**Target**

Multiplication of acquired - or collection material.



**1) General selection criteria**

The following categories of material are eligible to be multiplied:

a. Material with status 'received' (receipt number, INS-CGN-PG-003), originating from the acquisition process, from which it appears that insufficient seed is present (see INS-CGN-PG-002) to include the number directly in the genebank;

b. Material with status 'received' (receipt number, INS-CGN-PG-003), of which in

initially seemed to have enough seed present but of which after drying,

seed cleaning and germination determination has been established that the quantity (see INS-CGN-PG-002) and/or the quality (see INS-CGN-PG-005) of the seed lot does not meet the specified requirements;

c. material with status 'accessed' and already included in the genebank. Hereby

three situations occur):

* depletion of stock (insufficient seed present for release); a decision is made whether it is a user- or base-multiplication, see INS-CGN-PG-004.
* germination below set requirements, see INS-CGN-PG-005.
* multiplication necessary due to new phytosanitary regulations, see PRT-CGN-PG-601 Phytosanitary policy.docx).

d. material listed under a, b and c that could not be successfully propagated and

from which this multiplication procedure must be repeated (FOR-CGN-PG-002).

Several times a year, a summary showing the quality and quantity of seed material is made from GENIS (Collection management report).

**2) Multiplication planning**

Depending on the budget, the number of samples to be multiplied per crop and the urgency to multiply, the project leader decides which crops will be multiplied after consultation with the curators. For a number of crops, a minimum number of samples is needed to efficiently multiply. Preliminary consultations are then held with Unifarm, breeding companies and horticulturists regarding the necessary facilities, working hours and the presence of GMO varieties of crossable crops. If crossable GMOs are grown at the sites, multiplication will not take place there.

The following questions are important when performing the multiplications (INS-CGN-PG-005):

1. Do phytosanitary requirements apply to the crop? See PRT-CGN-PG-601 Phytosanitary policy.docx and multiplication protocols PRT-CGN-PG-101 to 130.
2. Is the crop (cf. species) propagated outdoors or in greenhouses/cages?
3. Is the crop (partially) cross-fertilising or self-fertilising?
4. Does cross-fertilisation take place via wind or insects, what is the necessary insulation?
5. How many plants should be propagated on?
6. Is pollination adequately ensured in cross-pollinated crops?
7. How large must the total seed production be for inclusion in the genebank?
8. Does the harvested seed have sufficient quality?

The multiplication protocols detail these aspects for each crop in outline. Non-binding multiplication advisory methods have also been drawn up for the implementing parties in addition to the binding multiplication protocols. By means of crop evaluation, suggestions for adjustment of the advisory methods are discussed and possibly implemented.

**3) Concluding agreements with implementing parties**

Separate multiplication protocols have been established for most crops and their wild relatives (see PRT-CGN-PG-101 to 130). The multiplications are carried out by Unifarm, horticulturists and breeding companies. In consultation between CGN and the breeding companies, through the Plantum crop groups, agreements in principle have been made on the multiplication of CGN collections by member companies. Multiplications can also be carried out by foreign breeding companies. The breeding companies are selected for their crop-specific knowledge. The multiplications by breeding companies are an 'input in kind' to the CGN. If applicable, the number of accessions to be multiplied is agreed annually, either verbally or in writing, with the breeding companies, with which agreements in principle have been made via Plantum, or with foreign companies.

To reserve facilities from Unifarm, the "Request for Trial Facilities" forms should be completed annually. For Unifarm, the cost of facilities and Unifarm staff will be budgeted for each crop and recorded in a written agreement.

Agreements made with horticulturists are set out in a cultivation agreement (FOR-CGN-PG-046).

**4) Implementation of multiplications**

Samples that are propagated have the status 'received' (not yet in the collection) or 'accessed' (in the collection) - see point 1. The curators collect the necessary seed material. If the multiplication involves material with the status received, material has already been selected from source material that is stored as material in the hold for multiplication under a yellow label (see UIT-CGN-PG 6.2.41 '*Identification and traceability'*). If the multiplication involves an accession number, INS-CGN-PG-004 'Multiplication in case of depletion of seed stock' is followed regarding the type of bag from which multiplication is to be carried out. The seed bags used for multiplication are registered in GENIS by scanning the barcode on the passport list and the samples to be issued. It is thus known which sachets were sent to which multiplication address.

The curators/collections management project staff compile seed lists of the material to be multiplied in a given year (FOR-CGN-PG 002 '*Multiplication log'*). The material, provided with these lists and multiplication protocols, is sent to the parties to be exported. Parties other than Unifarm must sign a Standard Material Transfer Agreement (SMTA) and, if necessary, the material must be provided with disinfection advice, a phytosanitary declaration and/or an EU plant passport (see INS-CGN-PG-007). If the material sent is used exclusively for multiplication, signing FOR-CGN-PG-036 "MOU for multiplication of CGN accessions by third parties" or a cultivation agreement (FOR-CGN-PG-046) is possible as an alternative to signing the SMTA. If a user bag is multiplied, it should be entered in GENIS as for a release.

The samples to be propagated are given a field number. These numbers are maintained throughout the multiplication process. Among other things, these numbers are used in the records in the multiplication logbook (FOR-CGN-PG-002), on the labels in the field and in the greenhouse and on the bags used during all post-harvest procedures. Only when the seed sample enters the process inclusion in the genebank, the original receipt and accession numbers are used.

Multiplications are carried out according to the Multiplication Protocols

(PRT-CGN-PG-101 to 130). These protocols contain the essential requirements for each crop (number of plants, method of pollination, method of harvesting and phytosanitary requirements). If necessary, additional multiplication advice methods are added for performers.

Material may be removed from a multiplication only after written communication between curator and the responsible foreman.

The harvested seed lots from multiplications must be sufficiently dried before they can be fine-cleaned. Cleaning procedures vary from crop to crop and are described in general protocols (see PRT-CGN-PG-201 and PRT-CGN-PG-202). If necessary, multiplication advice methods are given to Unifarm.

**5) Transfer of samples to trustees**

After cleaning, the seed lots are marked with crop name, year of multiplication or acquisition and the name of the CGN. The seed is then transferred to the trustees. The breeding companies are requested, if the seed has not been transferred at the end of a multiplication cycle, to still transfer the seed as soon as possible (within 6 months at the latest).

The trustees visually check the purity (INS-CGN-PG-005) of the seed samples and the presence of phytosanitary papers if applicable (PRT-CGN-PG-601). If the purity does not meet the criteria, post-cleaning takes place.

**6) Deviations observed during multiplication**

During the execution of the multiplication work, there is the possibility of recording the activities carried out in the Multiplication Log (FOR-CGN-PG-002) and/or in the logbooks used by third parties. If the execution of the multiplication by third parties deviates from the requirements in the multiplication protocols, this should be recorded by the executing parties and reported to the trustees.

Deviations are recorded by the curators in the Multiplication Log after the multiplication work is completed and evaluated so that remedial action can be taken. If the deviation is caused by non-compliance with the instructions, as provided to the performing party, this is included in the supplier assessment procedures. If an abnormality is so severe that the identity and genetic integrity (see INS-CGN-PG-005) of a propagated seed sample cannot be guaranteed (e.g. due to mixing of different samples during pollination, harvesting or cleaning), the propagated sample is rejected and destroyed. Based on a cause analysis, possible corrective and/or preventive measures are identified and - if possible - implemented by the quality officer. The person responsible for the process where the deviation has been identified should check whether the measure has actually been effective. The cause and the measures identified and/or taken are recorded in the logbook. In addition, a Supplier Assessment (FOR-CGN-PG-016) is completed annually for each propagator.

**7) Storage in drying room**

The seed bags marked with field numbers are placed for further drying in the drying room of CGN-PGR's storage facilities in crates marked with a blue label. If the seed was delivered by breeding companies in sealed aluminium bags, these are opened and repacked into paper bags.

Seed samples from the multiplication process are transferred to the Seed Manager. The curator renumbers the field numbers into receipt numbers or as possible accession numbers. If the field numbers do not match the field numbers of the multiplication list, the seed material should be considered deviating (swapped).

The seed quantity is determined. The results are recorded in the Multiplication Log (FOR-CGN-PG-002).

**8) Check whether seed material from previous multiplication is available**

It is checked whether material from a previous multiplication (no more than 5 years ago) is available from the same number (access number or receipt number). This information is extracted from GENIS and/or previous Multiplication Logbooks. This seed is marked as divergent seed material with a red label. If sufficient seed harvested from sufficient plants from the current multiplication is available, seed from a previous multiplication is not needed and can be removed. If seed material from a previous multiplication is available, it is examined whether this seed material can be used to arrive at sufficient seed for storage.

If the seed from the previous multiplication is needed, it is combined with the currently multiplied seed. Here it is important that the seed is mixed well, for example using the seed divider or by spreading the new seed in a large tray after which the seed from the previous multiplication is scattered over it.

**9) Assess whether (total) batch or recent multiplication is sufficient**

The quantity of the (aggregated) seed material is determined. The weight is recorded in the Multiplication Log on the harvest list. In the case of aggregation, the total number of plants multiplied is noted.

Using the seed quantities instruction (INS-CGN-PG-002), it is assessed whether sufficient seed has been generated for a user or base multiplication (see INS-CGN-PG-004). In exceptional cases, the Collection Management project leader may decide to include a seed lot harvested from too few plants in the collection.

If the seed quantity is not sufficient or/and if seed has been harvested from too few plants (see multiplication protocols), the seed is marked with a red label and stored as deviant seed material.

**MEASURING POINTS PROCESS EFFECTIVENESS & CAUSE ANALYSIS**

4. Germination. Percentage of accessions from a crop multiplication that meet germination requirements (80% for cultivars; 60% for wild relatives). Standard: at least 80%

*Measurement point: annual analysis crop logs after quality assessment*

5. Genetic erosion. The number of accessions from a crop multiplication included despite a multiplication with a harvest on too few plants. Standard: maximum 5%

*Measurement point: annual analysis crop logs*

6. Cost efficiency. On average, 75% percent of multiplications in one round should result in uptake according to the applicable criteria (except potato 60%)

*Measurement point: annual analysis crop logs*