Plate assay for quantification of Root System Architecture of Arabidopsis seedlings

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1 Works for me

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ABSTRACT

This is a standard assay for examining Root System Architecture of Arabidopsis seedling in response to hormone treatment or any other stress that can be easily "added" to agar media (such as salt, osmotic and such).

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

This experimental set up was used in the following papers:

Capturing Arabidopsis Root Architecture Dynamics with root-fit Reveals Diversity in Responses to Salinity, Magdalena M. Julkowska, Huub C.J. Hoefsloot, Selena Mol, Richard Feron, Gert-Jan de Boer, Michel A. Haring, Christa Testerink, Plant Physiology Nov 2014, 166 (3) 1387-1402; DOI: 10.1104/pp.114.248963

Genetic Components of Root Architecture Remodeling in Response to Salt Stress, Magdalena M. Julkowska, Iko T. Koevoets, Selena Mol, Huub Hoefsloot, Richard Feron, Mark A. Tester, Joost J.B. Keurentjes, Arthur Korte, Michel A. Haring, Gert-Jan de Boer, Christa Testerink, The Plant Cell Dec 2017, 29 (12) 3198-3213; DOI: 10.1105/tpc.16.00680

MATERIALS

NAME	CATALOG #	VENDOR
Murashige & Skoog medium including B5 vitamins	M0231	Duchefa Biochemie
MES, free acid, monohydrate	MB0341.SIZE.25g	Bio Basic Inc.
Sucrose	S7903	Sigma Aldrich
Daishin agar	9002-18-0	Duchefa Biochemie

Sterilize the seeds of Arabidopsis lines that you wish to use for the RSA assay and keep them for at least 48 h at 4°C to ensure higher germination rates

1 Sterilize the seeds by putting them first in 50% household bleach (the one you can buy at the supermarket - but no "extra strenght") for 10 minutes, then wash the seeds 5-10 times with 1 ml of sterile MQ water and finally put them in a sterile 0.01% agar to keep at 4°C. The 0.01% agar solution will prevent all the seeds sinking to the bottom, ensuring the equal exposure to the cold treatment, and later will help with putting the seeds on the plate.

Prepare 1/2 MS media for germination.

2 For 1L of media:
- 2.2 g of Murashi-Skoog powder
- 5 g sucrose
- 1 g of MES monohydrate buffer
- adjust pH to 5.8 with KOH (NEVER use NaOH!)
- pour the liquid into a 1 L bottle containing 10g of Daishin agar
- sterilize the media in the autoclave
- in the laminar hood pour the media into square petridishes (12 x 12 cm) - around 40-50 ml per plate
- let the media 'dry' in the laminar hood for 2 h
- store the plates in the plastic bags (the same as they were packed) in cold room untill necccessary

Put the seeds on agar plates in the laminar hood

3 If you have the seeds stored in 0.01% agar, then you can use a 1000 uL pipette to put the seeds on the plate - the tip opening is big enough to get the seed through.

The best is to keep the pipette tip, containing the seeds swimming in 0.01% agar, close to the media surface. Push the seeds out of the pipette by slowly reducing the pipette volume. Let the plate dry untill all the "water" around the seeds is evaporated. Seal the plate using the leukopor tape, or any other kind of surgical tape.

Place the agar plates with seeds under 70 degree angle in the growth chamber and leave for four days for germination.

4 The conditions for the growth chamber are up to you, but I usually germinate the seeds at 22°C, 60% humidity and long day conditions (16h light / 8h dark).

Also - I noticed that the seedlings grow very poor if they are very close to the light source. I usually try to keep the plates around 30 cm below the light source

Prepare treatment plates

5 For 1L of media:
- 2.2 g of Murashi-Skoog powder
- 5 g sucrose
- 1 g of MES monohydrate buffer
- IF you are ading salts / osmotic agent (like PEG or mannitol) add it at this stage of media preparation. If you want to add agents that are suseptible to degradation at high temperature (like hormones, antibiotics and such) add the filter-sterilized stock to the media just before pouring the plates
- adjust pH to 5.8 with KOH (NEVER use NaOH!)
- pour the liquid into a 1 L bottle containing 10g of Daishin agar
- sterilize the media in the autoclave
- in the laminar hood pour the media into square petridishes (12 x 12 cm) - around 40-50 ml per plate
- let the media 'dry' in the laminar hood for 2 h
- store the plates in the plastic bags (the same as they were packed) in cold room untill necccessary

After 4 days of germination, transfer the seedlings to the treatment plates

6 I usually use sterile yellow pipette tip (2-200 uL) to transfer the seedlings. It is less likely that you accidentally "strangle" the seedling than if you would be using foreceps. Also - it is easier to keep the seedlings sterile. The best way to transfer the seedling is to insert the tip tiny bit into the agar, just under the hypocotyl of the seedling, and then lift it together with the entire seedling into the treatment plate.

If you wish to observe Root System Architecture for longer periods (longer than 4 days), I advice to transfer no more than four seedling per plate.

Also - I advice to put two different genotype per plate - one on the left side, other on the right side of the plate.

Scan the plates containing the seedlings at 200 dpi at 0, 2, 4, 6 and 8days after transfer to treatment plates

7 You can also choose to scan for longer periods of time - this is entirely up to you and also depends on how quickly your plants are growing. In the growthchamber I normally use, the RSA of the seedlings grown at control conditions is usually very dense at 12 days after germination (= 8 days after transfer), and there is no point in scoring it for longer.

Analyze the plate images by hand or using your favourite root image analysis software such as EZ-Rhizo or SmartRoot

8 You can find more tools to analyze your root architecture data here: https://www.plant-image-analysis.org/