

## **Growth Conditions for Arabidopsis in Soil (from Scholl et al., Arabidopsis Protocols, p.1-30) (adapted by M.Raizada)**

### **General Notes**

1. preferably autoclave soil mixes 30 min.
2. use metromix 350 (well drained soil) or other peat moss-based mix (1:1:1 perlite, fine vermiculite and sphagnum moss)
3. use constant 25C temperature
4. Humidity less than 50%
5. Arabidopsis requires long-days to flower, so >12hr photoperiod; Arabidopsis also grows fine under continuous light, though the plants flower earlier, and produce fewer seeds.
- 6/ Not necessary to supplement plants with nutrients, but adds vigour.

### **Sowing seeds:**

1. moisten soil first and (optional) apply a systemic insecticide (eg. INTERCEPT) by spraying onto the surface.
2. sow seeds individually with a wetted spatula or the fused end of a glass capillary tube (using seeds soaks in water), or distribute seed from a folded card (detailed description, page 3)
3. 4 x 4 inch pots hold >10 plants.

### **Germinate and Growing Seeds**

1. cover pots with clear plastic wrap; cut several small slits. Place pots in the fridge for >2 days. Certain accessions require up to 7 cold days.
2. Alternative: Sterilize seeds with ethanol/bleach, suspend in .4-.8% agarose, keep in fridge for 2-4 days, pipette onto sterile medium.
3. After cold treatment, place pots in growth area. Remove plastic wrap and maintain 2cm of water around base of pots for 4-6 days (during germination). For plants in a growth chamber, leave plastic wrap on and do not add additional water during this period.
4. In a growth chamber (but not greenhouse), can place pots in a larger tub, keep 1-3cm of water in tub and cover whole tub with plastic wrap, undisturbed until all plants have expanded cotyledons. Perforate the wrap for aeration.
5. Germinate at 22C +/- 2C day, and 18-20C night (optimal day is 25C but chambers fluctuate in
6. temperature, so best to keep lower).
7. lighting: 150 uE/m<sup>2</sup>/s cool white or very high output (VHO/SHO) fluorescent lamps supplemented by incandescent lights in growth chambers. In greenhouses, use 60% shade cloth and 16 hr photoperiod.
8. Water frequently during first few days to avoid drying until first 2 true leaves have started to expand, then reduce to 1-2 waterings per week until plants flower. During silique fill, dramatically increase watering (daily).
9. If algae, dry pots and scrape off algae; indication of too much moisture.

10. Though not absolutely necessary, apply mild mineral nutrient solution every two weeks, especially during later phase of growth. Use commercial slow-release fertilizer that mimicks micro and macronutrient solutions on page 3.
11. Stake the plants.
12. Keep different genotypes 20cm apart.
13. After 3-4 weeks, siliques form; takes 2 weeks for them to mature during which time they turn from green to yellow/brown, and can shatter. Add cellophane bags (45 x 190mm) over plants as the first silique ripens and secure at bottom of bag by sticky tape.

### **Insect Control**

1. Use yellow strips to monitor insect types.
2. A basic systemic insecticide is INTERCEPT 70WG applied to soil prior to sowing seeds.
3. For aphids, use Talstar or Tame+Orthene aerosols.
4. For fungus gnats, keep soil surface dry using turface.
5. For Thrips, use Margosan-O + Avid
6. For Whiteflies, use Talstar+ Enstar applied weekly.
7. For spider mites, apply avid weekly or orthene aerosol.

### **Harvesting**

1. Before harvesting, stop watering 1 week in advance.
2. Harvest when siliques have completely browned.
3. Cut off silique at base, place in 1 gallon plastic bag, dry inside the bag for a few days before threshing out the seed. Sieve seeds to get rid of other dry matter.
4. Use Aracons (Lehle Seeds) for effective single-plant harvesting.
5. Alternative: Before any siliques have begun to brown, bag entire pot with a 4L transparent bag, but keep widely open at all times to prevent condensation. Wait until siliques have browned, then cut off entire inflorescence at base and shake seeds into the plastic bag. Seeds will fall out if the siliques are gently pressed after being completely dry.
6. Cleaned seed are placed in open, labeled glass jars (do not use plastic due to static effects) to allow seeds to dry. Air dry seeds at room temperature for 1-3 weeks.

### **Seed Storage**

1. Store in tightly sealed containers, such as cryovials. 1250 seeds = 25mg = 50uL.
2. Store at 4C; very long term storage at -20C; store vials in a larger container with dessicant, but not necessary if vials are closed tightly.
3. Removal of vials from cold is a dangerous step. Vials should be warmed to room temperature before opening them (place vial at 37C water bath for 10 minutes). If sees exposed to too much moisture, leave vials open in a dry location before returning to cold storage.
4. A germination viability test is described on page 9.