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

<table>
<thead>
<tr>
<th>Machine/Product</th>
<th>Reference (Company, Type, …)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volumetric flasks</td>
<td>250 ml, 1 L, 2L</td>
</tr>
<tr>
<td>Hotplate/Stirrer</td>
<td>Jenway Model 1100</td>
</tr>
<tr>
<td>Potassium Nitrate</td>
<td>Merck 60410</td>
</tr>
<tr>
<td>Calcium Nitrate</td>
<td>Vel 6560</td>
</tr>
<tr>
<td>Ammonium Phosphate</td>
<td>Merck 1.01126</td>
</tr>
<tr>
<td>Magnesium Sulfate</td>
<td>Merck 105886</td>
</tr>
<tr>
<td>Ferrous Sulfate</td>
<td>Merck 1.03965</td>
</tr>
<tr>
<td>Na₂-EDTA (disodium salt dehydrate) 99 %; 372.2 g.mol⁻¹</td>
<td>Sigma ED2SS</td>
</tr>
<tr>
<td>Boric Acid</td>
<td>Baker 0501</td>
</tr>
<tr>
<td>Manganese chloride</td>
<td>Merck 1.05927</td>
</tr>
<tr>
<td>Copper sulfate</td>
<td>Baker 0104</td>
</tr>
<tr>
<td>Molybdic Acid</td>
<td>Aldrich 232084</td>
</tr>
<tr>
<td>Zinc sulfate</td>
<td>UCB1884</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Macro elements</th>
<th>1 L /10 L</th>
<th>H 1:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>g.mol⁻¹</td>
<td>g / 2 L</td>
</tr>
<tr>
<td>Ca(NO₃)₂•4H₂O</td>
<td>101.11</td>
<td>20.4</td>
</tr>
<tr>
<td>MgSO₄•7H₂O</td>
<td>236.15</td>
<td>14.16</td>
</tr>
<tr>
<td></td>
<td>246.48</td>
<td>9.8</td>
</tr>
</tbody>
</table>
Phosphorus
\[ \text{NH}_4\text{H}_2\text{PO}_4 \]
115.03 4.6  20

Iron Solution
\[ \text{FeSO}_4\cdot7\text{H}_2\text{O} \]
g / 250 ml 6ml / 10L
278.02 1,9  27
\[ \text{Na}_2\text{-EDTA}\cdot2\text{H}_2\text{O} \]
372.2 1,25  13

Micro elements
\[ \text{H}_3\text{BO}_3 \]
61.83 2,86  46
\[ \text{MnCl}_2\cdot4\text{H}_2\text{O} \]
197.91 1,81  9.1
\[ \text{CuSO}_4\cdot5\text{H}_2\text{O} \]
249.68 0,08  0.32
\[ \text{H}_2\text{MoO}_4 \]
161.97 0,09  0.55
\[ \text{ZnSO}_4\cdot7\text{H}_2\text{O} \]
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 an 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

<table>
<thead>
<tr>
<th>Machine/Product</th>
<th>Reference (Company, Type, …)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eppendorf Microtubes</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>PE containers</td>
<td>Fisher Bioblock</td>
</tr>
<tr>
<td>Fitting, Perforated Lids</td>
<td></td>
</tr>
<tr>
<td>Agar No.2 “Bacteriological”</td>
<td>Lab M Ltd. MC006</td>
</tr>
<tr>
<td>Paper tape</td>
<td></td>
</tr>
</tbody>
</table>
**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.

**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.**

<table>
<thead>
<tr>
<th>Container type</th>
<th>Useful volume</th>
<th># holes in lid</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 L</td>
<td>2900 ml</td>
<td>9x9 = 81</td>
</tr>
<tr>
<td>1.5 L</td>
<td>1400 ml</td>
<td>9x4 = 36</td>
</tr>
<tr>
<td>1 L</td>
<td>960 ml</td>
<td>4x4 = 16</td>
</tr>
</tbody>
</table>

**Agar Seed Bed**

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**

<table>
<thead>
<tr>
<th>Machine/Product</th>
<th>Reference (Company, Type, ...)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disposable Petri Dish</td>
<td>90 mM diameter</td>
</tr>
<tr>
<td>Filter Paper</td>
<td>S&amp;S blue ribbon 5893</td>
</tr>
<tr>
<td>Fine-tipped tweezers</td>
<td></td>
</tr>
<tr>
<td>Small diameter tubing</td>
<td></td>
</tr>
<tr>
<td>Peristaltic Pump</td>
<td></td>
</tr>
<tr>
<td>Tubing for Peristaltic Pump</td>
<td></td>
</tr>
<tr>
<td>Climate Chamber</td>
<td>Snijders</td>
</tr>
<tr>
<td>Commercial Bleach</td>
<td>![LODA Javel &lt;5% Cl 10°](Merck 20429.320)</td>
</tr>
<tr>
<td>Nitric Acid, concentrated</td>
<td>![LODA Javel &lt;5% Cl 10°](Merck 20429.320)</td>
</tr>
<tr>
<td>HNO₃ (65 %; 1.41 g.ml⁻¹; 63.01 →14.55 mol.l⁻¹)</td>
<td>![LODA Javel &lt;5% Cl 10°](Merck 20429.320)</td>
</tr>
</tbody>
</table>

**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 μmol.m², 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>
<thead>
<tr>
<th>Process step</th>
<th>ChambE</th>
<th>HmdtyE</th>
<th>illumE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChambS</td>
<td>HmdtyS</td>
<td>illumS</td>
<td>Time</td>
</tr>
<tr>
<td>1. All but 24</td>
<td>22 °C</td>
<td>65 %</td>
<td>65 %</td>
</tr>
<tr>
<td>22 °C</td>
<td>65 %</td>
<td>65 %</td>
<td>05:30</td>
</tr>
<tr>
<td>2. All but 24</td>
<td>18 °C</td>
<td>65 %</td>
<td>10 %</td>
</tr>
<tr>
<td>22 °C</td>
<td>65 %</td>
<td>65 %</td>
<td>00:30</td>
</tr>
<tr>
<td>3. Dark</td>
<td>18 °C</td>
<td>65 %</td>
<td>10 %</td>
</tr>
<tr>
<td>18 °C</td>
<td>65 %</td>
<td>10 %</td>
<td>09:30</td>
</tr>
</tbody>
</table>

Table 2: Climate chamber program for Arabidopsis thaliana

15:00 → 20:30
20:30 → 21:00
21:00 → 06:30
### 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 (HNO₃ 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.