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A costum-made hydroponic culture system to study plant roots during root infection with Plasmodiophora brassicae

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bm3mk8k6

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ABSTRACT

Plant roots are fascinating organs that anchor the plant in the ground, take up water and nutrients and are the interaction zone for plants with their surrounding microbial rhizosphere. In contrast to easily accessible leaves, the study of these belowground organs is more difficult as harvesting the tissue is time-consuming and - when sampled as whole - kills the entire plant. The time-consuming and often disruptive process of root harvest can hamper molecular studies that require for samples to be harvested in a narrow time frame in order to rule out negative sample bias such as circadian gene regulation or effects that stem of the sample handling rather than the actual assay.

For our studies of the soil-borne pathogen *Plasmodiophora brassicae* in interaction with soil microbes in roots we needed a plant cultivation method that allows quick and easy harvest of root tissue while at the same time allowing the roots to form the pathogen-specific clubbed root structures.

We therefore came up with an easy custom-build setup, that can be easily implemented in other laboratories using similar materials. Our setup requires clear square plastic containers high enough to keep our desired plant species (Arabidopsis and Brassica) and a custom-build rack with holes that fit 10ml pipette tips. This setup allows easy cultivation of plants in sand and hydroponic medium and largely damage-free root harvest within several seconds for each plant. This method allowed us to harvest samples for a large microarray experiment as well as countless experiments for root transcript studies.

Since the height of the box and the extend of the pipette tips is a limiting factor we suggest to use this system only for relatively small plants such as Arabidopsis or young stages of larger plants such as *Brassica rapa* or *Brassica napus*.

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PROTOCOL CITATION

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GUIDELINES

Read the "Before start" to get instructions on how to build a hydroponic culture system for cultivating Arabidopsis and Brassica in sand with liquid medium.

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MATERIALS TEXT

MATERIALS

see also "Guidelines" and section "Before you start" for instructions on how to build a hydroponic box.

Finntip™ Pipette Tips, 10mL, sterile **Thermo Fisher Catalog #9402163**

- the tips described above are similar to the ones we use and are not obligatory, you can use other brands and types though we recommend to use 10 ml tips

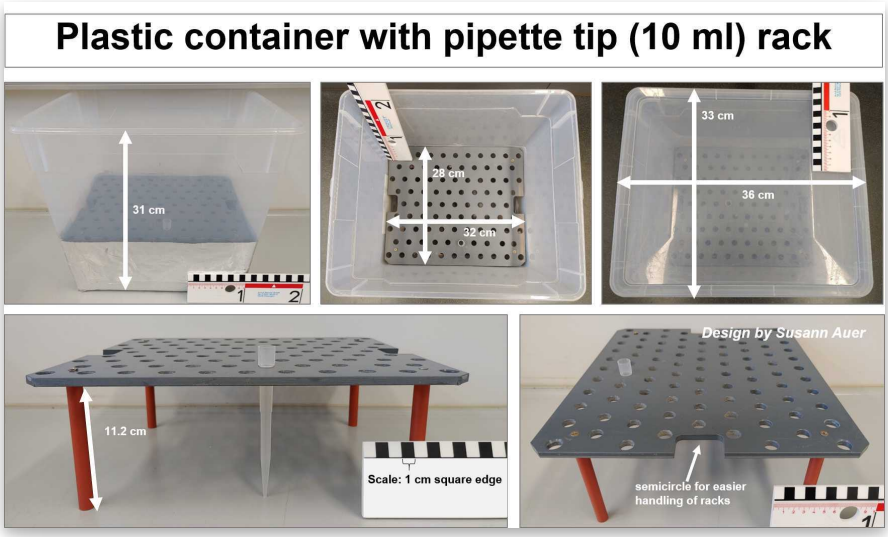
- liquid medium for hydroponic Arabidopsis cultivation according to Smeet et al. 2007 (Plant Physiology and Biochemistry 46 (2008) 212e218):
0.505 mM KNO_3, 0.15 mM $Ca(NO_3)2{\times}4H_2O$, 0.1 mM $NH_4H_2PO_4$, 0.1 mM
MgSO4×7H2O, 4.63 mM H_3BO_3, 0.91 mM $MnCl_2{\times}4H_2O$,
0.03 mM $CuSO_4{\times}5H_2O$, 0.06 mM $H_2MoO_4{\times}H_2O$, 0.16 mM
$ZnSO_4{\times}7H_2O$, 1.64 mM $FeSO_4{\times}7H_2O$ and 0.81 mM Na_2-EDTA
- children´s play sand, free of toxins

- transparent plastic box with loose fitting lid from a hardware store, ours was from the company Rotho [Rotho Kunststoff AG, CH-5303 Würenlingen, Switzerland]; dimensions: 31-36x26-33x31cm (LxWxH)

- durable plastic plate in gray, with 1.2 cm wide holes drilled in to fit 10 ml pipette tips, dimensions of ours: 32x28cm, for ease of handling semicircles are cut at the edge of the shorter side of each tip rack; the center of each hole is approximately 3 cm apart from the next hole; Attached to the plastic board are 4 durable plastic feet that were reinforced with screws from the top to avoid breaking off. plastic feet that were reinforced with screws from the top to avoid breaking off.

- plastic rods cut to 11.2 cm length and drilled into the edge of the plastic plate; plastic rods are attached to the plastic board with glue and reinforced with screws from the top to avoid breaking off

- tin foil is glued at the bottom part of the plastic container to minimise light exposure to roots



SAFETY WARNINGS

No safety warnings.

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Germination rate of seeds on sand is often poor. Some plants might die during the experiment. Consider that before you start your experiment and provide a sufficient amount of backup plants.

BEFORE STARTING

We study the interaction of plants (Arabidopsis, Brassica) with the clubroot pathogen, a soilborne protist that causes swelling of the roots and other microbes. A normal experiment in our setup contains 4 treatment groups so we use four boxes with 93 pipette tips each, amounting to roughly 400 plants in total. For our studies we need large amounts of root material that need to be harvested quickly to allow for comparisons across all treatment groups. We therefore came up with this hydroponic cultivation system that allows complete, non-destructive root harvesting within seconds for each plant.

The system described here was made from materials that should be available in hardware stores. We used hardware store plastic storage boxes that are 31-36x26-33x31cm (LxWxH) in dimension and have a loosely-fitting lid that allows some air circulation, see details under "Materials".
The pipette racks were built to fit inside these boxes and each can hold 93 pipette tips. They consist of a durable plastic board (32x28cm) with holes drilled in it and 4 feet to stand inside the box. The holes have a diameter of 1.2 cm and hold a 10 ml pipette tip. When the pipette tip rack is placed inside the box, each pipette tip reaches to the bottom of the box which allows low levels of medium, as low as 1 l total volume, and thus reduces the weight of each box during the experiment.
In our setup we can fill 4 l of medium into the containers with no problem. However since the boxes are moved throughout the experiment we recommend to use smaller amounts of volume to ease handling.

Setup of the system 2h

1 Place tip rack in the clean container and fill 10 ml pipette tips with sand to the top.

1.1 Tap the tip lightly on a hard surface to make sure the sand does spread evenly in the tip. Fill tip up again to the top if necessary. You don´t want the sand to be packed too tightly as this might cause problems with ventilation of the roots later.

2 Place pipette tip in the rack of the container and continue with the next tip until you have all pipette tips filled with sand.

3 Fill liquid sterile medium in the box containing the tips and let the medium be soaked up by the sand. Since soaking up medium will lower the level of medium in the box make sure you fill in enough medium to cover at least the lower third of your pipette tips that reach into the medium.

3.1 The soaking will take a few seconds or minutes. Make sure all tips are fully soaked and the medium reaches to the top of the sand surface. If not, lightly tap the tip to spread the sand better and continue soaking. You want to make sure that all tips soak up the medium so that your plants have access to water and nutrients.

Sowing of seeds 2h

4 Sow seeds on the surface of the sand. Before sowing, make sure the sand surface is wet with medium so the seed has enough liquid to germinate.

4.1 When using Arabidopsis seeds you can seed 2 seeds per pipette tip a few millimeters apart from each other. After germination you remove one of the seedlings. When using Brassica seeds one seed per tip is enough. Alternatively use Brassica seeds that are pre-germinated with the radicle already emerging. Place these seeds gently on the sand and make sure the radicle points downwards.

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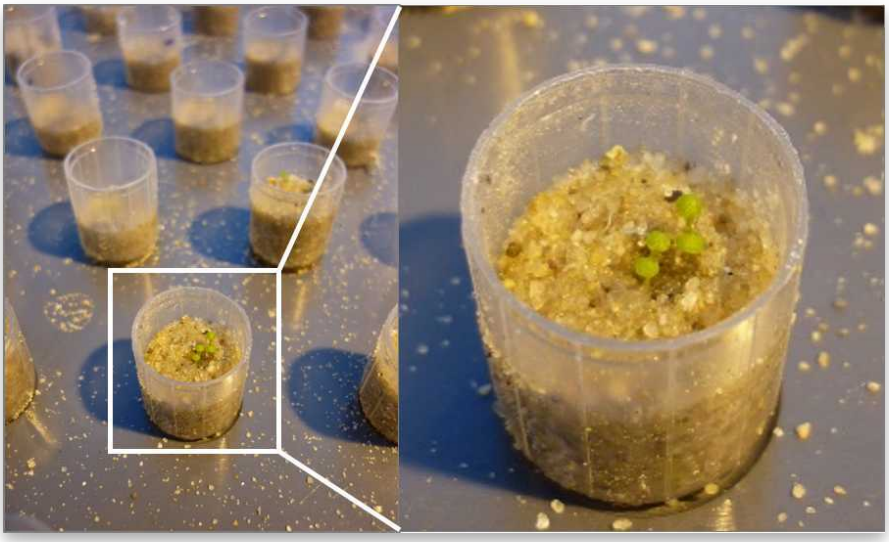

Arabidopsis seedlings, 2 seeds were placed in one tip. One seedling will be removed before the start of the experiment.

Maintenance during experiment 6w

5 Cover the plastic box with a lid and place the box with your tips in a suitable environment for germination. We use a greenhouse or climate chamber with longday conditions: 16 hours light at 23 °C, 8 hours dark at 18 - 23 °C. Keep the lid on during the whole experiment except for medium changes and harvests.

5.1 For Arabidopsis and Brassica the best results were obtained under somewhat natural light conditions in a greenhouse that has an average temperature of 20 °C and is located on the top of the Biology building at TU Dresden, Germany. Average light intensity was 120 µmol/s×m² on cloudy days and up to 700 µmol/s×m² on sunny days.

6 Monitor the development of your plants in the first few days closely. To avoid drying out of the sand surface within the first few crucial days of germination it helps to daily spray the tips from above with sterile nutrient solution until the sand surface is thoroughly wet. The spraying should be done until the plants have reached the 2 true leaf stage or when they appear stable and large enough to reach with their roots deeper in the sand.

6.1 Germination rate on sand is usually worse than on soil and you might lose one third of your plants within the first days. To avoid this, check on them daily and make sure the sand stays moist with nutrient solution. Once the plants are large enough to reach the nutrient solution and do not flood up when you spray the sand from above they will likely survive. To prevent microbial contamination of your plants always use gloves, open the box only as long as necessary and always use autoclaved fresh nutrient solution. Wiping down the insides of the box with ethanol from time to time can prevent contamination.

7 Start your pathogen treatment at the desired growth stage. We inoculate Arabidopsis plants 10 to 14 days after sowing and Brassica plants 3 to 5 days after sowing with 200µl of 10^7 spores/ml of *Plasmodiophora brassicae* in 50 mM KH_2PO_4, depending on the developmental stage of the plants.

8 Maintain your cultures throughout the experiment by changing the medium every week to fresh, autoclaved medium. Should plants struggle to reach the medium, carefully pipette a small volume of medium up to 200 ml on top of the sand surface taking care to not uproot the plant.

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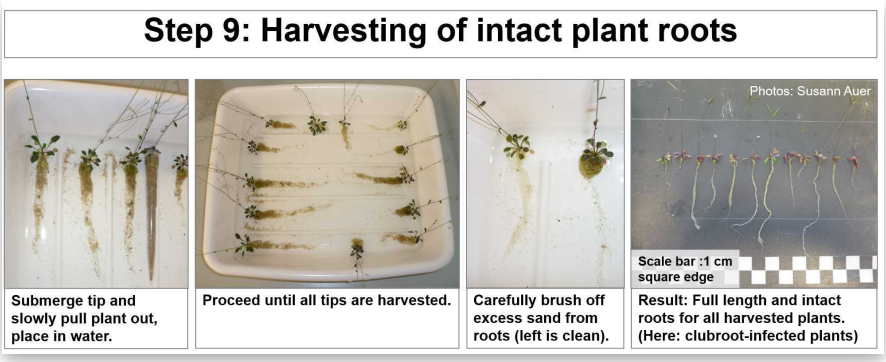
8.1 After pathogen inoculation we continue cultivation of Arabidopsis in this setup up to day 40 after sowing and of Brassica up to day 24 after sowing.

Arabidopsis, 40 days old. Each tip contains 2 to 3 plants, this was a backup for the original experiment.

Harvesting of plants 2h

9 To harvest your plants first prepare two shallow containers and fill them with tap water at room temperature. Fill the first container with water almost to the top and the second with a small amount of water, just enough to cover the bottom. Take each individual tip carefully out of the box and submerge in water of the first container. Then carefully, under water, pull at the plant on top to slide the whole plant out of the tip taking care to not break off the roots. Use a paint brush to remove residual sand from the surface of the roots and then put this plant in the second container, making sure the roots are in the water and leaves float on the surface. Repeat this process with all tips. Each harvest should only take a few seconds.



Optional adjustments and hacks

10 Aeration of medium: If necessary, you can aerate the liquid medium using an aquarium pump. We did not observe better

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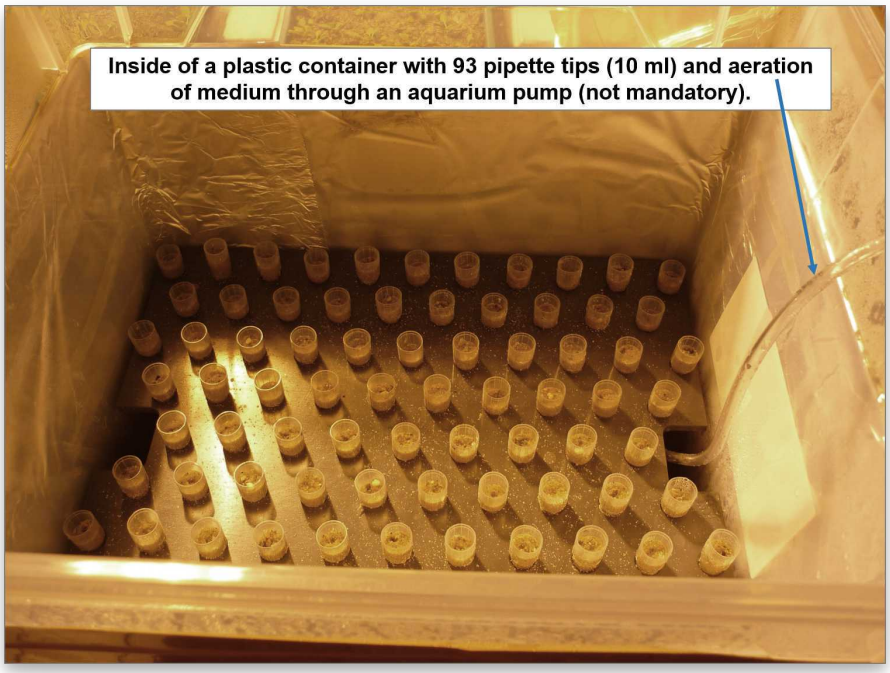
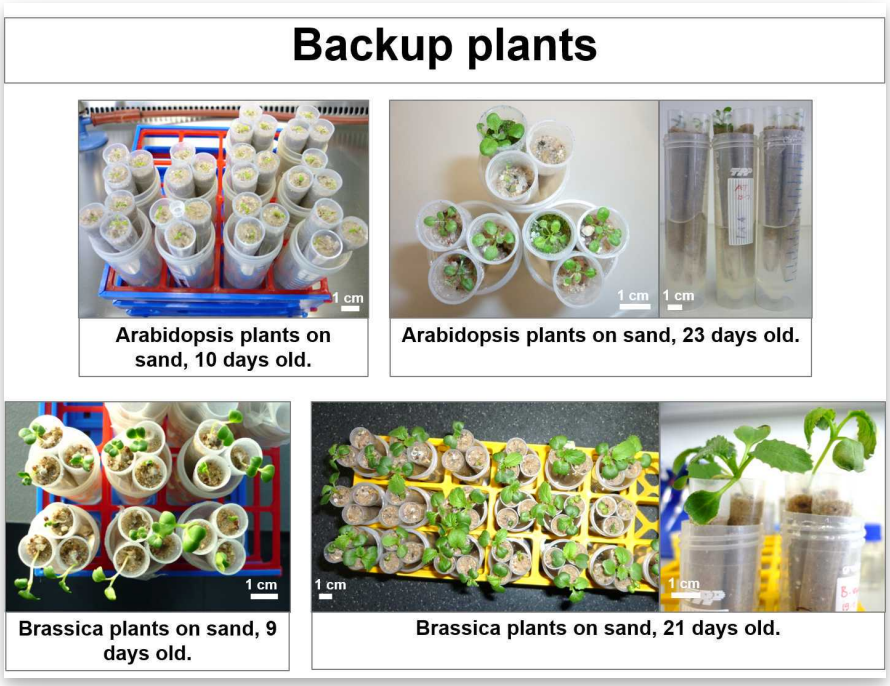
growth with aerated medium so we don´t do it.



Backup seedlings: Germination rate on sand is in our experience often lower than on soil. We therefore prepare extra tips with seeds in a similar manner as described above. Three tips are then placed together in one 50 ml falcon tube that is filled with 5 ml of medium. Several falcon tubes are placed in a falcon rack and covered with a transparent hood and located at the same growth conditions as the larger containers. These backup tips replace tips in the larger container where no seeds germinated on the surface. For a small scale experiment to test out medium and growth conditions this approach can work as well. Medium should be exchanged twice a week. Also, watch the falcons closely for contamination with algae and microorganisms.



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