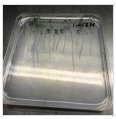

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Studying Root System Architecture changes in tomato (S.lycopersicum and S.pimpinellifolium) in response to stress

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Salt Lab KAUST


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ABSTRACT

This is a simple protocol, adapted from the Arabidopsis protocol (https://dx.doi.org/10.17504/protocols.io.zkqf4vw) for studying salt-stress induced changes in Root System Architecture in tomato species. This protocol uses the in-vitro culture to germinate and grow the tomato seedlings and observe (relatively fast) changes in response to stress and how they affect RSA.

MATERIALS

NAME	CATALOG #	VENDOR
Murashige & Skoog medium including B5 vitamins	M0231	Duchefa Biochemie
Sodium Chloride	PubChem CID: 5234	
MES, free acid, monohydrate	MB0341.SIZE.25g	Bio Basic Inc.
Sucrose	S7903	Sigma Aldrich
Daishin agar	9002-18-0	Duchefa Biochemie
Potassium hydroxide	1050121000	Sigma Aldrich

Experiment preparation

1 Prepare the germination media

Per 1L of media: 1.1 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 (12x12cm) 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 Prepare the salt stress media

Use the basic media concentration as described in Step 1 - but now add salt - make media containing 0, 75 or 100 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.

Seed germination

3 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 hood and wash 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 (doesn't work that well for tomato as for Arabidopsis).

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

4 Put the seeds on the plate (approximately 5-6 seeds per plate)

IMPORTANT NOTE: Perform all of the steps in the laminar hood!

Get the seeds from 4C and transfer both the 1/4 MS agar plates (germination plates) and the seeds into a clean laminar hood.

Use the forcepts (STERILE!) to put the seeds on the plate. Make sure to be gentle and not squeeze the seeds too much.

What works best is to pour water & seeds "suspention" onto the sterile empty petridish, and pick from the petridish.

Leave the seeds on the surface of the plate for 10-15 minutes for the water around the seeds to evaporate - leave the plate open for this step and do NOT staple the plates on top of eachother - this will increase chances of bacterial/fungal infection!

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

5 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 26/24 C, 12/12 light/dark cycle with 60% of humidity.

It is important that the light is at least 50 cm above the plates - having the light source too close to the plates will result in short root in some cases.

Salt treatment

6 Three days after germination (usually - I put the plates in the growth chamber for germination on Thursday and transfer on Sunday) - transfer the germinated seedlings to new plates containing 0/75/100 mM NaCl

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

Transfer the seedlings with sterile foreceps, picking the plants gently at the hypocotyl.

Seal the plates with leukopor tape.

Each plate contain only one seedling - as the plants tend to get very large very quickly

7 Put a dot with a permanent marker to mark the position of main root tip

Alternatively scan the plates before putting them back into the growth chamber / Percival

8 Scan the plates every day until the plants are 8-10 days old (or whenever the root architecture becomes too complex). Scan the plates at 300

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)

10 Analyze the data using R - an example of the analysis of dynamic changes in tomato seedling's Root System Architecture can be found here