Identification of iron pools in the roots of cucumber during recovery from iron deficiency

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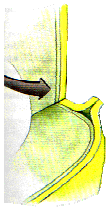
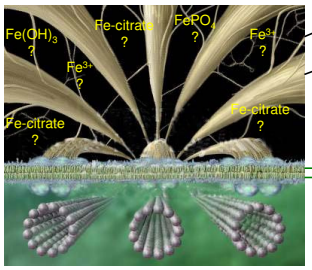
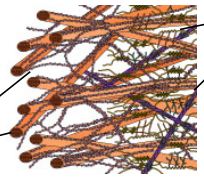
Capsule: Mössbauer spectroscopy was applied in order to identify chemically distinct iron compounds in the roots of cucumber. The formation of ferrous iron has been validated and the decrease of reducing activity has been monitored during recovery from iron deficiency.

Background

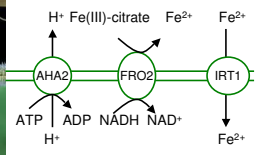






pectin binds metal ions with its carboxyl groups

extensin



High affinity iron uptake reactions in Strategy I plants [1]

The modified Hoagland nutrient solution contains:

1.25 m*M* KNO_3
1.25 m*M* $Ca(NO_3)_2x4H_2O$
0.5 m*M* $MgSO_4x7H_2O$
0.25 m*M* $KH_2(PO_4)$
11.6 μM H_3BO_3
4.6 μM $MnCl_2x4H_2O$
0.19 μM $ZnSO_4x7H_2O$
0.12 μM $Na_2MoO_4x2H_2O$
0.08 μM $CuSO_4x5H_2O$
10 µM Fe(III)-citrate

Iron chemical species in the nutrient solution:

2.8% $FeHPO_4^+$
1.1% Fe-citrate
71.8% Fe[citrateOH]$^-$
24% $[Fe_2Citrate_2(OH)_2]^{2-}$

Predicted by MinteqA2 (US Environmental Protection Agency, Washigton DC) in [2].

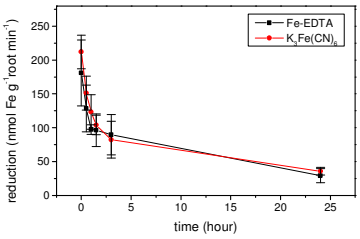
Objectives

The aim of this work was to
- detect the chemical forms of iron basically in the root apoplast,
- check and follow the iron reduction process.

Results

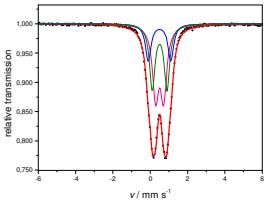
Iron deficient roots adsorbed 30.4 nmol Fe-citrate (17.1 nmol g^{-1} FW) in one hour 59% of which was removable as Fe(II)-bipyridyle. After a week 5.54 nmol Fe-citrate remained on the roots 27.6% of which was still removable. The rest was translocated to the shoot.

Iron adsorption and uptake was measured with ^{59}Fe-citrate labelling using a γ-scintillation counter. Washing procedure was made as in [3] using bipyridyle+dithionite.



Fe-EDTA and $K_3Fe(CN)_6$ reduction of 18-day-old cucumber roots grown in the above nutrient solution without iron and supplied with 0.5 mmol Fe-citrate for 0, 0.5, 1, 1.5, 3 and 24 hours, respectively, was measured photometrically as Fe(II)-$BPDS_3$. Analytical reactions for $K_3Fe(CN)_6$ reduction measurements were made as in [4]. Data are means±SD, n=5.

Root ferric chelate reductase activity decreased sharply for 1 hour whereas ferricyanide reduction did so for 1.5 hours as a response to Fe supply. After 24 hours both reached the iron sufficient level (7.5±3.7 and 36.0±11.4 nmol Fe g^{-1} min^{-1} for Fe-EDTA and FeCN, respectively). The measurement of the samples show that the Fe-EDTA and FeCN reducing activities are almost identical.

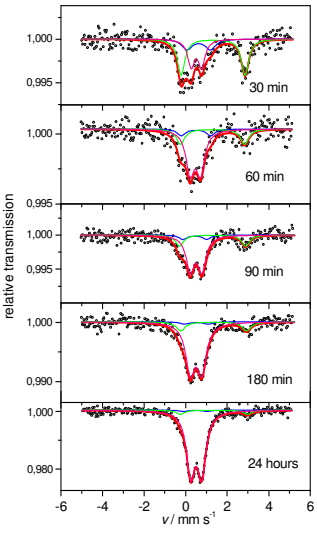


Mössbauer spectra of iron sufficient cucumber roots supplied with 10 µM ^{57}Fe-citrate for the 3-week growth period. (red line)

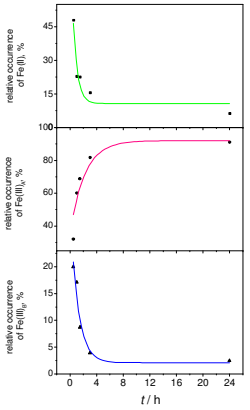
The main iron component $Fe(III)_A$, is a high-spin Fe^{3+} in octahedral coordination with δ=0.50 mm/s, Δ=0.44 mm/s (doublet shown with pink line). Its relative content is 44%.

The second iron component ($Fe(III)_B$) has a significantly higher quadrupole splitting: δ=0.50 mm/s, Δ=1.22 mm/s (blue doublet) with 21%.

The third iron component ($Fe(III)_C$) exhibiting δ=0.50 mm/s, Δ=0.80 mm/s. It can be assigned to ferritin and its relative amount is 35 % (olive doublet).



Mössbauer spectra of iron deficient cucumber roots supplied with 0.5 mmol ^{57}Fe-citrate for 0.5, 1, 1.5, 3, 24 hours. (red line)



Changes in the relative content of the components indicated in the Mössbauer spectra

In the iron deficient samples, an Fe^{2+} component is well detectable while in other studies under iron sufficient conditions, only Fe^{3+} was shown in different roots [5,6,7].

The Fe^{2+} component can be assigned as a ferrous hexaaqua complex based on its characteristic Mössbauer parameters (δ=1.34±0.04 mm/s, Δ=3.17±0.12 mm/s, indicated with green line). Its relative content is decreasing with the time of iron supply from 48% to 6%.

The main iron component ($Fe(III)_A$, 32% after 30 min and 91% after 24 hours) is a high-spin Fe^{3+} in octahedral coordination with δ=0.49±0.03 mm/s, Δ=0.48±0.04 mm/s (doublet shown with pink line).

The third iron component ($Fe(III)_B$) has a significantly higher quadrupole splitting: δ=0.48±0.04 mm/s, Δ=1.13±0.02 mm/s (blue doublet) and its relative content decreases from 20% to 3%.

The exponential change of the Fe(II) into $Fe(III)_A$ refers to the reoxidation and assimilation of iron.

The reduced iron is probably not associated to the carboxyl groups in the cell wall, it remains in hexa-aqua coordination.

The $Fe(III)_B$ species can be attributed to jarosite $KFe_3(OH)_6(SO_4)_2$, or its analogous compounds, since the parameters agree with those of jarosite [7,8], and the nutrient solution contained suitable amount of sulfates. Jarosite is assumed to be loosely connected with the cell wall.

Conclusions

- Four different chemical forms of iron was found in the roots of iron sufficient and iron deficient cucumber plants using Mössbauer spectroscopy: (A) a high-spin Fe^{3+} compound in octahedral coordination, (B) jarosite, (C) ferritin and (D) a ferrous hexaaqua complex.

- The formation of Fe^{2+} was directly evidenced with Mössbauer spectroscopy in iron deficient roots and the recovery upon iron supply could be followed due to the simultaneous decrease of Fe^{2+} form and increase of Fe^{3+} forms.

References

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