

Operational Genebank Manual

Centre for Genetic Resources, The Netherlands (CGN)

In this manual, at several places a reference to the ISO Quality Management System of CGN is made. This system, that complies to NEN-EN-ISO 9001:2015, is documented in detail in a quality manual written in the Dutch language. Large parts of the manual have been translated and are available on the CGN website, however these can be several years old. The latest Dutch quality manual is available on request.

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1 Germplasm Acquisition and Accessioning

Genebanks can obtain the germplasm they want to conserve through a number of different ways. Conducting collecting missions is possibly the best way of acquiring germplasm material in the most reliable manner. Germplasm exchange with other genebanks is a second route to add genetic diversity to the collection. Obtaining and storing germplasm from researchers and plant breeders is another route to acquire genetic material. Such acquisitions should be guided by a formal mandate that the genebank concludes with its host organization or government and that provides the basis for a genebank acquisition policy. The actual accessioning of acquired germplasm samples, i.e. formally including it into the collection with its unique accession number, is a complex process during which the curator has to check a number of aspects such as the verification of the identity of the material, the health status, the availability of pertinent information, etc. It is further understood that also legal aspects form part of this activity, e.g. was the material collected/obtained in legal manner, are there any restrictions on its use, etc.

1.1 Germplasm Acquisition and Accessioning

GA1 Briefly describe any formal mandate that your genebank might have concluded with or received from your "mother organization" (e.g. institute, governmental body).

This description should include details on:

- a) which species you conserve and make available;
- b) who decides on what your mandate is and, if different,
- c) from whom do you received the mandate;
- d) the main aspects of the mandate; and
- e) legal considerations on PGR as foreseen in national legislation).

The Centre for Genetic Resources, The Netherlands (CGN) is an organization hosted by Wageningen University and Research Centre (WUR) and was created to fulfil the legal obligations of the Dutch government in the field of genetic resources conservation. It has clusters dealing with animal genetic resources (AnGR), forest genetic resources (FGR), and plant genetic resources (PGR). This PGR cluster is largely, for about three quarters, funded by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV). Additional funding has to be approved by the ministry of LNV to assure CGNs independence from its stakeholders. To assure the quality of its operations CGN has adopted a quality management system according to NEN-EN-ISO 9001:2015.

CGN concentrates on crops that are of importance to the Dutch agriculture and breeding industry, and tries to avoid duplication of efforts of other genebanks. As a result it focusses on horticulture crops (including potato) suited to the Dutch climate or cultures in greenhouses. CGN maintains its material on behalf of the Dutch government, who decided that all material maintained by CGN should be, as far as legally possible, in the public domain; all material is, as far as legally possible, available under the terms and conditions of the SMTA of the International Treaty, including non-annex I material. The Dutch

government doesn't deem it necessary to exercise its national sovereignty regarding access and use of genetic resources occurring in the Netherlands in national legislation.

Further information about CGN and its mandate is provided on the CGN website (www.cgn.wur.nl).

GA2 Specific agreements. Does your genebank have any specific formal agreements with other genebanks regarding the conservation of specified germplasm?

This should include:

- a) whether or not your genebank has any international agreements to conserve specified germplasm on behalf of other countries,
- b) a specific region, and/or
- c) the world), and
- d) which crops or genebanks fall under these agreements?

Based on a formal agreement of 1994 between the Federal *ex situ* Genebank of Germany and The Centre for Genetic Resources, The Netherlands (CGN), the two institutions share the responsibilities for a defined set of germplasm (potatoes, sugar beet and chicory). This agreement has not been terminated, revised or updated since 1994. In practice, the collaboration is not pursued, but CGN still maintains the bilateral potato collection, as far as it concerns the seed regenerated material.

GA3 In case your genebank has a germplasm acquisition policy, what does the policy entail?.

- a) please specify which crops or which geographic area, if applicable.

The acquisition policy of CGN is pragmatic and spelled out in its ISO Quality Management System. If a need arises (based on the judgement of the curator or on feedback received from the user community) and there is an opportunity, CGN will request and/or collect material of the crops for which it maintains collections, regardless its geographic origin. The materials acquired mainly consist of obsolete varieties (including farmers' varieties) and wild related species of the CGN crops. All acquisition activities are done in close consultation with experts, crop scientists and breeders when it concerns varieties or experts from local universities and institutes when it concerns collecting missions. In every step all legal requirements are met, and the 'Guiding principles for the development of future harvest centres, policies to address the possibility of unintentional presence of transgenes in *ex situ* collections' are also followed.

The logistics of the acquisition of new material is part of the ISO Quality Management System of CGN.

GA4 How do you verify the identity of the germplasm material received (e.g. relying on the donor's information, comparing material with other accessions, involving (taxonomic) expertise, etc.)?

Although no protocols are in place to check the identity of received material, every possibility for checking this is used, including feedback from crop experts and breeders during regeneration or evaluation of material, feedback from users, or targeted molecular analysis of (parts of) CGN collections. When possible mis-identification is tracked, this will be verified and corrected when needed.

CGN has done research and published about the authenticity of genebank material (e.g. Crop Science 51: 736).

GA5 Describe if and how you conduct an assessment of the various quality aspects of the seeds, tissue culture or plant material received.

This description includes:

- a) quality aspects related to the correct identification of a given accession, but also
- b) health
- c) purity aspects of the sample/accession), and
- d) use of a quality control system (e.g. ISO).

The new material always follows any phytosanitary regulation applicable to it. When required it is regenerated and subjected to the regular CGN procedures regarding viability and quantity of the seeds, as described elsewhere in this document.

GA6 Describe whether and how the SMTA is being implemented

- a) Extent of materials covered by SMTA (crops, numbers of accessions)
- b) Ways of SMTA implementation and documentation of transfers of PGR
- c) Other aspects (e.g. monitoring, supervision)

All CGN material, including the non-annex 1 material, is only distributed with a SMTA, with the exception of material distributed for direct use or repatriation. Distribution of CGN material for non-food/feed uses are negotiated on a case-by-case basis, respecting the provisions of the Nagoya Protocol. The SMTA transactions are administered appropriately and are being reported to the ITPGRFA secretariat.

Besides the 'regular collection', the main collection of the CGN funded by the Ministry of LNV, that follows the procedures of the ISO Quality Management System and whose protocols are described in this Genebank Manual, CGN also maintains a number of 'special collections', that are funded by the user community of each special collection. Material of these special collections can have MTA's (other than the SMTA) or, such as for example for the lettuce SSD collection, a simple contract added to the SMTA.

GC1 Describe here the details of the strategy that you follow in implementing germplasm collecting missions.

This description should include:

- a) general aspects of planning and implementing a collecting mission,
- b) the criteria you use for priority setting;
- c) the actual strategy followed in sampling material from farmers' fields, from nature, etc.; and
- d) how your germplasm acquisition policy underpins the mission.

Recent collection missions have been initiated by CGN following requests from the user community regarding the arising need for specific breeding material. Once such a need is identified, CGN will explore the possibilities to obtain the material from existing germplasm collections. If these do not exist, possibilities to collect the needed material *in situ* are evaluated, and contacts with local institutes or universities are established, legal matters are *handled* and a collecting mission is organized. CGN only undertakes a collecting mission after signing an MoU with the authority concerned in which the authority of the country where the collecting takes place agrees with the subsequent distribution of collected material by CGN under the terms and conditions of the SMTA, thus assuring that all ABS matters are appropriately dealt with.

There is no explicit strategy for the actual sampling of material during collection missions; the collector tries to sample the population as good as possible, i.e., assuring proper representation of the population and not causing damage to the population or the environment.

Obviously, all collecting activities have to follow from, or at least fit in the CGN acquisition policies, in terms of crops, numbers of accessions and accessibility.

GC2 Provide any additional information on the germplasm collecting activities of your genebank, including the collaboration with others.

Most recent CGN collecting activities have been financially supported by the user community, usually a group of plant breeding companies organized in the Dutch sector organization Plantum. In these collaborations, the collected material is made exclusively available to the funding companies for a period of three to five years, after which the material becomes part of the regular CGN collections and thus publicly available.

2 Ensuring Security

This chapter refers to the security of the genebank structure itself (i.e. its physical security), the safety of its germplasm (i.e. the maintenance of viability) as well as the institutional and personnel security, aspects which together will ensure the long-term conservation of the entire collection.

2.1 Physical Security

To ensure the physical security of the collections, the following aspects are regarded as essential elements for achieving the objective:

2.1.1 Safety Duplication (of long-term conserved germplasm)

SD1 Please describe how your genebank implements the safety duplication of your germplasm material.

This description should include the following aspects:

- a) The type of safety duplication (e.g. black-box; no specific arrangement; other);
- b) The location(s) where you store your safety duplicates (country; genebank);
- c) Whether or not you are using a formal agreement with the genebank(s) that store your duplicates?
- d) Whether the safety duplicates are stored under conditions comparable to your own? Please provide details;
- e) Do you maintain safety duplicates from other genebanks at your genebank? If so, do you know any details of that material?

As described in the ISO Quality Management System of CGN, all material is duplicated under black box arrangements in colleague genebanks outside the Netherlands. These arrangements are spelled out in a Memorandum of Understanding that is concluded between CGN and each of the hosting genebanks. Individual accessions in laminated aluminium seed bags are packed in cardboard boxes and stored locally at temperatures used in the hosting genebank for long term seed storage, usually -20°C or -18°C and thus similar to the conditions at CGN.

The CGN collections have also largely been triplicated in the Svalbard Global Seed Vault.

Reciprocally, CGN stores duplicates from colleague genebanks under black box arrangements.

SD2 Do have a safety duplication policy? If so, please provide essential details.

The policy is described largely in the previous paragraph, and comprehensively in the ISO Quality Management System of CGN.

2.1.2 Structure

SS1 Please provide details on how your genebank building has been designed to resist natural disasters (e.g. earthquakes; flood; storm).

The threats to the CGN storage facilities are limited, as it is located in a moderate climate without earthquakes or hurricanes. Flooding is the only threat - this risk is accepted given that the complete collection is safety duplicated, and >80% is triplicated, so there is no risk of losing the PGR.

SS2 Please describe the security arrangements that you have in place to protect your genebank against burglars, fire and others.

Please include details on the following arrangements, as applicable:

- a) Fences;
- b) Security doors;
- c) Alarm system;
- d) Fire detectors;
- e) Standby generator;
- f) Others (please specify).

The specifications of the CGN storage facilities are spelled out in the ISO Quality Management System of CGN. It includes all regular elements such as access policy, backup power supply, automatic temperature and humidity monitoring and alarm systems, an argon based fire extinguishing system, etc. The facilities can be considered (although they are old) state of the art, heavily tested and fully reliable.

SS3 Please provide information on any other structural security aspects that you might have in place.

CGN staff, when entering the cooled storage rooms alone, are required to carry a device that detects non-movement for two minutes, and automatically notifies security officers when this happens. These devices are regularly checked.

2.1.3 Security Equipment

SE1 Provide details on the kind of emergency (back-up) equipment or arrangements that you have in place to ensure permanent electricity and cooling.

Aspects to consider are:

- a) "back-up" compressors for your cold rooms;
- b) generator;
- c) regular maintenance and trial runs;
- d) other.

Every cold room has a 'back-up' compressor and the entire storage unit has a 'back-up' generator. These facilities are tested twice a year by the Facility Services of WUR.

SE2 Describe how you monitor temperature and relative humidity in your cold stores and drying room?

Temperature (in all rooms) and relative humidity (in the drying room) are monitored electronically. If the measured values exceed the defined limits, security staff is automatically alarmed after 15 minutes. The temperature in the 'household freezers' (for the bags for distribution to users) in the room with 4°C is not monitored centrally, the freezers sound an alarm in case of malfunctioning – this alarm is detected when someone enters the room.

2.1.4 Institutional and Personnel Security

IPS1 Provide details on the "institutional security", in particular with respect to the provision of financial means to operate the genebank

Aspects to consider are:

- a) timely transfer of funds from the "mother" organization to the genebank;
- b) do you have direct access to the "mother" organization that provides the budget?;
- c) internal "security" of accessing these funds;
- d) long-term security and stability of funding (compensation of inflation rates, avoiding variation in years)
- e) any other observations that are relevant in this context.

Since CGN is organized as a statutory task of the Dutch government and hosted by a foundation under public law, all financial arrangements are completely transparent. Issues such as 'security of accessing funds' are irrelevant to an organization as CGN.

Regarding continuity and level of funding, CGN is funded on the basis of five-year agreements. The level of funding is re-negotiated with the Ministry of LNV every five years. Outcome of these negotiations will obviously depend on many factors, including economics and politics. However, a basic level of funding is guaranteed based on the legal tasks that CGN performs.

IPS2 Describe how you secure adequate staffing of your genebank?

The ISO Quality Management System of CGN, which is audited twice yearly, includes provisions regarding adequate staffing. CGN staff largely has permanent labour contracts. Much of the work is outsourced.

2.1.5 Contingency Plans

CP1 Describe the kind of emergency or contingency plan that your genebank has in place to cope with disaster situations.

The ISO Quality Management System of CGN provides procedures for most occurring situations. There is no explicit contingency plan for disasters.

CP2 Provide information on the kind of training, security drills and other activities that your genebank gives to its staff to deal with emergency situations, if any.

All CGN staff is informed regularly regarding the safety procedures. In the hosting organisation (WUR) a system with Emergency Response Officers is functional, assuring that help is available when needed.

3 Germplasm Maintenance

This chapter deals with key aspects of managing germplasm in a genebank, i.e. the maintenance of the viability, the genetic integrity, the availability of the conserved germplasm as well as the management of the corresponding information. Given the fact we are covering seed, in vitro cultures and entire plants it might well be that not all aspects are covered by one and the same genebank. In those cases it is suggested that only the applicable sections are completed. Accordingly, at the beginning of each section of this chapter you will find a “navigation box” (highlighted in yellow) that will help you as user of the template to complete the correct section(s).

3.1 Maintenance of Viability

This section refers to the maintenance of the longevity of the seeds or of tissue cultures or living plants in storage. A high initial viability is the most important pre-condition for achieving the longest lifespan of seed accessions in storage, hence maximum efforts need to be taken to ensure that seeds to be stored have the highest possible viability. Optimum growing conditions when multiplying/regenerating the accessions, efficient management of the preparatory steps before storing the germplasm, adequate storage conditions as well as proper monitoring of the viability are critically important.

Navigation on Maintaining Viability section

Seed - If applicable, please complete the section on Maintaining Viability for the activities related to seed genebanks (i.e. boxes 3.1.1.A - 3.1.3.A)

In vitro cultures - If applicable, please complete the section on Maintaining Viability for the activities related to in vitro culture (i.e. boxes 3.1.1.B - 3.1.3.B)

Cryopreservation - If applicable, please complete the section on Maintaining Viability for the activities related to cryopreserved collections (i.e. boxes 3.1.1.C - 3.1.3.C)

Field genebanks - If applicable, please complete the section on Maintaining Viability for the activities related to field genebanks (i.e. boxes 3.1.1.D - 3.1.3.D).

As CGN only has very few accessions not maintained as seeds (apple and cabbage, a total of < 250 accession), this Operational Manual will only cover the seed storage facilities and procedures.

3.1.1.A Initial seed viability

IV1 Describe the procedures or practices that you have in place to ensure the highest possible initial viability of your seed, in particular during regeneration and post-harvest (e.g. cultivation practices, pollination aspects, use of specific equipment as shelters, storage of harvested seeds, cleaning, etc.).

Regenerations are done, according to the protocols in the ISO Quality Management System of CGN, at a high level of quality. The period between harvesting and freezing the seeds is kept as short as possible (at this point CGN is trying to further improve the procedures to further limit this interval). During this period the seeds are cleaned, dried and the initial viability is determined.

IV2 Describe procedures how you deal with a) dormancy and b) hard seeds?

In the cases where the viability tests are hampered by dormancy or hard seededness, appropriate measures are taken, as far as these are known. These include treatment with gibberellic acid or KNO_3 , cold stratification, cutting, sandpaper or other scarification methods.

IV3 Please provide any other information on procedures that you follow to ensure highest possible initial viability.

In case the viability is not high enough, sometimes the seeds are cleaned more carefully.

3.1.2.A Seed Viability Monitoring

VM1 Describe the routine seed viability monitoring system that you use.

The monitoring system should include the following aspects:

- frequency of testing;
- sampling method applied;
- any thresholds that you use;
- whether you apply different procedures for crops/species with erratic initial viability or irregular viability lifespan;
- etc.

All details regarding the germination testing procedures are described in the ISO Quality Management System of CGN. It includes the principle that the initial viability of every sample must be tested before it can enter the genebank, and is retested after 25 years. After the first retest, the interval of retesting depends on the crop (15 years in case of wheat and barley and 10 years for all other crops) or the result of the test (the interval is decreased to 5 year in case of signs of aging – score 2 (see below)). The viability testing is done with in-house developed protocols (based on ISTA protocols and other sources).

The germination threshold applied is 60% germination for the wild species, and 80% for the cultivated species.

Viability tests result in scores that indicate that the seed lot is (1) very healthy and well above the threshold, (2) above the threshold, but showing signs of aging and in need of more frequent monitoring, (3) on or just below the threshold and in need of regeneration but not very urgently, (4) poor germination below threshold and/or showing severe signs of aging and thus in need of urgent regeneration.

If the initial viability is below the threshold (i.e., scores 3 or 4), the seeds cannot enter the genebank, the seed lot needs to be cleaned better, or the accession needs to be regenerated again. Only in very few cases exceptions can be made (following a well-defined procedure for approval) for seed lots that cannot meet the requirements for good reasons, but should be accepted anyway.

VM2 Please describe the information “system” that you might have in place that allows you to make more species or even accession-specific decisions when the next monitoring should take place.

The results of the germination tests are stored in the CGN documentation system Genis. The planning of the germination tests is done on the basis of lists that are generated automatically, but decisions are taken ‘manually’ on the basis of these lists, using Excel spreadsheets, allowing better planning over the years, optimising the facilities.

VM3 Please provide information on non-specific thresholds that you might use for viability of seeds (i.e. percentage of germination) and for the amount of seeds left of an accession to initiate regeneration? In case you differentiate between self- and outbreeding species, please answer for each category separately.

There are no thresholds regarding viability, apart from the ones described in VM1. There are thresholds regarding the amount of seeds: (1) in case the number of seeds is not more than what is necessary to create two bags for multiplication a regeneration is started to create a new generation of the base sample (2) in case the number of seeds is too low to create a (sub-)crop specific number of bags for distribution to the users (usually four) a regeneration is started to create more seeds for distribution (a user-regeneration). The number of seeds required for the regeneration or user bags is (sub-)crop specific and specified in the ISO Quality Management System of CGN.

3.1.3.A Seed Storage Conditions (for the different types of collections, i.e. short/medium- or long-term storage)

SC1 Please provide details on temperature and relative humidity conditions of your storage and drying rooms. In case they vary from room to room, please provide details for each.

Seeds are dried to an equilibrium in a room with a temperature of 15°C and a RH of 15%. After packing and sealing the seeds under light vacuum in laminated aluminium bags, the dry seeds are stored at -20°C.

SC2 Provide details on the type of containers and the packaging procedures (and the corresponding equipment, if any) that you use.

After drying the seeds are packed in laminated aluminium bags that consist of an inner layer of 75 µm polyethylene (for the sealing), a middle layer of 12 µm aluminium and an outer layer of 12 µm polyester.

Depending on the purpose of the bag (distribution, germination, multiplication/safety duplication, or rest container) and the crop (lettuce has small, faba bean has large bags) four different sizes of bags.

In case of sharp seeded species (spinach) an extra cardboard inner layer is inserted in the bag to avoid piercing of the bag.

The bags are sealed under light vacuum with an industrial sealing device: air is extracted to a level where possible leaks in the bag or seal can be detected, but not that far that the seeds would damage the bags.

SC3 What is the range of seed moisture contents (smc) of your stored seeds of different species; what measures do you apply to keep and/or monitor the (low) moisture level? Do you treat different species differently?

Seeds are dried in at least eight weeks to an equilibrium in a room with a temperature of 15°C and a RH of 15%. The equilibrium MC that can be reached at 15% RH of the environment depends on the species, and varies between 3-7%. All species are treated the same way.

SC4 Provide data on the total storage capacity (number of containers, number of accessions) and an estimated percentage to which extent this capacity has been filled.

CGN uses two freezing rooms at -20°C with fixed shelves and a total surface of 80m², and ca. 250m³ contents, and one cool room at +4°C with 33m² surface floor. In the cooling room numerous household freezers are operated at -20°C.

The storage capacity is largely used, probably at 90%.

Plans for a new building, with double drying and storage capacity have been formulated and are under consideration.

SC5 Please include any other aspects regarding storage conditions at your genebank that you regard as important (e.g. anticipated lifespan of freezing and drying equipment and related prudent financial management).

None.

3.2 Maintaining Genetic Integrity

Maintaining the genetic integrity of an accession can be achieved by minimizing genetic drift which may occur predominantly during the process of regeneration, due to too small numbers of individuals being planted, sub-optimal pollination and/or the introgression of alleles from other accessions or commercial crops or crop wild relatives. The following aspects are important and for achieving the objectives of maintaining genetic integrity and should be briefly described. Please note that a distinction should be made between seed numbers for an accession and seed numbers for sub-samples per accession. The latter only applies if the seeds of a given accession are being stored and distributed as sub-samples. As genetically modified materials get more widely distributed and as it might have specific (legal, technical, administrative) requirements a separate box on this type of material is included.

For in vitro cultured and cryopreserved material, which are normally maintained as clones, genetic stability is as important as genetic integrity of the seed-stored material.

Navigation on Maintaining Genetic Integrity section

Seed - If applicable, please complete the section on Genetic Integrity for the activities related to seed genebanks (i.e. boxes 3.2.1.A - 3.2.5.A)

In vitro cultures - If applicable, please complete the section on Genetic Integrity for the activities related to in vitro culture (i.e. boxes 3.2.1.B - 3.2.3.B)

Cryopreservation - If applicable, please complete the section on Genetic Integrity for the activities related to cryopreserved collections (i.e. boxes 3.2.1.C - 3.2.3.C)

Field genebanks - If applicable, please complete the section on Genetic Integrity for the activities related to field genebanks (i.e. boxes 3.2.1.D - 3.2.3.D)

As CGN only has only very few accessions not maintained as seeds (apple and cabbage, a total of < 250 accession), this Operational Manual will only cover the seed storage facilities and procedures.

3.2.1.A Seed Containers and Sample Size

SCSS1 Do you document the initial number of seeds of individual accessions (either as received from collecting missions or through exchange)?

The initial number of received seeds, prior to the first multiplication, is not recorded in the documentation system Genis.

SCSS2 Please describe what kind of containers (and equipment) you use, the procedure you follow with respect to sub-sampling, seed numbers per container, etc.

As indicated in SC2, the seeds are packed in laminated aluminium bags that consist of an inner layer of 75 µm polyethylene (for the sealing), a middle layer of 12 µm aluminium and an outer layer of 12 µm polyester.

The amount of seeds is counted per bag with an automated seed counter. The remaining seeds, stored in the 'rest bag' are weighed.

The number of seeds per bag differs per type of bag and per species, the details are given in the ISO Quality System of CGN, but in general the bags for germination testing contain 100 seeds, those for regeneration/safety back-up contain 50-4000, and for distribution to the user between 25 and 300 seeds. The actual numbers are determined on the basis of criteria listed in paragraph SPP5 below.

SCSS3 What is the number of seeds that you use as the minimum threshold per accession? Are these seed numbers of a given accession based on genetic parameters (such as reproduction biology; heterogeneous samples)? Please provide URL of your protocols if these are on-line available

Generally a minimum of four bags for germination, four for regeneration and four to ten for distribution to the user are required for each accession before it can be accessed. Depending on the species, this amounts to 750 (fruit vegetables) to 14,200 seeds (flax) per accession. Details are given in the ISO Quality Management System of CGN.

SCSS4 Please provide details on other aspects that are important in this context.

None.

3.2.2.A Pollination Control

PC1 Please describe the regeneration procedures that you follow for self- and outbreeding species.

Please include in your description the following aspects:

- Any control measures to minimize or avoid cross pollination between accessions;
- The use of pollination cages for insect pollinated species;
- The use of specific pollinators for insect pollinated species;
- Strategies to ensure that males and females participate equally in the reproduction).
- Strategies to avoid any genetic drift (minimum number of plants, minimum number of plants at flowering stage before pollinators introduction, similar quantity of seeds harvested from each plant, etc.

The regeneration protocols differ strongly per crop or species,. In this context only some general principles will be described. For the details please refer to the ISO Quality Management System of CGN.

Cross pollination between cross pollinating species is avoided in different ways, depending on the species: spatial by isolating in a high crop or physical in separate greenhouse compartments. Some crops require insects for pollination.

The minimum number of plants that have to contribute to the next generation differs per crop based on the expected heterogeneity. Only in a few crops the contribution of the male and female parent is regulated (maize with chain pollination and potato with the separate collecting of pollen).

A substantial proportion of the regeneration performed by CGN is done by collaborating breeding companies, always on the basis of the CGN regeneration protocols (part of the ISO Quality Management System of CGN).

PC2 - Provide any other relevant information on procedures that you apply to control pollination of your germplasm.

None.

3.2.3.A Regeneration Environment and Procedures

RE1 Describe the regeneration environment and conditions that you apply. If applicable, you might want to distinguish between different types of germplasm (e.g. wild relatives, landraces, modern varieties, breeding material, genetic stocks, etc.).

Consider the following aspects:

- a. In how far are the environmental conditions of the current regeneration of individual germplasm accessions comparable to the environmental conditions that existed at the original collecting or breeding site?;
- b. Do you use controlled environments?;
- c. Do you collaborate with other genebanks in Europe?;
- d. others.

Since almost all regeneration is done in greenhouses, the environment for regeneration is usually controlled. Also isolation cages are being used. In specific cases the regenerations are done abroad, like short-day onions that are multiplied in Australia. This usually has to do with the inability of the accessions concerned to flower and produce seed under Dutch conditions.

Collaboration with other genebanks in Europe is currently being set up for the regeneration. Furthermore, there is extensive collaboration with Dutch breeding companies, who sometimes organise their regeneration for CGN abroad.

RE2 Please include any other relevant points on regeneration environment.

None.

3.2.4.A Seed Processing Procedures

SPP1 Describe the protocol(s) that you use for threshing and seed cleaning. .

All the harvested material is threshed and cleaned. Care is taken that the accessions are identified in a clear way. The field number given before sowing is kept with the sample until the end of the cleaning process. Any selection during the seed cleaning is avoided as far as possible. After cleaning the used equipment is cleaned to avoid mixing up the seeds.

Fruit vegetables: seed treatment after harvest; after 2-7 days the seeds are removed from the fruits. Seeds of potato, eggplant and pepper are extracted with water. Seeds of cucumber, melon and tomato are extracted with HCL (cucumber and melon 2% and tomato 1%)

SPP2 Describe the protocol(s) that you use for seed drying, including whether you use different drying procedures for different types of species.

Seeds are at least eight weeks to an equilibrium in a room with a temperature of 15°C and a RH of 15%. This procedure is applied to all species, the only variable is the duration that the seeds are dried.

SPP3 Please describe how you keep the time between harvesting and the actual (long-term) storage of seeds as short as possible.

Seeds need to be threshed and cleaned and send to CGN as soon as possible but ultimately 6 months after harvesting. After at least eight weeks of drying in the CGN drying room the seeds are temporarily stored at -20°C. After receiving the germination results from the accessions which meet the CGN standards are repacked for permanent seed storage.

SPP4 Please describe how and where you store (in a temporary manner) newly harvested seeds.

Please provide details on the temperature and relative humidity of the storage room/space; what type of containers do you use, if any.

After the harvest the seeds of most crops (not the fruit vegetables) are dried in a drying cabinet for some days. The temperature is kept below 27°C. After drying, before threshing and cleaning, the seeds are stored at 15°C and 30% RH.

SPP5 Describe the criteria you use to decide on seed quantity per accession for the long-term storage.

The criteria to decide the number of seeds to store, that resulted from the actual protocols for seed storage, are based on the need (1) to properly back-up and, in time, regenerate the sample, (2) to monitor the viability over time, (3) to distribute the sample on request to users. Since usually also one or more bags with remaining seeds is stored, in cases where seed production is not an issue, the number of stored seeds will exceed the minimum number needed to meet these needs. However, for the species with a complex seed production or with very large seeds, the decisions regarding the minimum number of seeds can be influenced by pragmatic consideration.

3.2.5.A Genetically Modified Material

GMM1 In case you treat GMO material differently from “normal germplasm”, please provide here the details for each of the deviating procedures (and equipment).

So far, CGN has not accessed any GMO's in its collections. Furthermore, the ‘Draft guiding principles for the development of future harvest centres, policies to address the possibility of unintentional presence of transgenes in *ex situ* collections’, or the next versions of these guidelines, will be followed.

GMM2 Describe the policy and procedures (if any) in your genebank, related to ensuring that distributed samples are not containing GMOs.

As indicated, so far CGN has not accessed any GMO's.

3.3 Ensuring Availability

An important objective of conservation efforts is to facilitate the effective utilization of germplasm accessions by researchers, breeders and farmers. Thus, ensuring the ready availability of stored germplasm is an important principle. It refers to the ability of genebanks to supply and distribute the stored germplasm, together with any associated information, in an adequate way to users. Aspects that can affect the availability include: (a) policies, (b) seed stock, (c) health status of accessions, and (d) distribution quantity. Although most of the questions are not relevant in the ECPGR/AEGIS context, it was decided to keep the questions and to allow for a comprehensive genebank manual that can be used “globally”.

Navigation on Ensuring Availability

Seed - If applicable, please complete the section on Ensuring Availability for the activities related to seed genebanks (i.e. boxes 3.3.1.A - 3.3.4.A)

In vitro cultures - If applicable, please complete the section on Ensuring Availability for the activities related to in vitro culture (i.e. boxes 3.3.1.B - 3.3.4.B)

Cryopreservation - If applicable, please complete the section on Ensuring Availability for the activities related to cryopreserved collections (i.e. boxes 3.3.1.C - 3.3.4.C)

Field genebanks - If applicable, please complete the section on Ensuring Availability for the activities related to field genebanks (i.e. boxes 3.3.1.D - 3.3.4.D)

Seed Collections

3.3.1.A Ensuring Availability of Germplasm - Policy Aspects

AGP1 Describe the germplasm distribution policy that you follow at your genebank.

You might want to consider in your response the following aspects:

- a) crop/species specificity;
- b) whether or not sufficient seed stock is available; who the requestor is;
- c) what the purpose of the germplasm request is;
- d) any restrictive conditions and/or

- e) the total amount of accessions sent per request for distribution of germplasm;
- f) use of a formal agreement to distribute the germplasm.

Any user can request germplasm from CGN via the website. However, CGN only guarantees access under the terms and conditions of the SMTA of the International Treaty. Requests for material for other purposes than the ones specified in the ITPGRFA, such as for a hobby garden or for industrial purposes, are dealt with on a case-by-case basis. Individual requests for more than 50 samples will be investigated by CGN, and can be followed up with the requestor to assure all material is needed.

AGP2 Do you have as part of your service rendering policy aspects such as a "maximum time" between receiving a germplasm request and distribution of the germplasm?

Since the processing of seed requests, including signing of the SMTA, is largely automated, the vast majority of requests are processed immediately. On the CGN website it is indicated that three weeks is the maximum period of attending to a request. If, due to whatever reason, this is not realized and the requestor inquires about the situation, an internal complaint is made that is treated according to the procedures as defined in the ISO Quality Management System: causes are traced, if possible remedies are implemented, and all actions are documented (usually the causes are outside the reach of CGN, having to do with import officials, local postal services, etc.).

AGP3 Describe how you treat "related information" about the requested accessions that you make available to the requestor, i.e. provide details on the typical information you send out with the germplasm.

As all information is readily available via the internet, the information supplied as hard copy with the seeds is limited to the basic identification (number, name, species) and an acknowledgement card and when relevant, instruction about how to handle the seed (sowing instructions, disinfection advice and dormancy breaking methods).

3.3.2.A Ensuring Availability of Germplasm - Seed/Germplasm Stock Aspects

AGSS1 Please provide details on the minimum/maximum amount of seed, plant, in vitro samples that you distribute (where relevant, differentiated by species groups, i.e. self-pollinating, cross-pollinating and/or whether an accession is homo- or heterogeneous).

crop	type	pollination	seeds for user-sample
Lettuce	cultivated	self	100
	wild		50
Spinach	cultivated	cross	200
	wild		100
<i>Allium</i>	cultivated	cross	300
	wild		100
Crucifers	cultivated	cross	300
	wild		100
Potato	landraces/wild	cross/self	50
Small grains	cultivated	cross/self	100
	cultivated/landraces		50
	wild		25
Fruit vegetables	cultivated/wild	cross/self	25
Pulses	cultivated	cross/self	40
Forages	cultivated/wild	cross	200
Meadow grass	cultivated/wild	apomict	100
Flax	cultivated	self	300
	wild		50

AGSS2 Describe how you store the seeds/etc. of a given accession with respect to the use of single or multiple bags or containers per accession.

All the seeds are pre-packed and stored as user bags at -20°C. CGN stores four to ten user bags per accession depending on the (sub-)crop of the accession.

AGSS3 Describe how you manage the availability of adequate seed/etc. stock per accession, including the use of an absolute lower minimum of seeds per accession as the threshold to decide to regenerate.

Once a year an overview is made of the all the accessions which do not meet the CGN requirement of having sufficient seed to make, depending on the crop, four to ten user bags. Accession with too few seeds are earmarked to be regenerated as soon as possible.

AGSS4 Provide here information on any other aspects that are relevant to manage seed/etc. stocks.

None.

3.3.3.A Ensuring Availability of Germplasm - Health Aspects

AGHA1 Describe how you store seed/other germplasm with respect to germplasm health considerations, including whether you have a "policy" of storing only "disease free" (as far as you can see or determine) accessions, at least for the quarantine pests and diseases.

CGN is storing only seeds free of quarantine pest and diseases, as far as known. In contact with the Netherlands Food and Consumer Product Safety Authority (NVWA), all required tests are done to assure the material is free from any quarantine pests and diseases. In case CGN is aware of the (possible) presence of non-regulated diseases in the seeds, the user of the material is informed.

AGHA2 Describe how you follow plant quarantine rules and regulations when exporting germplasm abroad (especially to countries at another continent).

During regeneration plants are inspected by the Netherlands Food and Consumer Product Safety Authority (NVWA) or a delegated organisation for quarantine diseases or another certified organization.

AGHA3 Describe if and how you distribute germplasm accompanied by a phytosanitary certificate or a "plant passport".

The guidelines of the Netherlands Food and Consumer Product Safety Authority (NVWA) are followed. When required by regulations in the country of the the requestor, CGN sends the seeds with a phytosanitary certificate and/or plant passport.

AGHA4 Provide any other relevant information on procedures that you follow with respect to germplasm health aspects.

None.

3.3.4.A Germplasm Supply

GS1 Describe the policy of your genebank with respect to the sample size that you use for distribution purposes, including whether you differentiate between germplasm from self- or outbreeding species, heterogeneous accessions, and possibly other aspects.

See information at 3.3.2.

GS2 As GS1 above, but in case your germplasm samples do not possess the minimum viability, would you increase the number of seeds?

No, if there are complains about the viability of the seeds, we retest the seed quality and send new, and if possible more seeds. The viability of the user samples is not routinely checked. Information/feedback from our users is appreciated.

GS3 Please provide information on any other aspects related to seed supply.

None.

4 Providing Information

The lack of adequate information on a given accession may well decrease the value of that accession to the user. The information on individual accessions should be as complete as possible in order to facilitate the identification of duplicates and/or to select accessions with desirable characteristics. A genebank should have a documentation system in place that allows to optimize management of the collections as well as to provide access to information about the collection to users.

4.1 Genebank Documentation System

GD1 Please provide details on the technical aspects of the genebank information management system(s) that you use.

- a) On which software is the system based (i.e. Oracle, Fox Pro, MS Access, MS excel, MS Word, other?).
- b) In case you use a manual information management system, please provide details.
- c) In case your "internal" database(s) is/are different from the publicly available database(s), please provide details on both,
- d) Describe which activities of the genebank are covered by the system.

CGN uses a state of the art Oracle based documentation system called Genis for documenting its accessions. This system is described for users and colleague genebanks, in the Genis Data Dictionary.

The publicly accessible database is a mirror of the Oracle database, updated at least every two months, stored in an SQL .net environment. It contains most of the internal information, with an exception of the distribution data, but it also includes 'synthesized C&E data' that can be searched online. All non-synthesized C&E data are available for download in Excel spreadsheets. The public system includes additional functionalities such as the core selector and the online ordering system.

Logistics of the operations of seed regeneration and characterization are not covered by Genis, although there is a flag in Genis indicating that an accession is 'in the process of being regenerated'.

GD2 Provide details on which types of data you handle in your documentation system, e.g. passport data, characterization & evaluation data, cultivar data, material distribution etc.

Genis contains all accession related data: passport data, C&E data, viability data, utilization data, and seed storage logistic information.

GD3 In case your internal database(s) is/are different from the publicly available database(s), please provide details on both.

The differences are indicated under GD1.

GD4 Describe in which form you send accession specific data (e.g. as hard copy, electronically - if the latter, please specify (in plain text) which file format, i.e. Excel, Access, others is used).

All passport and C&E data can be downloaded in Excel spreadsheets from the CGN website. For the passport data the MCPD format is used. All spreadsheets are updated every two months, and contain detailed information about the format and codes used.

GD5 Provide information on how technical support for development and maintenance of the documentation system is arranged

The management of the hardware of Genis and the public interface is outsourced to the Facility Services of WUR. The same applies to the application management of the public interfaces. For the Oracle application, the technical application management is outsourced to an external IT firm, the user support and functional management is handled internally by CGN, this includes data entry, and the generation of custom made output.

GD6 Describe your genebank policy with respect to backing-up of the database contents, including with which frequency?

The back-up procedures of the hosting institute WUR is followed. These include daily incremental back-ups, and daily copies of the virtual machines.

GD7 Provide any other information on your information management system that is not covered in one of the above questions.

None – at the moment.

4.2 Information Exchange

IE1 Please describe how you make your passport data available to users (i.e. as hard copy; via the internet; other?).

Most passport data are on-line searchable and all passport data are downloadable in MCPD format in Excel files via the CGN website.

IE2 Please indicate if your data is available as machine to machine web-services. In case it is, describe

- a) what types of data (passport data, characterization & evaluation data etc) and
- b) which web-service interfaces are available (i.e. GBIF IPT, BioCase, TapirLink).

The passport data of the CGN collections are accessible via web-service interfaces. A BioCase wrapper has been installed several years ago, and the GBIF-IPT has been installed and is operational since early 2012. Work is on-going to make the C&E data also available via the IPT.

IE3 Please indicate if your data is published to EURISCO. Describe which data is published to EURISCO and at which intervals.

All passport data in Genis is uploaded every two months to EURISCO.

IE4 Please provide any other information on information exchange that is important for others to know.

All data (except details about the identity of the recipients) is available via the web search interface, via downloadable Excel spreadsheets and/or via web services. The data is also accessible via EURISCO, GBIF and several central crop databases.

IE5 Describe the kind of information you distribute together with the germplasm to persons that request germplasm?

Please consider the following data types: Passport, Characterization; Evaluation, and/or Germplasm management data (e.g. viability percentage; protocols followed for routine operations; etc..

As all information is readily available via the internet, the information supplied as hard copy with the seeds is limited to the basic identification (number, name, species), and when relevant, instruction about how to handle the seed.