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BRIEF COMMUNICATION

Localization of cadmium in the root cells of *Allium cepa* by energy dispersive X-ray analysis

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Abstract

Allium cepa L. roots were exposed to 0.1 and 1.0 mM Cd for 6, 24 and 48 h and the localization of Cd in the root tissue was investigated. Scanning electron microscopy (SEM) and energy dispersive X-ray analysis（EDXA）were performed on frozen-dried tissues of roots. No Cd was detected in the roots treated with only 0.1 mM Cd, while after exposure to higher Cd concentration (1.0 mM) Cd was observed in cell wall and in cytoplasm in the epidermis, cortex and vascular tissues in the roots.

Additonal key words: cell wall, cytoplasm, scanning electron microscopy (SEM).

Cadmium is an important environmental pollutant with high toxicity to animals and plants. Cadmium causes a decrease of mitotic index, inhibits cell division and cell proliferation, and has toxic effects on chromosomal morphology including c-mitosis, anaphase bridges, and chromosome stickiness of *Allium cepa* and *A. sativum* (Liu *et al*. 1992, 2003). Cadmium alters the synthesis of RNA and impedes ribonuclease activity in rice (Shah and Dubey 1995). Although the effects of Cd on plants were widely discussed at biochemistry and cell physiology levels (Rauser 1999, Sanità di Toppi and Gabbrielli 1999, Cobbett and Goldsbrough 2002, Hall 2002, Kamnev and Lelie 2000), conflicting results were reported for the storage site of Cd in the cells. Ernst (1975), Lignell *et al*. (1982), Khan *et al*. (1984) and Vázquez *et al*. (1992a) indicated that cell walls are the main site of Cd accumulation. The data obtained by electron energy loss spectroscopy (EELS) by Liu and Kottke (2003, 2004) showed that no Cd was detected in the cell walls. Energy dispersive X-ray analysis (EDXA) following freezing of the plant sample allows the precise localization of many elements in the cell walls (Vázquez *et al*. 1992a), and thus can provide information on mechanisms of metal toxicity and tolerance (Tsezos *et al*. 1997, Barceló and Poschenrieder 1999, White and Gadd 2000, Küpper *et al*. 2001). The objectives of this investigation were to increase our understanding of Cd accumulation sites at the cellular level in the root of *A. cepa.*

Healthy and equal-sized onion (*Allium cepa* L.) cloves were selected and germinated in plastic containers by dipping the base of the bulb and the emerging root tips in Hoagland's nutrient solution (Stephan and Prochazka 1989). The plants were cultivated in greenhouse at 20 °C with 15-h photoperiod at photosynthetic photon flux density of 150 μmol m^{-2} s^{-1} and relative humidity of 65 %. When the roots reached a length of 1 cm, 6 seedlings per treatment were selected and exposed to different concentrations of Cd in Hoagland's solution for 6, 24, and 48 h. Cadmium was applied as $CdCl_2 \cdot 2\,H_2O$ at concentration 0.1 mM and 1.0 mM. Hoagland's nutrient solution was used for the control. The solutions were continuously aerated with an aquarium air pump and changed every day.

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Abbreviation: EDAX - energy dispersive X-ray microanalysis; EDXA - energy dispersive X-ray analysis; EELS - electron energy loss spectroscopy; SEM - scanning electron microscopy; SIMS - secondary-ion mass-spectrometry.
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For energy dispersive X-ray analyses (EDXA) ten root samples of 1 cm length were cut from the root tips (as young root tissue including the meristem) and basal parts of root segments (as mature tissue) of each treatment and rapidly frozen in liquid nitrogen and lyophilized. Cross-sections of the roots (about 1 mm) were coated by gold using the sputter/coater (*AGAR Scientific Ltd*., Stansted, Essex, England). The energy dispersive X-ray microanalytical studies were carried out using scanning electron microscopy (*Philips XL30*, Eindhoven, The Netherlands) provided with *EDAX DX-4 eDXi* System, version 2.11. EDAX spectra were collected between 0 and 20 keV and X-ray detector equipped with a super ultra thin window. The collection time of spectra was 120 s.

The results indicate that there was a direct correlation in the amounts of Cd in root cells of different regions with

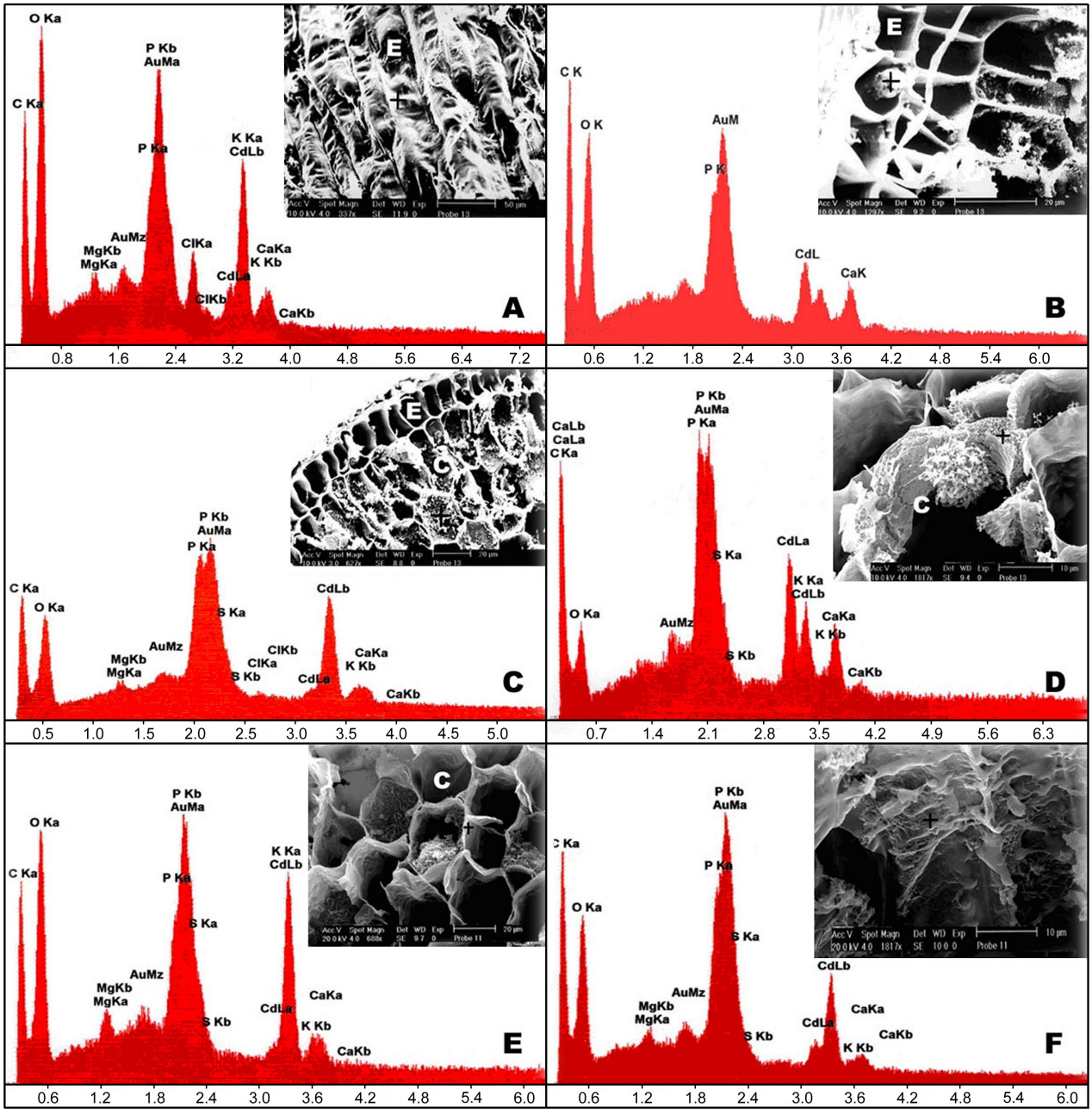

Fig. 1. EDXA spectrum taken from the site of the SEM micrographs, showing Cd localization in root cells of *A. cepa*. *A* - Cd on the surface of epidermis of basal parts of roots (1 mM Cd; 48 h). *B* - Cd in cytoplasm of the epidermis of the basal parts of roots (1 mM Cd; 48 h). *C* - Cd in the third cortical cells (0.1 mM Cd; 48 h). *D* - Cd in cytoplasm of the cortical cells (1 mM Cd; 48 h). *E* - Cd on cell walls of the cortical cells (1 mM Cd; 24 h). *F* - Cd in secondary walls of vessel (1 mM Cd; 24 h). C - cortical cell. E - epidermis. "+" site of the analysis; x-axis - energy [keV].

increasing Cd concentration in the solution and treatment time.

No Cd could be observed in roots treated with 0.1 mM Cd for 6 and 24 h. Only trace amounts of Cd were detected after exposure of 48 h. In roots treated with 1 mM Cd for 6, 24 and 48 h, Cd was found in the epidermal cell wall of root tips and basal parts of roots. Cadmium ions were localized on the surface of epidermis (Fig. 1*A*) and in cytoplasm in the epidermis of the basal parts of root (Fig. 1*B*) after 48 h treatment.

Cd was found in all cortical cells exposed to 1 mM Cd for 6, 24 and 48 h and to 0.1 mM Cd for 48 h, while no Cd was found at 0.1mM Cd for 6 and 24 h. EDXA spectrum revealed that Cd was accumulated in several layers of cortical cells adjacent to the epidermis after treatment with 0.1 mM Cd for 48 h (Fig. 1*C*). At 1 mM Cd, Cd ions were present in cytoplasm (Fig. 1*D*) and in cell walls (Fig. 1*E*).

The vascular tissues were also storage sites of Cd. Cd was found in the parenchyma, and vessels. Cd appeared in the secondary walls of the vessel (Fig. 1*F*).

EDXA as an analytical technique is very useful for localization of elements in biological specimens at subcellular level. The present studies clearly indicate that Cd is accumulated in the tissues and the cell walls. The results of this investigation are in line with the findings of other workers that cell walls are the main site of Cd accumulation (Ernst 1975, Lignell *et al*. 1982, Khan *et al*. 1984, Vázquez *et al*. 1992a). However, conflicting results on the subcellular compartmentation of Cd have been reported. The results of our previous electron energy loss spectroscopy (EELS) studies using chemical fixation showed that Cd was localised mainly in the vacuoles and nucleoli of cortical cells of differentiating and mature root tissues in *Allium sativum* treated with 10 mM Cd for 9 d (Liu and Kottke 2003) and in *Allium cepa* exposed to 1 mM and 10 mM Cd for 2 and 3 d (Liu and Kottke 2004), while no Cd was detected in cell walls. Generally speaking, EELS is a good method and can present a better energetic resolution and a higher sensitivity than X-ray micro-analysis (Castaing 1987). EELS is more sensitive than both energy dispersive X-ray microanalysis (EDAX) and secondary-ion mass-spectrometry (SIMS) (Huxham *et al.* I999). Why was no Cd found on cell walls using EELS in our previous investigations and was Cd detected in cell walls using EDXA in the present study? According to the results of previous studies by other research workers and us, the method of sample preparation can be involved and thought to be very important. In our previous studies, chemical fixed material was used. Due to an increasing permeability of membranes during the chemical procedure, losses and dislocations of mobile electrolytes cannot be excluded (Hall and Gupta 1984). Rauser and Ackerley (1987), using EDAX in conventionally fixed material, could not find Cd on cell walls of maize and *Agrostis* roots. Former works on samples not prepared by freeze-substitution revealed the absence of Cd in root cell walls (Vázquez *et al*. 1991, 1992b). Rauser and Ackerley (1987) indicated that one reason for lack of Cd signals from regions other than the electron-dense granules could be due to loss of Cd ions during the preparation of the tissue for electron microscopy. Cryofixation of the material can overcome some of the problems arising from chemical fixation (Bücking *et al*. 1998). According to Vázquez *et al*. (1992a) X-ray microanalysis on freeze-substituted samples of the Cd hyperaccumulator plant *Thlaspi caerulescens* revealed that high amounts of Cd, Ca and Fe were found in cell walls, whilst in samples fixed with glutaraldehyde in presence of sodium sulphide (Danscher 1981) no significant amounts of Cd were generally detected in these cell walls. Therefore, method of sample preparation is a very important link. Both sensitive and accurate analytical means and perfect sample preparation method are needed in identification of sites of localization of heavy metals at the subcellular level in cell organelles, cytoplasm or cell walls.

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