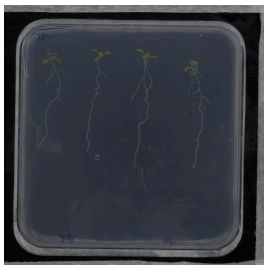
Mar 28, 2019

Quantification of salt-induced changes in Root System Architecture in Arabidopsis



DOI
dx.doi.org/10.17504/protocols.io.zkqf4vw

Magdalena M Julkowska[1], Christa Testerink[2]

[1]Boyce Thompson Institute; [2]Wageningen University, University of Amsterdam,

Magdalena M Julkowska
Boyce Thompson Institute

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.zkqf4vw

Protocol Citation: Magdalena M Julkowska, Christa Testerink 2019. Quantification of salt-induced changes in Root System Architecture in Arabidopsis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.zkqf4vw

Manuscript citation:
Julkowska et al., 2014 Plant Phys DOI: https://doi.org/10.1104/pp.114.248963

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

Created: March 28, 2019

Last Modified: March 28, 2019

Protocol Integer ID: 21872

Keywords: Root System Architecture, Arabidopsis, salt stress,

Abstract

The protocol was developed for observing salt-induced changes in Root System Architecture of Arabidopsis and other small plants. The seeds are germinated in control conditions, and exposed to stress 4 days after germination. As the agar plates are transparent, this system provides quick, easy and non-destructive method to observe development of roots. This protocol was used to produced the data in two manuscripts from our lab - **Julkowska et al., 2014 Plant Physiology** and **Julkowska et al., 2017 Plant Cell**.

Guidelines

This assay is working well for Arabidopsis seedlings and other small plants. Please make sure that the plant you are working with is not inhibted in its germination by light exposure.

Materials

MATERIALS

- Potassium hydroxide **P212121**
- Murashige & Skoog medium including B5 vitamins **Duchefa Biochemie Catalog #**M0231
- MES, free acid, monohydrate **Bio Basic Inc. Catalog #**MB0341.SIZE.25g
- Sodium Chloride **Fisher Scientific Catalog #**S271-3
- Sucrose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S7903
- Daishin agar **Duchefa Biochemie Catalog #**9002-18-0

Make sure that you have:
- flatbed scanner **(Epson Perfection V700)**
- square petridishes **(Greiner dishes, square dish, 120 × 120 × 17mm, vented)**
- trays to put the plates in - either assembled in the workshop or use black trays to grow the plants in - with ribs on the bottom
- Growth Chamber - or other plant growth facility. Whever you are using, make sure that the light is not too close to the plate - this inhibits the roots growth. Try to have at least 20-30 cm distance between the plates and the light tubes.

Safety warnings

not applicable

Before start

Prepare the agar plates in advance - make sure to pour them in the sterile conditions in the laminar hood - preferably one that is not used for handling bacteria / fungi. You can store the poured plates at 4C in sealed plastic bags for couple of days / weeks.

1 Prepare germination media.

Per 1L of media: 2.2 g Murashi-Skoog, 5 g sucrose, 1 g MES Monohydrate, pH at 5.8 with KOH.

After adjusting the pH add 10g Dashin agar directly into the bottle. Make sure you add it AFTER measuring pH - it can clog the pH meter otherwise.Autoclave using liquid sterilization cycle.

Pour 40 ml per square plate (12×12cm) and let it dry in the laminar hood for at least one hour (can be up to three hours) - long drying time will reduce the condensation at the plate surface.

Put the plates (still in the laminar hood) in the plastic bags that they came in - put the tape to close the plastic bags and write your name & date ⇒ store at 4C

2 Sterilize the seeds.

Soak the seeds in 50% Bleach (50% from the original household bottle - diluted in 1:1 ratio with MQ) for 10 minutes - DON'T vortex but gently shake once in a while.

Transfer the work into the laminar hoodWash the seeds with sterile MQ using 1ml pipette 5-8 times.

After sterilization - store the seeds in the 4C for overnight to ensure equal germination.

NOTE: Do not store the seeds at 4C for longer than a week - after that, they start to germinate in the dark and you end up with etiolated seedlings - NOT good for the experiment

3 Put the seeds on the plate (approximately 150-200 seeds per plate)

Get the seeds from 4C and transfer both the 1/2 MS agar plates and the seeds into a cleaned laminar hood.

Use the pipette with the manual control of volume (the ring) and suck the seed “suspension” into the 1ml pipette tip (STERILE!)

In the laminar hood - put the seeds onto the plate by gently touching the plate surface with the pipette tip - if they are not coming out - reduce the volume while touching the plate surfaceLet the plates dry in the laminar hood until all the water droplets evaporate - put the agar plates on the top of the plate lid for 5 minutes

Close the plates and wrap the plate ende using the leukopor tape.

4 Put in the plates in the growth chamber

usually, we grow the plates under +/- 70 degrees angle stacked in a ribbed tray used as a bottom for normal plant pots.

Let the seeds germinate in the growth chamber set at 16h light, 8h dark, 22C and 60% humidity.

5 Prepare the “treatment” plates:

Use the basic media concentration as described above - but now add salt - make media containing 0, 75 and 125 mM NaCl (added after pH measurement - pH should be ok because of the MES buffer).

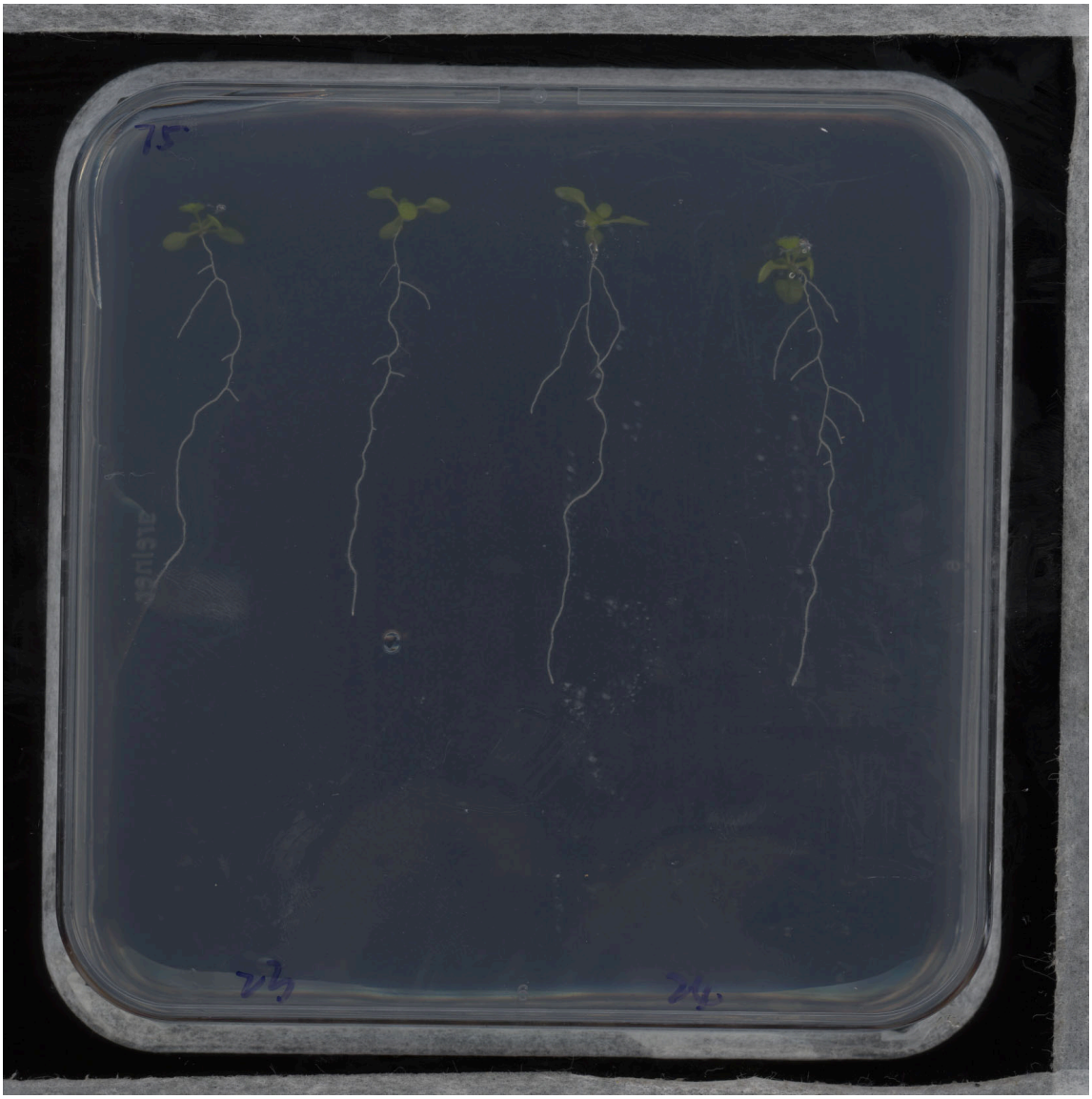
Pour the plates according to the instructions above and let dry in the laminar hood between 1 and 3 hours.

6 Four days after germination (usually - I put the plates in the growth chamber for germination on Thursday and transfer on Monday) - transfer the germinated seedlings to new plates containing 0/75/125 mM NaCl

Usually, we aim to have at least 12 replicas per condition per genotype.

Transfer the seedlings with sterile (or freshly opened) yellow pipette tips.Seal the plates with leukopor tape.

Each plate gets divided into two halves - and each half will contain 2 seedlings of each genotype. Please see the picture below:



The typical design of the plate. In the upper left corner there is a plate number, in the upper right corner there is a plate composition (0 / 75 / 125 mM NaCl), and the lower two numbers are corresponding to the plant genotype - encoded and written in the lab journal.

7 Put a dot with a permanent marker to mark the position of main root tip / scan the plates before putting them back into the growth chamber / Percival

8 Scan the plates every two days until the plants are 12 days old (or whenever the root architecture becomes too complex). Scan the plates at 200 dpi resolution - this will optimize the file size and still allow you to identify the emerging lateral roots.

IMPORTANT - make sure to scan the plants with the black background.

9 Analyze the root system architecture using the **Smart Root** or **EZ-Rhizo (windows only)**