ACCUMULATION AND DISTRIBUTION OF CHROMIUM IN TOMATO PLANTS: STUDIES USING SIMS AND ELECTRO PROBE X-RAY MICROANALYSIS.

P. MANGABEIRA[1-], I. MUSHRIFAH [2], F. ESCAIG[1], A-A.F. ALMEIDA[1], D. LAFFRAY[3], M.I.G.SEVERO[1], A.H. OLIVEIRA[1] and P. GALLE[1].

1. Department of Biology, Universidade Estadual de Santa Cruz, Km 16 Rodovia Ilhéus-Itabuna, CEP 45600000, Ilhéus, Bahia, Brazil.

[-]paom@jacaranda.uescba.com.br

2. Department of Botany, Faculty of Life Sciences, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

3. Laboratoire de Physiologie Végétale ,Faculté de Science, Université Paris XII - Val-de-Marne, Créteil, 94000, France

Abstract

The tissular distribution of chromium in roots, stems, leaves and fruits of *Lycopersicon esculentum* was studied with Secondary Ion Mass Spectrometry (SIMS) and electro-probe X-ray microanalysis. Ion microscopy SMI 300 CAMECA (first generation of SIMS Microscopy) was used to obtain direct analytical images. One-month old plants were transplanted and grown in nutrient solution in the presence of 25, 50 and 100 $mgCr^{-1}$ at pH 5.8. After 90 days Cr had accumulated in the plant tissues; mostly in the vacuoles of xylem cells. The greatest concentration is present in the roots and progressively less in stems and leaves. No chromium was detected in fruits. The high Cr levels in the roots demonstrates the poor mobility of this plant ion.

keywords: Cr localization, *Lycopersicon esculentum*, SIMS, X-ray microanalysis.

Introduction

The phytotoxicity of chromium has been reported by several authors (Vazquez, Poschenrieder and Barcelo, 1987 ; Donghua, Jiang and Li, 1992). However, this element is beneficial to plant growth at low concentrations (Zheng *et al*. 1987). There are conflicting views on the uptake and localization of chromium by plant roots (Skeffington, Shewry and Peterson, 1976). The purpose of the present work was to study the uptake, transport and localization of Cr in tomato plants using SIMS microscopy and EPMA microanalysis.

Material and Methods

Seeds of *Lycopersicon esculentum* Mill. were germinated on moist paper for one week, transplanted to pots containing quartz sand were they grew in a half strength nutrient solution (pH 5.5). After one week the plants were placed on a full-strength nutrient solution, which was changed weekly. The composition of the nutrient solution has been described elsewhere (Moral et al 1996**)**. The experiment was conducted in a growth room at 28°C, and photoperiod 12 h fluorescent white light at 500µm^{-2} s^{-1} and 12 h darkness. The four control plants received only the basic nutrient solution, while the remainder also received 25, 50 and 100mg CrL^{-1} as $CrCl_3$ $6H_2O$. The plants were placed in randomized positions. Four plants were selected in each sample for each treatment to investigate the effects of the dosages of 50mg Cr^{-1}.

The samples (roots, stems and leaves) were taken 90 days after exposure time, cut and fixed in 3.0% glutaraldehyde in sodium cacodylate buffer (0.1M, pH 7.2) for 2 hours at room temperature, then washed in the same buffer and postfixed in 1% osmic acid (OsO_4) with similar cacodylate buffer for 3 h at room temperature, dehydrated in an alcohol series ranging from 50% to 100%. After embedding in Spurr's low-viscosity epoxy resin (Spurr, 1969). The specimen were sectioned (2µm) and (65 nm) using a Reichert-Jung Ultracut E Ultramicrotome and placed on square gold plate for analysis by SIMS, and on glass slides for a contingent correlation using light microscopy. A Philips EM 300 microscope was used to study the ultrastructure of tissue sections was performed using EPMA microanalyser.

Image analysis was performed on the Secondary Ion Mass Spectrometry (CAMECA SMI 300) using O_2^+ primary beam at a tension of 10kV. Under bombardment, sample surface atoms or atom groups are pulverised into ionic form. They constitute the secondary ion beam which is directed toward a mass spectrometer which then separates the different ion species according to their specific mass/ charge ratios. Secondary ions, selected by the mass spectrometer, can be converted by a metallic cathode into secondary electrons. The images representing the distribution of a given element can be observed on a fluorescent screen and recorded on photographic emulsion. This sensitive method permits the detection of elements that are present in very low concentrations or even at trace levels (1ppm). The field observed is about 250µm in diameter, the spatial resolution 1µm. All the elements and their isotopes can be separately imaged and analysed. The distribution of sodium ($^{23}Na^+$) and calcium ($^{40}Ca^+$) was used to reveal the topography of the tissues examined. However the images of distribution in a epon section of diffusible ions such as Na^+ Ca^+, and K^+ do not correspond to their distribution in the living cells (Galle,1985). The most abundant chromium isotope was chosen (chromium 52) to obtain the images representing their microscopic distribution.

Results and Discussion

Sodium and calcium ion distribution (Fig 1A, 1B, 1D, 1E, 2A, 2B, 3A and 3B) are valid

only in orienting the analyst to the morphological features being imaged and is not a reflection of the sodium and calcium distributions in the original material (Stika *et al,* 1980).

The secondary ion images provided a map of the distribution of $^{52}Cr^{+}$ across the sections of the roots, stems and leaves. These images showed a high level of chromium inside and around the secondary root xylem (Fig. 2C), whereas a low level was detected in the cortex and surface tissue. High precipitation of Cr in the root cells may be responsible for the lower level of Cr in the leaves. Cr was mainly accumulated in roots and poorly transported up the root to other organs. The chromium used principally the apoplastic transport across the root surface. The high level detected in roots might be partly due to physical adsorption onto the cell walls associated with the protoplasmic fraction (Myttenaere and Mousny 1974). The low level of Cr was detected in leaves (Fig. 3C) might be due to the retention of Cr III on the multiplicity of cation binding sites in cells and cell walls and only a small amount was transported to the upper parts of the plant in the xylem transpiration stream (Fig. 1C, 1F, 2C and 3C) (Vàzquez *et al* 1987). In the leaves Cr was mainly found in the cell walls of the vascular bundle (Fig. 1C). Ultrastructural studies of young leaves exposed to 50 and 100 mg/l showed marked changes in their chloroplast organization (Fig. 4B), while degeneration of their cellular membranes was evident. The spectra obtained on the EPMA microprobe confirm the secondary ion emission microanalysis data (Fig. 4A and 4C). Cr was detected in decreasing order of concentration in roots, stems and leaves. No chromium was detected in the fruits. Molecular and cellular knowledge of these processes will be necessary to improve plant metal resistance. However, fundamental knowledge of the biological systems controlling metal uptake by plants and the mechanisms involved in the resistance of plants to metal toxicity are of primary importance (Briat *et al* 1999).

References

Briat, J-F. and M. Lebrun. 1999. Plant responses to metal toxicity. *Comptes rendus de L'Academie des Sciences, Série III*, *Sciences de la Vie* –Life Sciences, Elsevier Editions, 322, 1, 1-18.

Donghua,L., J. Wusheng and L. Maoxie. 1992. Effects of trivalent and hexavalent chromium on root growth and cell division of *Allium cepa*. *Hereditas,* **117:** 23-29.

Galle P. 1985. La microscope ionique analytique des tissus biologiques*. Ann. Phys. Fr.* **10**: 287-305.

Moral, R., I. Gomez, J. Navaro Pedreno and J. Mataix. 1996. Adsorption of Cr and effects on micronutrient content in tomato plant (*Lycopersicum esculentum* M.*)*. *Agrochimica.***Xl,** n- 2-3: 133-138.

Myttenaere C and Mousney, J.M. 1974. The distribution of chromium-51 in lowland

rice in relation to the chemical form and the amount of stable chromium in the nutrient solution. *Plant and soil.***41**, 65-72.

Skeffington, R.A., Shewry, P.R., Peterson, P.J. 1976. Chromium uptake and transport in Barley seedlings (*Hordeum vulgare* L.*). Planta(Berl.).* **132,** 209-214.

Spurr, **A.R**. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *J.Ultrastruc.Res.***26,** 31-43.

Vazquez, M.D., Poschenrieder, C.H and Barcelo ,J. 1987. Chromium VI induced structural and ultrastructural changes in bush bean plants (*Phaseolus vulgaris* L.*) Annals of Botany*, **59,** 427-438.

Stika, K.M., Bielat, K.L and Morrison G.H. 1980. Diffusible ion localization by ion microscopy : a comparison of chemically prepared and fast-frozen, freeze-dried, unfixed liver sections.

Figures

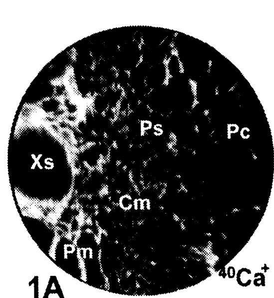
Images obtained with the ion microscope SMI 300 CAMECA

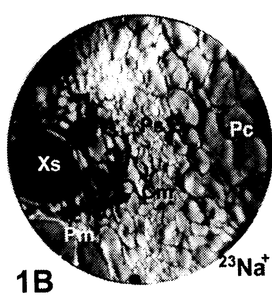
Figure 1 A-F. Images (field diameter = 250 µm) show the histological structure of the cortex in root cross sections. Figure 1C shows the high signal of Cr in xylem cell. Figure 1F shows the Cr associated with calcium carbonate inside cortex cell.

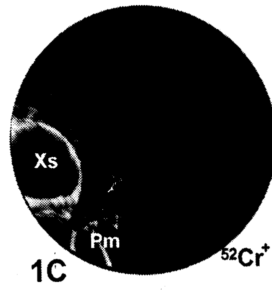
Figure 2 A-C. Secondary ion image (field diameter = 250 µm) representing the vascular bundle in stem cross section. The ion images showed a high level of Cr in the xylem cells (Fig 2C).

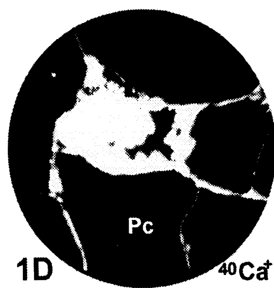
Figure 3 A-C. Elemental distribution maps (field diameter = 250 µm) of the leaf cross section. The images show a high level of Cr in the xylem cell walls (Fig 3C).

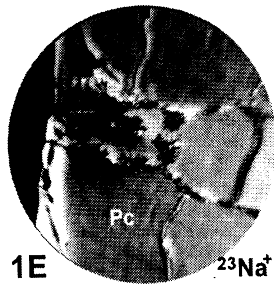
Figure 4A. Structural detail of cortex cells in tomato root showing numerous deposits with different osmiophilic vacuolar precipitates (X 10500). The sectrum confirms the presence of Cr.

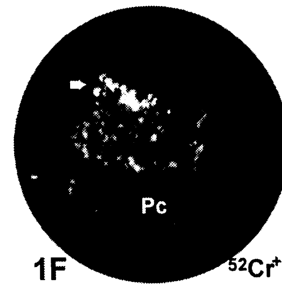

Ps
Pc
Xs
Cm
Pm
1A
$^{40}Ca^{+}$

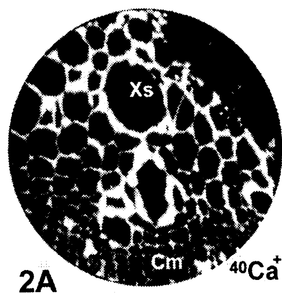

Pc
Xs
Pm
1B
$^{23}Na^{+}$

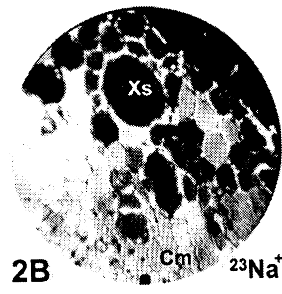

Xs
Pm
1C
$^{52}Cr^{+}$

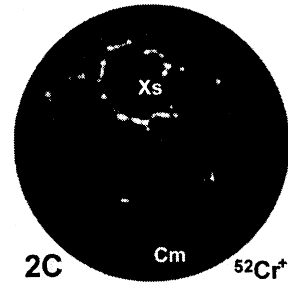

Pc
1D
$^{40}Ca^{+}$

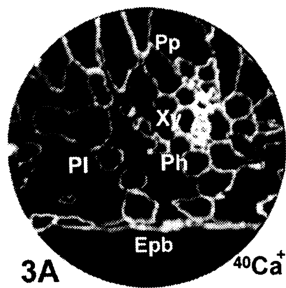

Pc
1E
$^{23}Na^{+}$

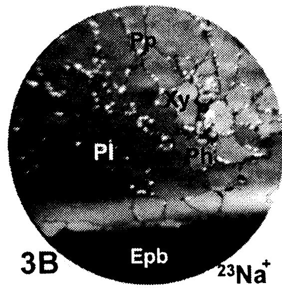

Pc
1F
$^{52}Cr^{+}$

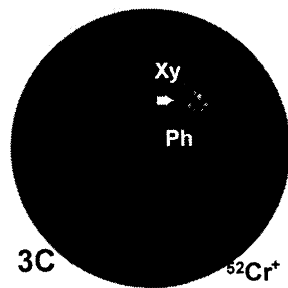

Xs
Cm
2A
$^{40}Ca^{+}$

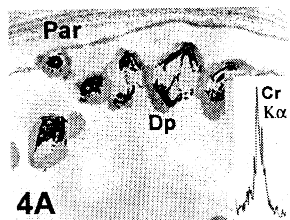

Xs
Cm
2B
$^{23}Na^{+}$

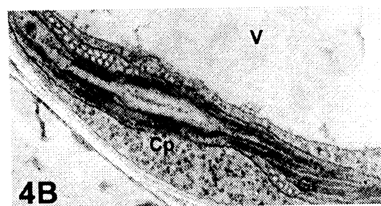

Xs
Cm
2C
$^{52}Cr^{+}$

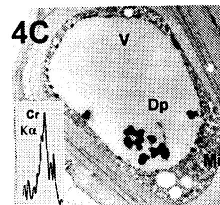

Pp
Xy
Pl
Ph
Epb
3A
$^{40}Ca^{+}$


Pp
Xy
Pl
Ph
Epb
3B
$^{23}Na^{+}$


Xy
Ph
3C
$^{52}Cr^{+}$


Par
Dp
Cr Kα
4A


V
Cp
4B


4C
V
Dp
Mi
Cr Kα