Appendix 3. Additional seed preparation information

This appendix provides additional information to prepare seeds for long-term storage at the USDA-ARS National Laboratory for Genetic Resources Preservation. It provides information about seed extraction, drying, conditioning and packaging. For long-term seed storage, it is important to check the cleanliness of the containers and tools at every step of the process to avoid mixing or contaminating samples. If there are questions about seed processing procedures, please contact NLGRP-Blackbox@usda.gov.

Seed extraction

- Dry seed can generally be easily removed from the plant. It is recommended to remove as much non-seed plant material as possible at harvest for most efficient and uniform drying.
- Wet seeds:
 - Seeds harvested from fleshy fruit (such as pumpkin, cucumber, melons and tomatoes) should be scooped out with surrounding pulp. Wash the seeds repeatedly in cold water, removing as much of the fleshy coating as possible. This is best achieved by placing the seeds in a mesh strainer that is fine enough so that seeds don't slip through and running water over the seeds, allowing the pulp to fall through.
 - After pulp is removed, let seeds dry on a mesh or screen to ensure good air flow.
 - For smaller seeds in firmer pulp or in berries (such as horned melons or strawberries), the fruit (with any harder outer skin removed) can be processed in a blender with added water to break down the pulp and then poured onto a fine mesh to rinse off remaining pulp pieces.
 - Cautions on collecting wet seeds:
 - Leaving wet seeds to dry on paper towels will usually result in the seeds sticking to the paper. Drying on mesh or screen will ensure the seeds stay free flowing.
 - Some sources will suggest "floating" seeds in water to quickly determine which seeds are empty. Air pockets around the seed coats can make the results unreliable, and it is not preferred.

Seed drying

- When seeds are free from pods or fruit, spread them out on mesh or screen laid on a flat surface in a dry (15-25% relative humidity (RH)), cool (below 25°C/77°F) room.
- Spread seeds out so air can circulate around seeds.
- Drying time will correspond to drying temperature, ranging from less than 1 week for drying at 25°C/77°F to 1 month for drying at 5°C/40°F.
 Seeds can be dried effectively using a dehumidifier in a small air-tight space. Lay seeds out in thin layers on mesh or screens and place in a space with a dehumidifier at the maximum level. Drying time depends on variables such as the dehumidifier power, the size of the room, the quantity of seeds to dry and the moisture content of the seeds.
- Silica gel beads can also be used to dry seeds, especially in a humid environment. Loose silica gel beads are available from craft stores and are useful for drying seeds. The silica gel will change color when it has absorbed moisture (typically blue = dry and pink = saturated). Silica gel can be placed in the oven or microwave to reactivate it.
 - To dry seeds using silica gel:
 - 1. Weigh the seeds to be dried.
 - 2. Place the same amount by weight of silica gel into the bottom of a glass screw-top jar.

- 3. Separate the seeds from the silica gel. Put seed in a paper bag or place a small piece of screen on top of the gel and place the seeds on top of the screen.
- 4. Spread seeds out as much as possible so they sit in a thin layer.
- 5. Put the lid on the jar and keep it sealed for 7 to 10 days. Large seeds, like squash or pumpkin may need a few extra days.

Seed conditioning. Seed conditioning is the process of removing inert matter (e.g., dirt, sticks, glumes, empty seeds), and contaminates from the sample of desired seed.

- Inert matter and contaminants should always be removed from the sample.
- It is usually best to condition seed after drying.
- Seeds can be sorted by size, shape, and/or weight. Conditioning techniques should be decided on a case-by-case basis and multiple conditioning techniques can be used on a single sample if necessary.
 - Weight
 - Winnowing
 - Winnowing separates a sample by weight by allowing natural air movement or a fan on low to blow away light material as the sample is carefully tossed into the air flow.
 - \circ This method can be used anytime the desired material will not be moved by the air flow.
 - General Blower:
 - Blowers are specialized equipment that separate a seed sample by weight but allow the fan to be set at a variety of speeds, confine the sample so no material is lost, and provide a catch for the portion of the sample that is blown off.
 - This technique is most often used to remove empty seeds with intact seed coats that are not immediately obvious to the sorter.
 - When using a blower, the original sample is placed in the drawer or cup located over the fan output. Once the drawer/cup is in place, the fan is turned on. Start the fan on a low setting to allow the seed to be moved individually. If the fan is turned to maximum to start, the whole sample will lift and carry a disproportionate amount of material into the air. Slowly increase the speed of the fan until the desired speed is reached. Let the fan run for at least 3 minutes before turning the fan off. At this point, the part of the sample that has blown off is called the 'light portion" and the part of the sample that remains in the initial drawer/cup is called the "heavy portion"
 - The desired portion of the sample depends on the seed being sorted and what material is being sorted out. For example, for carrots, the light portion should be empty seeds and pedicle pieces, and the developed seeds should remain in the heavy portion. With very small seeds, like Amaranth, the seeds will be picked up by the fan and pushed to the light portion. The heavy portion will be the inert matter to be discarded.
 - To find the best fan speed for the sample, run the sample through the blower at a trial setting, then check the light and heavy portion for full seeds. This can be done using a "cut test." This means cutting a seed open to see if the embryo is developed inside. Typically, ten seeds are picked to be cut from each portion. When there are 3 or less developed seeds in the portion with undesired material, or 7 or more developed seeds in the portion of desired

material, the fan speed is generally regarded as good. Depending on the quantity of seed available, these proportions can be changed at the discretion of the sorter.

• Removing material by weight may result in a mixture of seeds and inert matter with discernable size and shape differences. At this time, the sorter may want to sieve the desirable portion from the blower to sort by size or shape.

• Size and shape

- Sieves or screens come with a variety of hole sizes and shapes and can be used to sort seeds, inert matter, or contaminants by size, shape, or both. A collection pan should always be placed below the sieve.
- Material should not be forced through the sieve because this could cause damage to the seed.
- Both sieve and pan should be vibrated or shaken lightly together to agitate the sample enough for material to fall through into the pan, but not so hard that material is shaken out. After agitating, the collection pan should be examined to assess if the desired sorting was achieved and decide if further sorting is needed.
- Depending on the variation in the sample, multiple different sieves/screens may be used in succession. For example, imagine a sample that contains both large chaff, medium seed, and small contaminants. Sorting on a sieve with small holes will allow the contaminants to fall through and they can be discarded. The portion remaining on top of the sieve will still include large chaff with the seeds, so the sample is not yet clean. The remaining portion will be put through a second sieve with larger holes than the first, to allow the seeds to fall through, but not large enough for the chaff to pass. In this case, the material in the collection pan would be the desired portion of the sample to be saved and the chaff on top of the sieve could be discarded.

• Hand Conditioning/Seed Picking

- Conditioning by hand is more time consuming than other methods, but it is the most accurate method for sorting visible differences in samples.
- The sample is poured out on a clean flat surface and any undesirable material is manually sorted out. If it is possible for light to pass through the seed, sorting can be done on a surface lit from below, which allows the sorter to see if the seed is properly developed or empty. This is mostly done with grass species but can be done with many other seeds as well.
- A lot can be sampled as many times or with as many methods as necessary until the sorter has the desired seed sample. Once the desired sample is reached, it is ready for packaging

Seed germination

An initial viability test for seeds being sent for long-term storage is optional but recommended. Feel free to contact NLGRP to discuss methods for testing viability of seed for long-term storage.

Seed packaging

- Seed samples need to be sealed in a suitable airtight container for long-term storage.
- Ideally seed will be packaged and sealed in a foil bag that will keep moisture out and help store seeds safely.

 An example of a bag that will store seeds safely: <u>Amazon.com: 100 Pack Mylar Bag 3.5</u> <u>Gram,4.7 Mil Thickness Smell Proof Bag,Stand-up Packaging Pouch,Resealable Ziplock Foil</u> <u>Food Storage Baggies Safe Material,4x5 Inches,Matte Black : Home & Kitchen</u>

Some recommended references:

Genebank Standards for Plant Genetic Resources for Food and Agriculture

<u>Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and</u> <u>Agriculture: Conservation of orthodox seeds in seed genebanks</u>

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