CUCUMBER AND NANOPARTICLES: CAN *C. SATIVUS* UTILISE NANOFERRIHYDRITE AS IRON SOURCE?

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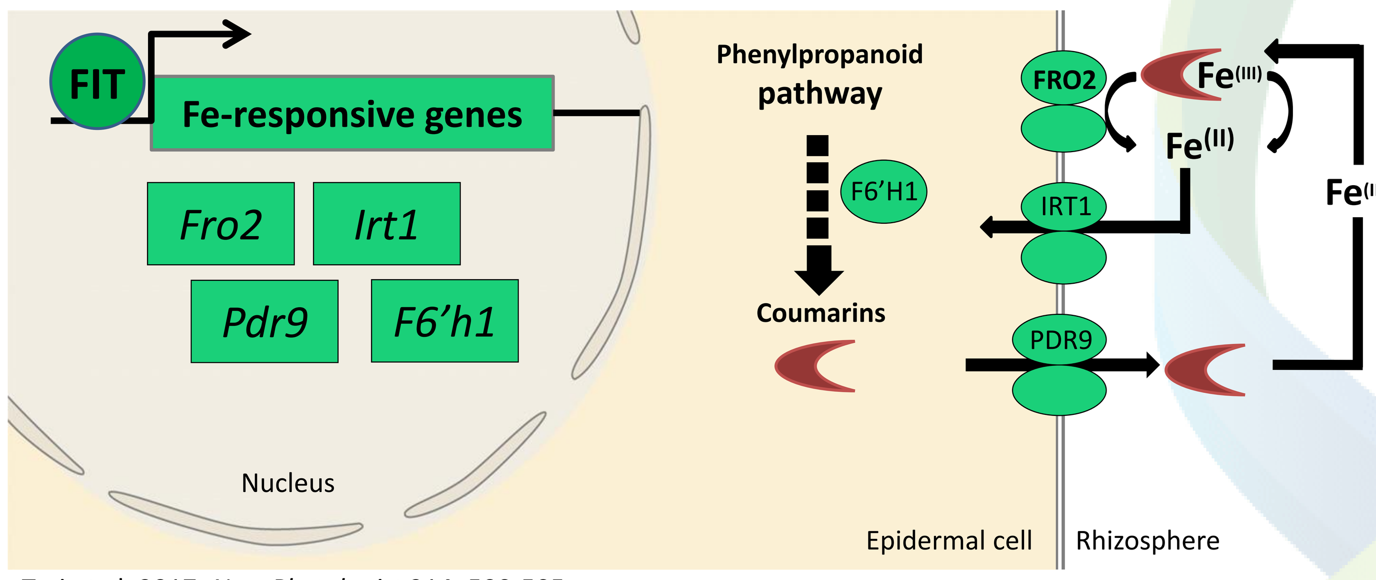
Introduction

The iron deficiency caused by alkaline soil is a permanent problem worldwide. Nanoparticles, like the studied nanoferrihydrite can provide the solution of iron precipitation on alkaline condition. Due to it's $Fe^{(III)}$ stabilizing effect it has a high potential to become an ingredient of fertilizers. Plants acquire iron from the rhizosphere in different ways. Cucumber (*Cucumis sativus*) uses the reduction-based strategy (Strategy I). Root ferric chelate reductases (belongs to the Fro family) are an iron deficiency-inducible membrane bounded enzymes that are responsible for reduction of iron at the root surface.

To investigate the effects and bio-utilisation of the nanoferrihydrite on cucumber iron uptake iron deficient plants were grown and regenerated in hydroponics. The short time expression profiles of *Fro* genes of root and the reductase activity of Fro were analysed.

IRON UPTAKE UNDER IRON DEFICIENT CONDITION
(A. thaliana)

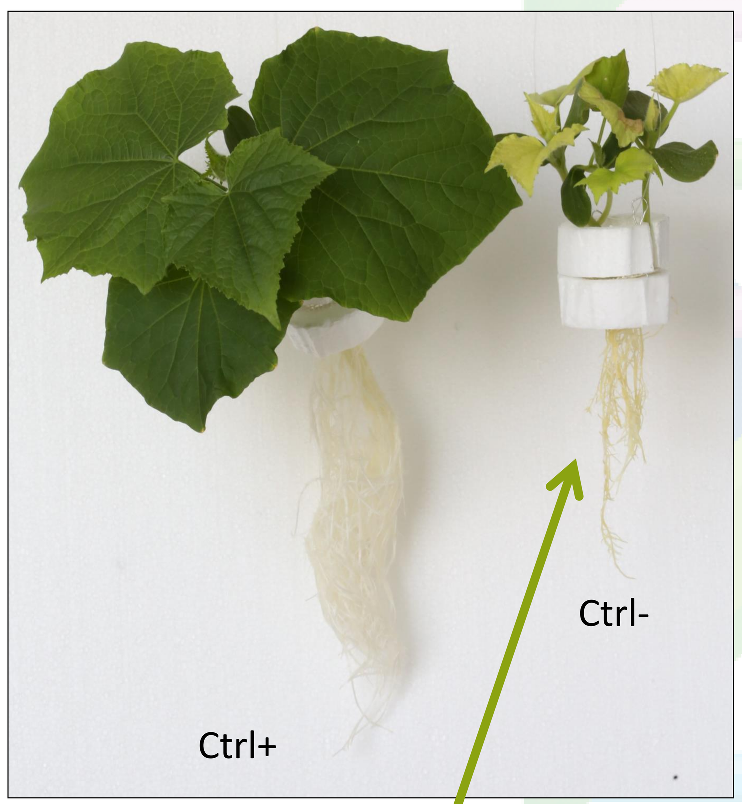


Tsai et al. 2017, *New Phytologist* 214: 500-505.

Methods

Plant material

- 18 days old *C. sativus* cv. Joker F1
- climate chamber
- ¼ Hoagland- solutions:
  - Ctrl+: 10µM $Fe^{(III)}$ -citrate
  - Ctrl - : without Fe
  - **dFe+NFH:** 20µM nanoferrihydrite NH-2015/001/4x pH=1,5



- reduced, clorotic already first leaves
- reduced, „fishbone" structure root

qRT-PCR

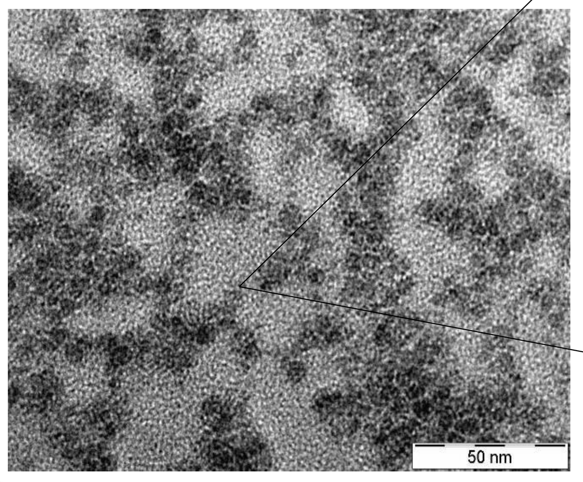
- GenoVision mRNA Isolation Kit
- ABI StepOne Plus Instrument
- ICGs: ***Act*** (actin), ***Cacs*** (chlatrin adaptor complex subunit)
- GOI: ***Fro1*** and ***Fro4*** (iron-chelate oxidoreductase-1 and -4).

Root Fe (III) chelate reductase activity assay

- **Fe (III) -EDTA** 500 µM
- **BPDS** (bathophenanthroline disulfonic acid) 400 µM
- **MES** (morpholinoethanesulfonic acid) 5mM → pH=6
- **½ Hoagland**

The Nanoferrihydrite

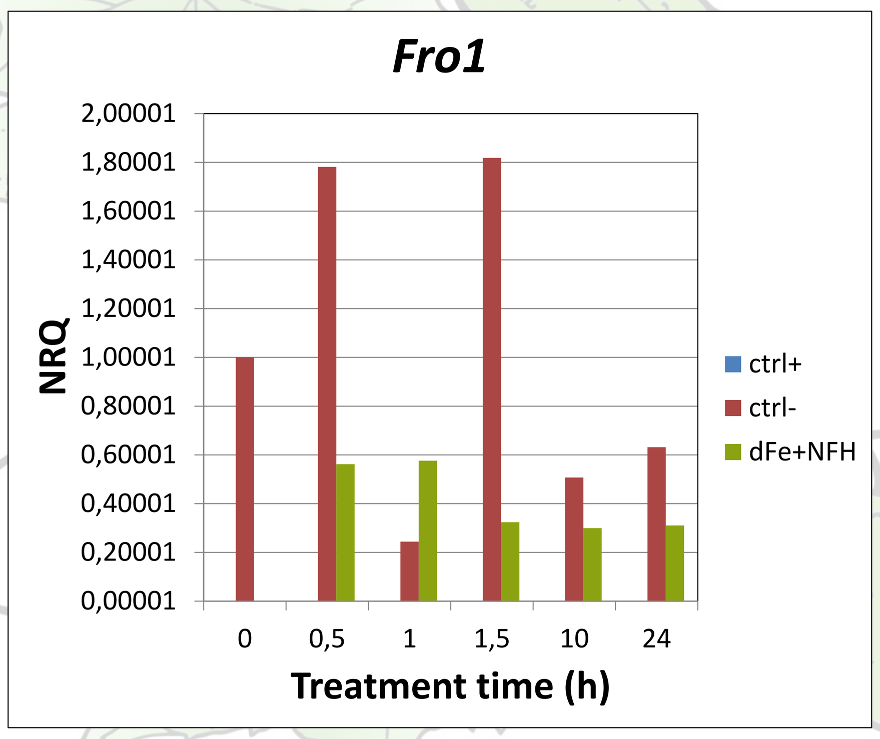
(α-Fe_2O_3)
NH-2015/001/4x

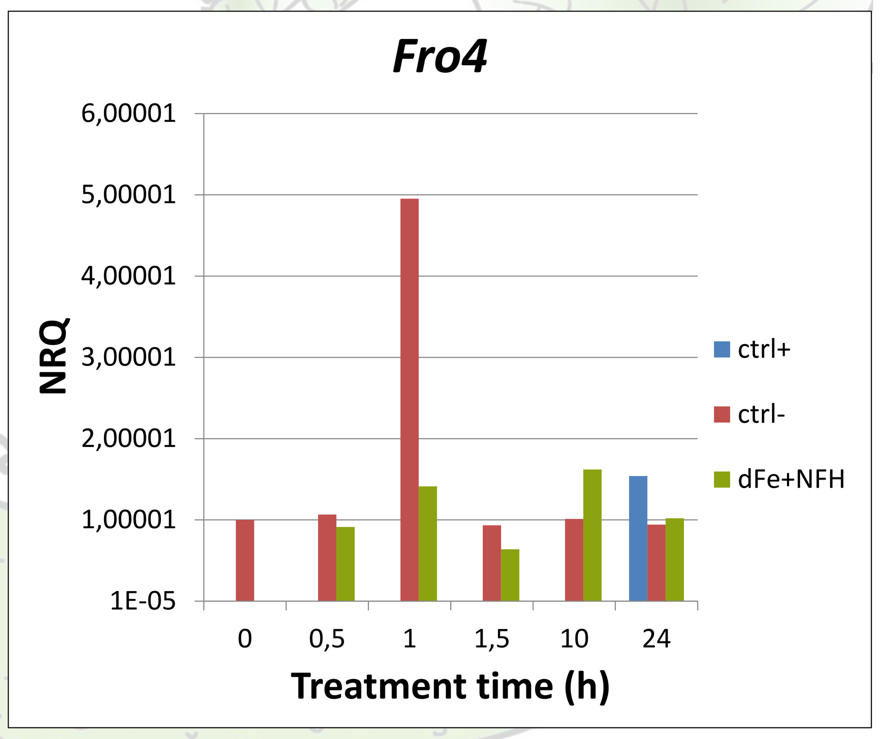


Colloidal suspension with 5-8 nm particle size $Fe^{(III)}$ with organic envelope → protect against Fe-precipitation→ stabile complex in the soil

Results

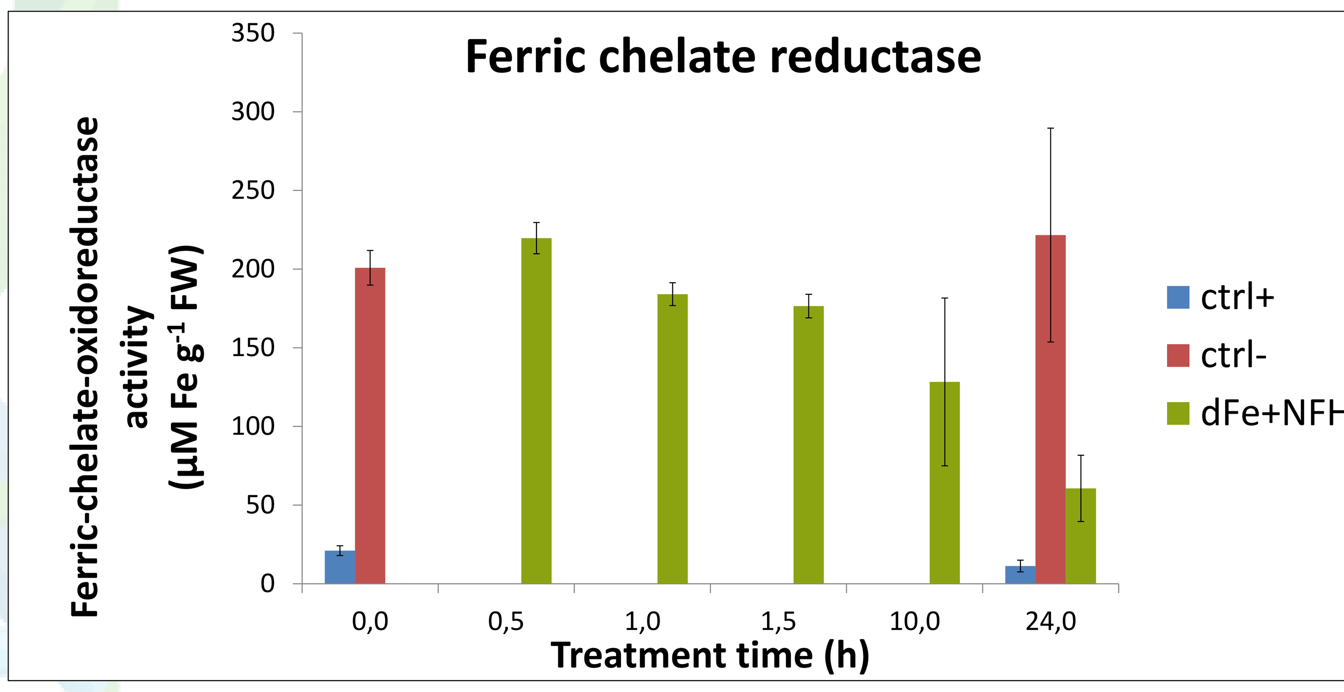
***AtFro*-like genes were identified in cucumber genome by sequence homology (http://www.icugi.org/).**
Two possible homologues Csa5g175770 as *Fro1;* Csa3g183380 as *Fro4*.





The *Fro1* expression constantly decreased following the treatment.

The *Fro4* expression did not show significant changes during the experiment.



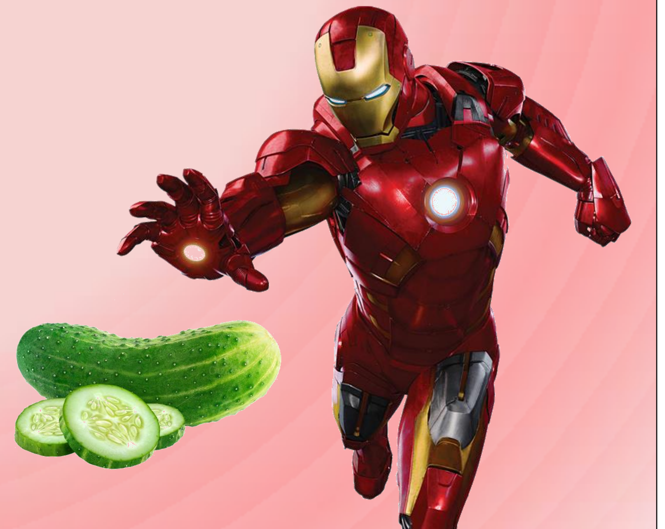
The functional assay measures the whole reductase potential of the roots, but does not distinguish between different reductases. Total Fro enzime activity shows similar charasteristics like *Fro1* gene expression, reflecting the role of this protein.

Discussion

Nanoferrihydrite treatment induced comparable changes on both transcription and enzyme activity levels. One theory assumes that roots take up the whole nanoherrihydrite particle, in contrast others suggest that the nanoparticle is disaggregated in the rhizosphere. As a consequence mobilised iron complexes are available for the root Fe uptake system. Our observations support the latter hypothesis, since both in the *Fro1* gene expression and in the reductase activity rapid changes occurred after $Fe^{(III)}$-EDTA treatment.

The sharp decrease in the gene expression and reductase activity indicates that using nanoferrihydrite as iron source, Strategy I. plants such as cucumber are able to regenerate. These results are promising in iron biofortification.

This investigation contributes to the understanding of the effects of nanomaterials as potential fertiliser ingredients, providing the production of iron-fortified vegetables, preventing iron deficiency-induced anemia.



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This work has been supported by the grants NKFIH-OTKA PD-112047, PD-111979 and NKFIH-OTKA K1 115913, K115784.
Á. Solti was also supported by the Bolyai János Research Scholarship of the Hungarian Academy of Sciences (BO/00207/15/4).

Green For Good IV., June 19-22, 2017, Olomouc, Czech Republic