

Appendix III. Standardized minimum protocol for seed regeneration and seed storage of Solanaceae

(Revised and updated February 2012: addition of section 7.1)

This protocol provides general guidelines for successful seed regeneration and seed storage. Environmental conditions and available equipment can vary according to the partner.

1. Disinfection of seeds

- 1.1. If seed disinfection is carried out at the time of seed extraction, after seed cleaning, put the soaked seeds in a solution of sodium hypochlorite (NaClO) at 1.2 chlorometric degrees for 30 minutes; then rinse carefully and dry. This method could, however, negatively affect longevity of the seeds. Commercial bleach is available at various concentrations, expressed as “chlorometric degrees”. Example: for a commercial bleach at 48 chlorometric degree, dilute it 1/40 (1 volume for 39 volumes of water) to obtain a solution at 1.2 chlorometric degrees.
- 1.2. If seed disinfection is carried out just before sowing, several methods are possible:
 - For any crop, bleach can be used against fungal, bacterial and viral contaminations. Soak the seeds in a solution of NaClO at 6 chlorometric degrees for 15 minutes, then rinse carefully and sow.
 - For pepper, seeds can be disinfected against TMV by soaking in a 10% solution of sodium triphosphate (Na₃PO₄) for 15 minutes up to a maximum of 1 hour. Thoroughly rinse the seeds with (tap) water and sow them immediately afterwards.
 - Tomato seeds can be disinfected against TMV by thermotherapy. Maintain the seeds at 80°C for 24 hours, after which they can be germinated. This treatment must not be applied to very freshly harvested seeds, but only to well dried seeds aged at least 2 months.
 - Fungicides with the active compound “mancozeb” (e.g. Titane 445) can be used against fungal diseases. Sow the seeds immediately after disinfection.

2. Identification

Plants must be labelled clearly with a unique number during the regeneration procedures to prevent mix-up of accessions. Use the same number for one accession from sowing until harvest. This can be a field number or, better, the accession number.

3. Number of plants regenerated

- 3.1. For self-pollinating species, use at least 5 plants. For heterogeneous accessions or cross-pollinating species at least 10 plants should be used to preserve genetic diversity. If possible, use more plants.
- 3.2. Try to regenerate as few times as possible because every time a heterogeneous accession or a population is regenerated, an involuntary selection is made, which

causes loss of genetic diversity. Regeneration cycles can be minimized if seeds are processed and stored under optimum conditions (long-term storage).

4. Transplanting of seedlings

From the seedlings, the requested number of plants for regeneration must be picked without making a selection, except:

- Seedlings that are not vigorous enough to grow and reproduce can be skipped.
- The very vigorous seedlings in self-pollinating species can be hybrids, so it is better to reject them.

5. Isolation

Though cultivated Solanaceae are considered a self-pollinating species, in some climatic conditions they can display a variable spontaneous outcrossing rate due to insect activity. This occurs in particular in eggplant, pepper, *Physalis* and *Cyphomandra*.

- 5.1. For self-pollinating species, to prevent accidental outcrossing, isolate each accession. This can be done by keeping accessions in an insect-free greenhouse, by isolating accessions with gauze nets, or by bagging the flowers. Vibrating the flowers or hand-pollination improves seed set.
- 5.2. For cross-pollinating species such as some wild relatives of cultivated Solanaceae, geographical isolation is possible. The minimum distance indicated between two accessions is 1 km, depending on the local conditions (climate, topography, insect population, etc.). Cross-pollinating species grown in isolated greenhouses can be pollinated by hand with a mixture of pollen from all plants of that accession.

6. Harvest

- 6.1. Harvest an equal number of comparable fruits per plant to ensure that each plant of a given accession contributes equally to the seed harvest.
- 6.2. Harvest only healthy fruits from healthy plants to obtain a good seed lot with high germinability.
- 6.3. Harvest fully ripe but not over-ripe fruits to obtain a good seed lot with high germinability. Sometimes, seeds start germinating in the fruit when it is over-ripe.
- 6.4. Harvest as many seeds as possible because this reduces the frequency of seed regeneration.

7. Seed cleaning

Seeds can be cleaned in different ways depending on the species and local facilities.

Example: Water method. Cut the fruits and remove the seeds by squashing the fruits in a bowl, or by using a spoon. Add a surplus of water and mix well. Add water and pour it off several times along with the fruit pulp. The pulp and empty seeds will float. The good seeds sink to the bottom.

A variant of this method can be used for tomato, the seeds of which are coated with gel. Instead of water, add a solution of HCl at 2% to the pulp, mix well together and leave for one hour. Rinse carefully.

- 7.1. Special attention must be given to cleaning seeds during collection expeditions. When seeds are not removed in time, fungi can start to grow, which affects seed quality.
 - Dried fruits of *Capsicum* can be stored for longer time.
 - Fresh fruits of tomato, eggplant and pepper can be stored for a few days if they are not damaged, after which time remove the seeds, clean them and dry them immediately.
 - When fresh fruits are damaged, remove, clean and dry the seeds immediately to prevent growth of fungi on the seeds.

8. Desiccation

- 8.1. Seeds must be dried as quickly as possible. Spread the seeds as much as possible and ensure there is an air current for rapid drying. If seeds are dried outside, always put them in the shade as very high temperatures can affect the germination rate of the seeds. The temperature should never exceed room temperatures.
- 8.2. After a first drying, the seeds can be packed in paper bags for further drying with silica gel or in an air-conditioned room.
- 8.3. The best way to store seeds is in airtight containers under cool conditions. Frozen seeds will keep their germinability even longer. If seeds are frozen they must first be well dried. A seed moisture content of 4-7% is good; 6% can be reached for eggplant, pepper and tomato by drying outside when the relative humidity is 30%.

9. Storage

- 9.1. Well dried samples can be stored in sealed plastic or aluminium foil bags, or in sealed tins or glass jars. In this way seeds will retain their germinability for a long time. Caution: storage in paper bags at ambient temperatures may cause rapid loss of germinability. If the moisture content of the seed is not easy to establish before storage, a small packet of silica gel put in the airtight container can ensure that the seed is sufficiently dry.
- 9.2. The best storage temperatures for long-term storage range from -15°C to -20°C and for medium-term storage from +4°C to +5°C.
- 9.3. Always have a safety-duplicate of each accession stored under good conditions in another location.