Arabidopsis thaliana Culture in Hydroponics

Principle

Culture of *Arabidopsis thaliana* on a hydroponics system enabling experiments where roots can be harvested relatively easily and without excessive damage.

Hoagland Solution and variants

Equipment and reagents

Machine/Product	**Reference (Company, Type, …)**
Volumetric flasks	250 ml, 1 L, 2L
Hotplate/Stirrer	 Jenway Model 1100
Potassium Nitrate KNO_3	 Merck 60410
Calcium Nitrate $Ca(NO_3)_2 \cdot 4H_2O$	 Vel 6560
Ammonium Phosphate $NH_4H_2PO_4$	 Merck 1.01126
Magnesium Sulfate $MgSO_4 \cdot 7H_2O$	 Merck 105886
Ferrous Sulfate $FeSO_4 \cdot 7H_2O$	 Merck 1.03965
Na_2-EDTA (disodium salt dehydrate) 99 %; 372.2 $g.mol^{-1}$	 Sigma ED2SS
Boric Acid H_3BO_3	 Baker 0501
Manganese chloride $MnCl_2 \cdot 4H_2O$	 Merck 1.05927
Copper sulfate $CuSO_4 \cdot 5H_2O$	 Baker 0104
Molybdic Acid H_2MoO_4	 Aldrich 232084
Zinc sulfate $ZnSO_4 \cdot 7H_2O$	 UCB1884

Hoagland Stock Solutions

Weigh off the different reagents for each stock as given in table below:

Macro elements			1 L /10 L	H 1:1
	g.mol-1	g / 2 L		mM
KNO_3	101,11	20.4		100
$Ca(NO_3)_2 \cdot 4H_2O$	236,15	14.16		30
$MgSO_4 \cdot 7H_2O$	246,48	9.8		20

Phosphorus			1L / 10L	
$NH_4H_2PO_4$	115,03	4.6		20
Iron Solution		g / 250 ml	6ml / 10L	
$FeSO_4•7H_2O$	278,02	1,9		27
Na_2-EDTA•$2H_2O$	372,2	1,25		13
Micro elements		g / 1 L	10 ml /10 L	
H_3BO_3	61,83	2,86		46
$MnCl_2•4H_2O$	197,91	1,81		9.1
$CuSO_4•5H_2O$	249,68	0,08		0.32
H_2MoO_4	161,97	0,09		0.55
$ZnSO_4•7H_2O$	287,54	0,22		0.76

Note: Heating can be necessary to dissolve molybdate in the micronutrients solution. Work on a laboratory hotplate with constant stirring.
Note: Stock solutions are autoclaved after being prepared to prevent growth of algae and microfauna.
Note: Macro-elements stock solution tends to precipitate when staying at 4°C for longer periods. The precipitate can be dissolved by bringing to room temperature and putting in a ultrasonic bath for a few minutes.

Hoagland Work Solutions

Hoagland 1:10

Macro elements without phosphorus ..... 100 ml for 10 L
Phosphorus solution ..... 100 ml for 10 L
Iron Solution ..... 0.6 ml for 10 L
Micro elements ..... 1 ml for 10 L

"HP" Hoagland

Macro elements without phosphorus ..... 100 ml for 10 L
Phosphorus solution ..... 50 ml for 10 L
Iron Solution ..... 0.6 ml for 10 L
Micro elements ..... 1 ml for 10 L

"LP" Hoagland

Macro elements without phosphorus ..... 100 ml for 10 L
Phosphorus solution ..... 12.5 ml for 10 L
Iron Solution ..... 0.6 ml for 10 L
Micro elements ..... 1 ml for 10 L

Hydroponic device set-up

Machine/Product	**Reference (Company, Type, …)**
Eppendorf Microtubes	1.5 ml
PE containers	Fisher Bioblock
Fitting, Perforated Lids	
Agar No.2 "Bacteriological"	 Lab M Ltd. MC006
Paper tape	

Autoclave	Tuttnauer
Hoagland Solutions	See 0 *Hoagland Work Solutions* above
Peristaltic Pump	
Climate Chamber	Snijders

Pre-treatment of hydroponics containers

Plastic containers are made opaque. First they are coated with primer spray paint (Motip Primer Grey Eriks+Baudouin Catalog#04054) and the with black spray paint (Motip 4006 RAL9005 mat black; Eriks+Baudouin Catalog#04006).
When used for the first time the containers are prewashed and rinsed with 10 % HCl, followed by deionized water, to remove every wall bound metal to prevent metal contamination in the nutrient solution.
Fitting lids are made in opaque gray plastic (PE) with holes of approx. 11 mm diameter.

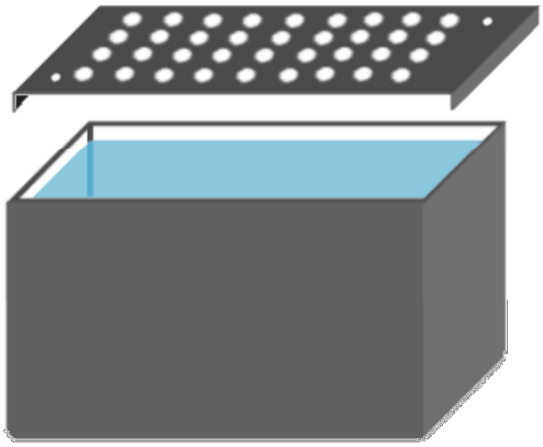

Figure 1: Example of Hydroponics container and lid

Table 1: Types of containers mostly used for experiments with their nominal volume, their useful volume(max volume with lid + microtubes) and number of holes that could be fitted in the corresponding lids.

Container type	Useful volume	# holes in lid
3 L	2900 ml	9x9 = 81
1.5 L	1400 ml	9x4 = 36
1 L	960 ml	4x4 = 16

Agar Seed Bed

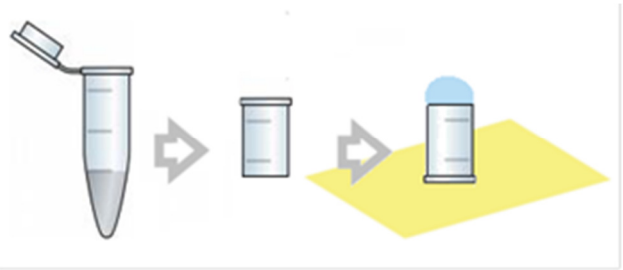

Figure 2: Making and filling the agar seed bed devices

Cut off the lid and the bottom half of standard 1.5 ml Eppendorf tubes
Autoclave the tubes at 121 °C
Place the tubes upside down on a strip of paper adhesive tape

Prepare the agar solution from “LP Hoagland” with 6 g Agar No2 per liter (0.6 %)
Autoclave the solution and leave it to cool to about 40-45 °C

 Fill the tubes with agar solution and make sure they are filled over the brim “with a bubble” as pictured in Figure 2 above. Use a pipette or a
Let cool down to room temperature (± 15 min) and transfer to the cut-out lids of the hydroponics containers.
Transfer the lids to the hydroponics containers filled with the appropriate pre-culture solution and place in a high-humidity environment (65 % RH).

Arabidopsis thaliana culture

Machine/Product	**Reference (Company, Type, …)**
Disposable Petri Dish	90 mM diameter
Filter Paper	S&S blue ribbon 5893
Fine-tipped tweezers	
Small diameter tubing	
Peristaltic Pump	
Tubing for Peristaltic Pump	
Climate Chamber	Snijders
Commercial Bleach	 LODA Javel <5% Cl 10°
Nitric Acid, concentrated HNO_3 (65 %; 1,41 $g.ml^{-1}$; 63.01 →14.55 $mol.l^{-1}$)	 Merck 20429.320

Vernalization (cold treatment)

Cold shock is a germination improvement technique where an incubation period of at 4 °C is applied to the seeds to synchronize germination.
For *Arabidopsis thaliana* the seeds are placed on wet filter paper in a closed Petri dish for 3 days at 4 °C in the dark before sowing.

Sowing, Germination and Climatic Growth conditions

The seeds are transferred in pairs to the agar with fine tweezers seed beds placed in nutrient solution. The germination conditions are 14/10 day/night cycle at 165 $\mu mol.m^{-2}$, with respectively 22 °C / 18 °C and a constant relative humidity of 65 %. (see Table 2 below)
It takes about 3 days for the seeds to germinate and after about 1 week the agar seed beds can be “thinned out”, which means only one seedling is left per tube and the excess is removed using fine tweezers.

Table 2: Climate chamber program for Arabidopsis thaliana

Process step				**ChambE**	**HmdtyE**	**illumE**	
ChambS	**HmdtyS**	**illumS**	**Time**				
1. All but 24				22 °C	65 %	65 %	
22 °C	65 %	65 %	05:30				*15:00 → 20:30*
2. All but 24				18 °C	65 %	10 %	
22 °C	65 %	65 %	00:30				*20:30 → 21:00*
3. Dark				18 °C	65 %	10 %	
18 °C	65 %	10 %	09:30				*21:00 → 06:30*

4. All but 24				22 °C	65 %	65 %	
18 °C	65 %	10 %	00:30				*06:30 → 07:00*
5. All but 24				22 °C	65 %	65 %	
22 °C	65 %	65 %	08:00				*07:00 → 15:00*
6. Repeat				0.0	0.0	0.0	
1.0	0.0	99.0	00:00				

Maintenance of the Hydroponics Culture

Once the Arabidopsis plants are a week old, the nutrient solution has to be replaced twice a week. For experimental pre-culture, "Hoagland HP" is usually used.
From the time the plants are 1 week old, the nutrient solution also has to be aerated. This is achieved by placing a piece of small-diameter diameter about 5 cm in the nutrient solution and bubbling air by means of a peristaltic pump placed next to the growth cabinet.

Dismantling and cleaning the hydroponics setup.

Tubes:

- Remove the agar from the Eppendorf tubes. Place the tubes in a 2 L PE bottle and fill with a detergent solution and a dash of household bleach. Shake overnight.
- Rinse the tubes with hot tap water, then with demineralized water. Put the clean tubes in a glass beaker and steam-sterilize them in the autoclave.

Containers and Lids:

- Remove all labels and writings from the containers and lids. (Do not use acetone as it corrodes the plastic from the lids). Wash them thoroughly in hot water and detergent. Make sure to scrub the corners and the bottom since precipitates accumulate there most easily.
- [optional] If the experiment carried out contained heavy metals or radionuclides, the containers have to be rinsed with acid solution (HNO_3 1M = 70 ml /L). Use a shallow platter for this.
- Rinse once with tap water and rinse once with dilute household bleach (<5%). Use a shallow platter for this.
- Thoroughly rinse with demineralized water to remove any trace of bleach.