NASC Protocols

Growing *Arabidopsis*

There are many possible ways to grow *Arabidopsis* using different composts, plant maintenance routines and seed collection systems. Here we describe only the protocols adopted at NASC with a few alternative suggestions.

Compost :

After trying out several compost mixes we have found a variety of commercially available composts that give very good results. Water control is important, so we recommend pre-mixed or home-mixed composts that include perlite. Our current compost is a pre-mixed compost that includes John Innes and perlite components.

Pest Management

For routine control of scarid fly, and other insect pests, a solution of Intercept systemic insecticide is prepared at $0.2gl^{-1}$. More information on Intercept is available on our 'products that NASC uses' page. 50ml of this solution is then added per litre of compost mix. Thorough mixing is important and can be done in a large container (dustbin / trash can), or in smaller quantities (plastic bag - e.g. autoclave type).
The final compost mix should be moist but not soggy (individual soil crumbs should still be evident) - if necessary you can add water as required during the mixing procedure.

Tobacco 'bombing' with nicotine shreds can be used to clear greenhouse space of insect pests quite efficiently BUT it is important to bear in mind that this will almost certainly make some or all of your arabidopsis plants temporarily infertile. For this reason, crosses or silwet / vacuum transformations should not be attempted for 1-2 days preceding, or for 1-2 day after tobacco treatment.

To control mildew and some other fungal infections, general plant cleanliness: i.e. prompt removal of dead plant material and regular replacement / cleaning of the growing area is recommended. If mildew has taken hold, then sulphur vapour treatment is very effective and has not been harmful to arabidopsis growth or fertility in our experience.

Sowing :

1. Fill your pots with compost (clay, plastic or polystyrene - we use 3.5 or 5.5 inch diameter disposable plastic pots for efficiency). Settle the soil by giving the pots a sharp tap and compress very lightly to give a firm bed.
2. Sow the seed onto the surface of the compost by scattering them carefully from a piece of folded card (for even distribution and ease of handling for large volumes of seed it can be useful to mix them with clean sand).

3. Place the pot in a flower sleeve then wrap the sleeve around the pot (effectively forms a mini propagator for each pot) then place them in a tray and transfer to a cold (4°C) dark room for days - this stratification improves germination rate and synchrony. Traditional plant propagators can be used in place of the flower sleeves.
4. Transfer to the greenhouse. Our Greenhouse is maintained as close as possible to 22°C (18-28°C) and has a 24h photo-period provided by 10,000 lux globe (Son-T) lighting. Alternatively, Arabidopsis can be germinated and grown for part, or all, of it's life cycle in a growth room.
   For standard lines we do not find it necessary to supplement the growth medium.
   Pots are placed on raised benching which supports capillary matting covered by perforated black polythene (to reduce algal growth and ease-cleaning / prevent clogging of the capilliary matting. Replacement of the black polythene alone extends the life of the capilliary matting and thereby reduces costs).
   When the seeds have germinated and the seedlings reach the four leaf stage (7-10 days) the sleeves are unfurled to form a 'personal propagator' figure 1. which keeps the plants of one line isolated from neighbouring plants (ensuring that absolutely no cross-pollination can occur), and provides support to reduce sprawl and conserve growing area space.
   The pots are then watered from below via the capillary matting. If any shoots reach above the lip of the sleeve, they can be trimmed with large scissors efficiently in one cut.
5. Plants are watered until at least 90% of the seed pods have dried completely and then the plants are allowed to dry slowly in situ for maximum viable seed production (the sleeves physically prevent cross-contamination by pod-shatter).

Stratification (Breaking seed dormancy):

For many lines this is not necessary. However, lines will normally germinate more uniformly (synchronise) if placed in the cold (4°C) for 1-5 days once sown, i.e. they are cold treated as they imbibe. Over this range, the period of stratification correlates to the degree of uniformity.
Vernalisation is a different process, whereby plants may be induced to flower earlier as the result of an extensive cold period during the initial vegetative growth period.

Plant isolation and staking:

Although Arabidopsis is predominantly self-fertilising it is a good idea to plant material far enough apart to prevent flowers from different lines coming into contact with one another or preferably isolate the plants from each other using a system such as the plastic sleeves, mentioned above, or the commercially available Aracon sleeves. If for some reason you need access to the plants while they are growing it may not be practical to contain the plants inside an isolation system (e.g. if you are making crosses) the plants may therefore require staking. Stakes can be made from commercially available garden wire and pea rings or alternatively: plastic rods (resistant to bleaching!) and disposable wire bag ties.

Harvesting:

Because we grow the plants inside plastic sleeves this makes seed collection straightforward. When the plants are completely dry you can:
Grasp the bottom of the sleeve (to catch any falling seeds)
Cut the base of the sleeve away from the pot
Place the bottom of the sleeve into a bag.
Currently we use cellophane bags which were custom made but any non-static bags would do.
The outside of the bag is massaged to separate the seeds from the dry vegetative material.
Seeds are then collected directly from the bags by cutting a small hole in the corner.
This means that we do not have to sieve the material and so reduce the risk of mixing or contaminating seed lines.

Storage:

Seeds will remain viable for at least two years if stored in a dry atmosphere at room temperature.
For medium term storage we place in a controlled environment maintained at 15°C, 15% relative humidity (the seeds will dry to 5-6% moisture content under these conditions).
For long term storage we dry the seeds to ~5-6% water content in the controlled environment described above and then place them in Sarstedt screw top 1ml vials at -20C. We do not store the seed under desiccant.

Following our move in 2001 to our new location at Sutton Bonington, we have moved our seed stores out of the bespoke cabinets that we had previously used, and into a humidity and temperature controlled room. This allows us to both store and handle seeds in an atmosphere conducive to long term viability.
Our cabinets utilised an externally mounted heated and rotating drying drum with independent compartments of hygroscopic gel. The dry gel extracted the moisture from the cabinets and was then dried by heating with moist air vented to the outside of the cabinet. The current room works on a similar heating and external venting mechanism.
Most commercial 'stand-alone' dehumidifiers reduce humidity to a maximum of 20% which should be a reasonable environment for medium term storage of seed.

We are in the process of conducting long term storage and accelerated aging trials in collaboration with Kew/Wakehurst place. Currently we predict that stored in the manner described here, we expect the seed to be viable for more than 100 years.