

HANDLING ARABIDOPSIS PLANTS AND SEEDS

Methods used by the Arabidopsis Biological Resource Center

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The methods used by the ABRC for handling plants and seeds are outlined below. These procedures are designed to generate healthy plants that give maximum set of pure seeds and to preserve these in the safest and most convenient manner. Many other approaches may be equally as good, especially in specific experimental situations.

GROWTH OF PLANTS

Arabidopsis can be grown in a variety of environmental settings including growth rooms, window ledges, outdoors, growth chambers and greenhouses.

Peat moss-based mixes, commercial greenhouse mixes, relatively inert media watered with nutrient solutions, and defined agar media can all be employed as plant substrates.

Our focus will be on growth of plants on agar and soil in growth chambers and greenhouses. The plant and seed management methods are discussed in the chronological order in which they would normally be utilized.

Growth of plants in sterile conditions

It is necessary to use sterile conditions to grow Arabidopsis for specific experiments such as selection of transformed plants, drug resistant plants, early root and shoot phenotypes, lethal mutants, etc. Otherwise, contaminants can essentially take over plant cultures.

Various shapes and sizes of containers such as petri dishes, 'Magenta' boxes, or culture tubes can be used, depending on the required length of the growing time (2-3 weeks or to maturation) and characterization of phenotypes (shoot or roots). We will emphasize the

use of petri dishes. All procedures should be accomplished in a sterile hood or environment.

The most commonly used media is 0.5x or 1x Murashige and Skoog (MS) mineral salts with 0.8-1% BactoAgar™. Optional 0-3% sucrose and vitamins can be added to the media. Preparation of 0.5x MS agar media is as follows:

1. Add 4.31 g of MS Salts to 1.8 L of distilled water and stir to dissolve.
2. Check and adjust pH to 5.7. Adjustments can be made with 1M KOH.
3. Dilute to final volume of 2 L and add agar (10 g / L).
4. Autoclave 15 minutes at 15 psi, 121°C.
5. Optional sucrose and vitamins should be added after agar media cools, before pouring solution into container (e.g. petri dishes, Magenta boxes, culture tubes).

Seeds can be surfaced sterilized by soaking for 8 min in bleach (5.25-6.15% Sodium hypochlorite) with 0.05% Tween 20 and rinsing the seeds 3-5 times with sterile, distilled water. Be sure that all bleach residue is removed. Maintain seeds in a small amount of water in a watch glass and plant immediately.

There are several methods for placing the seeds on medium, depending on the preferred plant density and type of container used:

- a.) For planting of individual seeds in low density, a small pasteur pipet with a latex bulb on the upper end can be used. Exhaust air from the pipet, submerge its tip and use slow release pressure on bulb to draw a single seed into the end of the pipet. The seed can be dropped at the desired location by carefully exhausting of the pipet. Do not draw seeds beyond 1-2 cm into the pipet. Repeated pipetings are used for the remainder of the seeds.
- b.) For planting at high densities with uniform distribution on agar, mix seeds in sterile distilled water (or 0.1% cooled top agar), pour onto dish, and swirl to distribute seeds evenly. A sterile Pasteur pipet tip can be used to move seeds around to adjust the distribution, and to remove excess water. Allow the water or top agar to dry slightly before replacing lid.

After planting seeds on Petri dishes with agar, replace cover and seal with Parafilm to prevent desiccation. Place dishes at 3-4°C (refrigerator temperature) for at least 2 to 4 days to break dormancy, if needed. Dishes can be placed directly into the growth environment. A temperature of 23-25°C, 130-150 (E m-2 sec-1 illumination are suitable.

Growth of plants on soil

- **Planting on soil**

Different mixtures and media can be utilized for growing Arabidopsis. Growth of plants on soil includes all media that can be successfully used for non-sterile growth of plants in pots or other similar containers. Mixtures of soil that have substantial peat moss with some perlite and vermiculite for aeration can be used successfully. Peat-based commercial mixes represent a convenient and reliable base for growing plants. Mixes such as "Sunshine LC1 mix" support healthy Arabidopsis growth and have fertilizer added so that fertilization is not necessary in the very early growth phases.

Seeds can be planted in various ways, however, strict control of numbers of seeds planted can be maintained, and separate rows of different lines can be planted in the same pot for critical comparisons with the techniques described here. The density of planting depends on the genetic material, the purpose of the plants and availability of seeds. For seed production, high yields are achieved utilizing densities of 10 to 20 plants per 10 cm square pot. Larger populations of plants do not necessarily reduce yield, but production per plant is reduced inversely. Larger populations are necessary for maintenance of representative proportions in a segregating population, and this can be achieved with more dense plantings in one or two 10 cm pots or in flats (approx 26 cm x 53 cm).

Preparation of pots and planting can be accomplished as follows:

1. Thoroughly wet soil with tap water and apply a commercially available extended time release fertilizer such as Osmocote 14-14-14 (14% nitrogen, 14% phosphate, 14% potassium) which feeds up to 3 months from planting (apply in amounts according to the label). Alternatively, nutrient solution can be used to wet the soil. Mix well with trowel or large spoon. Soil can be autoclaved to eliminate pests, but this is not usually necessary.
2. Place soil loosely in pots or flats, level without compressing to give a uniform and soft bed. Pots are ready for planting.
3. When planting many seeds in a pot, scatter them carefully from a folded piece of filter paper (weighing paper or other paper) distributing seeds evenly onto the surface of the soil.
4. When planting individual seeds in low density, use a Pasteur pipet with a latex bulb on the upper end. Exhaust air from the pipet, submerge its tip and use slow release pressure on bulb to draw a single seed into the end of the pipet. The seed can be dropped at the desired location in the pot by carefully exhausting of the pipet. Repeated pipetings are used for the remainder of the seeds.
5. Planted seeds should not be covered with additional soil, because *Arabidopsis* seeds need light for germination.
6. If several pots are planted, they can be placed in a tray or other similar container and covered with clear plastic wrap. In all cases the plastic wrap should not be allowed to contact the soil surface. Cut several small slits in the plastic with a knife in order to provide some aeration, but still maintain enough humidity for germination and also avoid seed desiccation. Clear plastic domes are available for covering flats, but should not be tightly sealed.
7. Pots can be placed at 3-4°C (refrigerator temperature) for at least 2-4 days to eliminate any dormancy, improve germination rate and its synchrony. The use of

a cold treatment to break dormancy of seeds, also called stratification, is very important for plantings utilizing freshly harvested seeds, which have more pronounced dormancy. Most widely used lines have moderate dormancy, and cold treatment may not be required when planting older seeds of these lines. For certain lines, as many as 7 days of cold treatment is necessary. Cold treatment of dry seeds is normally not effective in breaking dormancy.

8. After cold treatment, place pots in growth area (growth chamber, growth room, greenhouse, etc) and maintain approx 2 cm of water around base of pots during the germination phase. Leave plastic wrap on for plants grown in growth chamber.

- **Growth conditions**

In general, the growth and development of Arabidopsis plants, including time to flowering and time to harvest depend on several growth conditions in addition to the genetic background. Management of water, nutrition, light and temperature will ensure that healthy plants develop and produce high quality and quantity of seeds. Under continuous light, 25°C, good water supply and good nutrition, seeds of the commonly used lines germinate within 3-5 days, bolt and flower around 3-4 weeks, and can be harvested within 8-12 weeks.

Water and nutrition

Maintenance of soil moisture is imperative for successful germination of seeds. This can be ensured in one of two ways: a) leaving the plastic with small perforations over the pots or tubs, or b) placing the pots in flats without the plastic cover and maintaining a depth of 1-3 cm of water, which is maintained continually until all plants germinate and have expanded cotyledons. We prefer the former for growth chamber and the latter for the greenhouse. The first method is dangerous in the greenhouse setting, due to the potential for overheating underneath the plastic covering on sunny days, killing the germinating seedlings.

After germination, plants are watered as needed to avoid water stress. Water is best applied by sub-irrigation when the soil begins to dry. Sub-irrigation can be achieved by placing pots into flats or trays, allowing proper drainage of the soil. Over-watering should be avoided due to the potential for algal or fungal growth on the soil surface. Over-watering of greenhouse plants also provides favorable soil conditions for fungus gnat larvae. More frequent watering may be necessary during the first few days, as it is necessary to avoid any drying before the first two true leaves begin expanding. After plants have developed true leaves, watering frequency may be reduced to as low as once or twice per week until the plants flower. The water requirement of plants increases dramatically during silique filling. Daily watering at this stage is necessary for good seed production.

Water requirement is strongly influenced by relative humidity. Arabidopsis plants, including seedlings, tolerate low humidity (e.g., 20-30%) although increased humidity (e.g., 50-60%) greatly reduces the risk of accidental drying of the soil surface and subsequent desiccation of the fragile, germinating seedlings. Very high humidity (more than 90%) can induce the formation of mold. Low humidity (less than 50%) is desirable when siliques begin to mature.

Poor nutrition can lead to rapid flowering, short growth period and low seed set. If an extended time release fertilizer was not utilized before, a mild mineral nutrient solution can be applied to the pots at 2-week intervals (5 mM KNO₃, 2.5 mM KH₂PO₄ (adjusted to pH 6.5), 2.0 mM MgSO₄, 2.0 mM Ca (NO₃)₂, 50 microM Fe-EDTA, 70 microM H₃BO₃, 14 microM MnCl₂, 0.5 microM CuSO₄, 1 microM ZnSO₄, 0.2 microM Na₂MoO₄, 10 microM NaCl, 0.01 microM CoCl₂, pH 6.5).

Light

Optimum light is approx 130-150 uE m⁻² sec⁻¹. Very high output or cool white (VHO or SHO) fluorescent lamps, supplemented by incandescent lighting are used for growth chambers. Older plants tolerate higher light intensity, up to full sun, although the use of 60% shade cloth in summer greenhouses helps with light intensity control and temperature regulation. Supplemental evening and morning light is provided in the greenhouse during winter since the plants generally require a long photoperiod (at least 12 hours) for flowering. Photoperiods of 16 hours work well for greenhouse growth. Plants flower rapidly under continuous light or long days, while under short days flowering is prevented or delayed, favoring growth of vegetative tissue. Continuous light is well-tolerated and can be used to accelerate the reproductive cycle.

Temperature

The optimum growth temperature range for Arabidopsis is 23-25°C. In general, high temperatures favor a reduced number of leaves and flowers, and fertility is reduced. At lower temperatures, growth is slow and flowering is delayed. Lower temperatures are permissible, but higher temperatures are not recommended, especially for germination through early rosette development. Older plants tolerate higher temperatures, at least up to 30°C. It is advisable to set the greenhouse temperature at 21-23°C to avoid fluctuations to higher temperatures. It is recommended that night temperatures be maintained 2-4°C lower than the day temperature. Some late flowering natural accessions (ecotypes) require an additional 4°C incubation (vernalization) of young rosettes for 3-4 weeks to induce flowering.

- **Control of pests**

Several insects can cause substantial damage to or even kill Arabidopsis plants in the greenhouse and growth chambers. The main pests we have encountered are thrips, aphids and fungus gnats; other minor pests are whiteflies and spider mites.

Despite any precautions (avoiding transfer of insects from contaminated areas to growth space), even the most carefully managed growth spaces occasionally become infested by pests. The best strategy for eliminating the infestation is emptying the room, destroying all plants growing in the area (if possible), thoroughly cleaning the room and heating the space to 40C for at least 24 hours to kill insect eggs and larvae.

An important aspect of insect control is detection and identification before populations multiply. Traps (yellow cards with adhesive) are vital in this regard for catching winged insects. Fresh traps should be placed in greenhouse rooms and growth chambers frequently and monitored continuously. Always identify a pest before embarking on treatment.

Although chemical or biological treatments can reduce the population of insects, such remedies cannot make a room completely pest-free while allowing Arabidopsis plants to survive.

Where local governmental regulations permit and infestation is highly probable, application of insecticide as a preventative measure can be very effective in assuring plant health throughout growth. This helps to avoid heavy use of chemicals that may be necessary after infestations have occurred. The following procedure may prevent infestation of thrips, aphids, fungus gnats and white flies.

1. Add 1.2 ml of "Enstar", 1.2 ml of "Tempo" and 1.2 ml of "Conserve" to 3 gal of water. Mix well. Spray lightly on rosettes prior to bolting stage - before placement of any isolation devices for the plants.
2. "Marathon" (granular) can be added to the soil surface as per product label. However, applications for small plants can be made in the tray with water in cases where the pots are being sub-irrigated. This practice reduces risk of damage to the plants by the insecticide.

Listed below are the main pests and the possible insecticides used for their control once insects have been observed.

Thrips: these insects feed on developing buds and leaf primordia, visual symptoms of damage are: brown spots in young leaves, withered inflorescence tips, crumpled and curled leaves, sterility and plant death. Effective pesticides to control thrips are "Conserve", "Mesurol", "Duraguard", or "Marathon".

Aphids: severe infestation can produce stunted plants or even plant death. Insecticides like "Marathon", "Mesurol", "Duraguard" are useful.

Fungus gnats: the larvae of this insect inhabit the soil around the plants, especially in over-watered pots, and can cause severe root and leaf damage, destroying plants; adults are easily detected (2-3 mm long). Larvae populations can be controlled by reduced watering or by treating the soil with the highly selective biological insecticide "Gnatrol" (*Bacillus thuringiensis*), or the microencapsulated Chlorpyrifos "Duraguard" for time release control. "Marathon" is a systemic alternative.

Note that the use of brand names does not constitute an endorsement of product nor does it imply that other approaches may be necessarily inferior. The chemicals listed are for information only. Also, when these or any other pesticides are employed, check the label instructions of the manufacturer before purchase or application for registered usages of the product and recommended application rates and frequencies. Label instructions of pesticides must be strictly followed, and the product applied only by individuals with currently valid licenses. All applications of pesticides should be made in evening hours, and greenhouse rooms flushed with fresh air before the next morning to minimize exposure to workers. Notice of application should always be posted.

Many predator species are currently marketed for control of some of the above pests. Some of these are effective, but others are less successful. We have used predators to eradicate spider mites. However, some others have been less successful in our hands, and when it becomes necessary to apply chemicals to control one pest, all predators are often lost. We have also experienced difficulty in keeping some predators on hand for quick response to pest immigration. However, as technology relating to this type of pest control advances, their use will surely increase.

- **Plant isolation and harvesting**

It is necessary to avoid seed mixing among adjacently growing lines and to prevent loss of seeds due to shattering while assuring quality of the harvested seeds. It is essential to keep plants of one line isolated from neighboring plants to ensure that absolutely no cross-contamination can occur. It is useful to keep inflorescences from sprawling for maximum use of growth area. Various means and devices, such as "AraconsTM", plastic floral sleeves and plastic bags can be employed to achieve these goals. In case you need access to the plants while they are growing e.g. if you are making crosses, it may not be practical to contain the plants inside an isolation system, therefore plants can be supported on wire stakes or plastic rods using disposable wire bag ties.

Watering of pots should be discontinued several days prior to harvest so that pots are dry when harvest is conducted. It should be noted that delays in harvesting following physiological maturation of the plant result in seed deterioration, especially under non-optimal environmental conditions.

Commercial seed collectors: Aracons™ (Lehle Seed Co. Catalog) placed soon after bolting are effective for single plant harvesting, preventing cross pollination and seed contamination, but are not necessary when simple bulk production is desired. Harvesting after pots have been allowed to dry is accomplished easily by carefully cutting off the inflorescence under the cone device, placing the Aracons™ plus contents carefully on a large plastic bag (approx 4 liter) or a large piece of paper, removing the plant material from the plastic cylinder, and then shaking the seeds into the bag or paper. Alternatively, if plants are totally dry, the plant material can be placed directly onto threshing sieve (see threshing section below).

Use of plastic floral sleeves: For bulk seed production using individual pots, the pots can be placed into transparent clear plastic (i.e. polypropylene) floral sleeves near the bolting time. These sleeves fit snugly around a 10-cm pot, extend 50-60 cm upward, and are wider at the top allowing for expansion of the developing inflorescences, maintain upright stiffness, and tear easily for harvesting. All plant inflorescences are maintained within the sleeve, forming a propagator for each pot. At harvest, the sleeves can be cut or torn, the inflorescences cut off at the base, and the plant material placed into plastic bags or, if plants are totally dry directly onto threshing sieve. This method is very effective for achieving high densities of lines while maintaining productivity and purity of each pot.

Bagging inflorescences by pot: Inflorescences can simply be trained into an approx 4 liter transparent plastic bag before any siliques begin to brown. The bag, however, may potentially collect moisture from transpiration or careless watering and provides a haven for insects when greenhouses are sprayed. To reduce these possibilities, the tops of these bags should be kept widely open at all times. Wait until the inflorescence has browned before harvesting. This method is conducive to strict isolation of the lines, and the bag serves to collect shattered seeds. Harvesting is accomplished by carefully cutting the entire inflorescence off at its base after all seeds have matured and shaking the seeds into the plastic bag.

Bulk production on the open bench: For bulk seed production, the best method is to simply grow the plants on the open bench, keep all lines separated by adequate space, avoid disturbance of maturing inflorescences, and harvest when all siliques are dry. The entire inflorescence is cut off at its base and carefully placed into an approx 4 liter or larger transparent plastic bag, depending on the size of the bulk of plants. This is compatible with the goals of high seed quality, maximum seed yield, and good pest protection. Some seeds may be lost, but the remainder are almost always healthy, and result in vigorously germinating seedlings. After harvest, the entire contents of the bag are allowed to dry in preparation for threshing.

Early harvest of individual siliques: Seeds from individual siliques can be harvested after the siliques have turned completely yellow, if rapid turnover is required. However, such seeds do have high levels of germination inhibitors. For

normal seed production, seeds are harvested only after the siliques have completely browned, and when pressed with fingers, do not compress (if the silique has dried even further, the silique may shatter at this point). At this stage, seeds are completely formed. Since formation and maturation of siliques occur over time, early siliques can be harvested before later ones mature to avoid seed loss. However, it is usually recommended to wait until the entire inflorescence has browned before harvest.

SEED HANDLING AND PRESERVATION

The longevity of seeds can be affected by a) genotype, b) pre-storage environment, such as conditions during seed maturation, harvesting and seed handling and c) seed storage conditions. A slow process of deterioration begins as soon as seeds mature on a plant. Therefore the sooner seeds are placed into storage, the better. Harvested seeds should be processed promptly (including threshing, cleaning, drying and packaging) and then placed into storage.

Preservation of seeds involves adherence to a few simple principles. Hence, it is not a difficult task although deviations can result in damage to seeds. We treat Arabidopsis as oil seeds which means that the most careful, and conservative handling procedures must be applied. The following procedures form a sequence that ensures that the seeds will be conserved in the best possible condition.

Threshing

Hand rather than machine threshing and cleaning of the small Arabidopsis seeds is recommended mainly because the threshing machines need rigorous cleaning between lines to avoid sample cross-contamination, require very careful adjustment and do not accommodate the variable size of Arabidopsis seeds well.

If seeds are collected in a plastic bag, the harvested plant material should be allowed to dry for a few days in the opened bag before threshing, since threshing is easier when the inflorescences are dry. Seeds should be threshed when the moisture content is approx 10%, to minimize seed damage during threshing. This moisture content will be reached when all material in the bag appears to be dry. The plastic bags containing dried inflorescences can be gently hand-pressed from the outside, and the seeds will fall to the bottom of the bag. Most of the dry inflorescence can be removed from the bag by hand before seeds are sieved to separate them from chaff.

Hand sieves with graded mesh sizes (i.e. No. 40) are recommended to remove debris, with seeds passing through the mesh and collected on clean paper. Totally dry plants from AraconsTM and sleeves can be placed directly onto the sieve. After sieving, the seeds are still likely to be mixed with soil and residue. A combination of additional sieving, blowing and visual inspection can be employed to clean the seeds completely. Small samples can be cleaned by hand with the aid of a pointed tool on an opaque glass plate illuminated from below. Cleaned seed samples are placed in open, carefully labeled

glass jars (do not use plastic due to static effects), or in small manila "coin" envelopes to allow seeds to dry.

Seed drying

The moisture content of Arabidopsis seeds after threshing is usually around 10%. The seeds should be dried to 5-6% moisture, prior to storage. This is verified by moisture testing, as outlined in the protocol below, on samples that can be disposed. Higher moisture content can cause seed deterioration. There are many methods available for drying seeds. The safest method is to air-dry the seeds at room temperature for 1-3 weeks. Low relative humidity (20-30%) is necessary for seeds to reach the desired moisture content. The lower the humidity, the faster the seeds will dry and the lower their final moisture content. If after testing, the moisture content is not low enough, continue to dry further and check again.

Seed moisture content determination

General Considerations

Moisture testing is necessary to verify that seeds are dry enough for storage. Seed moisture content can be determined by several methods. The method outlined is a destructive method, and the seeds employed for testing will no longer be viable.

1. The total weight of seeds used for a moisture content determination should be sufficient to make the test accurate and yet not be wasteful of seeds. The sample should be fully representative of the accession and a minimum of 100 mg should be used to prepare the samples for the test. Accurate results were obtained using approximately 200 mg of seeds.
2. The lower the weight of seed used, the more accuracy is required to achieve a true result. Small samples should be weighed with an analytical balance to four decimal places using light-weight dishes (small aluminum dishes or petri dishes), so that the ratio of the weight of the seeds and the dish is not too disproportionate.
3. It is suggested that a minimum of three replicates of 100 mg of seeds or two replicates of 200 mg of seeds per sample be used for the moisture content determination.
4. Always work with care and finish one sample at a time. Do not leave the dishes open in the laboratory between weighings because the seeds will either lose or absorb water from the air and small changes in weights can result in large differences in the calculations when the amount of seed used is small.
5. High temperatures cannot be used to determine the moisture content because the oil will also vaporize and give a false result of water plus oil content. Temperatures of just over 100°C allow evaporation of water and minimal vaporization of oils.

Equipment:

Heat resistant dishes with cover, analytical balance, forced draft oven, desiccator with silica gel, tongs and oven cloth.

Method:

1. Weigh one clean numbered dish and cover accurately to 4 decimal places using an analytical balance. Write the weight (W1) in the notebook.
2. Add approximately 100 or 200 mg of seeds distributed evenly over the base of the dish, replace the cover and accurately weigh the dish and cover. Write this weight (W2) in the notebook.
3. Place the dish in a safe place and continue to do the second and/or third replicates in the same way.
4. When all samples have been weighed into numbered dishes, place each dish on top of its numbered lid in the oven at 100-105°C.
5. Wait for the oven to reach this temperature and heat the samples for 15-17 hours.
6. Remove the dishes from the oven, replace their covers and place in a desiccator to cool for 30 to 45 minutes at room temperature. After heating, make sure that the dishes are put directly into the desiccator so that the dry seeds do not absorb more moisture.
7. Remove the dishes one by one from the desiccator and immediately weigh each dish and cover, and write the weight (W3) in the notebook. Do not leave the desiccator open during the weighings.
8. Moisture content is calculated as the loss in weight as a percentage of the original weight of seeds. This is known as wet basis or fresh-weight basis, and is expressed to one decimal place. Algebraically, if W1 is the weight of the dish, W2 the weight of dish and seed before drying, and W3 the weight of dish and seed after drying, then:

$$\% \text{ Moisture Content} = 100 \times (W2 - W3) / (W2 - W1)$$

Seed packaging for storage

After seed moisture content is within the safe storage limits, dried seeds should be placed in tightly sealed and impermeable containers to prevent rehydration. Cryovials (with threaded lids and gaskets) are convenient for storage. They hold large numbers of seeds, seal tightly and can be resealed many times.

In packaging seeds, each container should be labeled with relevant information including date of storage using a waterproof permanent marker, or a suitable printed label. In determining seed quantities, approx 1250 seeds = 25 mg = 50 microliters. Seal the

container immediately after filling, and visually check. During storage, check the containers at regular intervals to assure that they remain in good condition.

Seed storage and preservation

The major factors influencing seed longevity are storage temperature and seed moisture content. The higher the value of either, the shorter the lifespan of the seeds. Seeds left at ambient temperature and relative humidity lose viability relatively quickly, although they may be viable for about two years if stored in a dry atmosphere at room temperature.

For sealed cryovials or any moisture proof container, where seeds already have 5-6% moisture content, there are two storage options.

1. For active collections which are stored for short to medium terms and are accessed often, a convenient temperature is approx 4°C (regular refrigerator temperature).
2. For base collections where seeds are placed in long-term storage without disturbance, a temperature of -20°C is appropriate.

The arrangements of vials in storage can vary, but it is important to record the exact location of each line. Codes can be used to indicate boxes, racks, trays, and refrigerators/freezers.

For open containers such as envelopes, the seeds can be stored at 15-16°C, with a relative humidity of 15%. Under this controlled environment, the seeds will maintain suitable low moisture content.

Removal of vials from storage to access seeds represents a potentially very dangerous step. Vials must be warmed to room temperature before opening. Rapid re-warming (placing vial in a 37°C water bath for approx 10 min) serves to minimize freeze/frost damage that can occur during this process. Working in a low relative humidity (20-30%) environment, if possible, also aids in prevention of hydration. If it is suspected that condensation has occurred in a vial during storage or opening, the vial should be left open in a dry location until seeds have desiccated before returning them to cold storage.

Seed viability

Seed viability is the condition in which seeds are alive, have the potential to germinate and develop into normal reproductively mature plants, given the appropriate conditions. Factors that affect viability include the initial viability of the seeds at the start of the storage, seed moisture content and storage environment. Viability should be monitored at regular intervals. It is anticipated that viability of Arabidopsis seeds should remain high for long storage periods, assuming proper conditions.

A viability test for Arabidopsis seeds can be conducted in 3 to 6 days. Tests should be carried out before seeds are packaged and stored, so that poor quality seeds can be

recognized. A germination test is the best method of estimating seed viability. Arabidopsis seeds may fail to germinate because they are dormant or because they are dead. Dormant seeds typically remain firm and in good condition during the germination test while dead seeds soften and are attacked by fungi. Imbibing seeds with water at low (refrigerator) temperatures can usually break dormancy.

The following method to test seed viability is suitable for Arabidopsis:

1. Place two layers of filter paper (free from chemical residues that could interfere with the germination of the seeds) firmly in the bottom of a 10-cm diameter petri dish, labeled with line number and date.
2. Moisten the paper with distilled water. The paper should be totally saturated, but no excess water should be left in the dish.
3. Distribute 100 seeds uniformly on the surface of the paper. Replace the lid and seal the dish with Parafilm or clear tape, to preclude desiccation.
4. Cold treat seeds by placing petri dishes in the refrigerator for 2-4 days.
5. Place the dishes on an illuminated shelf (or in a growth chamber) under standard light and temperature conditions for Arabidopsis.
6. After 3 to 6 days, count germinated vs. un-germinated seeds, and record germination percentage.

These methods are used by the ABRC for handling plants and seeds. If you have any questions concerning the above procedures, feel free to contact us at abrc@arabidopsis.org.

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