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

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Protocol status: Working
We use this protocol and it's working

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Abstract

Aim of this experiment: examine the activity of Ferric reductase oxidases like FRO2. This is achieved by qualitative observation of colour changes. You might also see spatial localization of the ferric reductase activity in the different root zones.

Image Attribution

The photo was taken after 4h (photo taken by Jannik Hornbergs).

Exemplary picture: Comparison of +Fe Col-0 plants (left side) vs. -Fe Col-0 plants (right side). The photo was taken after 4h (photo taken by Jannik Hornbergs).

Materials

Needed materials:

- Intact Arabidopsis plants (Please note that the assay is performed with living and intact plants)
- Ferric reductase plates (see recipe)
- plastic forceps (iron-free, don't use metal/ steel!)
- A camera/ photo station

Compound table:

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

Before start

Preparations in advance:

Grow your plants in the desired system:

- 6 days vertical growth on Hoagland +Fe plates (50 µM final concentration of 10 mM FeNaEDTA stock solution) or Hoagland -Fe plates (no FeNaEDTA is added)
- 14+3 days system: Plants are grown for 14 days vertically on Hoagland +Fe plates, then all plants are transferred to fresh +Fe / -Fe Hoagland plates and grown vertically for three additional days.

Think of proper controls!

For analysis 5 plants are grouped in a bundle, at least 2 bundles are required = at least 10 plants are needed.

Bundles are needed to have a stronger staining of the reduced Fe in the agar plates.

Protocol for the day of the assay:

1 Always prepare the plates freshly on the day of the assay.

2 Boil water with 0.5 mM $CaSO_4$ and 0.7 % (w/v) agar in the microwave until the agar is completely dissolved.

3 Let the medium cool down to approx. 50°C.

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

5 Pour your plates under the sterile hood and keep them in the dark before the assay (in the dark to avoid light-triggered Fe reduction in agar plates).

6 Transfer the plants to the assay plates with plastic forceps; we previously used at least 2 bundles of 5 seedlings per genotype and growth condition which worked well.

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

8 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.

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Exemplary picture: Comparison of +Fe Col-0 plants (left side) vs. -Fe Col-0 plants (right side). The photo was taken after 4h (photo taken by Jannik Hornbergs).