



General guidelines for regeneration, processing and storage of cucurbit species

Developed by the ECPGR Working Group on Cucurbits

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This protocol gives general guidelines for successful regeneration, processing and storage of seeds. Conditions and available equipment can vary.

Disinfection of seeds

These treatments can be used for disinfection of cucurbits seeds (depending on the crop, other treatments are possible).

Cucumber

Before sowing, seeds may be disinfected. Disinfection can be done by maintaining seeds at a temperature of +76°C for 72 hours using dry heated air. Before the heat treatment seeds must be dried to a moisture content not higher than 6% (seeds with a higher moisture content may be damaged during the heat treatment).

Cucurbita, melon and other cucurbits

Seeds can be disinfected with sodium hypochlorite, 150 ml/l commercial bleach, for 5 minutes.

Identification

Plants must be properly labelled with a unique number during regeneration to prevent any mix-up of accessions. From sowing until harvest the same number will be used for one accession.

Number of plants regenerated

At least 10 plants per accession should be used but more is better. For heterogeneous accessions at least 15 plants must be used to ensure the preservation of genetic diversity.

Transplanting seedlings or elimination of seedlings in field plots

From the seedlings, the needed number of plants for regeneration must be picked without making any selection. Only seedlings that are not fit enough to grow and reproduce can be skipped or removed.

Isolation, pollination and seed production

Most cucurbits are naturally self- as well as cross-pollinating. To prevent outcrossing, each accession must be isolated. This can be done by putting accessions in an insect-free glasshouse, or by isolating accessions with gauze nets or single buds by tying them closed with cotton tape, or planting in isolation fields, or by bagging the flowers.

Pollination procedure

The best time for pollination of all cucurbits is up to 12:00 am.

Cucurbita maxima, moschata and pepo

Male and female flowers are selected to be used the following day and flower buds are tied to prevent contamination with unwanted pollen. An appropriate flower will have its closed corolla yellowish at the apex. The next morning, a staminate flower is removed, opened, and the dehisced pollen rubbed on to the stigma of the previously tied female flower.

Melon (monoecious types) cucumber and watermelon

A bright-coloured male flower is picked. The corolla is removed. In this way a kind of natural brush is formed. The stamens of the male flower are rubbed gently against the stigma of a female flower. Pollen must be of good quality and dry (for cucumber). The best time for pollination is between 10:00 and 12:00 am.

When it is difficult to obtain fruits/seeds with hand pollination, bumble bees or honeybees can be used. The plants are placed inside a gauze net inside a greenhouse or in plastic tunnels. When plants start flowering a few bumble bees are placed in the cages.

Pollen (of melon or cucumber) can be stored, but the general practice is to use fresh pollen. Pre-anthesis flowers (buds) kept for a few days at low temperature and high humidity can provide pollen.

On parthenocarpic samples of cucumber, treatment with gibberellin or silver nitrate to induce male flower production should be performed. Sometimes parthenocarpic cucumber seed production is like in other types. In the case of a very long ovary the number of seeds produced per fruit may be low.

Melon (andromonoecious types)

Anthers need to be manually excised from each perfect flower and that is why hand cross-pollination of andromonoecious melons proves to be more difficult than that of monoecious plants. On the afternoon before anthesis the anthers of perfect flowers are usually removed and then, in order to prevent insect pollination, the emasculated flower is enclosed. It is possible to close the male flowers before anthesis, or to pick them off as near mature buds and keep them indoors overnight at high humidity. From these flowers pollen is transferred the following morning. Pollen can also be transferred to the emasculated female flower from male flowers taken from the plant on the day of pollination without being stored. Hermaphrodite flowers pollinated by insects tend to show a higher rate of successful pollination.

Seed production

For seed production several alternative methods can be used:

- To collect and mix pollen from all plants of one accession and use it to pollinate all plants. This method does not prevent selfing completely, but the probability of selfing is low.
- The so-called chain pollination, carried out by hand. For each accession plant 1 is crossed with plant 2, plant 2 with plant 3, etc.
- Accessions in isolation cages may be pollinated by insects such as bumble bees or honeybees.

Treatments during regeneration

For the plants producing predominantly male flowers, female flowers can be obtained by pruning. If pruning does not prove effective, ethylene can be applied at the early stage of growth (seedling with 2-3 leaves). Male flower production can be induced in plants with only female flowers by treatment of sprouts with 250-1000 ppm silver nitrate (or gibberellin). During regeneration, a plant should be removed only if it is very clear that it is an off-type because a mix-up has taken place.

Harvest

To be sure that each plant of the accession contributes equally to the seed lot, on average an equal number of comparable fruits per plant must be harvested. Do not make selections during fruit harvesting.

Processing and storage

Cucurbit seed development will go on even after the fruit is removed from the vine. If, because of frost or other unfavourable circumstances, fruits are harvested before they have matured, they should be stored for 1 or 2 months before seed extraction. In general germination rates will increase if harvested fruits are stored for one week before extracting seeds.

The seeds of pumpkins or some melons which form in the hollow cavity of the fruit require separating from the placental tissue, washing and drying.

Embedded seeds of watermelon, cucumber, melon, wax melon and squash can be removed by chopping the fruits. When water is added to the mixture, seeds will sink and the flesh debris, which floats, can be poured off.

Fermentation helps with removing the persistent placental material encasing cucumber and bitter melon seeds. The water and seed mixture is left for 1-2 days at a temperature of 20-35°C. When seeds settle to the bottom of the container and the placental material floats, the fermentation is complete. Then the seeds are rinsed and left to dry.

Hydrochloric acid or ammonia solution can be used for more effective cucumber seed cleaning. By adding some enzyme (e.g. Rapidase® Smart) it is possible to stimulate tissue fermentation. Some 30 minutes of vigorous stirring will free the seeds of flesh. Before drying seeds require thorough rinsing.

Mechanical seed cleaning is not recommended for bitter melon seeds or necked seeds of *Cucurbita pepo* which are fragile and can easily get broken.

Seeds of loofah and bottle gourd, which are extracted from dry fruits, require little or no processing before storage. However, mature seeds of most cucurbits have adherent, wet flesh which has to be removed.

Seeds should be dried as quickly as possible. Cucurbits seeds are typically spread out to dry under warm (less than 35°C), arid conditions. Drying at higher temperatures or in full sunshine will reduce the storability. After the first drying, the seeds can be packed in paper bags for further drying with silica gel or in an air-conditioned room with controlled air humidity and temperature. The storage conditions for dry seeds (5-6% moisture content) involve airtight containers kept in the dark at about 5°C and 25 RH. Silica gel can be added to absorb moisture. Under such conditions cucurbit seeds can remain viable for at least 10 years. Frozen seeds (first dried down to safe moisture content) will keep their germinability longer.