Environmental and Experimental Botany 100 (2014) 10–19

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Localization and chemical speciation of cadmium in the roots of barley and lettuce

Mst. Fardausi Akhter[a,1], Christopher R. Omelon[b], Robert A. Gordon[c], Desmond Moser[d], Sheila M. Macfie[a,*]

[a] Department of Biology, Western University, London, ON, Canada N6A 5B7
[b] Department of Geological Sciences, The University of Texas at Austin, Austin, TX 78712-1722, USA
[c] Pacific Northwest Consortium Synchrotron Radiation Facility, Argonne National Laboratory, IL 60439, USA
[d] Department of Earth Sciences, Western University, London, ON, Canada N6A 5B7

ARTICLE INFO

Article history:
Received 28 October 2013
Received in revised form
30 November 2013
Accepted 4 December 2013

Keywords:
Cadmium
Electron spectroscopy
X-ray spectroscopy
Chemical speciation
Hordeum vulgare
Lactuca sativa

ABSTRACT

Plants have the potential to accumulate toxic amounts of cadmium (Cd), and understanding how and where Cd is stored in plants is important for ensuring food safety. Previous experiments have determined that a greater amount of Cd is translocated into the leaves of lettuce (*Lactuca sativa*) as compared to barley leaves (*Hordeum vulgare*). Preferential retention of Cd in root of barley would explain this difference. Hence, the purpose of this study was to determine the localization and coordination environment of Cd (i.e., the ligands to which Cd was bound) in the different root tissues of lettuce and barley using histochemical staining, electron microscopy and micro X-ray spectroscopy. Retention of Cd in barley roots could be explained by accumulation of Cd at the endodermis, comparatively higher amounts of Cd sequestered in the symplast of cortical cells and binding to xylem cell walls. Increased translocation of Cd to lettuce shoots seemed to be due to a less effective barrier at the endodermis and less sequestration of Cd in the cortex. Regardless of the tissue type, most of the Cd^{2+} was bound to S ligands in the roots of barley, possibly reflecting accumulation of Cd–phytochelatin and Cd–S molecules in the vacuoles. In lettuce roots, Cd was more evenly distributed among ligands containing S, O and NO_3 groups, which is indicative of proportionately more Cd binding to the cell walls, relative to barley. These results will be useful in uncovering the mechanisms of differential Cd-tolerance and sequestration in lettuce and barley.

1. Introduction

The mechanisms of cadmium (Cd) uptake and tolerance in plants have been studied extensively (reviewed in Sanità di Toppi and Gabbrielli, 1999), but a clear understanding of what controls the translocation of Cd to aboveground tissues is lacking. One approach to better understanding the factors that control Cd accumulation and distribution is to determine where Cd is bound as it travels from the root surface to aboveground parts.

For some plants, the primary defense against Cd toxicity is exclusion of Cd^{2+} from active tissues and sequestration in non-active tissues. For example, Ouariti et al. (1997) showed that 98% of total Cd was retained in the roots of *Phaseolus vulgaris* with only 2% translocated to the shoot; presumably, much of the Cd in the root was in the apoplast or the vacuoles. When comparing Cd uptake and toxicity in *Pisum sativum* and *Zea mays*, Lozano-Rodriguez et al. (1997) found that the two species had equal concentrations of Cd in their roots and shoots but *P. sativum* exhibited more severe toxicity symptoms compared to *Z. mays*. The Cd in *Z. mays* was bound to the cell walls, which could explain increased Cd-tolerance. In plants such as *Arabidopsis thaliana* (Ager et al., 2002, 2003) and the Cd-hyperaccumulator *Biscutella laevigata* (Pielichowska and Wierzbicka, 2004), some of the Cd that is translocated is ultimately sequestered and rendered non-toxic in trichomes.

To understand better the distribution of Cd within a plant, it is insufficient to measure the concentrations of Cd in bulk tissues only (e.g., root, shoot, leaf, stem, etc.). Instead, the cellular and/or subcellular distributions of Cd in plant tissues could be determined using a variety of histochemical, imaging and physical fractionation methods. Histochemical methods include using Cd-specific dyes (Seregin and Ivanov, 1997; Vollenweider et al., 2006; Vieira da

* Corresponding author at: Department of Biology, Western University, 1151 Richmond St. N., London, ON, Canada N6A 5B7. Tel.: +1 519 661 2111; fax: +1 519 661 3935.
E-mail addresses: fardausi@gmail.com (Mst.F. Akhter), omelon@jsg.utexas.edu (C.R. Omelon), ragordon@alumni.sfu.ca (R.A. Gordon), desmond.moser@uwo.ca (D. Moser), smacfie@uwo.ca (S.M. Macfie).
[1] Present address: Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada S7N 5B4.

0098-8472/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.envexpbot.2013.12.005

Cunha et al., 2008; Hu et al., 2009). However, it is possible that Cd is redistributed among and within cells during sample preparation, especially if tissue sections are immersed in an aqueous solution that could cause leaching of Cd. Images of the distribution of Cd in plants can also be obtained by using scanning electron microscopy (SEM) or transmission electron microscopy (TEM) along with energy dispersive X-ray spectroscopy (EDS, Solís-Domínguez et al., 2007; Hu et al., 2009) or energy dispersive X-ray (EDX) microanalysis (Rauser and Ackerley, 1987; Wójcik et al., 2005; Van Belleghem et al., 2007; Vázquez et al., 2007; Cocozza et al., 2008). Other imaging techniques include secondary ion mass spectrometry (SIMS, Migeon et al., 2011), micro-particle-induced X-ray emission (μ-PIXE, Ager et al., 2002; Vogel-Mikuš et al., 2008), and micro-autoradiography (Cosio et al., 2006). While the techniques listed above are able to detect Cd-specific signals in particular regions within a tissue, the low concentrations of Cd in the cells and the proximity of the emission spectra from Ca or K in the sample can make analysis difficult. In addition, unless tissue sections are fixed chemically prior to dehydration and embedding, Cd could be redistributed during sample preparation. Others have used subcellular fractionation to separate Cd-containing tissues and organelles (Weigel and Jäger, 1980; Wu et al., 2005; Wang et al., 2008); however, the centrifugation, filtering and washing steps are designed to rupture cells, which could release Cd and redistribute it among the fractions.

Synchrotron radiation-based analytical techniques have also been used to detect Cd at the cellular and subcellular levels (Isaure et al., 2006; Naftel et al., 2007; Fukuda et al., 2008; Harada et al., 2010; Terada et al., 2010). Micro-synchrotron X-ray fluorescence (μ-XRF) has a micron-scale beam spot size and highly sensitive detection systems that can provide spatially resolved maps of elements at the cellular scale. When added to micro X-ray absorption near-edge structure (μ-XANES) and micro X-ray absorption fine structure (μ-XAFS) spectroscopy, the system can also provide information on element-specific oxidation state(s) and coordination environment(s) of metals inside the cell. These techniques can be used to determine both where and to what Cd is bound within plant tissue.

Despite these robust methodologies, results from studies of the Cd distribution at the cellular and subcellular levels are not consistent. For example, Cd was not detected in the cell walls of *P. vulgaris* grown in 0.5 μM Cd for 6 d (Vázquez et al., 1992) or *Allium cepa* grown in 10 mM Cd for 3 d (Liu and Kottke, 2004), whereas the cell wall was found to be a very important site for binding Cd in *Hordeum vulgare* grown in 5 μM Cd for 25 d (Wu et al., 2005) and *Lupinus albus* grown in 150 μM Cd for 35 d (Vázquez et al., 2007). These differences could be due to differences among plant species or concentrations of Cd in the growth medium as well as different sample preparation techniques and different methods of detection.

In most of the studies mentioned above, the distribution of Cd was determined in hyperaccumulator plants, which can accumulate high concentrations of Cd (>100 μg/g leaf dry weight, Bert et al., 2002) in their aboveground biomass without showing visible symptoms of toxicity. Those studies have provided useful information on qualitative imaging of cellular and subcellular Cd and, in a few cases, Cd speciation, and have also expanded our understanding of the mechanisms of Cd accumulation in hyperaccumulator plants. However, low metal-accumulating plants (such as agricultural crops) are expected to use different mechanisms to regulate their intracellular concentrations of Cd since lesser amounts of Cd tend to be taken up by these plants. Only a few studies have been conducted on the distribution of Cd in agricultural crops (Weigel and Jäger, 1980; Rauser and Ackerley, 1987; Seregin and Ivanov, 1997; Naftel et al., 2007; Vieira da Cunha et al., 2008; Terada et al., 2010), and none of these studies provided information on Cd speciation in the plants.

To address these knowledge gaps, we will apply multiple techniques including histochemical staining, SEM-WDS (wavelength dispersive spectroscopy), μ-XRF and μ-XANES on samples taken from individual plants grown under identical experimental conditions, thereby providing a more consistent answer to the questions of where and in which chemical form Cd is localized. Lettuce (*Lactuca sativa*) and barley (*H. vulgare*) were chosen because we previously found that approximately 80% of the total Cd was translocated to leaves of lettuce, whereas only 20% of the total Cd was translocated to barley leaves (Akhter and Macfie, 2012). This led to the hypothesis that barley and lettuce have different mechanism(s) to either store Cd in the root or translocate Cd to the leaves. Our objectives are (1) to determine the proportion of Cd bound within specific tissues of the root and (2) to use information about the coordination environment of Cd to predict the ligands to which Cd^{2+} is bound.

2. Methods and materials

2.1. Germination and growth conditions

Lettuce (*L. sativa* L. cv. Grand Rapids) and barley (*H. vulgare* L. cv. CDC McGwire, hulless 2-row feed barley) seeds were germinated in reverse osmosis (RO) water and grown in hydroponic culture for 28 days (d) under the same conditions as reported in Akhter et al. (2012) except, on day 7, individual seedlings were transplanted into 1.4 L jars filled with nutrient solution (adjusted to pH 6.0) to which either 0 or 1.0 μM $CdCl_2$ was added. Three replicate jars per experimental treatment were established.

In a preliminary experiment, we determined that concentrations of Cd in plants grown for 28 d with 1.0 μM $CdCl_2$ were below the detection limits of electron and X-ray fluorescence spectroscopy. In an effort to increase the concentrations of Cd in the root tissues, we tried placing the plants in solutions containing 10, 50, 500, 5000 or 10,000 μM $CdCl_2$ for 1 h prior to harvest. Concentrations of Cd were above the detection limits of electron spectroscopy only in plants from the two highest concentrations. We compared the distribution of Cd in roots grown in 1.0 μM $CdCl_2$ for 28 d and harvested immediately to those that were soaked in 5 or 10 mM $CdCl_2$ prior to harvest, using histological staining (see Section 2.2.1), and found no visible differences. Therefore, on the 28th day in hydroponic culture, the lettuce and barley plants from the 1.0 μM $CdCl_2$ treatments were transferred into fresh nutrient solution (pH 6.0) with 5.0 mM $CdCl_2$ and 10.0 mM $CdCl_2$, respectively, for 1 h. A comparatively higher concentration was selected for barley since it could accumulate higher amounts of Cd in the roots, compared to lettuce, without showing symptoms of Cd-stress (Akhter and Macfie, 2012). At harvest, fresh weights of roots and shoots were recorded, roots were rinsed in RO water and a 3.0 g subsample of root was immediately fixed in 2% (v/v) glutaraldehyde (Electron Microscopy Sciences (EMS), Hatfield, PA, USA) and kept at room temperature overnight, to be used for microscopic analysis (Section 2.2). The remainder of the root and shoot samples were oven-dried (60 °C) to constant weight and analyzed for total Cd content following the hot acid digestion and inductively coupled plasma optical emission spectroscopic (ICP-OES) methods described in Akhter and Macfie (2012).

2.2. Procedures for microscopic studies

2.2.1. Light microscopy

The localization of Cd in root tissues was studied using the histochemical method developed by Seregin and Ivanov (1997), which involves staining with dithizone, a reagent that produces an insoluble red salt, Cd-dithizonate, in the presence of Cd. Approximately