Plant Stress Physiology

STUDY OF THE PLANT IRON FERTILIZATION WITH SYNTHETIC FERRIC CHELATES BY MASS SPECTROMETRY

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INTRODUCTION

The ferric chelate of the synthetic ethylendiamine di(*o*-hydroxyphenylacetic) acid (Fe(III)-*o,o*EDDHA) is one of the most efficient fertilizers to correct Fe deficiency in calcareous soils. Commercially available Fe(III)-*o,o*EDDHA fertilizers contain a mixture of approximately 1:1 of two groups of stereoisomers, a *meso* form [(R,S-Fe(III)-*o,o*EDDHA)] and a *d,l racemic* mixture [(R,R-Fe(III)-*o,o*EDDHA) + (S,S-Fe(III)*o,o*EDDHA)], that have different stability constants with Fe (*meso* K = $10^{34.15}$ and *racemic* K = $10^{35.86}$ [1]). Previous studies dealing with the relative efficiency of the two Fe(III)-*o,o*EDDHA stereoisomers to supply Fe to plants showed that Fe from the *meso* isomer was preferentially depleted from nutrient solutions [2,3] and was more easily reduced by root chelate reductase than the *racemic* isomer [4].

In this study the uptake and distribution of the Fe simultaneously provided by both groups of Fe(III)-*o,o*EDDHA stereoisomers was monitorized in Fe-deficient sugar beet plants. For that, plants were treated with *meso* and *racemic*-*o,o*EDDHA isomers, each one labelled with a different Fe low-abundant stable isotopes (^{54}Fe or ^{57}Fe) to differentiate among the Fe provided by each isomer and the plant native Fe (mainly ^{56}Fe). Also, the occurrence and the allocation of the chelating agents (*meso* and *racemic*) inside the plant was determined by a highly sensitive mass spectrometry method recently developed by our group [5].

MATERIAL AND METHODS

PREPARATION OF Fe STABLE ISOTOPE LABELLED Fe(III)-*o,o*EDDHA CHELATES: *Meso* and *racemic* Fe(III)-*o,o*EDDHA isomers were separated by selective Mg precipitation according to [1], then Fe was removed and the *o,o*EDDHA isomers were further chelated with ^{54}Fe and ^{57}Fe.

PLANT MATERIAL: Three-week old sugar beet (*Beta vulgaris* L. cv. 'Orbis') plants were hydroponically-grown in absence of Fe for 2 weeks. Some of these Fe-deficient plants were Fe-resupplied for 3 and 6 hours with:

i) 30 µM *racemic* ^{54}Fe(III)-*o,o*EDDHA : 30 µM *meso* ^{57}Fe(III)-*o,o*EDDHA or

ii) 30 µM *racemic* ^{57}Fe(III)-*o,o*EDDHA : 30 µM *meso* ^{54}Fe(III)-*o,o*EDDHA.

Leaves and roots were sampled from both classes of Fe-deficient plants (Fe-resupplied and non Fe-resupplied). All plant material samples were divided in two aliquots before analysis preparation. An aliquot was immediately liquid N_2 frozen and o,oEDDHA extracted as it is indicated in [4]. The other aliquot was dried at 60 ºC, ground in a zirconium oxide-ball mill and microwave acidic digested.

IRON STABLE ISOTOPES DETERMINATION: Iron-54, ^{56}Fe, ^{57}Fe and ^{58}Fe contents in all plant material samples were determined by inductively coupled mass spectrometry, using isotope dilution analysis. Iron remobilization was estimated comparing the native Fe contents occurring in both Fe-resupplied and non Fe-resupplied Fe-deficient plant.

*o,o*EDDHA DETERMINATION: *meso* and *racemic*-*o,o*EDDHA were determined in plant extracts by high performance liquid chromatography coupled to time-of-flight mass spectrometry using the method described in [5].

RESULTS

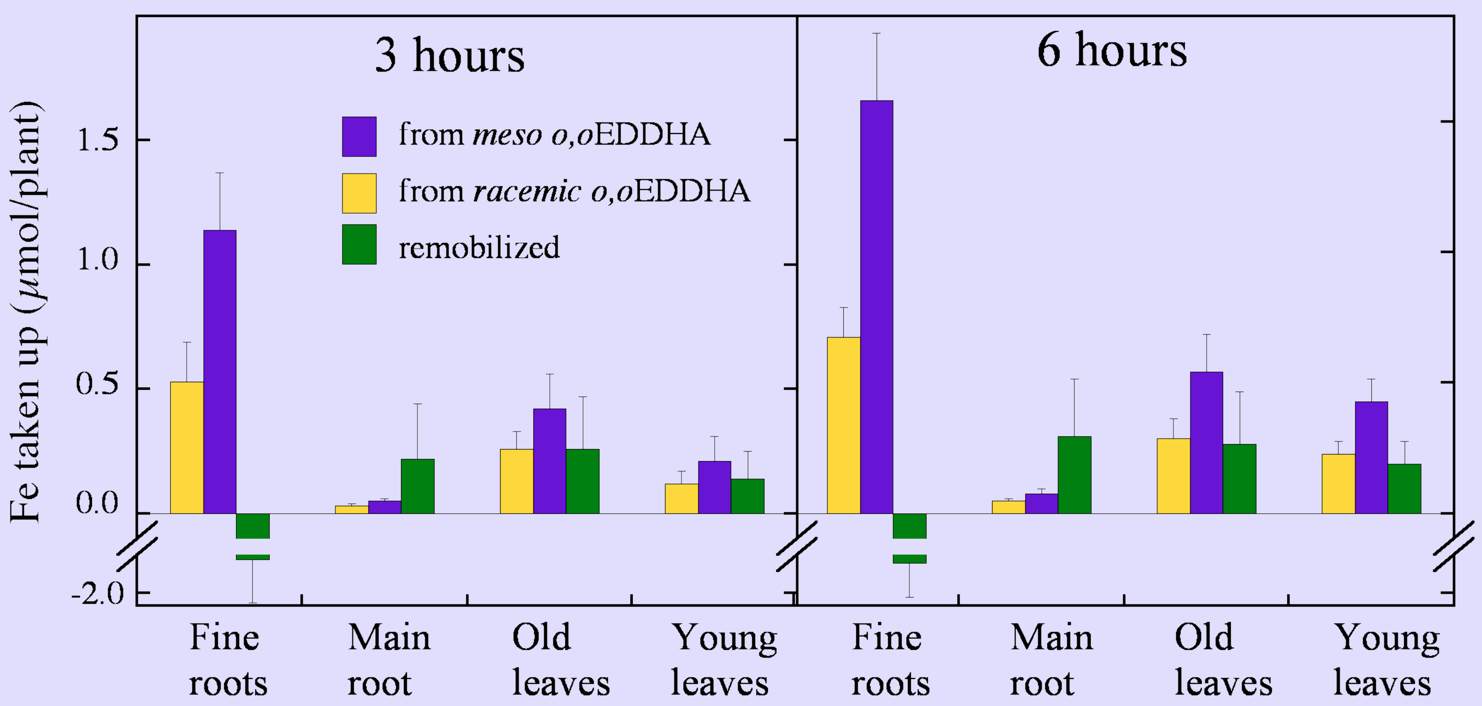

Figure 1. Allocation inside the plant of Fe taken up after 3 and 6 h of Fe-resupply with both Fe(III)-o,oEDDHA isomers. Data also include the Fe remobilized from roots to shoots. Data are means ± SE (n = 8).

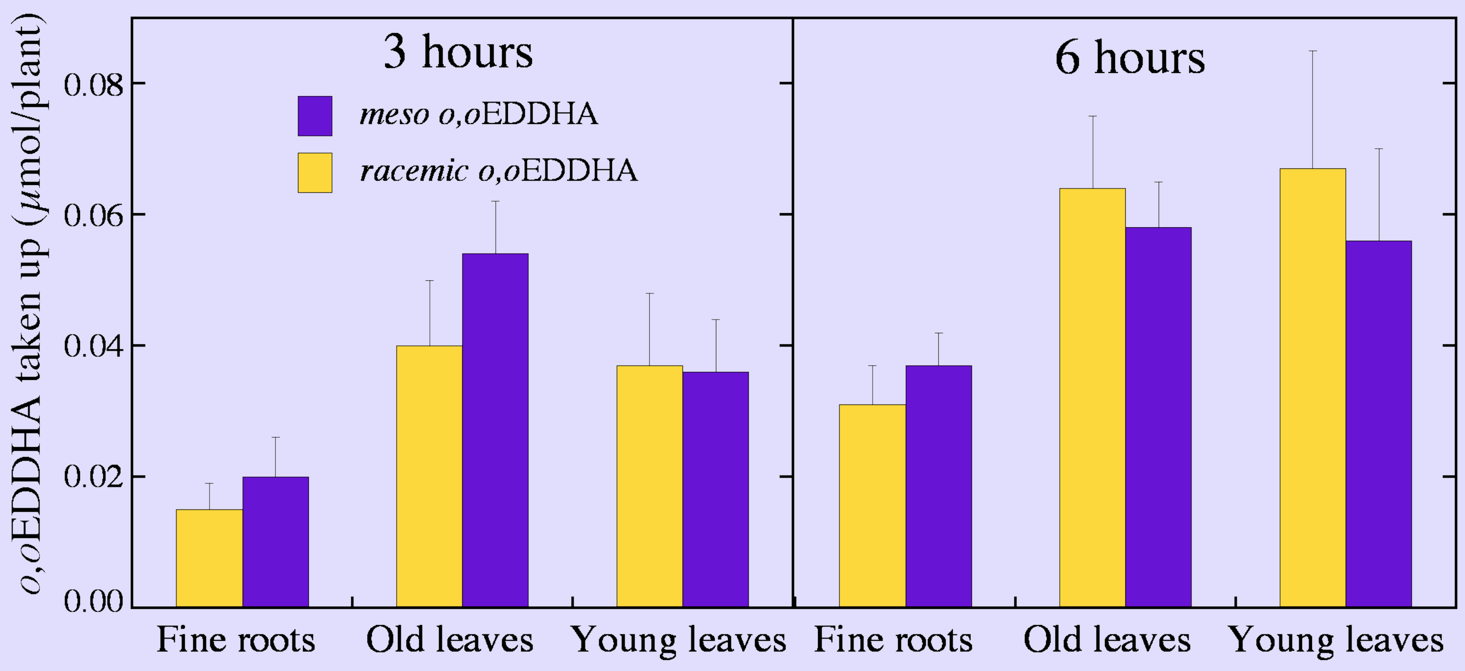

Figure 2. Allocation inside the plant of racemic and meso o,oEDDHA after 3 and 6 h of Fe-resupply with both Fe(III)-o,oEDDHA isomers. Data are means ± SE (n = 8).

IRON CONTENTS

- Plant contents of Fe come from the *meso* isomer were 1.5-3.0 fold higher than those come from the *racemic* one.
- Among plant materials the highest differences between contents of Fe come from each isomer were found in fine roots.
- Fe-resupply with both Fe(III)-*o,o*EDDHA isomers caused a remobilization of the native Fe from roots to shoots in sugar beet Fe-deficient plants.
- Old and young leaf contents of Fe come from either of both Fe(III)-*o,o*EDDHA isomer were 2.7-4.2 fold higher than those come from native Fe remobilized from roots.
- Plants treated for 6h had 1.7-fold higher Fe contents than those of plants treated for 3h.

*o,o*EDDHA CONTENTS

- For all plant materials, *meso o,o*EDDHA contens were not significantly different of *racemic o,o*EDDHA contents and the values of the *meso*-to-*racemic* ratio were in the range of 0.9–1.3.
- The highest *o,o*EDDHA contents were allocated in leaves with values of 108 ± 12 and 98 ± 18 nmol per plant for old and young leaves, respectively.
- The treatment period only affected the *o,o*EDDHA contents in fine roots that increased twice at 6h compared to those found at 3h.

REFERENCES: [1] Yunta *et al.* (**2003**) Inorg Chem 42: 5412; [2] Hill-Cottingham and Lloyd-Jones (**1965**) J Exp Bot 16: 233; [3] Cerdán *et al.* (**2006**) J Agric Food Chem 54: 1387; [4] Lucena and Chaney (**2006**) J Plant Nutr 29: 423; [5] Orera et al. (**2009**) Rapid Commun Mass Spectrom 23: 1694.

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