

ARABIDOPSIS PROTOCOL

Planting

Arabidopsis from seed

1. Place 1801 insert into a flat without holes. Break off and remove one of the corner pots, which helps for watering purposes.
2. Fill pots with Profile FPX. Do not compact soil, and brush excess soil off of tray so that soil is level with top of pots.
3. Moisten soil by filling flat half full of water, as well as by wetting soil from top. Once soil is fully saturated, you can dump out excess water.
4. Plant seeds by sprinkling over top of tray, or by your preferred method. Do not cover seeds.
5. Place dome on flat. This will be removed after the majority of the seeds have germinated.
6. Fill out the PTR information on the computer and place label in each flat.
7. Put your flats in your chamber or one of the multi-user ones, which are currently 214 and 216. If you want to vernalize the planted seeds, place the flats in chamber 222 (4°C) for the desired time before placing in the growth chamber.

Arabidopsis transplants

- 1-3. Same as above.
4. Transplant seedling(s) into each pot, making sure not to damage roots. Bottom leaves will be level with soil.
5. Place dome on flat. This will be removed after several days or once plants have re-established themselves from transplant.
- 6-7. Same as above.

Arabidopsis seed for selections

Depending on how many plants are needed, either 1801 inserts or 601 inserts may be used, or planting may be done directly into open trays with holes.

1. Fill pots with Profile FPX. Do not compact soil, and brush excess soil off of pots so that soil is level with top of pots.
2. If planting directly into a flat, use the flats with holes and fill at least half full of soil. Place a white tray under flat. Water in soil from the top and by filling white tray with water.
3. Plant seeds by sprinkling over top of tray, or by your preferred method. Do not cover seeds.
4. Put a "Bottom Water Only" sign in the front of the flat.
5. Place dome on flat. This will be removed after the majority of the seeds have germinated.
- 6-7. Same as above.

Watering

- RO water is used for all watering.
- Plants are checked twice a day for watering needs. Plants kept too wet will become purple and show signs of stress. Algae may grow on the soil and fungus gnats and/or shore flies will become more prevalent. Plants should be allowed to dry down prior to adding more water.

- Whenever possible, plants are top watered to prevent salt build-up.
- Small plants are bottom watered or misted until large enough to be top watered, unless a “Bottom Water Only” sign is present.
- Amount of water needed will vary according to pot size, plant age, and type of plant.
- Once plants have seed that has started to dry down, plants again are primarily bottom watered to prevent seed loss. Plants are allowed to dry down more than previously.
- Watering continues until a “Do Not Water” sign is in place or until the majority of seeds have turned brown. At this point plants are moved into Greenhouse 5A. The PTR location needs to be changed in the system at this time. If you do not wish to move your plants, greenhouse staff will move them for you.
- Open flats of soil with plants for selection are bottom watered.

Fertilizer Regime

- Arabidopsis plants are fertilized once per week with 15-16-17 at approximately 150 ppm N. This is usually done on Tuesdays and Fridays. Plants may appear drier on Mondays and Thursdays so that they are able to take up more fertilizer on Tuesdays and Fridays.
- Plants are fertilized from approximately 2 weeks of age until 2-3 months old, or when the majority of seeds are brown.
- When bottom watering, it takes about 4-6 seconds of running the hose into the flat. The amount of water needed will vary with how dry the soil is and how big the plants are. Excess water is dumped out of flat once soil is saturated.
- If pots are already fully saturated with water, they will not be fertilized until the next time to allow pots to continue to dry down before that time.
- If only a couple plants are dry in a flat, only those pots will top watered with fertilizer.

Staking/Harvesting

- It is the researcher’s responsibility to stake plants. This should be done once the seed heads begin to form.
- Researchers are also responsible for bagging their plants. Bags do not need to be placed on plants until seed heads are turning brown.
- Plants should be harvested as soon as possible to prevent seed loss. This is also the researcher’s responsibility. Cut off seed head and place in a bag, along with the label from the pot.
- To clean the seed, crush seed heads above a piece of paper. Pick off any plant waste. Blow lightly on the seed to separate it from the waste. The seed is heavier and will not move as much as the waste. Continue until the seed is clean and put in an envelope marked with label information. There are also several sieves available which can be used to separate out the seeds. A sieve may be used.
- Check out the PTR for each flat harvested.
- Plants should normally be harvested by 3-4 months old to prevent seed loss. If there are plants in the dry down area that are older than that, the staff will periodically email the owner to remind them to harvest their seed. The owner will also be notified that if the plants are not harvested in 2 weeks from the notice the plants will be disposed of, unless the owner has made special arrangements.

Pest Management

- The most common pests to watch for are thrips, fungus gnats, and powdery mildew. Pests can usually be controlled with regular pesticides. Note: Do not use Judo or Forbid on Arabidopsis as it will cause leaf and flower deformity.

- Currently, chambers with Arabidopsis are treated using beneficial insects for thrips and fungus gnats. Fungicides are sprayed as needed. If a pesticide or fungicide will interfere with the experiment, place a “No Fungicide” or “No Pesticide” sign in flat.

Growing conditions

Temp: 21-24° Celsius

Humidity: 50-70% (higher humidity levels encourage mildew growth)

Light: 200 micromoles

Day length depends on the scientist’s goal: Short day (8 to 12 hours) for vegetative growth

Long day (14 to 24 hrs) to promote rapid seed production

The multi-user chambers 214 and 216 are set at 22 degrees, 50% humidity, 200 micromoles, and 16 hour days.

Other guidelines for researchers

- When using signs such as “Bottom Water Only” or “No Pesticides,” put one sign in the front of each flat that the directions apply to. Even if multiple flats were planted at the same time, each flat needs a sign.
- Place only the same sized pots and the same aged plants into each flat. This will help with the plants getting the correct amount of water, especially when bottom-watered.
- If doing crosses, place a “Bottom Water Only” and a “No Dumping” sign in flat.
- Do not water your own plants unless you have a “Do Not Water” sign in place and are taking responsibility for all watering of your plants. Contact greenhouse staff if you feel your plants are too dry and will need water before our next rounds.
- Do not use a “Bottom Water Only” sign unless necessary for your particular experiment. Plants tend to do better when allowed to occasionally be top-watered. This also will reduce potential for over-watering.
- If the flat has pots that are free-standing in it, place pots in one of the holder trays so that they do not slide around when flats are dumped.
- If there are a large number of plants staked in a flat, such as when using 3601’s, use a flat with holes and put a white tray underneath. This will allow for easier dumping of water.
- Plants that are starting to set seed may be moved to the lower shelves by the staff, unless the owner specifies that their plants may not be moved. This is to prevent shedding seeds onto plants below.