LSOP Title	Hydroponic Procedure for Arabidopsis
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Location	UQ Node/Centre-wide
Policy/Procedure Link	*UQ- Equipment* *UQ -waste* *OGTR*
Risk Assessments	
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1.0 Scope

This procedure covers the hydroponic procedure for growing Arabidopsis.

This LSOP does not cover the growth of any other plants/species.

2.0 Definitions

Soln – Solution

$Ca(NO_3)_2$ - Calcium Nitrate

KNO_3 - Potassium Nitrate

NH_4NO_3 - Ammonium Nitrate

$MgSO_4$ - Magnesium Sulfate

KH_2PO_4 – Potassium phosphate, monobasic

KCl – Potassium Chloride

H_3BO_3 - Boric Acid

$MnCl_2$ – Manganese Chloride

$ZnSO_4$ - Zinc Sulfate

$CuSO_4$ – Copper Sulfate

H_2O – Water

$CoCl_2$ – Cobalt Chloride

$Co(NO_3)_2$ – Cobalt Nitrate

ddH_2O – Double Distilled Water

$(NH_4)_6Mo_7O_{24}$ – Ammonium Heptamolybdate

MoO_3 - Molybdenum Oxide

Fe-EDTA – Ferric EDTA

EDTA – Ethylenediaminetetraacetic acid

KOH – Potassium Hydroxide

NaOH – Sodium Hydroxide

MES - 2-(N-morpholino) ethane sulfonic acid, a buffering agent

3.0 Materials and Equipment

1. Hoagland's nutrient solution, pH 5.8 – 6.0 (see recipe below)
2. Rock wool
3. 1.5 ml microcentrifuge tubes
4. Sealed plastic boxes with transparent lid, dimensions 12x8x6.5 cm (LxWxH)
5. Rack to hold 1.5 mL microcentrifuge tubes.
6. Controlled environment growth chamber
7. Cork borer, 8 mm diameter
8. Forceps
9. Autoclave
10. Balance
11. Growth Cabinets

4.0 Prescribed Actions

1. Plan how many plants you will need for the experiment. 2-4 adult plants can be fitted per box (Figure 1; see appendix A). Make around 30% or more number of plants to what is needed in case of plant failure.
2. Using scissors or a hot scalpel blade, cut off the cap and the bottom 5 mm (or so) from each microcentrifuge tube (1.5 mL).
3. Cut slices of rock wool of 2 to 2.2 cm in height, flush them with abundant tap water and saturate with water at the pH wanted (5.8 – 6.0).
4. Make rock wool cylinders using a cork borer (8 mm diameter). Very gently bore the rock wool by rotating the puncher left and right. Make your cylinders where vertical patterns are clear. The cylinders need to be pushed from inside the borer with a suitable implement. Please do so carefully to avoid compaction. Make as many cylinders as microcentrifuge tubes (Figure 2; see appendix A).
5. Place cylinders in each tube, levelled with the top of the tube. Again, do not compress the rock wool.
6. Autoclave the tubes with the wet rock wool cylinders inside.

7. Pour 145 mL of 0.25X Hoagland's hydroponic solution per box. Excess of solution can flood the seeds and avoid root penetration. Fill all the racks with tubes containing rock wool (24 per box).

8. Place seeds into a rabbit packet and wet it with 100% ethanol inside a laminar flow. Place the packet vertically and change position to allow fast drying. Do not use ethanol in excess.
9. Sow seeds directly from the packet by gently tapping it and deposit 7 – 10 seeds onto the rockwool per tube. Alternatively wet the tip of a sterile toothpick or fine forceps, pick up seeds and deposit on the rock wool.
10. Close the boxes with sown seeds and stratify at 4°C for three days.
11. Move boxes to a growth cabinet at 60% RH, 21/16°C day/night, and 14-16 h light.

    NB: If possible use less than 100 µmol photons m-2sec-1 light intensity during the first week of growth. If you have access to a cabinet with multiple shelfs, turn off the closest top light and use light from one shelf upper.

    NB: From day 7 use light back to normal. Otherwise use 100-200 µmol photons m-2sec-1 for all the period.

12. Maintain lids closed and open gradually from day 7. This avoids seedlings to stress. Do so by placing a 1 mL pipette tip from the first line of small rack holes backwards each day for three days.

13. During the first 10 days the bottom of the tubes need to touch the solution. Use 145 mL of solution for full boxes and top up with 0.25X Hoagland's if solution is close to 115 mL during the first 10 days.

14. When lids are removed (day 10) replace the solution with 0.5x Hoagland's.

15. Thin plants with forceps to obtain one per tube. It can be done at day 10, but recommended at day 16 (Figure 2B; see appendix A). This also allows having longer seedlings.

16. After thinning change to 2 or 4 plants per box depending on your experiment. 165 mL of 0.5x Hoagland's per box is recommended. Change solution every 5 d, and from week 4 every 3 d.

17. In order to increase strigolactone production, phosphate (Pi) can be reduced to 10%. Start giving low Pi after roots are floating in the solution. If low Pi is given before roots are out of the tubes, plants are greatly affected and they do not recover. Do so when plants are bigger than 6 true leaves (around 3 weeks), their growth is not greatly affected and purpling of leaves appear when older.

5.0 Appendix A

Figure 1. Arabidopsis lbo plants (5 weeks old) grown in hydroponics using this protocol.

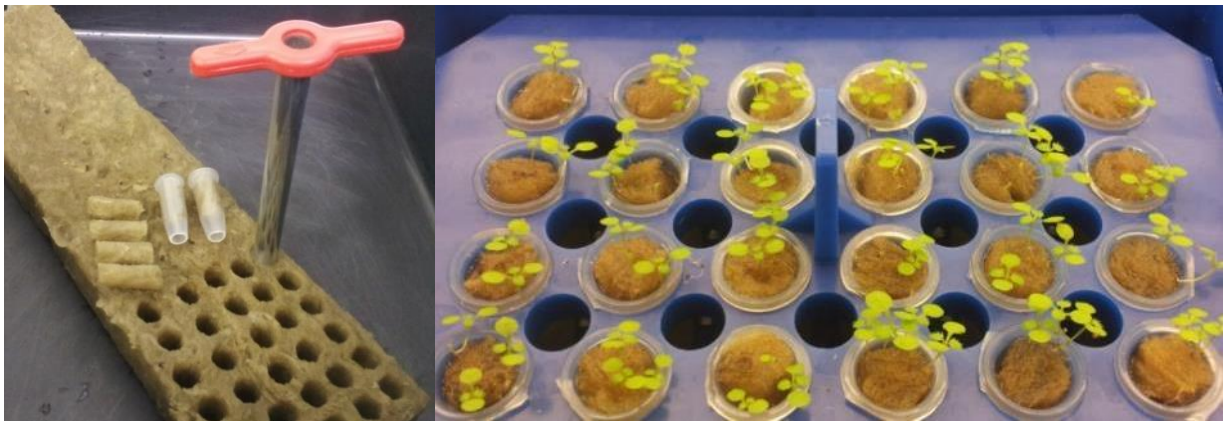

Figure 2. Set up of hydroponics using this protocol. A: Rockwool plugs obtained using a cork borer (8 mm diameter); B: Arabidopsis seedlings (ws-4; 14 days old) grown in hydroponics before thinning.

6.0 Appendix B

Hoagland's solution (0.5X)

- Use MilliQ water to prepare
- Store Solutions I and II at room temperature and all others at 4°C
- Autoclave all solutions (except solution I) using a cycle with 15 min @ 121°C

<u>Stock Solutions</u>

1. Stock solution I (500x): 1 M $Ca(NO_3)_2$ x 4 H_2O 236.2 g/L, <u>filter-sterilise</u>
2. Stock solution II (500x): 1 M KNO_3 101.1 g/L, autoclave
3. Stock solution III (1000x): 0.5 M NH_4NO_3 40.0 g/L, autoclave
4. Stock solution IV (500x): 0.25 M $MgSO_4$ x 7 H_2O 61.6 g/L, autoclave
5. Stock solution V (1000x): 0.25 M KH_2PO_4 34.0 g/L, autoclave
6. Stock solution VI (5000x): 0.25 M KCl 18.6 g/L, autoclave

<u>Micro element stock soln. (2000x):</u>

1. 0.004 M $MnCl_2$ x 4 H_2O 0.396 g
2. 0.004 M $ZnSO_4$ x 7 H_2O 0.575 g
3. 0.001 M $CuSO_4$ x 5 H_2O 0.125 g
4. 0.0003 M $CoCl_2$ x 6 H_2O 0.036 g or $Co(NO_3)_2$ x 6 H_2O 0.0758 g
5. 0.00015 M $(NH_4)_6Mo_7O_{24}$ x 4 H_2O 0.093 g or MoO_3 0.076 g
6. add 500ml ddH_2O, autoclave

<u>10 mM Fe-EDTA stock soln. (250x):</u>

1. 367.1 mg/ 100ml Fe-EDTA
2. Autoclave

3. Check pH if it is not dissolving and adjust pH to 8.0 using 5N KOH or 1N NaOH (if you want to get some Na+ ions into your solution).

<u>For 10 L of Hoagland's solution (0.5X):</u>

1. Dissolve 5 g MES in 8 L milliQ water and add, while mixing
2. Stock solution I 20 ml
3. Stock solution II 20 ml
4. Stock solution III 10 ml
5. Stock solution IV 20 ml
6. Stock solution V 10 ml

7. Stock solution VI 2 ml
8. Micro elements 5 ml
9. Fe-EDTA 40 ml

10. Adjust the pH of the solution with 5N KOH to pH 5.8 – 6.0.

NB: Further reading

Waters M.T., Nelson D.C., Scaffidi A., Flematti G.R., Sun Y.K., Dixon K.W., Smith S.M. (2012). Specialisation within the DWARF14 protein family confers distinct responses to karrikins and strigolactones in Arabidopsis. Development 139 (7): 1285-95.