Journal of Hazardous Materials 263 (2013) 398–403

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Exogenous auxin alleviates cadmium toxicity in *Arabidopsis thaliana* by stimulating synthesis of hemicellulose 1 and increasing the cadmium fixation capacity of root cell walls

Xiao Fang Zhu[a,b], Zhi Wei Wang[a], Fang Dong[b], Gui Jie Lei[b], Yuan Zhi Shi[c], Gui Xin Li[d,*], Shao Jian Zheng[a,b]

[a] *Key Laboratory of Conservation Biology for Endangered Wildlife of the Ministry of Education, College of Life Sciences, Zhejiang University, Hangzhou 310058, China*
[b] *State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou 310058, China*
[c] *The Key Laboratory of Tea Chemical Engineering, Ministry of Agriculture, Yunqi Road 1, Hangzhou 310008, China*
[d] *College of Agronomy and Biotechnology, Zhejiang University, Hangzhou 310058, China*

HIGHLIGHTS

- Cd reduces endogenous auxin levels in *Arabidopsis*.
- Exogenous applied auxin NAA increases Cd accumulation in the roots but decreases in the shoots.
- NAA increases cell wall hemicellulose 1 content.
- Hemicellulose 1 retains Cd and makes it difficult to be translocated to shoots.
- NAA rescues Cd-induced chlorosis.

ARTICLE INFO

Article history:
Received 8 June 2013
Received in revised form 7 September 2013
Accepted 10 September 2013
Available online 17 September 2013

Keywords:
Arabidopsis
Auxin
Cd^{2+} stress
Cell wall
Hemicellulose 1
Root

ABSTRACT

Auxin is involved in not only plant physiological and developmental processes but also plant responses to abiotic stresses. In this study, cadmium (Cd^{2+}) stress decreased the endogenous auxin level, whereas exogenous auxin (α-naphthaleneacetic acid, NAA, a permeable auxin analog) reduced shoot Cd^{2+} concentration and rescued Cd^{2+}-induced chlorosis in *Arabidopsis thaliana*. Under Cd^{2+} stress conditions, NAA increased Cd^{2+} retention in the roots and most Cd^{2+} in the roots was fixed in hemicellulose 1 of the cell wall. NAA treatment did not affect pectin content and its binding capacity for Cd^{2+}, whereas it significantly increased the content of hemicellulose 1 and the amount of Cd^{2+} retained in it. There were highly significant correlations between Cd^{2+} concentrations in the root, cell wall and hemicellulose 1 when the plants were subjected to Cd^{2+} or NAA + Cd^{2+} treatment for 1 to 7 d, suggesting that the increase in hemicellulose 1 contributes greatly to the fixation of Cd^{2+} in the cell wall. Taken together, these results demonstrate that auxin-induced alleviation of Cd^{2+} toxicity in *Arabidopsis* is mediated through increasing hemicellulose 1 content and Cd^{2+} fixation in the root, thus reducing the translocation of Cd^{2+} from roots to shoots.

1. Introduction

Cadmium (Cd^{2+}) is highly toxic to nearly all living organisms. Upon Cd^{2+} exposure, plants display rolled and chlorotic leaves, and reduced growth [1]. Plants have evolved several mechanisms to cope with Cd^{2+} toxicity, such as cell wall binding, chelation with phytochelatins, compartmentation in vacuoles, and regulation of distribution [2]. In plants, Cd^{2+} is believed to be taken up by roots through the same plasma membrane transporters/channels used for calcium (Ca^{2+}), iron (Fe^{2+}) and zinc (Zn^{2+}) [2], although no specific Cd^{2+} transporters in root cell plasma membranes have been identified.

Phytohormones are involved in Cd^{2+} toxicity/resistance. For example, an increase of endogenous abscisic acid (ABA) content is closely related to Cd^{2+} tolerance in rice (*Oryza sativa* L.) seedlings, and exogenous application of ABA reduces their transpiration rate, decreases Cd^{2+} concentration, and enhances Cd^{2+} tolerance [3]. In addition, salicylic acid application can partially protect barley seedlings against Cd^{2+} toxicity [4]. Gibberellic acid (GA) also can reduce the accumulation of heavy metals in rice shoots and

* Corresponding author. Tel.: +86 571 88981994; fax: +86 571 8820 6438.
E-mail address: guixinli@zju.edu.cn (G.X. Li).

0304-3894/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jhazmat.2013.09.018

alleviate the detrimental effects of Cd^{2+} and Pb^{2+} on broad bean, lupin and *Arabidopsis* [5–7].

Auxin is an important phytohormone that plays critical roles in the coordination of plant growth and defense. As one of the most abundant naturally occurring auxins, indole-3-acetic acid (IAA) influences a range of basic physiological and developmental processes, such as root elongation, root gravitropism, root hair development, and lateral root formation [8–12]. The level of IAA in plant tissues has been shown to increase upon exposure to environmental stresses such as Fe^{2+} deficiency in *Arabidopsis* [13], salt stress in tomato [14], and Cd^{2+} exposure in *Glycine max* [15]. Xu et al. [16] found that Cd^{2+} stress represses IAA accumulation by increasing the activity of IAA oxidase in the roots of *Medicago truncatula* seedlings, whereas exogenous nitric oxide can alleviate Cd^{2+} toxicity by maintaining the auxin equilibrium in the plant. As synthetic auxins have been reported to be more stable than the natural auxin IAA [17], α-naphthaleneacetic acid (NAA, an analogue of IAA) has frequently been employed by researchers. NAA enters plants mainly by passive diffusion and is metabolized more slowly than IAA [18,19]. NAA can rescue Cd^{2+}/Ni^{2+} toxicity in *Zea mays* and Cd^{2+} toxicity in rice [20,21], thus demonstrating a possible application for NAA in phytoremediation of heavy metal pollution. However, the physiological basis of how auxin (as IAA or NAA) modifies Cd^{2+} accumulation, transport, and toxicity remains unclear.

In the present study, we elucidated the physiological mechanism underlying auxin alleviation of Cd^{2+} toxicity by revealing a new connection between auxin, the Cd^{2+} binding capacity of hemicellulose 1 and the translocation of Cd^{2+} to the shoots in *Arabidopsis thaliana*.

2. Materials and methods

2.1. Plant material and growth conditions

The Columbia ecotype (Col-0) of *A. thaliana* was used in this study. Seeds were vernalized at 4 °C for 2 d and germinated on a sponge soaked with nutrient solution. Then, the seedlings were transferred to pots filled with nutrient solution. The nutrient solution consisted of the following macronutrients in mM: KNO_3, 6.0; $Ca(NO_3)_2$, 4.0; $MgSO_4$, 1; $NH_4H_2PO_4$, 0.1, and the following micronutrients in μM: Fe(III)-EDTA, 50; H_3BO_3, 12.5; $MnSO_4$, 1; $CuSO_4$, 0.5; $ZnSO_4$, 1; H_2MoO_4, 0.1; $NiSO_4$ 0.1 according to Murashige–Skoog salts with some modifications [22]. The final pH was adjusted to 5.6 with 1 M HCl or 1 M NaOH. The nutrient solution was renewed weekly at the seedling stage. Seedlings were kept under controlled environmental conditions at 22 ± 2 °C, 140 μmol photons m^{-2} s^{-1} and 16/8 h day/night periods.

2.2. Determination of Cd^{2+} concentration

Experiments were carried out using six-week-old plants cultured in the above nutrient solution. For Cd^{2+} treatment assays, 50 μM $CdCl_2$ with or without 0.05 μM NAA was added to the solution. For auxin concentration gradient experiments, 50 μM $CdCl_2$ with or without 0.005, 0.01, 0.05, 0.1, or 0.5 μM NAA was added to the solution. After 7 d of treatment, seedling roots were harvested and washed twice with deionized water. For time-course experiments, root samples were collected daily through 7 d. Plant materials were digested according to Gajewska et al. [23] with some modifications. The samples were incubated in 2 mL concentrated HNO_3/$HClO_4$ (4:1, v/v) at 90 °C for 4 h. After gentle shaking, the temperature was increased to 130 °C for about 24 h until the solution became transparent. Digestates were brought to 10 mL with ultrapure water and filtered. The total metal concentrations were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Fisons ARL Accuris, Ecublens, Switzerland). To evaluate the accuracy of the analytical methods, standard solution 56 for ICP analysis (Multi-Element calibration standard GSB 04-1767-2004 from China) was used in all determinations. Blanks were also prepared to determine reagent purity. Four replicates were run for each metal analysis.

Cd^{2+} in the cell wall was extracted by 2 N HCl for 24 h with occasional shaking. Cd^{2+} concentration in the extracts was determined by inductively coupled plasma-atomic emission spectrometry as described above.

2.3. Gene expression analysis

Plants were grown under the same conditions used for Cd^{2+} concentration measurement. Root tissues were collected after 7 d treatments and immediately frozen in liquid nitrogen before total RNA extraction. Total RNA was isolated using TRIzol (Invitrogen). cDNA was prepared from 1 μg total RNA using the PrimeScript RT reagent kit (Takara). For real-time RT-PCR analysis, 1 μL 10-fold-diluted cDNA was used for quantitative analysis of gene expression performed with SYBR Premix ExTaq (Takara) with the following pairs of gene-specific primers *IRT1*: forward: 5′-CGGTTGGACTTCTAAATGC-3′; reverse: 5′-CGATAATCGACATTCCACCG-3′; *ZIP1*: forward: 5′-AGACACCATAAAGCCACTCA-3′; reverse: 5′-TTTCCTGTAGCCTAAACCAC-3′; *ZIP3*: forward: 5′-TAGGAATCGTTGTGGGAATG-3′; reverse: 5′-AGGGTGCGTGAAATCTGC-3′; *ZIP4*: forward: 5′-TCTTTCAACTCGCATAGCCC-3′; reverse: 5′-AAGCCTCAAATTACAACTCATCCT-3′.

Each cDNA sample was used in triplicate reactions. Expression data were normalized to the expression of *tubulin* (forward: 5′-AAGTTCTGGGAAGTGGTT-3′; reverse: 5′-CTCCCAATGAGTGACAAA-3′).

2.4. Cell wall extraction and fractionation

Extraction of crude cell wall materials and subsequent fractionation of cell wall components were carried out according to Zhong and Läuchli [24] with minor modifications according to Zhu et al. [1]. Roots were ground with a mortar and pestle in liquid nitrogen, homogenized with 75% ethanol for 20 min in ice-cold water, and centrifuged at 4579*g* for 10 min, after which the supernatant was removed. The pellets were homogenized and washed with acetone, methanol:chloroform (1:1), and methanol for 20 min each, with all supernatant removed after each centrifugation. The remaining pellets, i.e. the cell wall materials, were freeze-dried and stored at 4 °C for further use.

Pectin was extracted from the above-mentioned cell walls (about 2 mg) by extracting three times with 1 mL water at 100 °C for 1 h each. The supernatants were combined in a 5-mL tube after centrifugation at 16,800*g* for 10 min. Then, the pectin extracted residue was further extracted with 1 mL 4% (w/v) KOH and 0.02% (w/v) KBH_4 twice at room temperature for 12 h. The two supernatants were combined in a 2-mL tube, and centrifuged at 16,800*g* for 10 min to obtain the hemicellulose fraction 1 (HC1) supernatant.

2.5. Uronic acid and total polysaccharide measurement

The uronic acid content in pectin was assayed according to Zhu et al. [1] using galacturonic acid (Sigma) as a standard. Briefly, 200 μL of the above mentioned pectin extracts were incubated with 1 mL 98% H_2SO_4 (containing 0.0125 M $Na_2B_4O_7{\cdot}10H_2O$) at 100 °C for 5 min. After chilling, 20 μL M-hydro-dipheny (0.15%) was added to the solution and after 20 min at room temperature, the absorbance was measured spectrophotometrically at 520 nm.

The total polysaccharide contents in the hemicellulose fractions were determined by the phenol sulfuric acid method [25] and