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Impact of galactoglucomannan oligosaccharides and Cd stress on maize root growth parameters, morphology, and structure

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ABSTRACT

Biologically active oligosaccharides, including galactoglucomannan oligosaccharides (GGMOs), affect plant growth and development. The impact of GGMOs is dependent on their concentration, and the plant species and plant parts affected. The aim of this article is to ascertain the effects of GGMOs, GGMOs + Cd^{2+}, on growth parameters, morphology, and the structure of maize (*Zea mays* L.) roots. We undertook this research because, in monocots, the effect of these oligosaccharides is so far unknown. In our study, GGMOs stimulated primary root elongation, induction and elongation of lateral roots, and biomass production. Their effect was dependent on the concentration used. Simultaneously, GGMOs moderated the negative effect of Cd^{2+} on root elongation growth. Besides, GGMOs affected the primary root structure, proven in the earlier development of xylem and Casparian bands, but not of suberin lamellae (compared to the control). The presence of Cd^{2+} shifted the apoplasmic barriers closer to the root apex in comparison to samples treated with GGMOs + Cd^{2+}. GGMOs do not inhibit Cd uptake into the root directly, but they moderate its effect, and therefore their influence at the structural and metabolic level seems possible. Their positive impact on plant vitality, even in contaminated conditions, strongly indicates their potential application in remediation technologies.

1. Introduction

The plant cell surface is protected by the cell wall, a dynamic structure, which beside this function participates in plant growth and development and in various defence processes, often as a source of signalling molecules (Albersheim et al., 1983; Cabrera et al., 2013; Field, 2009; Fry, 2004; Larskaya and Gorshkova, 2015). These oligosaccharidic derivatives of the cell wall polysaccharides can have a significant effect on gene expression related to cell wall metabolism; plant growth and development; stress responses evoked by biotic (Aziz et al., 2004; Laporte et al., 2007; Rasul et al., 2012) and abiotic factors (Ma et al., 2010; Zong et al., 2017; Zou et al., 2015); cell division and transcriptional control (González-Pérez et al., 2014). The regulation roles of these molecules are dependent on their degree of polymerization, their chemical composition and structure (Fry, 1989; McDougall and Fry, 1989; Ochoa-Villarreal et al., 2012).

Galactoglucomannan is one of the hemicellulosic components of the plant cell wall and, is apart from its other functions, a source of signalling molecules – galactoglucomannan oligosaccharides (GGMOs) thought to be involved in the regulation system of plants (Willför et al., 2008). These oligosaccharides stimulate or inhibit the above-mentioned processes in plants, depending on their concentration, the plant species, plant organ or cell ontogeny, and their interaction with plant hormones (Beňová-Kákošová et al., 2006; et al., 2005, 2006, 2009; ; Kollárová et al., 2009). They also reduce or moderate the negative impacts of biotic (TNV virus) (Slováková et al., 2000) and abiotic (Cd^{2+}) (Kučerová et al., 2014) stresses on plants, e.g. the reduction of elongation growth, the activity of enzymes, or the content of photosynthetic pigments. Therefore their utilization in certain phytoremediation techniques appears possible. The effects of GGMOs have until now been investigated in various dicots and gymnosperms (Auxtová et al., 1995; Kollárová et al., 2010), but not in monocots – including grasses. The latter plants are typified by their unique cell wall composition, different from all other plant species. The cell wall structure of grasses is characterized mainly by a lower pectin and xyloglucan content, a higher content of some hemicellulosic polymers (xylans, β-glucans) (Carpita and McCann, 2000) and so, to a certain extent, it is possible that these plants behave distinctly to exogenously applied GGMOs.

The aim of this paper is to ascertain the response of maize (*Zea mays* L.) plants, a representative of grasses, to treatment by GGMOs, expressed in biomass production, root growth parameters, root structure, and their reaction to the presence of cadmium cations.

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2. Material and methods

2.1. Galactoglucomannan oligosaccharides

GGMOs were prepared by partial acid hydrolysis of galactoglucomannan from spruce (*Picea abies* (L.) H. Karst.) cell walls according to Capek et al. (2000). The molar ratio of monosaccharides – galactose, glucose, and mannose – in the mixture of GGMOs was 1: 8: 33 and the average molecular weight was 827. The mixture of GGMOs, d.p. (degree of polymerisation) 4–8, was composed of consecutive oligomers: tetramers – 46%, pentamers – 28%, hexamers – 12%, heptamers – 9%, and octamers – 5%. All other chemicals and reagents were obtained from Sigma-Aldrich Co. (St. Louis, USA) and Slavus (Bratislava, Slovakia).

2.2. Plant material and cultivation

Maize seeds (*Zea mays* L., Novania, a hybrid sensitive to toxic metals), obtained from RWA Slovakia, spol. s r.o., Bratislava, were sterilised in 10% solution of the detergent JAR, a detergent with an anion-active component content of 5–10% (for 10 min). The seeds were thereafter rinsed in tap water (30 min), immersed in 0.47% sodium hypochlorite (SAVO) for 10 min, once more rinsed in tap water (30 min), and subsequently imbibed for 3 h in tap water in the dark at 25 °C. Then the seeds were placed onto perlite damped with distilled water and germinated for 3 days in the dark at 25 °C. After 3 days the length of the roots was measured. The plants were transferred to dark vessels which contained Hoagland solution (Hoagland and Arnon, 1950) enriched with GGMOs in various concentrations: 10^{-10}, 10^{-9}, 10^{-8}, and 10^{-7} M. In the experiment focused on the interactions between GGMOs and Cd^{2+} on plant growth and development, $Cd(NO_3)_2 \cdot 4H_2O$ in 5×10^{-5} M and GGMOs in 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M concentrations were added to the Hoagland solution. As a control, pure Hoagland solution was used. In each variant, the pH was adjusted to 6.2 and the solution was aerated throughout the whole cultivation. The plants were cultivated in a versatile environmental test chamber (MRL-351H, Sanyo Electric Co, Ltd., Osaka, Japan) at 25/20 °C, 16 h photoperiod, light intensity 130–140 μmol m^{-2} s^{-1}, and 70% air humidity.

2.3. Growth parameter determination

After 7 days of culture in hydroponics, the total length of the primary root and the length of the branched part of the primary root were evaluated. The number and length of lateral roots was determined. After these measurements, the fresh mass was weighed and dry mass was prepared by dehydration at 105 °C and likewise measured. Each experimental group consisted of 15 samples and each experiment was repeated at least three times.

2.4. Monitoring of Casparian bands, suberin lamellae, and xylem development

In primary roots, Casparian bands, suberin lamellae, protoxylem, early and late metaxylem were evaluated. The formation of Casparian bands, protoxylem, and early metaxylem were followed, up to 16% of the root length, in 2% length sections (Redjala et al., 2011; Vaculík et al., 2009). The formation of late metaxylem and suberin lamellae was observed from 10 to 100% of the root length in 5% length sections. For the visualization of protoxylem, early and late metaxylem staining with phloroglucinol (10 s.) was performed. The samples were rinsed in distilled water and then 36% HCl, for 5 s., was added (Vaculík et al., 2012). The samples were followed in bright field with the use of a DMI3000 B microscope (Leica Microsystems CMS GmbH, Wetzlar, Germany). For the visualization of Casparian bands stained with 0.2% berberine hemisulphate (60 min.) (Brundrett et al., 1988; Lux et al., 2005) and for the visualization of suberin lamellae stained with 0.2% Fluorol Yellow 088 (30 min), the methods of Brundrett et al. (1991) and Lux et al. (2005) were used. The staining was performed in the dark. Afterwards, the samples were rinsed in distilled water and stained with 0.025% toluidine blue (1 min). Then the samples were rinsed in distilled water and after in 0.1% $FeCl_3$ dissolved in 50% glycerol. For sample observations, an inverted fluorescent DMI3000 B microscope (Leica Microsystems CMS GmbH, Wetzlar, Germany) with excitation filter BP 450–490 nm, dichromatic mirror 510 nm, and emission filter LP 515 nm, was used. Measurement of the samples of each variant was repeated nine times.

2.5. Determination of cadmium and calcium cations content

The content of Cd^{2+} and Ca^{2+} in maize roots was determined in dry pulverized roots (200–300 mg from each of three repetitions) by flame atomic absorption spectroscopy (AAS) (PerkinElmer #1100, PerkinElmer, Boston, USA) in the Geoanalytical Laboratories, FNS, at Comenius University in Bratislava. The total content of Cd^{2+} and Ca^{2+} was calculated as their concentration (μg g^{-1}) in root mean dry mass.

2.6. Statistics

Each experimental group, consisting of 15 samples, was characterized by basic statistical parameters: mean value ± standard error (SE) of three separate experiments, when not stated otherwise. The data were analysed by analysis of variance (ANOVA). The differences between separate experimental groups were evaluated by LSD test (least significant difference) at $P < 0.05$, using the Statistica, statistical program, Version 9.1, Series 1009 (StatSoft, Tulsa, USA).

3. Results

3.1. Growth parameters of maize roots in the presence of GGMOs

Roots of 7 days old maize seedlings were at first characterized by growth parameters in the presence of GGMOs in various concentrations, because the effect of these oligosaccharides on monocots is as yet unknown. It was ascertained that GGMOs, in this case, affect the elongation growth of the primary root, and the induction and elongation of lateral roots. The stimulation of the elongation growth of the primary root was ascertained, at all concentrations of GGMOs used, with the highest values at the 10^{-9} M concentration (Table 1). The size of the primary root zone with induced lateral roots was significantly larger at two concentrations of GGMOs (10^{-10} M and 10^{-9} M), with a higher effectivity at the latter concentration. The stimulation impact of GGMOs on lateral root formation was established at all concentrations of GGMOs, followed by the lowest stimulation effect at the lowest (10^{-10} M) and the highest (10^{-7} M) concentrations. The highest stimulation of lateral root formation was noted at the 10^{-9} M concentration. The elongation of lateral roots was affected again mainly at the 10^{-9} M and 10^{-8} M concentrations, but the differences between them were statistically not significant. The differences between the control and the remaining concentrations of GGMOs were also not significant.

The fresh mass of roots increased significantly in the presence of all concentrations of GGMOs, except for the highest one. The dry mass was also positively affected by GGMOs. Again, both fresh and dry masses reached the highest values at the 10^{-9} M concentration (Table 1). This is supported by the root formation and elongation growth results mentioned previously.

For a detailed study of the impact of GGMOs on lateral root induction and elongation, the roots were sorted into three groups – short (< 10 mm), middle-long (10–40–mm), and long (> 40 mm). The GGMOs affected not only the induction of lateral roots, because of the high number of short roots, but simultaneously they stimulated their