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SOP 06. MULTIPLICATION/REGENERATION OF PLANT GENETIC RESOURCES

Objective

The multiplication/regeneration of samples in the Suceava Gene Bank collection is carried out when the seed quantity is insufficient for preservation and distribution or when the germination capacity of the conserved genetic material falls below the accepted standard (fewer than 1,000 seeds and germination below 60-85%, depending on the species).

Multiplication refers to increasing the seed quantity in the field such that the resulting genetic population retains the same traits as the original.

Regeneration entails renewing a seed sample by cultivating it in the field or protected spaces, ensuring that the resulting genotype, after harvest, possesses the same characteristics as the original. This process is applied when the germination percentage drops below the minimum standard set by FAO/IPGRI for seed genetic conservation

Procedure Description

Suceava Gene Bank works with a wide variety of botanical species grouped into crop categories. For most species, there are specific multiplication/regeneration guidelines.


Seed multiplication/regeneration is performed in experimental fields, greenhouses, and agricultural research and development institution fields in Romania, based on collaboration agreements signed at the beginning of each year.

The following aspects are considered to ensure the process complies with quality standards:

- Whether the species/crop should be multiplied/regenerated in open fields or greenhouses.
- Whether the species is allogamous, partially allogamous, or autogamous.
- The nature of pollination (anemophilous or entomophilous) and the necessary level of isolation.
- The number of plants required for multiplication/regeneration.
- The seed production volume needed to ensure sufficient seed quantity.
- Whether the harvested seeds meet quality standards.

The multiplication/regeneration process involves the following steps:

1. Selection of samples for multiplication/regeneration.
2. Requesting samples from the conservation sector for multiplication/regeneration.
3. Sowing samples in fields or greenhouses.
4. Monitoring the cultural condition of plants in experimental fields and greenhouses.
5. Establishing harvesting methods.

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6. Drying and conditioning of samples.
7. Finalizing the multiplication/regeneration process by introducing the samples into medium- and long-term conservation.

All samples for multiplication/regeneration, whether handled by the Bank or other research institutions, are prepared by the respective species curators.

1. Selection of Samples for Multiplication / Regeneration

Samples are selected based on the following criteria:


- Samples received or collected that, after conditioning and drying, do not meet the quantity and quality criteria for inclusion in the unit's collection.
- Seed samples received from various donors with a low seed count, making them unsuitable for inclusion in the collection.
- Samples in the Bank's collection with a seed count below the minimum threshold, even if the initial germination capacity exceeds the Bank's minimum standard.
- Samples in the Bank's collection with germination capacity below the standard used by gene banks (FAO).
- Samples multiplied/regenerated in the previous year but not accepted for conservation (e.g., insufficient seed quantity, mechanically or biologically damaged seeds). For re-multiplication original samples or those with the lowest multiplication count will be used.

2. Requesting Samples from the Conservation Sector for Multiplication/Regeneration

- Curators identify samples requiring immediate regeneration (e.g., germination below 85 - 60% or fewer than 300 seeds)
- Prioritization of multiplication/regeneration is based on available financial and human resources.
- Curators compile multiplication/regeneration lists and submit them to the Conservation Laboratory for sample release (*F.6.7.1.*).
- Conservation Laboratory staff package and transfer samples to the respective species curators.

3. Sowing (Planting) Samples in Fields/Greenhouses

- Seed bags are labelled with a field number, and samples are sown using different sowing schemes, depending on the degree of allogamy, seed quantity, etc.
- Each sown sample is labelled with its field number, as indicated on the seed bag and the multiplication/regeneration record for that species.
- For species propagated via seedlings (e.g., tomatoes, peppers, eggplants, medicinal, and aromatic plants), seeds are first sown in nutrient pots before transplanting to greenhouses or fields.

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- In greenhouses, soil treatments are applied before sowing or transplanting to prevent pests and diseases

4. Monitoring the Cultural Condition of Plants in Experimental Fields/Greenhouses


- During the growing season, samples are monitored by curators and phytosanitary protection officers.
- Curators regularly inspect the cultural condition of multiplication/regeneration parcels to remove weeds (mechanically, chemically, or manually) and apply phytosanitary treatments at optimal moments.
- For allogamous plants, appropriate isolation methods (e.g., protected spaces or inflorescence isolation) are used during and before flowering.
- Protective measures, such as netting to prevent birds from damaging plants, are implemented in experimental fields.
- Drip irrigation is used in greenhouses and experimental fields as needed, depending on species requirements.
- Minimum descriptors for multiplication/regeneration are recorded for each species during the growing season (*F.6.7.2.*).

5. Establishing Harvesting Methods

- All samples in fields and greenhouses are harvested manually at full maturity.
- For most species, fruits are harvested, while for others, only seeds are collected.
- After initial air-drying, samples are manually or mechanically processed as needed.
- For most species, small-scale threshing equipment is used, while sensitive seeds are processed manually.
- For certain vegetables (e.g., tomatoes, cucumbers, pumpkins), fruits are harvested, and seeds are manually separated under running water.

6. Drying and Conditioning of Samples

- After mechanical or manual conditioning, seed samples are packed in paper bags and stored on shelves with natural ventilation.
- Within 30-40 days, seeds are manually cleaned and labelled.
- Multiplication-specific labels accompanying each seed sample include the following information:
 - Field number.
 - Accession number.
 - Multiplication cycle.
 - Harvest year.
 - Phytosanitary approval.
- Samples without phytosanitary approval are discarded. Samples with insufficient seed quantity but good phytosanitary status receive a provisional label and are remultiplied

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the following year (using seeds from the original sample or the one with the lowest multiplication count) (*F.6.7.3.A.*).

7. Finalizing the Multiplication/Regeneration Process

- Labelled samples (*F.6.7.3.B.*) are transferred to the drying chamber by the curator. Once the moisture content falls below 8%, 100 seeds from each sample are taken for standard germination testing.
- After receiving germination test results, the curator records the germination rate on the sample label.
- Temporary Samples which meeting conservation criteria are transferred to the responsible person for documentation and registration, receiving a permanent entry number (*F.6.7.3.B.*).
- Only samples meeting the following three criteria accompanied by a specific list are transferred to the Conservation Laboratory: germination >60-85%, phytosanitary approval, and >500 seeds (*F.6.7.4.*)