Estonian Crop Research Institute

Operational genebank manual of the Estonian Crop Research Institute

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0. Date of compilation

Day/month/year: 03/10/2017

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 third 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.

Box 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 *ex situ* Genebank is organized as a department of the Estonian Crop Research Institute (ECRI) which is a research institution under the administration of the Ministry of Rural Affairs. The mandate of the Genebank is defined in the National Programme 'Collection and Conservation of Plant Genetic Resources for Food and Agriculture' coordinated by the National Commission on PGRFA.

The Minister of Rural Affairs has nominated the Genebank as the coordinator of long-term *ex situ* preservation in Estonia.

The mandate of the Genebank is collection, conservation, evaluation, characterization, documentation and utilization of plant genetic resources of agricultural crops of Estonian origin, thus providing an initial source for the future use of the genetic variation by Estonian plant breeders and researchers.

Seed-propagated material of agricultural crops (cereals, forage legumes and grasses, grain legumes, oil crops, vegetables) is conserved in the Genebank.

The *in vitro* culture collection is maintained at the Department of Plant Biotechnology.

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 genepools fall under these agreements?*

No.
With NordGen regarding safety-duplicates.

GA3 – In case your genebank has a germplasm acquisition policy, what does the policy entail?
Please specify which crops or which geographic area, if applicable.
The overall mandate of the Genebank is to conserve the material of Estonian origin or adapted to Estonian climatic conditions. Besides, forage grasses and legumes collected during the joint expeditions from the Baltic-Nordic region, as well as advanced breeding material of breeders' collections of the Estonian CRI not actively used in breeding programmes, are preserved in the Genebank.

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.)?

- Repatriated material from other genebanks – accepting donor's information.
- Material collected from natural habitats – breeders and representative of the Genebank attend the collecting missions and conduct evaluation of collected accessions.
- Germplasm material from breeders – tested in field nurseries and assessed in laboratory.
- Material collected from gardeners, farmers – relying on their information

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).*

Visual control of seeds; seed control and testing (estimation of viability, weight, moisture content).

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).*

The SMTA is implemented at the ECRI since March 2011. Initially, only material of Annex1 species was distributed with an SMTA (and non-Annex1 crops with a different agreement). Since January 2013, the SMTA is implemented for all material distributed. Information about signed SMTAs is recorded in spreadsheet files at the Genebank and reported when required.

Box 1.2. Germplasm Collecting

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,*

The collecting missions are planned to the biodiversity hotspots in Estonia, areas with high soil fertility and diversity of soil types. We target the areas which have not been recultivated for at least three decades, e.g. islets, former restricted military areas on the coastal line.

b) *the criteria you use for priority setting;*

Samples of traditionally cultivated forage and herbage legume species, rarely minor species are collected. Among the latter, turfgrass species are preferred over the forage grasses.

c) *the actual strategy followed in sampling material from farmers' fields, from nature, etc.; and*

Primarily, we sample for grasslands that have not been re-seeded for at least three decades and that are or have been subjected to grazing or cutting. This is based on the assumption that the survived plants have undergone selection for tolerance to frequent defoliation and wear, being thus more valuable from the breeding perspective.

The wild material that has evolved without any human intervention is also collected.

d) *how your germplasm acquisition policy underpins the mission*).

Accessions collected from nature form a vital source for plant breeding. Collected samples are characterised and evaluated and according to the results, the decision is made whether they will passively be stored or actively used (breeding, research projects).

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

Joint collecting missions have been carried out in Baltic countries in collaboration with Latvian and Lithuanian colleagues.

Supervision of the seed collecting activities in Estonia of IHAR (Poland) genebank collection holders.

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:

Box 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);*
Black-box. Agreement with the Nordic Genetic Resource Center (NordGen). Agreement for conserving safety-duplicates in Svalbard will be signed in 2017.

b) *the location(s) where you store your safety-duplicates (country; genebank);*
Sweden, NordGen.

c) *whether or not you are using a formal agreement with the genebank(s) that store your duplicates?*
Yes, Memorandum of Understanding between the Estonian Crop Research Institute and NordGen signed in 1997.

d) *whether the safety-duplicates are stored under conditions comparable to your own? Please provide details;*
Safety-duplicates are stored under the same conditions: seeds packed in laminated aluminium foil bags, stored in freezers at a temperature of -18^{o}C

e) *do you maintain safety-duplicates from other genebanks at your genebank? If so, do you know any details of that material?*
No.

SD2 – Do you have a safety duplication policy? If so, please provide essential details.
According to the mandate of the Genebank, only accessions of Estonian origin are stored at NordGen as safety-duplicates.

Box 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).
No danger of earthquake or heavy storm. There is a very low probability of short-term flooding, which shall not negatively affect the collections. Evacuation of the collections shall be carried out in case of serious disaster.

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*)

Alarm system by security service; temperature monitoring system in freezers with alarm to the mobile phone of staff, standby generator.

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

Box 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*).

Standby power system (generator) has been obtained.

SE2 – Describe how you monitor temperature and relative humidity in your cold stores and drying room?
Drying room: temperature and humidity are measured and recorded regularly by the monitoring device.
Freezers: the electronic temperature monitoring system automatically delivers an alarm message to the mobile phone of the staff.

Box 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*.

Funding is stable. The annual budget is allocated for the Genebank in the National Programme on PGRFA for a seven-year period. Funding is provided annually by the Ministry of Rural Affairs.

IPS2 – Describe how you secure adequate staffing of your genebank.
Staff is employed under permanent labour contracts.

Box 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 genebank has a plan for the evacuation of collections in emergency or contingency situations.

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.
Staff is informed how to act adequately in emergency situations.

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.

N.B. Sections on Cryopreserved collections and Field genebanks are not applicable for ECRI, therefore these sections have been removed from the document.

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.

A. Seed Collections

Box 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.).
Regeneration is carried out by experienced staff of breeding departments of relevant crops of the institute. In general, regeneration procedures are carried out in accordance with IPGRI descriptor lists, lists created by ECPGR working groups and descriptor lists created by other genebanks. Since the Genebank is relatively young (the first accessions are from 1999), the need for regeneration of the complete collection has not yet occurred.
Cleaning and drying of seeds is carried out promptly after harvesting.

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

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

Box 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:

a) *frequency of testing;*
b) *sampling method applied;*
c) *any thresholds that you use;*
d) *whether you apply different procedures for crops/species with erratic initial viability or irregular viability lifespan;*
e) *etc*.

The main procedures about seed viability testing are described in the Genebank protocol in Estonian language. ISTA standards are adopted and followed by the Genebank.
A germination test of each sample is conducted before storage and repeated after 5-15 years depending on species; also while delivering material for characterization and evaluation.

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 germination tests are recorded in the documentation system SESTO. Using search engine, once a year accessions are selected for regeneration in accordance with the probability of viability decrease.
In accordance with species-specific behaviour and empirical knowledge on the decrease of germination rates, germination tests are performed.

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.*
Regeneration takes place if germination rate is below 60-75% (depending on species).
No specific procedures are in use for regeneration of accessions for which the amount of seeds has decreased to a low level. However, this criterion will be added to the Genebank protocol.

Box 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.
Pre-drying to the moisture content 12-14%
Drying room 18°C, 15-20% RH
Long-term storage in freezers -18°C

SC2 – Provide details on the type of containers and the packaging procedures (and the corresponding equipment, if any) that you use.
Laminated aluminium foil bags sealed with industrial sealing device. Bags are of two different sizes depending on seed size and of the purpose (bulk bag and distribution 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?
4-8% depending on species
Electronic Moisture Analyzer is being used to determine moisture content.

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.
Nine upright deep freezers (temperature -18^{o}C) for ~3000 accessions; two freezers are reserved for emergency cases. Upon the need new freezers are purchased and installed.

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.

B. *In vitro* Culture Collections

Box 3.1.1.B. Initial viability

IV1 – Describe the procedures or practices that you have in place to ensure the highest possible initial viability of your plant material, in particular during culture of donor plants (e.g. cultivation practices [field, greenhouse], phytosanitary pre-treatments, like use of pesticides).
All initial material is tested for virus infection. When necessary, initial material is subjected to thermotherapy. This is followed by cultivation of meristem tips. If the first thermotherapy cycle is unsuccessful, then the second step is repeated. Disease-free meristem clones are tested for quality and yield and for the best characteristics.

IV2 – Describe procedures of explant isolation (organ source in the plant, manipulations) and sterilization (chemical and handling) of the explants.
After thermotherapy the tips of plants are sterilized in hypochlorite (5%) sodium for 30 sec, 2x washed with distilled water for 30 sec each. After that, the selected meristems are cut and cultivated on special regeneration medium worked out in our institute. The regeneration of plants from the meristem culture is done in special growing room with following conditions: light/dark period 16/8 hours, temperature 23^{o}C/18^{o}C, relative humidity 70%.

IV3 – Please provide any other information on procedures that you follow to ensure highest possible initial viability.
As source for explants, only healthy material is used. The detection of six common viruses is done by ELISA or PCR method according to EPPO protocol.

Box 3.1.2.B. Viability Monitoring

VM1 – Describe the routine *in vitro* viability monitoring system that you use.
The monitoring system should include the following aspects:

a) *regular control of contamination events,*
b) *control of hyper-hydricity,*
c) *control of health state (if different from a above),*
d) *etc*.

In vitro viability visual monitoring is performed regularly during transfer from one subculture to the other. Furthermore, visual control checks are conducted in the warm culture rooms every second week and in the cold room weekly. These checks cover visual controls on fungal or bacterial contamination. Hyperhydricity is excluded during transfers between the subcultures.

VM2 – Describe the information “system” (i.e. an “expert 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.
No specific system implemented. Experience and skill of the technical staff is crucial for special decision.

VM3 – Please provide information on non-specific thresholds that you might use for vigour of *in vitro* cultures (i.e. multiplication rates, loss by weak growth) and for the amount of culture vessels (tubes, jars) left of an accession to initiate additional multiplication measures.
Decisions on the cultivation temperature and multiplication regimes are taken according to the personal experience of the responsible staff members.
Decisions are made accession-specific and will be recorded in the running laboratory protocols. They cannot be published as standardized recommendations.

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

SC1 – Please provide details on light, temperature and relative humidity conditions of your culture and storage rooms, as applicable. In case they vary from room to room, please provide details for each.
In vitro culture growth room with controlled parameters: 16 h temperature 22 to 24°C, light intensity 30-40 µmol.m-2.s-1, humidity 70%; 8 h temperature 18°C, no light. The medium-term storage room: *in vitro* plants: 4°C, light (10 µmol.m-2.s-1), 70% humidity

SC2 – Provide details on the type of cultivation vessels (tubes, jars plastic vessels etc.) and the transfer procedures (including the corresponding equipment, if any) that you use.
In vitro cultures are maintained in glass tubes with cotton cap. Potato cultures are kept in test tubes, fruits and berries are kept in Erlenmeyer flasks or glass jars.

SC3 – Please include any other aspects regarding *in vitro* culture and storage conditions at your genebank that you regard as important.
No other information available.

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.

A. Seed Collections

Box 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)?
Sample weight is recorded for each accession.

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.
Laminated aluminium foil bags. Depending upon species, 50-200 seeds per distribution bag (each accession 5-10 bags); at least 1500/3000 (self-pollinating/cross-pollinating species, respectively) seeds per bulk bag; 500-1000 seeds per safety-duplication bag.

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 available on-line.
50-200 seeds in distribution bags; at least 1500/3000 (self-pollinating/cross-pollinating species, respectively) seeds in bulk bag.
The Genebank protocol is available in Estonian language.

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

Box 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:

a. *any control measures to minimize or avoid cross pollination between accessions;*
b. *the use of pollination cages for insect pollinated species;*
c. *the use of specific pollinators for insect pollinated species;*
d. *strategies to ensure that males and females participate equally in the reproduction*).
e. *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.)*

Regeneration of cross-pollinated species is carried out depending on species in isolation cabins or under isolation bags.
Isolation in space is used to significantly reduce eventuality of cross-pollination.
Number of plants of cross-pollinating crops has to be as large as possible to avoid genetic drift.

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

Box 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?;*

Regeneration is carried out in the fields and greenhouse of the Estonian Crop Research Institute. The majority of accessions are adapted to Estonian climatic conditions. Thus, environmental conditions of regeneration are favourable to a majority of accessions.

b. *do you use controlled environments?;*

In the greenhouse, air temperature, day length and humidity are controlled to create most adequate conditions for growing.

c. *do you collaborate with other genebanks in Europe?*

No.

d. *others.*

RE2 – Please include any other relevant points on regeneration environment.

Box 3.2.4.A. Seed Processing Procedures

SPP1 – Describe the protocol(s) that you use for threshing and seed cleaning.
Threshing and cleaning by machines and manually are conducted by breeding departments, the quality of procedures is estimated visually. The main goal is to retain purity of seed samples.

SPP2 – Describe the protocol(s) that you use for seed drying, including whether you use different drying procedures for different types of species.
Pre-drying of harvested material in fabric bags at 18°C, 20%RH.
After cleaning, seeds are packed into paper bags and final drying is carried out in the drying room (18°C, 15-20%RH) for four to eight weeks until the required seed moisture content (4-8%) is achieved.

SPP3 – Please describe how you keep the time between harvesting and the actual (long-term) storage of seeds as short as possible.
The time between harvesting and storage shall be as short as possible. Seeds are placed for drying in the genebank drying facility promptly after pre-drying and cleaning at crop departments.

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.
In case packing is not immediately possible after drying, seeds are temporarily stored in an airtight drying room with a temperature of about 18°C). Desiccators are used to reduce air humidity.

SPP5 – Describe the criteria you use to decide on seed quantity per accession for the long-term storage.
At least 1500 seeds of self-pollinating and 3000 seeds of cross-pollinating species, besides 5-10 distribution bags with 50-200 seeds per each accession. If seed amount is lower, all available seeds are conserved.

Box 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).
There are no GMOs in the Genebank.

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

B. *In vitro* Culture Collections

Box 3.2.1.B. *In vitro* Culture Vessels and Sample Size

SCSS1 – Indicate if you document the initial number of explants of individual accessions when culture is initiated (from one or from more clonal donor plants).
Each step of the working procedure, including the number of plants, used medium content, conditions, is documented.

SCSS2 – Please describe in general terms the type of culture vessels (as far not already done in section SC2 in Box 3.1.3.B), media and phytohormones you use as well as the procedures you follow with respect to cutting technique, callus exclusion, etc.

SCSS3 – Please indicate whether or not you use a minimum number of *in vitro* plantlets per accession.
Potato collections: minimum 5 accessions per variety are preserved. In the collection of fruits and berries, up to 30-40 micro-plants in 3-5 vessels are preserved.

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

Box 3.2.2.B. *In vitro* Culture Procedures

SPP1 – Describe the numbers of sub-clones you may cultivate per accession (assuming that this is not crop-specific).
The number of sub-clones is crop-specific.

SPP2 – Describe the sub-culture duration (if not crop-specific).
Established *in vitro* plant tissue cultures are renewed 2 to 3 times per year.

SPP3 – Describe the criteria you use to decide on *in vitro* plant quality (if not crop-specific).
In vitro cultures with visible growth abnormalities are eradicated.

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.

A. Seed Collections

Box 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.

One distribution bag with 50-200 seeds per accession is distributed (depending on the seed size). All requests are processed under the terms of the SMTA of the ITPGRFA.

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?

There is no special service policy applied. Material is distributed within the shortest time period, mainly depending on staff capacity.

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.

The URL link of the database or any information available is provided upon request.

Box 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).
50-200 seeds, depending on the seed amount in distribution bags. For most accessions 200 seeds are prepared in the distribution bags; *Pisum sativum*: 100 seeds, *Vicia faba*: 50 seeds.

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.
Seeds in distribution bags are pre-packed and stored at -18°C. The Genebank stores 5-10 distribution bags per accession.

AGSS3 – Describe how you manage the availability of adequate seed/other germplasm stock per accession, including the use of an absolute lower minimum of seeds per accession as the threshold to decide to regenerate.
If the number of distribution bags has decreased to less than two, another set of 5-10 bags is filled from the bulk bag, in case the amount of seed exceeds 1500/3000. Accessions with lesser amount of seeds will be regenerated.

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

Box 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.
No crop-specific tests are carried out. Yet, no seeds known to be infected with quarantine pests or diseases are preserved.

AGHA2 – Describe how you follow plant quarantine rules and regulations when exporting germplasm abroad (especially to countries at another continent).
Phytosanitary certificate is provided on seed delivery outside the EU.

AGHA3 – Describe if and how you distribute germplasm accompanied by a phytosanitary certificate or a "plant passport".
Instructions of the Plant Health Department of the Agricultural Board are followed. Seeds are shipped with phytosanitary certificate.

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

Box 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.
For most accessions 200 seeds are distributed, except the crops with seeds bigger in size: *Pisum sativum*: 100 seeds, *Vicia faba*: 50 seeds.

GS2 – As GS1 above, but in case your germplasm samples do not possess the minimum viability, would you increase the number of seeds?
Yes, although no relevant procedure for such samples exists. The decision is made on a case-by–case basis.

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

B. *In vitro* Culture Collections

Box 3.3.1.B. 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: is the user informed about the option to get provided with* in vitro *cultures and whether they are available all the time of the year, are* in vitro *samples an option or the only way to get material; who the requestor is; what the purpose of the germplasm request is; any restrictive conditions and/or the total amount of accessions sent per request for distribution of germplasm; use of a formal agreement to distribute the germplasm.*
In vitro cultures are distributed on request. Distribution for research purpose has the priority. Restrictive conditions/limitations for distribution to private users. SMTA is used as formal agreement to distribute the germplasm.

AGP2 – Indicate if you have as part of your service-rendering policy aspects such as a “regular or a maximum time” between receiving a germplasm request and distribution of the germplasm?
Time period is not fixed because of maintenance cycle requirements. Potato *in vitro* cultures are propagated for research purposes upon mutual agreement and as a priority; for private users only in spring.

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.
Accession number, name, and date. Potato *in vitro* cultures are also marked with meristem number.

Box 3.3.2.B. Ensuring Availability of Germplasm – Germplasm Stock Aspects

AGSS1 – Please provide details on the maximum amount of *in vitro* samples that you distribute.
The amount of samples distributed depends on how many were ordered and according to agreement.

AGSS2 – Describe how you store the samples of a given accession with respect to the use of vessels for culture and vessels for distributions (glasses of plastic bags).
Commonly the plants are distributed in glass vessels.

AGSS3 – Describe how you manage the availability of adequate plants per accession, including the use of an absolute lowest minimum of plants per accession as the threshold to decide to regenerate.
5 samples are maintained at all times. In case of order, plants are propagated from these.

AGSS4 – Provide here information on any other aspects that are relevant to manage stocks (e.g. transfer of material through greenhouse transfer phases in case a user cannot handle *in vitro* cultures).

Box 3.3.3.B. Ensuring Availability of Germplasm – Health Aspects

AGHA1 – Describe how you store 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.
A “policy” of storing only “disease-free” accessions, at least for the quarantine diseases and pests.

AGHA2 – Describe how you follow plant quarantine rules and regulations when exporting germplasm abroad (especially to countries at another continent).
For the export of the requested material outside the EU, germplasm is accompanied by a phytosanitary certificate issued by the Phytosanitary authority (Plant Health Department of the Agricultural Board).

AGHA3 – Describe if and how you distribute germplasm accompanied by a phytosanitary certificate or a “plant passport”.
Instructions of the Phytosanitary authority are followed. The distributed germplasm is accompanied by a phytosanitary certificate.

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

Box 3.3.4.B. Germplasm Supply

GS1 – Describe the policy of your genebank with respect to the sample size that you use for distribution purposes.
The size of the sample depends on the demand of the requestor, but the amount of the material is negotiated on a case-by–case basis.

GS2 – Please provide details of your routine methodology of containers etc. that you use to distribute *in vitro* cultures.
Commonly, the plants are distributed in glass vessels.

GS3 – Please provide information on any other aspects related to *in vitro* plant supply.

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.

Box 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?).

The data of both seed and *in vitro* collections are maintained in the data management system SESTO (Postgres SQL - database, accessible for users from a web interface, using php on an Apache webserver), which is hosted by NordGen. It includes botanical determination, collection data, passport data, storage and germination data.

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.

Recording above-mentioned data; search tool for public users and Genebank staff; updating EURISCO.

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.

Passport data, taxonomy, collecting data, seed storage data (incl. safety duplication data), germination, 1000 kernel weight, regeneration data..

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

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).

Selected information with SMTA (hard copy or electronically) and web address of SESTO. Any specific required information, e.g. characterization/evaluation data is provided in a spreadsheet file.

GD5 – Provide information on how technical support for development and maintenance of the documentation system is arranged
Technical support is provided by NordGen in close cooperation with the Genebank.

GD6 – Describe your genebank policy with respect to backing-up of the database contents, including with which frequency?
An automatic data back-up system is installed. A backup data file is compiled each night, saving the latest database information. Technically it is possible to store several backup files per year.

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

Box 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?).
Passport data are searchable and downloadable from the Report tool of the database.

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).

Passport data. GBIF.

IE3 – Please indicate if your data is published to EURISCO. Describe which data is published to EURISCO and at which intervals.
All data are published to EURISCO and updated twice a year via SESTO.

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

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..
Passport data - accession number and name, botanical name, country of origin, life form and germination. Available characterization and evaluation data upon request.