Micro-Synchrotron X-ray Fluorescence of Cadmium-Challenged Corn Roots

S. J. Naftel[1], R. R. Martin,[1] S. M. Macfie,[2] and T. K. Sham[1]

[1]*Department of Chemistry, University of Western Ontario, London, Ontario, Canada*

[2]*Department of Plant Sciences, University of Western Ontario, London, Ontario, Canada*

Introduction

Cadmium (Cd) is phytotoxic at high concentrations. Remarkably, the toxicity threshold for different plant species can differ by several orders of magnitude. Clearly, different plant species have different responses to exogenous Cd. In general, however, excesses of divalent cations are thought to inhibit the uptake and transport of other cations by competing for adsorption and uptake sites in the plant tissue. For example, Cd^{2+} has been shown to competitively displace Ca^{2+} from cell walls and transport proteins in the plasma membrane.[1]

Techniques that are capable of identifying the location of Cd within plant tissues and the changes in distribution of other elements as a result of exposure to Cd, will provide valuable insights into the mechanisms of Cd tolerance in plants. The spatial resolution and high sensitivity of synchrotron x-ray fluorescence microprobe analysis (XRM) and the ability to perform XRM under ambient conditions makes XRM a promising tool for the study of metals in plant tissues.

In this work XRM is used to study the distribution and relative proportions of Ca in transverse root sections of corn (*Zea mays*), grown with and without exposure to Cd.

Methods

Plants were grown in a greenhouse with 25/18º C day/night cycle set to 16 h days in acid-washed quartz sand. Plants were given a modified nutrient solution every second day and distilled water on alternate days. When the seedlings were 14 days old, they were given one of two treatments: 5 ml distilled water (control) or 5 ml of distilled water containing 500 µmol $CdCl_2$ (Cd-challenged). Alternating nutrient solution/distilled water feedings continued until the plants were 24 days old.

Sections of root tissue, 3-4 mm long and at least 1 cm from the root tip were fixed in 4% paraformaldehyde at room temperature for 2 h, then rinsed in distilled water. Following dehydration in a graded acetone series, samples were infiltrated overnight in a 1:1 mixture of Spur's (hard) and acetone, then infiltrated in pure Spur's for 72 h. The samples were then transferred to fresh Spur's and placed at 55º C for 24 h to polymerize the resin.

Two embedded tissue samples were chosen at random and hand sectioned to 20-50 microns using a razor blade.

The measurements were carried out at the 20-ID-B beamline (PNC-CAT) of the Advanced Photon Source (APS), located at Argonne National Laboratory. A pair of Kirkpatrick-Baez mirrors were used to focus the monochromatic x-ray beam to a spot size of 1.5 microns at the sample. A 13-element Ge detector was used

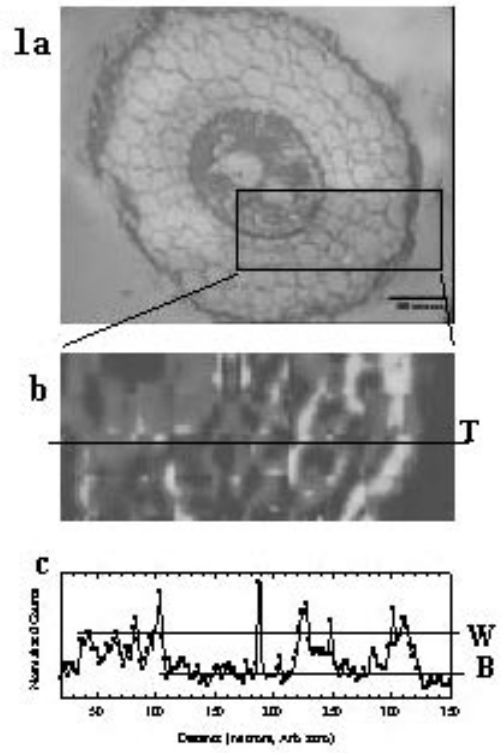

FIG 1. Cadmium-challenged root images: a) optical microscope image; b) Ca fluorescence intensity map; c) Ca intensity along line T. Line B represents the background Ca intensity and W represents the Ca intensity level in the cell walls.

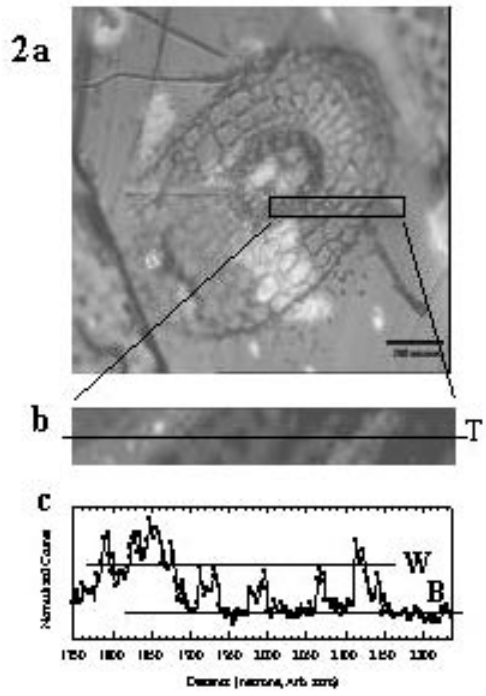

FIG 2. Control root images: a) optical microscope image; b) Ca fluorescence intensity map; c) Ca intensity along line T. Line B represents the background Ca intensity and W represents the Ca intensity level in the cell walls.

to monitor the emitted fluorescence x-ray intensities for Ca (Kβ) during each scan. The samples were mounted on an X-Y stage that allowed them to be scanned in 2 μm steps with a counting time of 2 s per point. The incident photon energy was set at 9000 eV.

Results and Discussion

Optical microscope photographs of the Cd-challenged and control roots are shown in Figs. 1a and 2a, respectively. The Ca Kβ x-ray fluorescence map obtained from each is shown in part b of each figure. Part c of the figures shows the Ca Kβ intensities obtained during a single scan across each root (at line T).

The Ca Kβ fluorescence intensity maps for both roots show that, in these samples, the Ca is localized in the cell walls. There appears to be a relative increase in Ca in the cortex of the control root as compared to the surrounding cell walls.

In order to determine if the Ca distribution is significantly different between the roots, we examined the individual line scans (part c of both figures).

In Figs. 1c and 2c there are two fluorescence intensities that can be identified with physiological structures. First, there is the background intensity from the embedding resin, labelled B in the figures. Second, the fluorescence intensity associated with the outer cell wall can de determined, labelled W in the figures.

With W and B labelled it is easy to see that, in the Cd-challenged root (Fig. 1c), the fluorescence intensity in the cortex (left side of image) is the same as W. In the control root, however, the fluorescence intensity in the cortex is higher than W. This suggests that the presence of Cd in the nutrient solution interferes with Ca transport in the plants.

Conclusions

In this study we found that Cd placed in the nutrient solution alters the uptake and/or transportation of Ca in corn seedlings. Micro x-ray fluorescence analysis is a promising technique for the study of metal mobility in soil/plant interactions.

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Reference

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