previously (Capek et al., 2000). The hydrolyzed mixture was separated on a column of Bio-Gel P2 into nine distinct fractions. Their degree of polymerization was identified by comparison with elution volumes of standard malto-oligosaccharides (Serva, Heidelberg, Germany). GGMOs (degree of polymerization 4–8) were combined and freeze-dried. Compositional analysis of the GGMOs revealed the presence of galactose (4.5%), glucose (21.1%), mannose (70.4%) and trace amounts of pentoses, xylose and arabinose. The oligosaccharides mixture was composed of tetramers (46%), pentamers (28%), hexamers (12%), heptamers (9%) and octamers (5%). Their number-average molecular mass (M_n) was calculated to be 827.

Primary root growth dynamics

The primary root growth dynamics were measured by scoring the position of the root apex on the back of the Petri dish once per day (for the first 7 days). The root growth dynamics were determined according to Hlinková (1991). The growth index (RI_j) and growth dynamics (ΔRI) were determined: $RI_j = (l_j - l_0)/l_0$, $\Delta RI_j = RI_j - RI_{j-1}$; RI_j – growth index over a certain time; $j = 1, 2, \ldots 7$ days; l_j – length of the primary root on j-th day, l_0 – length of the primary root at the beginning of the experiment.

Determination of photosynthetic pigments

Shoots of 21-day-old plants were collected (0.06 g of fresh weight per treatment) and photosynthetic pigments were extracted with 80% (v/v) acetone. Their concentrations were determined spectrophotometrically (Chl *a* at 663.2, Chl *b* at 646.8, and carotenoids at 470.0 nm), according to Lichtenthaler (1987). Chlorophyll and carotenoid absorption in the extract was measured using Libra S6 spectrophotometer (Biochrom Ltd, Cambridge).

Inductively coupled plasma mass spectroscopy (ICP-MS) analysis

The 21-day-old plants were collected. Their roots and shoots were rinsed thoroughly with distilled water. The samples were oven-dried at 40 °C for 7 days. Each sample was hydrolyzed in concentrated nitric acid in a PTFE pot of ZA-1 equipment. The samples were then boiled (160 °C) for 5 h. Afterwards, refrigerated samples were supplemented with deionized water. The Cd content of each sample was determined using ICP-MS (Perkin Elmer Elan 6000), with ^{111}Cd and ^{114}Cd isotopes and Rh as inner standard.

Cadmium staining

Cadmium staining in plant tissues (Cd and GGMOs + Cd treatment) was performed on days 1, 3, 5 and 7 of cultivation by histochemical techniques, according to Seregin and Ivanov (1997), with some modifications. In our work, whole plants were stained and vacuum infiltration of the dithizone solution (0.25 mg/ml) was used. To verify the accuracy of the method, control plants, as a negative control, were stained in every experiment. For positive control, 7-day-old plants of the cadmium hyperaccumulator *Noccaea caerulescens* (formerly *Thlaspi*), growing in the presence of