Arabidopsis Growing Protocol-A General Guide

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[Abstract] *Arabidopsis* as the model organism for higher plants is widely studied among plant biology labs around the world. However, taking care of this tiny plant may not be trivial. Here is a general guide used for the Heven Sze lab at the University of Maryland, College Park. A lot of effort has been taken by the Sze lab and fellow lab members to formulate a general guide for *Arabidopsis* plant growth in the lab.

Materials and Reagents

1. *Arabidopsis* seed
2. Miracle-Gro® Potting Mix with Fertilizer (2 cubic feet bag) (The Scotts Miracle-Gro Company, model: 74278300 from Lowe's, Item: 156581)

3. Miracle-Gro® 8 Qt. Miracle Gro® Perlite (The Scotts Miracle-Gro Company, model: 70752300, Lowe's, Item: 68468)
4. 0.1% Triton X-100
5. Deionized water (D.I. water)
6. Sterile D.I. water
7. 70% ethanol
8. Drierite
9. KNO_3
10. FeNa-EDTA
11. KH_2PO_4

12. $MgSO_4$
13. $Ca(NO_3)_2.4H_2O$
14. Sterilization solution (see Recipes)
15. 1/4 Strength Hoagland's solution (see Recipes)
16. Homemade MS (Murashige and Skoog, 1962) medium for *Arabidopsis* plants under different Ca^{2+}/Mn^{2+} conditions (see Recipes)

Equipment

1. The Scotts Miracle-Gro Company
2. Autoclave bin
3. Scooper
4. Fisher dishes
5. Pots (14 x 8.5 x 6 cm)
6. Aluminum foil
7. Humidome
8. Sealed container
9. Nylon mesh
10. Autoclave tape
11. Growth chamber
12. Microcentrifuge
13. Small Fisher dishes (100 x 15 mm size)
14. Whatman filter paper

Procedure

A. Preparing soil for planting soil

We use Miracle-Gro® Potting Mix with Fertilizer and Miracle-Gro® 8 Qt. Miracle Gro® Perlite. To make *Arabidopsis*-friendly soil, mix well 1 bag of potting mix (2 cb ft) with half bag of perlite (4 qt) in a big bin used to store soil.

Note: We always sterilize the soil before putting it into planting pots (see below).

1. Scoop dry soil into an autoclave bin. Fill until the soil is approximately one inch from the top after packing down with the scooper.
2. Cover the bins with aluminum foil and put a piece of autoclave tape on each one. Make sure the outsides of the bins are dirt free before putting in the autoclave.
3. Autoclave on normal/flash/quick mode for 30-50 min depending on how many bins are being autoclaved.

4. Once cool, the soil can be placed into pots, packing down lightly. Remember to cover the autoclaved soil well again to prevent any seed/fungi contamination. Once opened, autoclaved soil can be good for use for up to 2 weeks. Please don't autoclave more than needed.
5. Fill the flat with pots (not on the top of soil) with deionized water, cover with humidomes and let soak for 3-6 h or overnight. There should be standing water (~ 1 inch) after soaking, add more water if not.
6. The soil in pots is now ready for growing plants.

B. Seeds sterilization

1. Method A
   a. Put certain amount of seeds (wild type, mutant, or complements lines) into an eppendorf tube. Add 1 ml sterilization solution.
   b. Vortex at maximal speed for 20-30 sec.
   c. Stand for 7-10 min with occasional vortex.
   d. Spin in a microcentrifuge at 8,000 rpm for 5 sec.
   e. Pour off the water carefully.
   f. Add 1 ml sterile water and vortex to suspend the fluffy seeds.
   g. Spin in a microcentrifuge at 8,000 rpm for 5 sec and pour off the supernatant.
   h. Repeat steps 6-7 for another 4 times.
   i. Move the sterile seeds to small Fisher dishes with a wet Whatman filter paper (sterile or autoclaved) on its bottom.
   j. Transfer the seeds to agar medium plate or to soil with a tweezer that has been soaked in 70% ethanol for >30 min.
2. Method B
   a. Put certain amount of seeds (no more than 0) into 1.5 ml Eppendorf tubes. Add 1 ml 70% ethanol.
   b. Pour off water and add 5 ml 70% ethanol, incubate for 5 min.
   c. Rinse with sterile D.I. water 4 times.
   d. Add 0.1% Triton X-100 (or 30% Bleach + 0.1% Triton X-100) 10 min.
   e. Rinse thoroughly with sterile D.I. water (at least 5 times).
   f. Move the sterile seeds to small Fisher dishes with a wet Whatman filter paper on its bottom.

C. Planting

1. General concern

Please keep in mind your seeds can be contaminated with fungi and bacteria when they were produced on parental plants, seed sterilization is recommended for all plants (up to bolting time) and necessary for all flower-producing/seed-collecting plants and seeds from other labs. Chamber contamination is usually from dirty seeds in the lab (since we always use autoclaved soil). Flower parts are very attractive to insects and pests, please keep an eye on the flowers.

2. Grow healthy *Arabidopsis* plants

   Before start, the trays must be cleaned/brushed free of old seeds or fungus.

   a. Fill pots (14 x 8.5 x 6 cm) to the top with autoclaved soil.
   b. Add D.I. water to the tray which contains the pots, about 1/3-1/2 the height of a pot. It usually takes at least 3 h to let the soil soak thoroughly. Then spray the surface of the soil with a spray bottle (D.I. water), to make sure that the seeds will fall on a wet surface.
   c. Transfer seeds to soil (8-10 seeds/pot, to space seeds apart).
   d. After planting, cover the tray with a humidome, taping it to the tray, to keep the necessary humidity.
   e. Put the tray in 4 °C cold room for 3-5 days (5 day is better.) at dark.
   f. Move to growth chamber with the humidome still covering.
   g. Plants should germinate after 3-4 days in chamber with long day light cycle. About 5-7 days leave the humidome half-open for anther 2-3 days when 2 pieces of cotyledons have developed completely.
   h. Remove the humidome and water with D.I. water twice a week (Tuesday and Friday, for example).

Notes

1. *Arabidopsis* is sensitive to all stress conditions, so please water only when there is no standing water in the tray.
2. If the plants are desired to proceed to flowering, water with 1/4 strength of Hoagland solution once every other week until they start bolting (twice in total is enough). Hoagland is nitrogen-rich media that promotes the transition to reproductive growth.
3. Miracle-Gro potting soil has Micracle-Gro plant food (slowly-released fertilizer), which usually support plant growth for up to 3 months. So restrain the impulse to over-fertilize them.
4. Keep an eye when plants start flowering. Use any possible pest control as needed.

5. Aphids is a common insect problem. Spray 1:100 “Orthene” on the infected plants under hood, repeat spray for 3 days. Be careful not to over-spray them, otherwise it will kill the plants.
6. When plants stop flowering, move them out to a ventilated area for seeds to dry. Collect seeds promptly as soon as the siliques look yellow or brown, break with little or no applied pressure.
7. First, you should clean up some area free seeds of other species, to make sure all the seeds here are no contamination with other seeds you do not want. Rub your fingers around the silique, allow the seeds to fall on a big piece of paper below. Filter seeds through nylon mesh into another piece of paper. Store seeds in Eppendorf tubes, poke a hole on the cap of the tube. The collected seeds should be left at room temperature for one more week to dry. Then place them in a sealed container with Drierite.

Recipes

1. Sterilization solution
   20% Chlorox bleach
   0.05% Tween 20
   In sterile water, must be freshly made.
2. 1/4 Strength Hoagland’s solution (used to water *Arabidopsis* plant)

	FW	Stock	g/250 ml stock	final	ml stock/4 L final
KNO_3	101.11	2 M	50.6	1.25 mM	2.5
FeNa-EDTA	367.1	20 mM	1.8355	12.5 µM	2.5
KH_2PO_4	136.1	1 M	34.0	0.5 mM	2.0
$MgSO_4$	120.4	2 M	60.2	0.5 mM	1.0
$Ca(NO_3)_2.4H_2O$*	236.2	2 M	118.1	0.5 mM	1.0
Minors					1.0

*Note: * Add last when volume is almost full.*

Minors：

Chemical	FW	Stock (mM)	Final (µM)	g/100 ml stock
H_3BO_3	61.83	70	17.5	0.43 g
$MnCl_2$	197.9	14	3.5	0.28 g
$CuSO_4$	159.6	0.5	0.125	0.08 g
$ZnSO_4$	287.5	1	0.25	0.03 g
$Na_2MoO_4 \cdot 2H_2O$	241.9	0.2	0.05	0.005 g

Note: Start from >3.9 L water, then add every thing and stir thoroughly.
Ca^{2+} may precipitate with SO_4^{2-}.

3. Homemade MS medium for *Arabidopsis* plants under different Ca^{2+}/Mn^{2+} conditions

Stock:

a. Macronutrients (10x)		
mM (1x MS)	**Salt**	**10x stock (g/L)**
41.2	NH_4NO_3	16.5
18.8	KNO_3	19
3.0	$CaCl_2$·anhydrous*	3.3
1.5	$MgSO_4·7H_2O$	3.7
1.25	KH_2PO_4	1.7
*$CaCl_2$ is dropped out for Ca related medium.		
b. Fe-EDTA (100x)		
mM (1x MS)	**Salt**	**100x stock (g/L)**
Na 0.2	Na_2·EDTA	3.73
Fe 0.1	$FeSO_4·7H_2O$	2.78
c. Micronutrients (100x)		
μM (1x MS)	**Salt**	**100x stock (g/L)**
100.0	H_3BO_3	0.62
100.0	$MnSO_4·H_2O$	1.69
30.0	$ZnSO_4·7H_2O$	0.86
5.0	KI	0.083
1.0	$Na_2MoO_4·2H_2O$	0.025
0.1	$CuSO_4·5H_2O$	0.0025
0.1	$CoCl_2·6H_2O$	0.0025
d. 0.5 M $CaCl_2$		

Make the media

a. For 1/2 MS 1 L, add:

50 ml macronutrient 10x stock

5 ml Fe-EDTA 100x stock

5 ml micronutrient 100x stock

Proper $CaCl_2$ 0.5 M (add KCl to Ca medium as control for Cl^-)

0.5 g MES (always use 0.5 g for 1 L medium regardless of MS strength)

Adjust pH to 5.7, add agar to 1% or 0.8%

Autoclave for 20-30 min

b. Composition of plant media (mg/L, 1x)

Component	Gamborg's B5	Murashige and Skoog
Total weight	3,300	4,620
Inorganic salts		
$CaCl_2$ anhydrous	113.23	332.16
$CoCl_2 \cdot 6H_2O$	0.025	0.025
$CuSO_4 \cdot 5H_2O$	0.025	0.025
$FeSO_4 \cdot 7H_2O$	27.8	27.8
H_3BO_3	3	6.2
KH_2PO_4		170
KI	0.75	0.83
KNO_3	2,500	1,900
$MgSO_4 \cdot 7H_2O$	246	370
$MnSO_4 \cdot H_2O$	10	16.9
$NaH_2PO_4 \cdot H_2O$	150	
Na_2-EDTA		37.3
$Na_2MoO_4 \cdot 2H_2O$	0.25	0.25
NH_4NO_3		1,650
$(NH_4)_2SO_4$	134	
$ZnSO_4 \cdot 7H_2O$	2	8.6
Vitamins		
i-Inositol	10	100
Nicotinic acid	1	
Pantothenic acid.Ca-salt	0.874	
Pyridoxine·HCl	1	
Riboflavine	0.015	
Thiamine·HCl	10	0.4 *

** The original formulation contains 0.1 mg/L thiamine·HCl.*

References

1. Hoagland, D. R. and Arnon, D. I. (1950). The water culture method for growing plants without soil. *California Agric Exp Stn Circ* 347: 1-32.