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Qualitative Root Ferric Reductase activity assay with **Arabidopsis thaliana** seedlings

Forked from Protocol for Qualitative Root Ferric Reductase activity assay with Arabidopsis thaliana seedlings

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Abstract

Iron (Fe) is an essential micronutrient for plant growth and development but is often poorly available in soils due to its low solubility under aerobic conditions. To overcome this limitation, plants have evolved reduction-based mechanisms for Fe uptake that rely on membrane-bound ferric reductase oxidases (FROs), such as FRO2 in *Arabidopsis thaliana*. These enzymes reduce ferric iron (Fe^{3+}) to its bioavailable ferrous form (Fe^{2+}). The qualitative Root Ferric Reductase Assay enables visualization of this reduction process by detecting colour changes associated with Fe^{2+}–ferrozine complex formation.

Aim of the experiment:

The aim of this experiment was to assess the activity and spatial localization of ferric reductase oxidases in *Arabidopsis thaliana* roots under Fe-sufficient (+Fe) and Fe-deficient (–Fe) conditions. The method allows qualitative comparison of Fe reduction capacity between genotypes and visual observation of root zones involved in Fe uptake.

Open question:

While FRO2 is known to play a central role in Fe acquisition, the spatial dynamics and possible contribution of other reductase isoforms under Fe deficiency remain incompletely understood. This qualitative assay provides an initial, visual approach to explore these aspects before subsequent quantitative analysis.

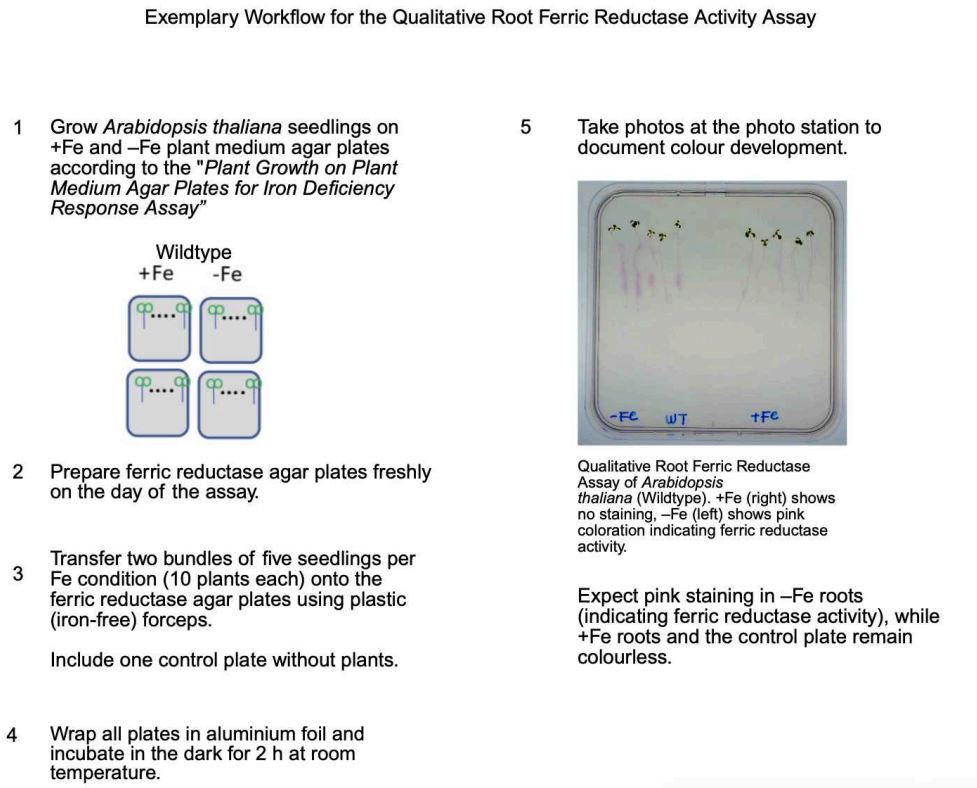
Conclusion:

Distinct pink to magenta staining patterns were observed in the root tips of Fe-deficient plants, indicating increased ferric reductase activity. In contrast, Fe-sufficient plants displayed little to no staining. The qualitative assay successfully demonstrated that Fe deficiency induces FRO activity, confirming its value as a diagnostic tool for Fe uptake competence in roots.

Image Attribution

Exemplary picture: Comparison of -Fe Col-0 plants (left side) vs. +Fe Col-0 plants (right side). The photo was taken after 2h (photo taken by Enya Becker).

Guidelines



Exemplary workflow for the Qualitative Root Ferric Reductase Assay with *Arabidopsis thaliana*.
The figure illustrates the experimental steps from plant growth to colour documentation. The photo, taken by Enya Becker after 2 hours of incubation, shows weak pink staining in +Fe roots and stronger pink coloration in –Fe roots, indicating enhanced ferric reductase activity under iron deficiency. The figure was created in https://BioRender.com.

Materials

Needed materials:

- Intact Arabidopsis plants (Please note that the assay is performed with living and intact plants), e.g. plants raised as described in Elke Wieneke, Enya Becker, Petra Bauer 2025. Plant Growth on plant medium agar plates for iron deficiency response assays. **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvrw84blmk/v1
- Measuring cylinders and glass beakers
- Microwave or heating plate (for dissolving agar)
- Pipettes and sterile pipette tips
- plastic forceps (iron-free, don't use metal/ steel!)
- Parafilm (for sealing plates)
- Aluminium foil (for wrapping plates during incubation; protects from light)
- Permanent marker (for labelling plates)
- A camera/ photo station

- Ferric reductase agar plates (120 × 120 mm (≈12 × 12 cm), volume ~50 ml medium per plate):

Compound	Stock concentration	Final concentration	100 ml (2 plates)	250 ml (5 plates)	500 ml (10 plates)
$CaSO_4$	10 mM	0.5 mM	5 ml	12.5 ml	25 ml
Plant agar	Powder	0.7 %	0.7 g	1.75 g	3.5 g
FeNaEDTA	10 mM	0.25 mM	2.5 ml	6.25 ml	12.5 ml
Ferrozine	50 mM	0.25 mM	0.5 ml	1.25 ml	2.5 ml
Millipore H_2O			92 ml	217.5 ml	435 ml

Composition of ferric reductase agar plates used for the qualitative Root Ferric Reductase Assay.
The table lists the required amounts of each compound to prepare 100 ml (≈ 2 plates), 250 ml (≈ 5 plates), and 500 ml (≈ 10 plates) of medium. The final medium contains 0.5 mM $CaSO_4$, 0.25 mM FeNaEDTA, and 0.25 mM ferrozine in 0.7 % (w/v) plant agar. Millipore water is added to the final volume. Plates should be poured under sterile conditions and kept in the dark to prevent light-induced Fe reduction.

Before start

Preparations in advance:

Grow *Arabidopsis thaliana* seedlings following the protocol *"Plant Growth on Plant Medium Agar Plates for Iron Deficiency Response Assays"* (doi: ...), e.g. using 6-day or 7–14+3-day growth systems on plant medium agar plates.

For analysis, 5 *Arabidopsis thaliana* (Col-0) seedlings were grouped in one bundle. Two bundles were placed per assay plate and per Fe treatment, corresponding to 10 plants per condition. In total, two plates were prepared for the +Fe and –Fe treatments (20 plants in total), plus one additional control plate without plants to monitor background colour development of the assay medium.

Prepare the ferric reductase agar plates

1 Prepare the ferric reductase agar plates freshly on the day of the assay.

1.1 Boil water with 0.5 mM $CaSO_4$ and 0.7 % (w/v) agar in the microwave until the agar is completely dissolved.
Let the medium cool down to approx. 50°C.

1.2 Add 0.25 mM FeNaEDTA and 0.25 mM ferrozine, fill up with deionized H_2O to the desired volume.

1.3 Pour plates and keep them in the dark before the assay (in the dark to avoid light-triggered Fe reduction in agar plates).

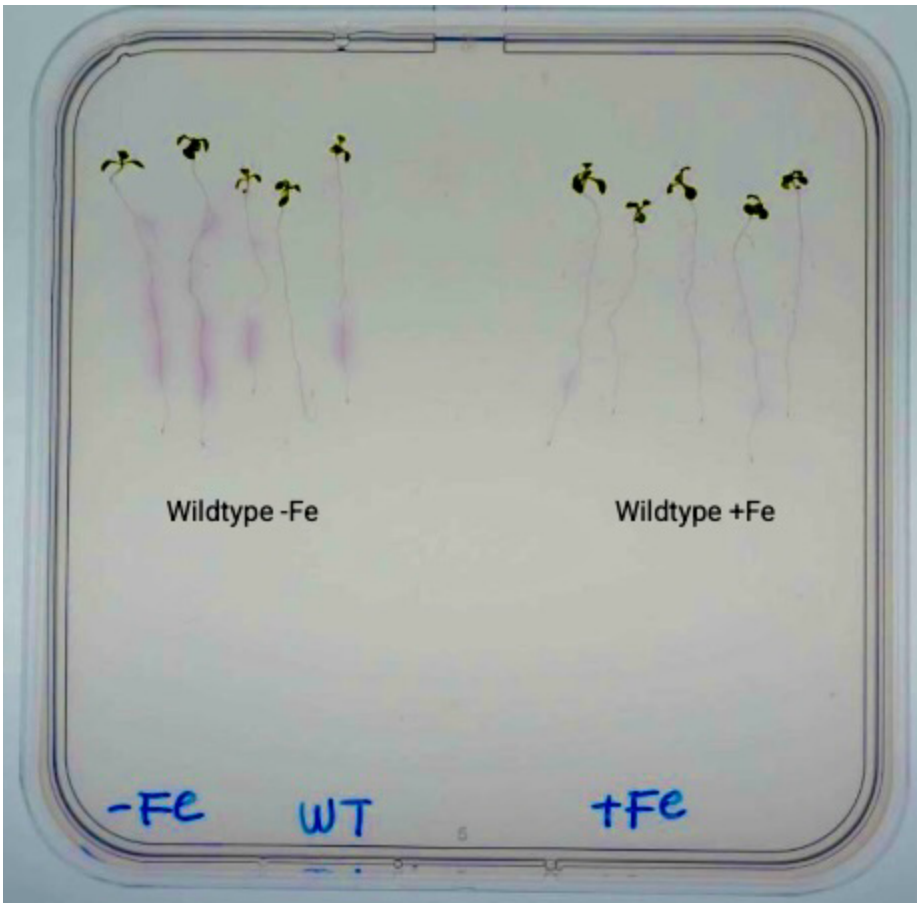
Start the Assay

2 Transfer the plants to the ferric reductase agar plates with plastic forceps.
We recommend to use at least 2 bundles of 5 seedlings per genotype and growth condition.

2.1 Wrap the plates with aluminium foil and incubate them in a drawer for approx. 2 hours (note down the time!).

Finish and document

3 Once the reaction is well-developed take photos with a camera stand, with low light from the bottom and the side. It might be useful to take photos after different time points, e.g. 2h, 4h, 24h.



Qualitative Root Ferric Reductase Assay of *Arabidopsis thaliana* wild type (Col-0). Seedlings were incubated for 2 hours on ferric reductase agar plates. Plants grown under Fe-sufficient conditions (+Fe, right) show only weak pink staining, whereas Fe-deficient seedlings (−Fe, left) display pronounced pink coloration along the roots, indicating enhanced ferric reductase activity under iron deficiency.
The photo was taken by Enya Becker and edited in https://BioRender.com.