Low temperature $^{57}$Fe Mössbauer study of cucumber root

K Kovács¹, E Kuzmann¹,², F Fodor³, Z Homonnay¹, L Machala⁴ and A Vértes¹,²

¹Institute of Chemistry, Eötvös Loránd University, P.O. Box 32, 1512 Budapest, Hungary
²Laboratory of Nuclear Chemistry, Chemical Research Center, HAS, P.O. Box 32, 1512 Budapest, Hungary
³Department of Plant Physiology and Molecular Plant Biology, Eötvös Loránd University, P.O. Box 32, 1512 Budapest, Hungary
⁴Centre for Nanomaterial Research, Palacky University, Svobody 26, Olomouc 771 46, Czech Republic

E-mail: kkriszti@chem.elte.hu

Abstract. Iron uptake and distribution in cucumber root were studied with the help of $^{57}$Fe Mössbauer spectroscopy at low temperature applying external magnetic field. Cucumber was grown in iron sufficient modified Hoagland nutrient solution. Mössbauer spectra of the frozen roots taken at 4.2 and 1.5 K, at 5 T external magnetic field support the identification of the main iron species (Fe$^{lll}$-carboxylates, hydrous ferric oxides, Fe$^{lll}$-sulfate-hydroxide) suggested according to its Mössbauer spectra taken between 35-200 K [1]. The magnetic ordering temperature of the hydrous ferric oxide and Fe$^{lll}$-sulfate-hydroxide was found to be in the range of 4.2-1.5 K, which suggests the incorporation of H$^3$O$^+$, PO$_4^{3-}$ and citrate into these minerals.

1. Introduction

The mechanism of iron uptake and the storage of iron in plants have great scientific interest since they can help to improve remediation techniques for iron chlorosis which is an old worldwide problem occurring in areas of calcareous and/or alkaline soils [2].

Iron is not readily available for plants due to the low solubility of iron oxyhydroxides at the pH of the soil. For this reason, plants have evolved different strategies for iron uptake. Non-graminaceous plants, just like cucumber, follow Strategy I that starts with reduction of iron chelates at the outer surface of the plasma membrane [3]. The ferrous chelate releases iron due to its much lower stability and the Fe$^{ll}$ ions are taken up by a divalent cation transporter which is located in the plasma membrane. For this iron uptake mechanism, we gave direct evidence by the help of Mössbauer spectroscopy [1]. Furthermore, three main iron(III) components (Fe$^{lll}$-carboxylate complexes, a ferritin-like hydrous ferric oxide and a jarosite-like Fe$^{lll}$-sulfate-hydroxide containing species) were
suggested according to its Mössbauer spectra taken between 35 – 200 K, exhibiting only three doublet subspectra [1].

The aim of the present work was to support the previously proposed iron bearing chemical species occurring in the root of cucumber applying Mössbauer spectroscopy below 4.2 K in external magnetic field.

2. Experimental
Cucumber (Cucumis sativus L. cv. Joker) was grown in modified Hoagland nutrient solution with 10 µM Fe^{III}-citrate (Fe was enriched in 90% with ^{57}Fe). After 3 weeks growth the plants were harvested and the roots were frozen and stored in liquid nitrogen. The Mössbauer spectra of the cucumber roots were recorded in a temperature controlled cryostat (Oxford) at 4.2 and 1.5 K without and with applied 5 T external magnetic field (perpendicular to the direction of γ-rays).

^{57}Fe Mössbauer spectra were recorded in the transmission geometry with a conventional Mössbauer spectrometer. γ-rays were provided by a 3×10^9 Bq ^{57}Co/Rh source. Isomer shifts are given relative to α-iron. The Mössbauer spectra were analysed by the least-squares fitting using Lorentzian lines using the MOSSWINN code [4].

3. Results and discussion
The Mössbauer spectra of the cucumber root taken at low temperature are shown in figure 1.

![Figure 1. Low temperature Mössbauer spectra of cucumber roots grown in 10 µM ^{57}Fe^{III}-citrate containing nutrient solution](image)

The Mössbauer spectrum of the cucumber root recorded at 4.2 K exhibits a ~70 % magnetically split subspectrum while one of the three doublet components, existing at higher temperatures and identified as Fe^{III}-sulfate-hydroxides, disappears. The remaining doublet with parameters of δ=0.5 mms⁻¹ and Δ=0.6-0.7 mms⁻¹ can represent the hydrous ferric oxides and paramagnetic Fe^{III}-complexes. At 1.5 K, which is below the magnetic transition temperature of Fe^{II}-sulfate-hydroxides and ferrihydrites, almost all species are magnetically ordered [5], [6]. The remaining paramagnetic species represented by the doublet can be easily transformed into magnetically split spectrum by applying 5 T external magnetic field.
The results can be interpreted in terms of the variation of the characteristic magnetic transition temperatures with the composition of the species. Namely, in the case of Fe$^{III}$-sulfate-hydroxides (jarosites with the composition of MFe$_3$(SO$_4$)$_2$(OH)$_6$ (M=K$^+$, NH$_4^+$, H$_3$O$^+$, ...)) the temperature of the paramagnetic-antiferromagnetic transition strongly depends on the stoichiometry, especially on the M$^+$ cation. It was shown in synthetic jarosites, where H$_3$O$^+$ units are built in the structure, that the transition took place even below 20 K and in a wide temperature range [7]. Taking into account that the iron uptake in Strategy I type plants is accompanied by significant proton release [8] and that high amount of sulfate ions are present in the nutrient solution, the formation of jarosites with various stoichiometry can be favored in the cell wall.

The magnetic properties of hydrous ferric oxides formed via biomineralization processes (as in the case of the iron storage protein ferritin) also strongly depend on its composition [6]. The high PO$_4^{3-}$ ratio and the incorporation of organic ions, such as citrate can lead to amorphous structure and to the decrease of the antiferromagnetic ordering temperature (< 4 K). These findings support that the magnetically split subspectra at 4.2 and 1.5 K can be attributed to Fe$^{III}$-sulfate-hydroxides and hydrous ferric oxides, respectively.

The paramagnetic component, which can be transformed to magnetically split spectrum by applying 5 T external magnetic field at 1.5 K, can be identified as ferric-carboxylates, since the parameters of the doublet ($\delta=0.5$ mms$^{-1}$, $\Delta=0.6$ mms$^{-1}$ present in figure 2 at 4.2 and 1.5 K) correspond with those of high spin Fe$^{3+}$ in octahedral O environment. The Fe$^{III}$-carboxylate component can be present both in the cell wall and in the cytoplasm, since citric acid was shown to be secreted into the rhizosphere and also to take part in the iron transport inside the cell.

4. Conclusions
Low temperature Mössbauer measurements supported the identification — based on the measurements between 35-200 K — of the iron species found in cucumber root. These main iron containing chemical species were found to be Fe$^{III}$-carboxylate complexes, a ferritin-like hydrous ferric oxide and a jarosite-like Fe$^{III}$-sulfate-hydroxide.

The paramagnetic-antiferromagnetic ordering temperature of Fe$^{III}$-sulfate-hydroxides and hydrous ferric oxides was found to be in the range of 4.2-1.5 K, which suggests the incorporation of H$_3$O$^+$ ions into jarosite and high PO$_4^{3-}$ and citrate content in the ferrihydrite species.

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References
[1] Kovács K, Kuzmann E, Tatár E, Vértes A and Fodor F 2009 Planta 229 271
[2] Lucena J J In: Barton J and Abadia J 2007 Iron Nutrition in Plants and Rhizospheric Microorganisms (Springer)
[3] Römheld V 1987 Physiol. Plant. 70 231
[4] Klencsár Z, Kuzmann E and Vértes A 1996 J. Radioanal. Nucl. Chem. 210 105
[5] Stevens J G 1958-2002 Mössbauer Effect Reference and Data Index (Interscience, N. Y)
[6] Chasteen N D and Harrison P M 1999 J. Struct. Biol. 126 182
[7] Kovács K, Kuzmann E, Homonnay Z, Vértes A, Gunneriusson L and Sandström A 2008 Hyp. Int. 186 69
[8] Schmidt W, Michalke W and Schikora A 2003 Plant Cell Environ. 26 361