Gene expression analysis of soybean plants treated with iron-based nanofertilizers

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Introduction

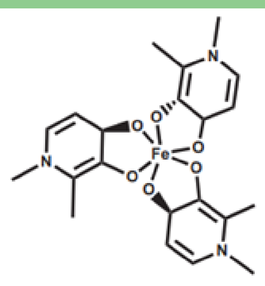

Figure 1- Iron (III) chelate of the 3-hydroxy-4-pyridinones (3,4HPO) class used in the produced nanoparticles.

In the current agronomic context, approximately 30% of the world's cultivatable land is composed by calcareous alkaline soils, which can induce iron deficiency chlorosis in certain crops, potentially diminishing their yields. The present study focuses on the development of nanofertilizers, consisting of polymeric nanoparticles carrying iron (III) chelates belonging to the 3-hydroxy-4-pyridinones (3,4HPO) class (Figure 1).
Previous research [1,2] has highlighted these chelates' efficacy in counteracting chlorosis in *Glycine max* plants.
There are three key genes responsible for absorption (*FRO2*), transport (*IRT1*), and storage (*Ferritin*) of iron whose expression can be analysed to make conclusions on the efficiency of these nanofertilisers. The primary hypothesis of this study is that these nanofertilizers can provide a consistent supply of iron to affected plants, being a more sustainable solution to current commercially available products.

Methods

In our recent investigation, *G. max* seeds were subjected to three distinct treatments:
a) Nanosuspensions with the newly produced nanoparticles containing FeDM, the iron chelate of the 3,4HPO class in the concentrations of 10 μM and 20 μM;
b) Iron chelate solutions in water, containing the same compound in the concentrations of 10 μM and 20 μM ;
c) A control group treated with water.

The seeds were planted in soil and grown for one month until reaching the V3 stage.
After harvesting, the plants' stems, roots, and trifoliates were separated, with the latter two being pulverized. RNA was then extracted from the pulverized root and trifoliate tissues, from which cDNA was synthesized. After this, a quantitative polymerase chain reaction (qPCR) reaction was performed, using the appropriate primers, to quantify the expression of the genes encoding *FRO2* and *IRT1* in the root tissue and *FERRITIN* in the leaf tissue.

Figure 2- Schematic representation of the process from the pulverization of plant tissues to qPCR reaction. Created with BioRender.com

Results

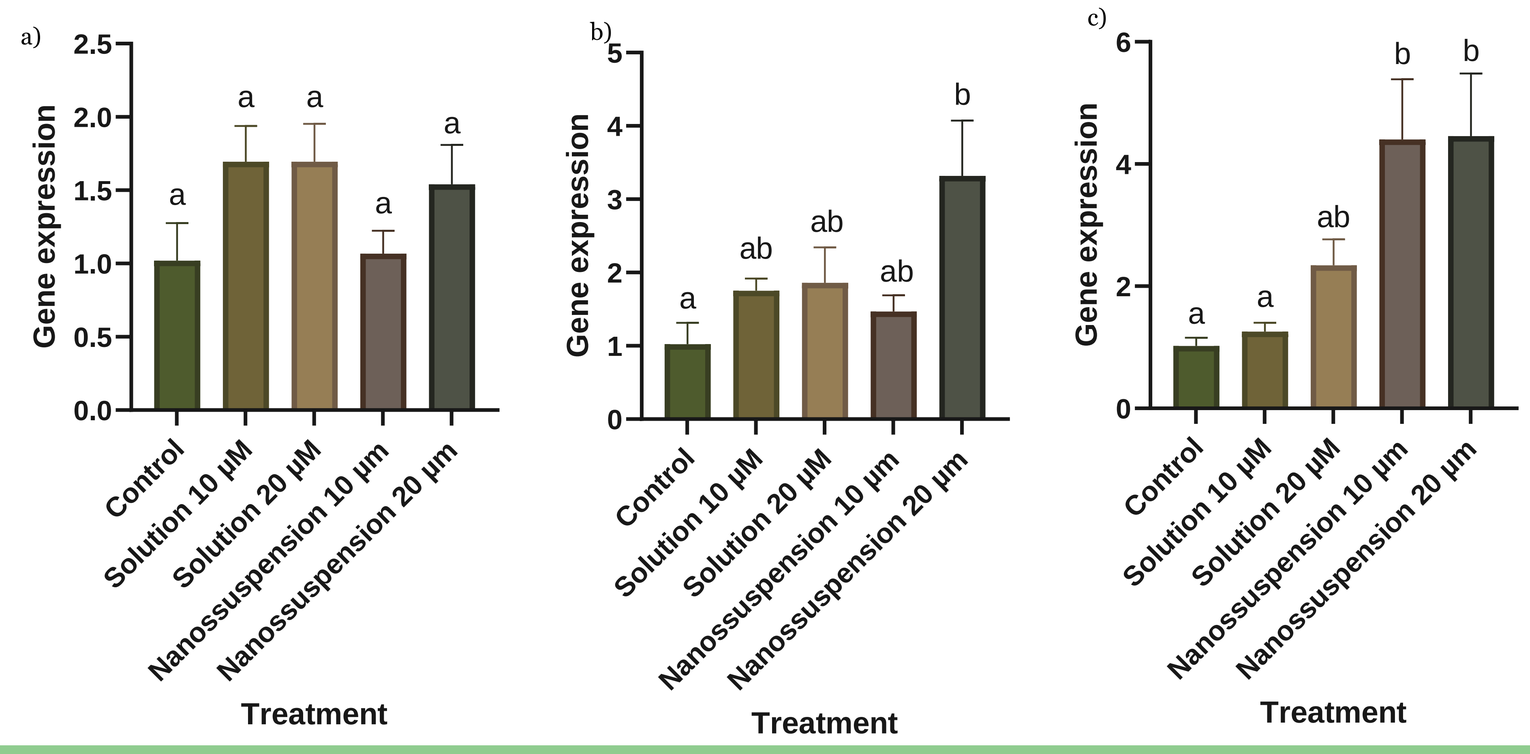

Figure 4- Chlorotic soybean leaves.

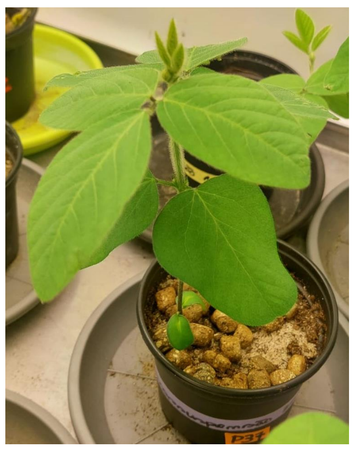

Figure 5- Healthy soybean leaves.

Figure 3- Effect of different Fe(III)-chelate treatments on:
a) *FRO2* relative gene expression;
b) *IRT1* relative gene expression;
c) *Ferritin* relative gene expression.
Different letters indicate significant differences ($p<0.05$) by One-way ANOVA with Tukey test.

Conclusion and future perspectives

- Plants that had their seeds exposed to nanosuspensions appeared to possess a higher amount of Fe stored in the leaves, suggesting that the nanoparticles were successful in providing the necessary amount of Fe to the plants. Also, plants treated with 20 μM nanosuspensions also seem to have enough Fe to induce the expression of *IRT1*.
- The forthcoming phase will encompass an in-depth mineral, enzymatic, amino-acid and Fourier-transform infrared spectroscopy (FTIR) analysis of both plants' tissues. In the end, we expect to get insight on efficacy of these nanoparticles in enhancing iron uptake through this type of application.

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References:

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[2] C. S. Santos, A. Leite, S. Vinhas, S. Ferreira, T. Moniz, M. W. Vasconcelos, M. Rangel, Plant Direct, 4 (2020) e00256.