UNIVERSITY THOUGHT
Publication in Natural Sciences, Vol. 8, No. 1, 2018, pp. 1-4.

doi:10.5937/univtho8-16360
Original Scientific Paper

HUMIRON® IS AN EFFECTIVE BIODEGRADABLE SOURCE OF CHELATED IRON FOR PLANTS: AN IRON-59 UPTAKE STUDY

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ABSTRACT

Here we tested plants' ability to use iron (Fe) from the water-soluble commercial product Humiron® (a complex of Fe with highly purified and solubilized humic acids extracted from leonardite) as a source of chelated Fe for both strategy 1 (e.g. cucumber) and strategy 2 (e.g. barley) plant species grown under low Fe conditions. Iron from radioactive ^{59}Fe-labelled Humiron® can easily be acquired by strategy 1 plant species via reduction of Fe^{III} by the inducible plasma membrane-bound reductase, similarly to the reduction of synthetic Fe^{III}-chelates. Strategy 2 plant species can also efficiently utilize Fe from Humiron® via ligand exchange between humates and phytosiderophores (PS). Moreover, the efficacy of Humiron® is comparable to Fe complexed with water-extractable humic substances obtained form peat. Being easily biodegradable this product can be used as an effective Fe source for organic crop production.

Keywords: Barley, Chlorosis, Cucumber, Humate Complex, Iron Root-acquisition Strategies, Transport.

INTRODUCTION

Even though the fourth most abundant mineral element in the Earth's crust, iron (Fe) in soils occurs mostly in the insoluble Fe^{III} forms which are unavailable to plants. The concentrations of available Fe from the Fe chelated compounds (e.g. Fe humates) in most well-aerated soils are often lower than required for an adequate plant growth, especially at neutral and alkaline soil pH (Römheld & Nikolic, 2006). According to Römheld & Marschner (1994), higher plants respond to a lack of Fe by developing two different strategies. Dicots and nongraminaceous monocots employ the strategy 1 response on low soluble Fe in the rhizosphere by: 1) release more protons, thereby decrease rhizosphere pH and increase Fe solubility (*AHA1* genes coding for a plasma membrane H^+-ATPase), and 2) induction of plasma membrane-bound Fe^{III}-chelate reductase (encoded by *FRO* genes), which is followed by the uptake of Fe^{2+} via an inducible IRT1 transporter. Strategy 2 plants, which include all grasses (Poaceae), release low-molecular-weight compounds, so-called phytosiderophores (PS) that chelate Fe^{3+} ions, and take up the Fe^{III}-PS complex via the root YS1 transporters (for review see Nikolic & Pavlovic, 2018).

The use of various commercial synthetic Fe-chelates, i.e. Fe complexed with ethylenediaminetetraacetate (EDTA) or ethylenediamine-di-o-hydroxyphenylacetate (EDDHA), as soil amendment is a frequent measure for remedy of for Fe deficiency chlorosis in agricultural practice (Römheld & Nikolic, 2006). However, their costs and the environmental risk of leaching and groundwater pollution by heavy metals may limit the interest for these products, particularly in sustainable agriculture. On the other hand limited number of organic acidic anions (e.g. citrate and malate) are able to form stable complexes with Fe^{III}, which are however highly degradable by soil microbes and/or also photo labile (Cesco et al., 2002; Römheld & Nikolic, 2006). Some naturally occurring Fe-chelates in soils, such as Fe chelated to the microbial siderophores (e.g. Fe^{III}- dihydroxamate; Hördt et al., 2000) as well as humic substances, could be an alternative to synthetic Fe-chelates. It has been demonstrated that the roots of both strategy 1 and strategy 2 plants can utilize Fe bound to the water-extractable humic substances (WEHS; e.g. Pinton et al., 1999; Cesco et al., 2002).

In this work we tested plants' ability to use Fe from the water-soluble commercial product Humiron® as a source of chelated Fe for both the strategy 1 and the strategy 2 plant species. Thus, the major objective was to study the possible mechanisms involved in utilization of Fe from the radioactive ^{59}Fe-labelled Fe-humate product in Humiron®, by cucumber (strategy 1) and barley (strategy 2) plants subjected to nutrient solution model experiments.

MATERIALS AND METHODS

Humiron® (kindly provided by HUMINTECH GmbH, Düsseldorf, Germany) is a complex of Fe with highly purified and solubilized humic acids extracted from leonardite (an oxidized form of lignite, a byproduct of coal mining).

The experiments were carried out according to the methodology previously described by Cesco et al. (2002). After germination in quartz sand moistened with saturated $CaSO_4$, cucumber (*Cucumis sativus* L., cv. Chinese long) and barley (*Hordeum vulgare* L., cv. Europa) seedling were transferred to the complete nutrient solutions as reported by Cesco et al. (2002), either without (–Fe) or with (+Fe) 50 μM Fe-EDTA. The nutrient solutions were renewed completely every 3 d and continuously aerated. Plants were grown for 7 d under controlled

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environmental conditions in a growth chamber with light/dark regime of 16/8 h, temperature regime of 24/20°C, photon flux density of approximately 300 μmol^{-2} s^{-1} at plant height and relative humidity of about 70%.

Determination of Fe^{III} reduction capacity by intact cucumber roots was carried out in an assay solution containing 0.5 mM $CaSO_4$, 1 µM Fe (supplied as either Fe-EDTA or Humiron®), 200 µM bathophenanthrolinedisulfonate (BPDS), and 10 mM Mes/NaOH (pH 6.0) or 10 mM Hepes/NaOH (pH 7.8) for 30 min in darkness. Reduction rates were determined as a formed red $Fe^{II}(BPDS)_3$ complex by measuring absorbance at 535 nm against blanks (without roots) and using an extinction coefficient of 22.14 mM^{-1} cm^{-1} for calculation (Nikolic et al., 2007).

In ^{59}Fe uptake experiments, Fe-chelates were labelled by mixing $^{59}FeCl_3$ with either Humiron® or Fe-EDDHA (specific radioactivity 0.2 µCi μmol^{-1} Fe) following the procedure previously described by Nikolic et al. (2000). The final concentration of Fe in the uptake solutions was 1 µM. For cucumber plants the ^{59}Fe-labelled uptake solution was buffered at pH 6.0 with 10 mM Mes/NaOH or at pH 7.8 with 10 mM Hepes/NaOH, and the uptake period was 6 h. For barley plants, the uptake lasted 4 h and was performed in the morning (period of high PS release; 2 h after onset of light) or in the evening (period of low PS release; 12 h after onset of light). Additionally, 2'-deoxymugineic acid (DMA) collected from the exudates of Fe-deficient barley roots and purified following the method of Awad et al. (1988) was added in the uptake solution at final concentration of 20 µM during evening experiment.

After the uptake periods, the plants were transferred to a freshly prepared ^{59}Fe-free nutrient solution for 10 min and then harvested. The extraplasmatic ^{59}Fe pool was removed by reductive incubation of roots with 1.5 mM bipyridyl and 7.5 mM sodium dithionite (Bienfait et al., 1985; Cesco et al., 2002). Roots and shoots were oven-dried at 80°C, weighed, ashed at 550°C, and suspended in 1% (w/v) HCl for ^{59}Fe determination by a liquid scintillation counting.

RESULTS AND DISCUSSION

Preculture of cucumber plants in Fe-free nutrient solution for 7 days induced Fe^{III} reductase activity in the roots for both Fe chelates (i.e. Fe-EDTA and Humiron®), which was 4-5 times higher compared to the plants grown in nutrient solution adequately supplied with Fe (Fig. 1). The root reduction capacity for Humiron® was about 20% lower than that measured for Fe-EDTA as a substrate. It has been shown that Fe^{III} complexed with WEHS can easily be reduced at the plasma membrane of both root and leaf cells (Cesco et al., 2000, 2002; Nikolic et al., 2003). Exposure of –Fe roots to high pH of the root medium decreased the Fe^{III} reduction capacity for both Fe chelates by 3-4 times as compared to the roots at low pH. The measured reduction rates were comparable to +Fe roots at pH 6.0. These findings undoubtedly confirm that Humiron® is a suitable Fe^{III} chelate substrate for Fe^{III} reduction via a plasma membrane-associated low-Fe-inducible Fe^{III} reductase.

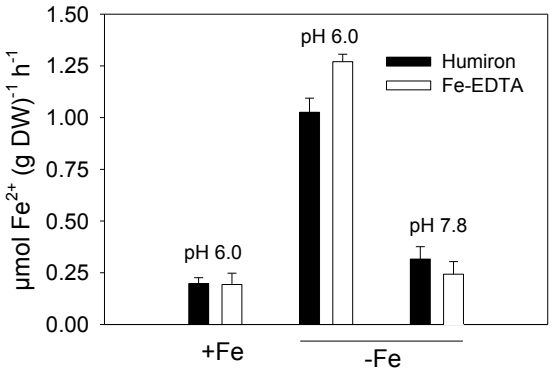

Figure 1. Fe^{III}-chelate reduction capacity of cucumber roots for Humiron® (Fe^{III}-humate complex) and Fe^{III}-EDTA (control) as affected by rhizosphere pH and Fe nutritional status.

Both high root pH (7.8) and addition of BPDS (strong Fe^{2+} scavenger) inhibited Fe uptake and root-to-shoot translocation of Fe from ^{59}Fe-labelled Humiron® in –Fe cucumber plants (Fig. 2), indicating that the preceding Fe^{III} reduction of Humiron® (see Fig. 1) was a necessary step for the uptake of Fe^{2+} by Fe deprived roots. On the other hand, significantly less ^{59}Fe was taken up by roots and thereby translocated to shoots of +Fe plants. Uptake and translocation of ^{59}Fe from Humiron® did not differ in principle from that of Fe-EDDHA complex (Fig. 2). Utilization of ^{59}Fe from ^{59}Fe-labelled Humiron® was strongly enhanced by a low pH of the root external solution, the conditions conductive for enhanced reduction of Fe^{III}-chelates and thereby root uptake of this micronutrient (Römheld and Marschner, 1994). However, the uptake and especially translocation rates were significantly higher for ^{59}Fe-EDDHA, while the extrapalsmatic Fe was found to be about 3-4 times higher in the roots exposed to ^{59}Fe-humate, indicating higher precipitation of Fe from Humiron® in the root apoplast (Fig. 3). This is in accordance with data of Cesco et al. (2002), which showed a large pool of extraplasmatic Fe formed in cucumber roots after supplying Fe-WEHS complex. Their study also demonstrated that cucumber plants were able to mobilize extraplazmatic ^{59}Fe precipitated after roots being incubated in Fe-WEHS supplied nutrient solution. Remobilization of extraplasmatic Fe was particularly evident at acidic condition (Bienfait et al., 1985; Zhang et al., 1991; Strasser et al., 1999). Hence, this extraplasmatic Fe pool can act as a good buffer for available Fe in the rhyzosphere which, depending of the capacity of strategy 1 plant species to acidify rhizosphere, allows the mobilization and translocation to the shoot of considerable amounts of apoplastic Fe.

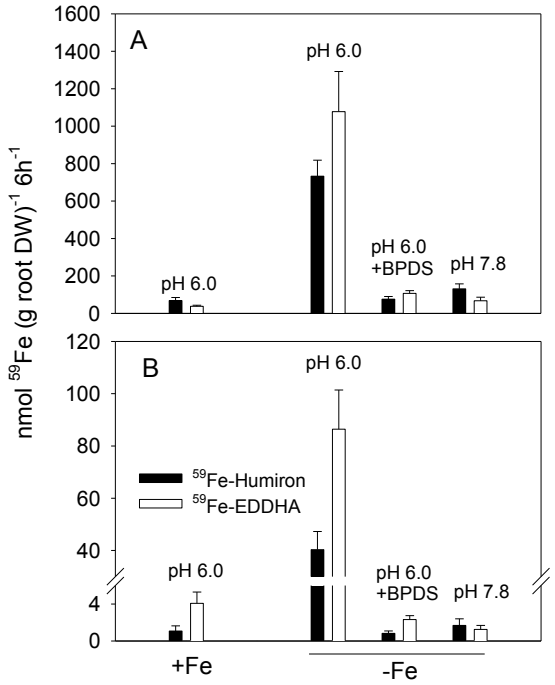

Figure 2. Root uptake (A) and root-to-shoot translocation (B) of ^{59}Fe in Fe-sufficient (+Fe) and Fe-deficient (-Fe) cucumber plants (strategy 1) after 6-h-exposure to ^{59}Fe-labelled Humiron® and ^{59}Fe-EDDHA (control). Translocation rate was calculated as a sum of root and shoot Fe content divided by the root DW.

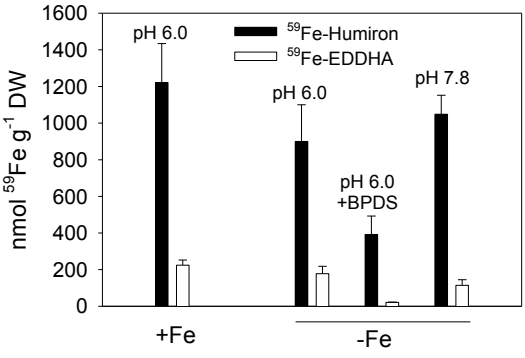

Figure 3. The concentration of extraplasmatic Fe of Fe-sufficient (+Fe) and Fe-deficient (-Fe) cucumber roots after 6-h-exposure to ^{59}Fe-labelled Humiron® and ^{59}Fe-EDDHA (control).

It is well known that the release of PS shows a diurnal rhythm with a morning maximum and an evening minimum. During the morning experiment barley plants precultured in –Fe nutrient solution took up about 4 times more Fe from ^{59}Fe-labelled Humiron® as compared either with +Fe plants (morning experiment) or with –Fe plants (evening experiment) (Fig. 4A). About 40% of the total Fe taken up by the roots from Humiron® was translocated to the shoots during 4 hours (Fig. 4B). Addition of deoxymugineic acid (DMA) in the uptake solution at final concentration of 20 μM during the evening experiment, increased both uptake and translocation rates of ^{59}Fe in –Fe plants approaching the levels recorded in the morning experiment (Fig. 4). Addition of DMA also significantly increased ^{59}Fe uptake and translocation by +Fe plants. Root extraplasmatic Fe in barley (not shown) was found to be about 2 times higher in the conditions with a lack of PS release (evening experiment) than in the uptake solution with high PS concentration (morning experiment or addition of 20 μM DMA in the evening).

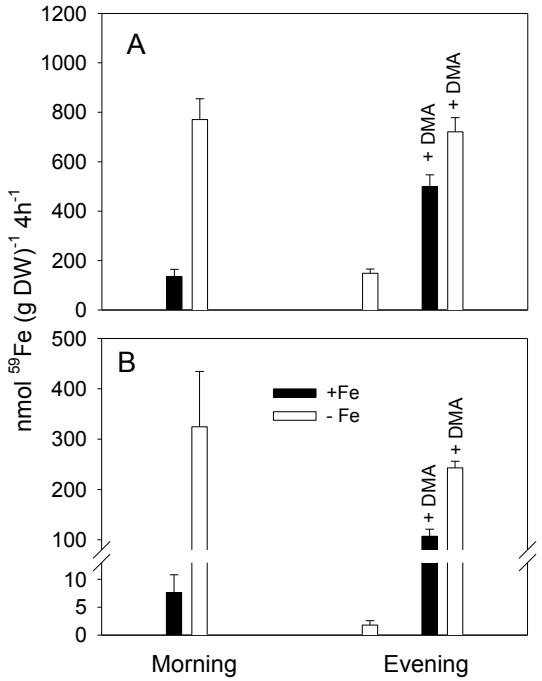

Figure 4. Root uptake (A) and root-to-shoot translocation (B) of ^{59}Fe in Fe-sufficient (+Fe) and Fe-deficient (-Fe) barley plants (strategy 2) after 4-h-exposure to ^{59}Fe-labelled Humiron®. To prevent latent Fe deficiency plants were sprayed with 0.3% (w/v) Fe-citrate twice daily. DMA was added to the uptake solution at the final concentration of 20 μM. Morning, period of high root PS release; evening, period of low root PS release. Translocation rate was calculated as a sum of root and shoot Fe content divided by the root DW.

CONCLUSION

Iron from Humiron® can easily be acquired by strategy 1 plant species (e.g. cucumber) via the reduction of Fe^{III}-humates by the inducible plasma membrane-bound reductase, similarly to Fe acquisition from synthetic Fe^{III} chletes such as Fe-EDTA or Fe-EDDHA. Strategy 2 plant species (e.g. barley) can also efficiently utilize Fe from Humiron® via ligand exchange between humates and PS released under Fe deficiency. Thus, utilization of Fe from Humiron® is comparable to the utilization

of Fe bound to water-extractable humic substances (extracted from peat) as has previously been reported by Cesco et al. (2002). There is a high potential for application of natural-based Fe-chelates such as Humiron® in organic crop production, as these chelates are more biodegradable than the synthetic ones, and therefore environmentally benign.

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