International Journal of Botany

ISSN: 1811-9700

science alert

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International Journal of Botany 4 (3): 340-343, 2008
ISSN 1811-9700

X-Ray Microanalysis and Ultrastructural Localization of Chromium in *Raphanus sativus* L.

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Abstract: Ultrastructural studies of root cortical cells of *Raphanus sativus* L. seedlings experimentally exposed to 0-7 mg L^{-1} Cr^{3+} revealed small electron dense granules especially in preplasmic zone. Root cortical cells of control seedlings grown in the absence of Cr^{3+} additions were void of any granules. X-ray microanalysis of the granules provided evidence that Cr was the predominant element while lower amounts of Fe, Zn and Mn were also measured in the granules. Presence of chromium deposit inclusions in the cells may be regarded as plant detoxifying mechanism to maintain relatively low cytoplasmic concentration of the element.

Key words: *Raphanus sativus* L., chromium, ultrastructural localization, TEM, X-ray microanalysis

INTRODUCTION

Chromium has long been established as an essential element for the nutrition of man and animals (Vincent, 2000; Ghosh and Bhattacharya, 2002; Anderson, 1998) but is apparently not essential for plant growth (McGraph, 1982; Mei *et al*., 2002). Nevertheless chromium is a ubiquitous element in plants and is actually accumulated to high levels by some plants (Lahouti and Peterson, 1979; Zhang *et al*., 2007). However agronomic plants in general contain only low concentrations of the element (McGraph, 1982; Hsiao *et al*., 2007; Toppi *et al*., 2002). Accumulation and localization of heavy metals including Cr, Cu and Pb have been studied in some plant species (Skeffington *et al*., 1976; Chatterjee and Chatterjee, 2000; Mei *et al*., 2002; Qian *et al*., 1999; Gwozdz *et al*., 1997). It was shown in an investigation that chromium was associated with cell wall fragments of *Hordeum vulgare* L. root cells treated with Cr^{3+} and CrO_4^{2-} (Skeffington *et al*., 1976). Although the percentage of chromium was dependent upon whether Cr was supplied as Cr^{3+} or CrO_4^{2-}. Nevertheless, differential centrifugation of cell organelles followed by TEM and electron microprobe microanalysis technique has demonstrated that little chromium was associated with those structures (Skeffington *et al*., 1976). Seedlings of *Brassica oleracea* L. supplied with ^{51}Cr have also been studied experimentally In which chromium-51 in the form of $^{51}Cr^{3+}$ and $^{51}Cr^{6+}$ was supplied for the plant seedlings in hydroponic cultures (Lahouti and Peterson, 1979). Then the radioactivity was determined in automatic γ-counter. Results of differential centrifugation of *Brassica oleracea* L. roots and shoots extracts showed that over half of ^{51}Cr was soluble in plant seedlings supplied with $^{51}Cr^{6+}$ whereas less Than half of the radioactivity was soluble in plants supplied with $^{51}Cr^{3+}$. Their results also showed that *Brassica oleracea* L. cell organelles contained small amounts of ^{51}Cr irrespective of whether chromium was supplied as $^{51}Cr^{3+}$ or $^{51}Cr^{6+}$ whereas larger amounts of radioactivity were recorded in the supernatants after organelle isolation from the Plants supplied with $^{51}Cr^{6+}$ whereas with the cell wall and debris fraction larger amounts of radioactivity were present in plants supplied with $^{51}Cr^{3+}$. Expressed as the percentage of chromium in the organs, there was more radioactivity in the leaf proteins of cauliflower than was present in the roots (Lahouti and Peterson, 1979). The results obtained were comparable with those reported for *Hordeum vulgare* L. (Skeffington *et al*., 1976) in which similar techniques were used. The aim of the present research was to study ultrastructural localization and X-ray microanalysis of chromium deposits in root cortical cells of *Raphanus sativus* L. supplied with different concentration of Cr^{3+}, also to carry out further investigation regarding detoxifying mechanism of plants under heavy metal stress and toxicity at the cellular level.

MATERIALS AND METHODS

Seeds of *Raphanus sativus* L. were germinated in lab. germinator at 20°C for 8 days seedlings were transferred to modified Hoagland nutrient solutions (Mei *et al*., 2002) supplemented with 0, 0.5, 1, 2, 3, 4, 5, 6 and 7 ppm Cr^{3+} for 4 weeks. The solution were changed every week and

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aerated continuously. Segments of tap roots (approximately 2×1 mm) were fixed in 2% ice cold glutaraldehyde in 0.1 M sodium cacodylate buffer to pH = 7. The segments were washed in cacodylate buffer and post fixed in 2% osmium tetroxide before embedding in Spurr's medium. Specimens were left unstained for examination under the electron microscope X-ray microanalyzer. Relatively thick sections of tap root tissue were prepared by ultramicrotomy and supported on uncoated grids where analyzed for Cr, Fe and Mn by X-ray microanalyzer to increase the accuracy of element location, the electron beam was focused to a narrow ellipsoid when examining deposits along the cell walls of root cortex cells. Cytoplasmic deposits and resin blanks were also analyzed with the same shaped beam. Conventional electron microscopy (TEM, LEO914 AB) was undertaken on unstained sections. Microanalysis of each element for a particular probe area is given as:

$$\frac{\text{Characteristic count for element}}{\text{While count}} \times 100$$

Time of counting 200 sec, all counts were corrected for background.

RESULTS AND DISCUSSION

TEM micrographs of root tissue of *Raphanus sativus* L. treated with 4, 5, 6 and 7 ppm Cr^{3+} showed relatively distorted cells with poorly defined plasmalemma (Fig. 1, 3, 5, 6) compared to the control (Fig. 2). Abundant dense bodies were observed in enlarged periplasmic zone of root cortical cells of the plant seedlings (Fig. 1, 3, 5, 6). Electron dense deposit were not noted in the cytoplasm. These inclusions were not seen in the control tissues (Fig. 2). Electron dense deposits ranged from 0.01 to 0.02 μ and occurred normally in small groups in the periplasmic zone along the cell wall and were seldom seen in the cytoplasm. Positive identification of Cr, Fe, Zn and Mn contained in the deposits was obtained by means of X-ray microanalyzer. Over 10 areas containing deposits were examined and compared with background areas of the cells. Moderate concentrations of Cr, Fe, Zn and Mn were recorded in cytoplasmic granules but these were not as high as those in periplasmic zone along the cell wall. Chromium was also measured in the non-granular regions of the cytoplasm but the analytical values were not significantly above the resin control (Table 1). Electron dense deposits were not observed in root cortical cells of *Raphanus sativus* L. Seedlings treated with 3 ppm Cr^{3+} (Fig. 4).

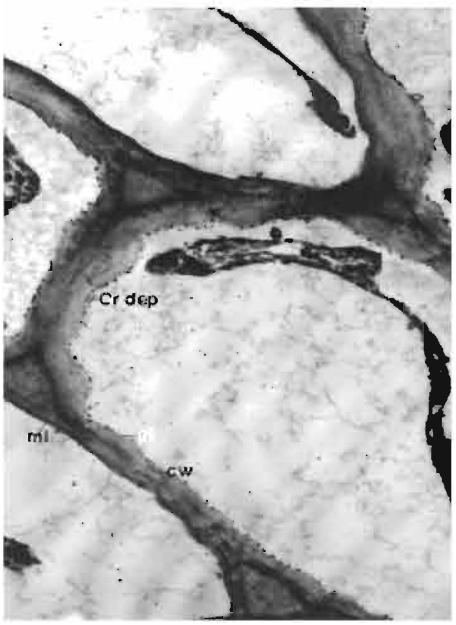

Fig. 1: TEM micrograph of *Raphanus sativus* L. root cortical cell (treated with 5 ppm Cr^{3+}) showing Cr granular deposits in periplasmic zone along the cell wall (x15800). (Cr dep = Chromium deposits, ml = middle lamella, cw = cell wall)

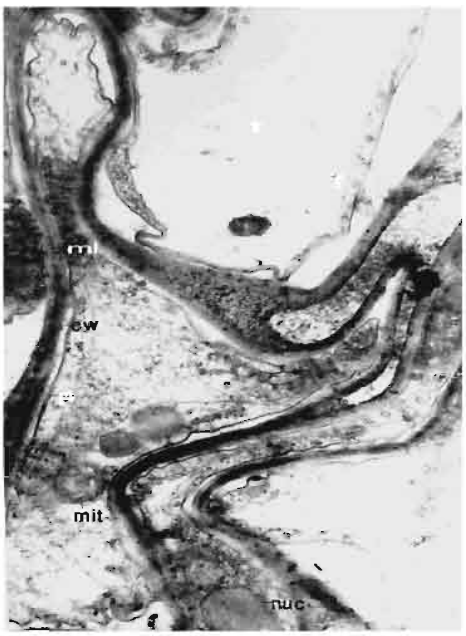

Fig. 2: TEM micrograph of *Raphanus sativus* L. root cortical cell (untreated) showing cytoplasmic organelles with no granular metal deposits (x9500). (mit = mitochondrion, cw = cell wall, ml = middle lamella, nuc = nucleus)

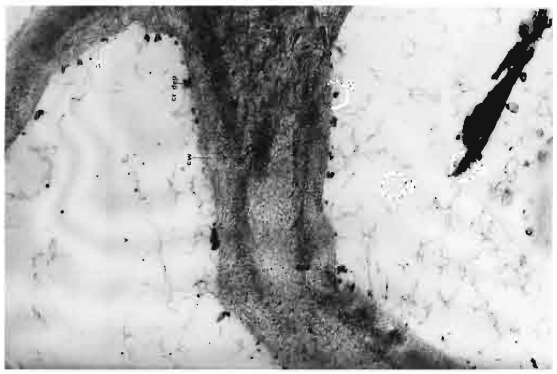

Fig. 3: TEM micrograph of *Raphanus sativus* L. root cell (treated with 6 ppm Cr^{3+}) showing Cr granular deposits in periplasmic zone along the cell wall (higher magnification x34500). (cw = cell wall, Cr dep = Cr deposits)

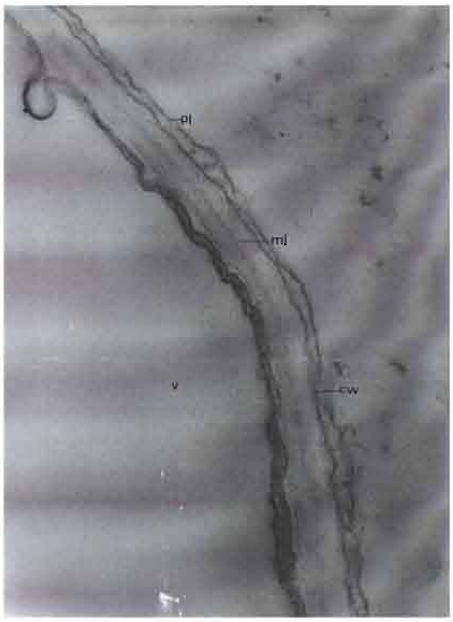

Fig. 4: TEM micrograph of *Raphanus sativus* L. root cortical cell treated with 3 ppm Cr^{3+} showing no granular deposits in priplasmic zone and along the cell wall (x17500). (cw = cell wall, ml = middle lamella, pl = plasmalemma)

Formation of metal deposits of various shapes and sizes is a characteristic feature of the ultrastructure of plant cells grown in the presence of high concentrations of metal ions (Chatterjee and Chatterjee, 2000; Jain *et al.*, 2000). However, the exact location of the electron dense deposits varies with the element and with the species investigated. Ultrastructural studies of algal cells exposed

Table 1: X- ray microanalysis of electron dense granules in *Raphanus sativus* L. root cortical cells grown in nutrient solution containing 7 ppm Cr^{3+}

Location of probe site	Electron dense granules	Microanalysis of each element			
		Cr	Fe	Zn	Mn
Periplasmic zone	Present	289.0±81	172.0±24	3.5±0.4	0.9±0.1
Deposited region in cytoplasm	Present	61.3±4.2	25.1±1.2	2.0±0.1	1.3±0.2
Nondeposited region in cytoplasm	Absent	1.2±0.1	1.0±0.1	1.0± 0.1	1.0±0.1
Resin control	Absent	0.9±0.1	0.9±0.1	0.9± 0.1	0.9±0.1

Values shown were the means of five measurements taken at different locations within the cell. In view of the low concentration of the elements in nongranular regions, values were presented to one significant figure only

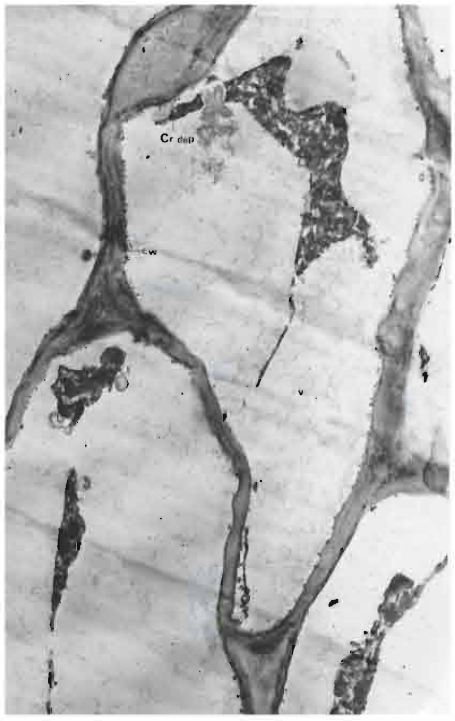

Fig. 5: TEM micrograph of *Raphanus sativus* L. root cortical cell (treated with 4 ppm Cr^{3}) showing Cr granular deposits in periplasmic zone along the cell wall (x14500). (cw = cell wall, Cr dep = Cr deposits)

to Cu revealed that this element mainly formed nuclear inclusions and only rarely were these deposits found in the cytoplasm (Silverberg *et al.*, 1976). Lead has been reported to be deposited within nuclei of moss leaf cells (Skaar *et al.*, 1973) and in some wetland plants (Qian *et al.*, 1999) and in corn seedling (Malone *et al.*, 1974). Results of the present investigation showed that chromium was mainly deposited in enlarged periplasmic zone along the cell wall and not within the cytoplasm and wall matrix. Although X-ray microanalysis revealed the presence of Fe, Zn and Mn in the granular deposits but Cr

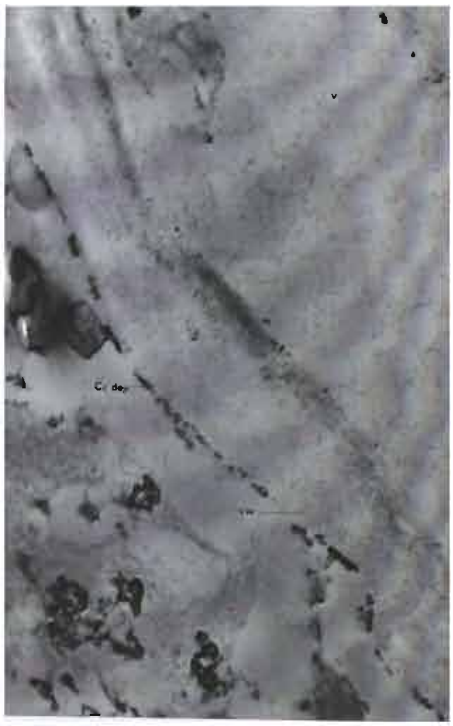

Fig. 6: TEM micrograph of *Raphanus sativus* L. root cortical cell (treated with 7 ppm Cr^3) showing Cr granular deposits in periplasmic zone along the cell wall (x26500) (cw = cell wall, Cr dep = Cr deposits)

was the predominant element. Present investigation revealed that Cr is another element which can be added to the list of metals deposited in granules within plant cells. The formation of deposited granules in the cells undoubtedly has the effect of maintaining a relatively low cytoplasmic concentration of the element and presumably reduces the toxic effects of the element on cellular metabolism as a detoxifying mechanism. Nevertheless, low chromium treatment (0.5 ppm Cr^{3+}) appeared to have stimulatory effect on the apparent growth and biomass of the plant seedlings under investigation.

ACKNOWLEDGMENT

The authors wish to thank Biology Department, Ferdowsi University of Mashad, Iran for financial aid and laboratory facilities for this research.

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