



NÁRODNÉ POĽNOHOSPODÁRSKE
A POTRAVINÁRSKE CENTRUM
VÝSKUMNÝ ÚSTAV RASTLINNEJ
VÝROBY



Operational genebank manual of National Agricultural and Food Centre - Research Institute of Plant Production Piešťany, Slovak republic



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0. Date of compilation

Day/month/year: 10.08.2022

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 Research Institute of Plant Production (hereinafter RIPP) Piešťany (without legal identity) is a part of the organization National Agricultural and Food Centre with headquarters in Lužianky (hereinafter NPPC). NPPC is directly managed by the Ministry of Agriculture and Rural Development of SR.

Plant genetic resources (PGR) are kept in the Gene Bank of the Slovak Republic, located at RIPP. The Gene Bank of the Slovak Republic operates as the central genebank for generatively propagated plant species maintained in Slovak crop collections belonging to the National Programme for Conservation of Plant Genetic Resources for Food and Agriculture (NP). The National Programme was created by Act 215/2001 and is supported by the Ministry of Agriculture. Slovak Republic is the first country to accredit a law on conservation of Plant Genetic Resources (PGR) in Europe. Mandate for coordination of the National Programme was given to the Research Institute of Plant Production (RIPP). Under the coordination of RIPP, other workplaces from the entire Slovakia participated in plant

genetic research activities. PGR material maintained in the Gene Bank of SR is available according to the SMTA, International Treaty for breeding, research, and education. Further information about the Gene Bank of SR and its mandate is provided on the RIPP website (www.vurv.sk).

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?*

The Gene Bank of SR has entered into a formal agreement with the CRI Czech Genebank in Prague on mutual storage of safety duplicates, which is valid for all generatively propagated crops.

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.

Seed samples acquisition is the responsibility of collection curators as legal bodies. Cooperating crop institutions and their responsibilities can be found at <http://griss.vurv.sk/>. Every cooperating institution enters into a contract with NPPC RIPP.

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.)?

Collection holders are responsible for the verification of plant material as well as the quality of accompanying information – including taxonomy. Collection curators are specialists with long-term experience in crop genetic resources. Collection curator is responsible for the inclusion of new genetic resources into the crop collection of the National Programme.

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*
- use of a quality control system (e.g. ISO).*

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 has been used since the beginning of 2013 and it is applied to all samples delivered from the genebank. Before 2013, the national short version of Material Transfer Agreement had been used. In addition to the MTA document, samples provided to non-EU countries must be documented also in Phytosanitary Certificate. All seed sample transfers from the Gene Bank of SR storage were documented in the central information system EVIDEN and currently in the information system GRISS.

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,*
- 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.*

Collecting expeditions are organized jointly with the cooperating institutions within the National Programme. They are focused on wild crop relatives of fodder crops and grasses, local cultivars of fruit trees, vegetables, cereals and medicinal plants. Collecting expeditions follow the document "International Code of Conduct for Germplasm Collecting and Transfer" (FAO 1994). Every expedition is carried out according to a prepared plan (floristic and herbarium data, map of collecting area, the occurrence of targeted species in consultation with local specialists for botany, ecology, pomology, and environment). The optimal period for collecting specific plant reproduction material (seeds, spikelet, fruits, in frutescence, grafts, scions, rhizomes, bulbs, rootstocks, whole plants, etc.) is selected. All collected materials are documented in a collecting book and subsequently relevant electronic tables (xls, dbf format) are created. Collecting data include a collecting number, collecting data, locality, description of the site, geographical coordinates, elevation, etc. The record structure follows MCPD descriptors related to the collected material; it also includes the description of soil type, geological substrate, slope orientation, plant abundance, phenology phases, etc. Exact localization is provided by a GPS device. Samples should represent existing population variability and their size is dependent on the abundance of plants (the recommended amount for genebank storage: 4000 seeds in self-pollinated and 12000 seeds in cross-pollinated species). Grafts or other vegetative plant parts should create homogeneous samples.

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

Collecting expeditions are organized with special attention to bordering areas, in cooperation with Czech, Hungarian, Polish, and Slovenian colleagues.

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);*
- 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?*

The Gene Bank of SR has an agreement with the CRI Czech Genebank in Prague on mutual storage of safety duplicates of seed samples. A black-box arrangement and a special location in the respective genebank are used for safety duplication in genebank storage in both countries. The seed samples are prepared for storage by the collection holder and kept without any external intervention.

All AEGIS seed accessions will be gradually sent to Svalbard.

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

Safety duplication should follow the rules for the base collection: under this policy, safety duplicates should be stored for all domestic material (originated in Czechoslovakia or in the Slovak Republic). All AEGIS accessions should be safety duplicated and gradually sent also to Svalbard.

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).

The Gene Bank of SR is situated in a safe location with very low probability of flooding or earthquakes. The building and refrigerating technology were completely renovated and reconstructed in 2014.

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 building is secured against fire by a fire damper consisting of a fire door. The building of the genebank is equipped with an alarm system and a standby generator. The primary and standby cooling system is secured by an electric generator. The building is secured against burglars by an electronic security system connected to the State Police of SR.

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

Entry into the storage rooms and laboratories of the genebank is only allowed to the genebank staff. All other persons or visitors can enter the genebank storage rooms only in the presence of an authorized person.

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.*

The refrigerating technology has a complete set of back-up compressors. The back-up devices are steadily ready for utilization. Back-up generator turns on after 5 min of power supply cut-off. Regular servicing and maintenance are secured by the technical staff of the institute. All devices (compressors and evaporators) are serviced once a year by an authorized company.

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

Temperature (20°C) and relative humidity (10%) are monitored in the drying room. Two storage rooms are kept at consistent temperature: two basic storage rooms at -17 °C, two active rooms at 0 – +4°C. This environment does not require monitoring of humidity. The corridors (manipulating space) are kept at +12 – +15°C. The temperatures in the cooling and freezing boxes and other rooms are controlled by a computer and the data are archived.

For selected species – vegetables, flowers, aromatic and medicinal plants – which have been stored at 0 – + 4 °C, shorter viability after 20 years of storage (1997 – 2017) was recorded.

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.*

The Gene Bank of SR is annually supported by the Ministry of Agriculture and Rural Development, through the task entitled "Operation of the Gene Bank of the Slovak Republic".

IPS2 – Describe how you secure adequate staffing of your genebank.

Minimal staff for storage and documentation: 1 Head, 1 laboratory technician, 1 database manager. All of them have permanent work 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.

In the Gene bank, the fire prevention and safety rules valid in RIPP are applied.

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.

No contingency plan available.

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 Box 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)

The period between harvesting and storing the seeds is kept as short as possible. During this period the seeds are cleaned, dried and the initial viability is determined. Crops for which the genebank department has a responsibility within the National Programme as a curator are treated according to the Methodology for the Gene Bank of SR which was created in accordance with ISTA and AOSTA (International rules for seeds testing).

***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)

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.).

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

Dormancy is usually broken by the placement of the sample at a temperature close to 0 °C for one or two weeks. One month of storage at +5 °C is sufficient to break dormancy. In case of hard seediness (particularly in the case of forage crops), we obtain data on viability directly from the collection curator, who is recording germination and hard seediness percentage, or a scarification test is used.

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

Seed drying is provided in a drying chamber at 10% R.H. and at +20 °C. High quality seeds are recommended for genebank storage – fully ripe, pure, healthy and viable (recommended standards are recorded in the Methodology for the Gene Bank of SR). If the seed sample does not meet the standards, it is returned to the collection curator to repeated regeneration. No chemical agents are used.

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.*

In the active collection (at temperature +5°C), viability monitoring is carried out every 5 years. In the base collection (at -17°C) the recommended frequency of viability monitoring is every 10 years. A major part of the stored accessions in the base collection are cereals and legumes, and their viability after 20 years (Gene Bank of SR has been active for 20 years) is sufficient. However, the time period actually used depends highly on the initial seed lot quality. A limiting factor to regular viability check could be a low amount of seeds in the sample. In this case the responsible crop collection curator is notified about the necessity to regenerate a sample and replenish the stock in the Gene bank storage. Collection curators follow the current viability of their samples via our online database GRISS, where samples with viability below the requested level are flagged.

After regeneration of the seed samples belonging to different seeds among of the same accession, these are not mixed or replaced, all of them are kept in parallel storage.

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

For selected species – vegetables, flowers, aromatic and medicinal plants – which have been stored at 0 – + 4°C, shorter viability after 20 years of storage (1997 – 2017) was recorded. In the following years, we will verify the correlation between field and laboratory germination in order to gain knowledge for longer preservation of germination of the selected species.

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.*

Germination level lower than the recommended standard is allowed in case of materials obtained from collecting expeditions or selected species (medicinal plants), which by their nature do not reach 100% germination level. Expedition materials that do not reach the recommended germination level are regenerated; in case that the regeneration is not successful, materials are excluded from the active collection and transferred into the working collection. We are preparing a table, which will serve as our internal prescription, for germination thresholds and quantities for depositing samples in the active collection, specifically wild species.

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.

Short and medium storage at 0 - +4°C and 60% RH, long-term storage at -18°C. The drying chamber works on a regime of 10% R.H. and at +20°C.

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

Seed samples are stored in glass containers (720 ml, 370 ml or 212 ml) covered with twist cap; a small bag with coloured dried silica gel is added to each sample. When the colour of the silica gel changes, it is known that the sample is at risk.

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?

The required moisture level of stored seeds depends on the species and varies from 4% to 7%.

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.

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).

The total capacity of the storage rooms is 50 000 glass containers; this capacity is presently used at 60%.

Currently, there are nearly 25 400 accessions stored, that is more than 50% of all generatively propagated crop collections within the National Programme.

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).

Donor material must be visibly healthy. Potatoes are usually pre-cultivated in pots in greenhouse conditions. The plant is properly fertilized, and protective measures are performed to prevent diseases. Hop is usually taken from agro-chemically treated plants in field conditions.

IV2 – Describe procedures of explant isolation (organ source in the plant, manipulations) and sterilization (chemical and handling) of the explants.

Hop: Apical buds from young shoots are washed in 97% ethanol for 1 min., rinsed in sterile water, then dipped in 4% NaOCl with detergent Tween 20 for 10 min. with gentle shaking, and are finally washed (4x) in sterile distilled water. Apical meristems are isolated under a stereomicroscope and inoculated onto nutrient medium.

Potatoes: Tubers are washed in 4% NaOCl with detergent Tween 20 for 15 min. Then segments of tubers with eyes are incubated in Petri dish wetted with water supplemented with 1 mg/l GA₃ and let to germinate in the dark at 20°C. Vigorous sprouts in length appr. 3 cm are cut and sterilized in 2 % NaOCl with detergent Tween 20 for 10 min and 4x washed in sterile distilled water. Upper halves of sprouts are cut and inoculated onto nutrient medium.

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

After the sterilization procedures, it is necessary to work in the laminar airflow cabinet to prevent contamination of the explants. The work must be done quickly, because the explants are sensitive to dehydration.

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 and visual contamination of shoot cultures are monitored twice a month. Hop cultures are also checked for endogenous bacterial contamination using Bacteria Screening Medium 523. Backup vessels are used to substitute contaminated or necrotized plants. Hyper-hydricity is checked during sub-culturing and such plants are excluded.

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 regarding 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 are made according to personal experience. Additional multiplication is usually made in cases when the amount of vessels with vigorous plantlets is lower than 3, but the final decision is accession-specific.

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.

Cultivation conditions for the hop and potato shoot cultures:
16 h light, $22 \pm 2^\circ\text{C}$, light intensity $50 \mu\text{mol}/\text{m}^2\text{s}^1$ / 8 h dark, $18 \pm 2^\circ\text{C}$.

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.

Cultivation vessels:

Hop – glass baby-food jars (205 ml) with metal cup, 3 vessels + 1 backup vessel per genotype.

Potatoes – glass test tubes (ø17x160 mm) with metal cup, 4 vessels + 1 backup vessel per genotype.

Transfer procedure: Well-developed shoots are cut into nodal segment on glass Petri dish (ø12 cm, heat-sterilized) using a scalpel and forceps. Nodal segments are then transferred onto fresh autoclaved medium. After each vessel used, tools are sterilized in glass-bed sterilizer, laminar box is sterilized by denatured ethanol.

SC3 – Please include any other aspects regarding *in vitro* culture and storage conditions at your genebank that you regard as important.

All *in vitro* material is endangered by endophytes, which cannot be removed completely from the explants by sterilization. In our genebank this is particularly relevant for accessions of potato. Effect of bacteria on plant vitality is dependent on genotype and cultivation conditions. In some cases, antibiotics are used to eliminate bacteria, but the dose that would be effective for complete eradication of bacteria often reduces plant vigour and growth or causes explant necrosis.

C. Cryopreserved Collections

Box 3.1.1.C. Initial viability

IV1 – Describe the procedures or practices that you have in place to ensure the highest possible initial viability of your cryopreservation explant (source: *in vitro* pre-culture or directly from *in situ* explants), sterilization and explant isolation.

Not applicable.

IV2 – Please provide any other information on procedures that you follow to ensure highest possible initial viability (e.g. elimination of virus diseases).

Not applicable.

Box 3.1.2.C. Viability Monitoring

VM1 – Please indicate whether (and if so when and how) you perform random viability tests after the initial viability test [see also VM3 below].

Not applicable.

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

Not applicable.

VM3 – Indicate for the initial regeneration control:

- a. what is the percentage of regenerated control explants relative to the total number of explants per accession;
- b. any thresholds that you use [e.g. discard the material as not storable below a certain regeneration rate of the control];
- c. whether you apply different procedures for accessions with erratic regeneration rates of the control [e.g. increase the amount of explants stored]; and
- d. what is the threshold number of remaining explants of a given accession under which you initiate regeneration for multiplication.

Not applicable.

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

SC1 – Please provide information on the general system used for cryopreservation (liquid nitrogen or vapour phase, automatic tank filling or filling by hand). In case they vary from tank to tank, please provide details for each.

Not applicable.

SC2 – Provide details on the type of cryopreservation tanks and storage system within the tank that you use.

Not applicable.

SC3 – Do you treat different species differently?

Not applicable.

SC4 – Please include any other aspects regarding storage conditions at your genebank that you regard as important.

Not applicable.

D. Field Genebank Collections

Box 3.1.1.D. Initial viability

IV1 – Describe the procedures or practices that you have in place to ensure the highest possible quality of your planting material, in particular during the growing from donor plants (e.g. cultivation practices in the field or greenhouse], phytosanitary pre-treatments, etc.).

Garlic: In order to use healthy cloves for planting, garlic is treated by a combination of fungicide substance as protection against soil pathogens, and the state of health is monitored during the vegetative period.

IC Medicinal plants: plants are maintained in field collections mostly as permanent crops, we use international standards for plant evaluation, genetic resources are regularly monitored and controlled for the occurrence of diseases and pests.

Grapevine: All accessions of the grape field collection are cultivated using standard techniques as standard conventional vineyard technology. The soil under the plants is kept free of weeds as required, chemically and mechanically. Protection of plants against pests and diseases is carried out as required, depending on the weather and the development of diseases. Field works are carried out manually or mechanically, harvesting is done by hand. Winter cut is carried out mostly between February and April, depending on the weather. Clipped shoots are left in the vineyard between rows; they are mechanically processed by mulching. Mulching between vineyard rows is done at least three times during a season.

Fruit trees: All accessions of the fruit trees collection are cultivated using standard techniques as standard conventional orchard technology. The soil under the plants is kept free of weeds as required, chemically and mechanically. Protection of plants against pests and diseases is carried out as required, depending on the weather and the development of diseases. Field works are carried out manually or mechanically, harvesting is done by hand. Winter and spring cut is carried out mostly between March and May, depending on the weather. Clipped shoots are left in the orchard between rows; they are mechanically processed by mulching. Mulching between fruit tree rows is done at least three times during a season. After the cut, the buds are used for the production of missing plants for the next year.

IV2 – Describe any particular procedures you use (e.g. which organ of the donor plant you use to reproduce the planting material).

Garlic: compound bulbs (cloves of garlic) are used for multiplication.

Grapevine: winter cut buds are used for multiplication. They are grafted onto rootstocks.

Fruit trees: buds are used for multiplication. They are grafted onto rootstocks.

Medicinal plants: seeds, cuttings, creepers, plants from in vitro propagation are used to propagate medicinal plants.

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

Box 3.1.2.D. Viability Monitoring

VM1 - Describe the routine field genebank monitoring system that you use.

The monitoring system could include the following aspects: regular control of disease or pest contamination, other types of damages to the plants, etc.

Plant health is checked once a week, pest and disease control measures are undertaken according to good agricultural practice.

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

The same system as in VM1.

VM3 – Please provide information on non-specific thresholds that you might use for the quality of the individual plants (e.g. loss by weak growth) and for the amount of plants of an accession left in the field before additional initiating multiplication measures.

Garlic: not applicable.

Fruit trees and vine: multiplication has to be initiated if the amount of plants per accession in the field Genebank collection is lower than 3.

Medicinal plants: the minimum number of plants is 10 plants in the field collection. When plants are discarded for health conditions, the number will be increased to the minimum number.

Box 3.1.3.D. Maintenance Conditions

SC1 – Please provide details on your cultural practices (e.g. cultivation practices; pruning; irrigation; protection against animals, etc.; pest and disease management; etc. applied to your field genebank material.

Garlic: the field with the garlic is fertilized during the autumn and spring periods. Irrigation is not used as a standard, only during a period of severe drought in the spring and summer period. Cloves that are in a good condition, i.e. without mechanical damage, pests or diseases, are used for planting. The garlic planting material (cloves) is treated by a combination of fungicide substances as a protection against soils pathogens. Garlic is transplanted into the field during the autumn, usually mid-October, spaced 30 cm x 10 cm, depth 5–7 cm, depending on the size of clove. Insecticides are used as protection against pests. Weed control is carried out by hand during the entire vegetative period. Harvest time is at the end of June, beginning of July. Harvested plants are dried. After 6–8 weeks the rest of the leaves and roots are cut and garlic is prepared for planting.

Fruit trees and grapevine: The same system as IV1

Medicinal plants are grown from seed in a greenhouse. When they grow, they are planted in the fields. They are grown under drop irrigation in field conditions. The health of the plants is checked regularly, if necessary, pesticides are applied. Each species needs specific conditions for ripening and drying of seeds.

SC2 – In the case of annual or sub-perennial species that cannot over-winter in the field genebank, what measures do you take?

Not applicable.

SC3 – Please include any other aspects regarding field genebank maintenance conditions at your genebank that you regard as important.

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 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 material gets 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 Box 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)

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)?

We document the weight (mass) of seeds in grams. The thousand seed weight (TSW) is also recorded, so we can calculate the seed number. The recommended standard amount of seeds is used for each accession – minimum 4000 for self-pollinated and 12 000 for cross-pollinated species.

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.

After drying, the samples are put into glass containers with silica gel and labelled outside and inside by identification label.

- For small seeds we use 120 ml glasses
- For medium seeds we use 270 ml glasses
- For bigger seeds we use 360 ml glasses

We use a vacuum packing machine.

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 online.

The minimum number of seeds in the regular active collection is the amount of at least two sowings. The amount is based on the heterogeneity of samples and species.

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:

- 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.*

Self-pollinated species follow sowing plans, where plots are designed with respect to taxonomy – different taxonomic varieties are grown around the plots, or different species, if necessary.

Cross-pollinated species are handled in isolation cages or by space isolation. Flowers and vegetables in isolation cages are pollinated manually or by insect. Curators follow their internal protocols.

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

Any visible admixtures are removed from the plot.

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?
- b) do you use controlled environments?
- c) do you collaborate with other genebanks in Europe?
- d) others.

Most of the accessions come from central European countries, therefore the conditions for regeneration are comparable to the environmental conditions at the original collecting or breeding site. We do not use controlled environment for the regeneration.

We collaborate with other specialized scientific organizations in Czech Republic and in Slovakia, which have more suitable conditions for regeneration of certain materials.

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.

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 dried to reach the storage moisture from 4 – 8 % in a drying room. The length of the drying procedure is different for different types of seeds and varies usually between 2–8 weeks. Large-seeded legumes can be dried even for 10–12 weeks.

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

The standard recommendation is to keep harvested seed samples at a temperature below +15 °C.

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.

Samples are temporarily kept in paper bags or paper boxes.

- Temperature: +5 – +10°C
- Humidity: 33 %

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

The recommended seed amount for the base collection is 4000 for self-pollinated and 12 000 for cross-pollinated species. The optimal active collection seed amount is two or three times higher than that of the base collection. Large-seeded samples (*Phaseolus*, *Faba*, etc.) have very high TSW (1500–2500 g) and thus, we cannot use this ‘optimal’ seed amount for reasons of space. The absolute minimum is 1000 seeds. Safety duplication follows the base collection’s rules.

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 not stored GMO seed samples in Gene bank of SR, therefore as they are not part of the National Programme collections.

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

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).

In potatoes, for each genotype there is one clone which has been selected and multiplied after chemotherapy. For hop, there are from 1 to 12 clones per cultivar; each clone originated from 1 shoot multiplied after chemotherapy.

SCSS2 – Please describe in general terms the type of culture vessels (as far as 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.

Genetic resources of hop and potatoes are stored as virus-free shoot cultures. The type of culture vessels is mentioned in section SC2 in Box 3.1.3.B.

Nutrient media:

Hop: MS macro and micro nutrients, WS vitamins, 20 g/l glucose, 3 g/l Gelrite

Potatoes: MS macro and micro nutrients, modified MS vitamins, 30 g/l sucrose, 10 g/l Agar, 20 g/l of growth retardant Daminozid (syn. Alar, Succinic Acid 2,2-dimethyl hydrazide).

SCSS3 – Please indicate whether or not you use a minimum number of *in vitro* plantlets per accession.

The minimum number of plantlets per accession is 28 (hop), or 5 (potatoes).

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).

We may cultivate two or more sub-clones of each accession.

SPP2 – Describe the sub-culture duration (if not crop-specific).

Duration of subculture is 3 months (potatoes) or 3–4 months (hop).

SPP3 – Describe the criteria you use to decide on *in vitro* plant quality (if not crop-specific).

Plants should be healthy looking, well growing, without vitrification, callus formation or visual contaminations.

Box 3.2.3.B. 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).

We do not store GMO 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).

We do not store GMO material.

C. Cryopreserved Collections

Box 3.2.1.C. Cryopreservation Containers and Sample Size

SCSS1 – Indicate if you document the initial number of explants of individual accessions.

Not applicable

SCSS2 – Please describe what kind of cryopreservation vessels (and equipment) you use (only if they differ from the corresponding answers in previous boxes), the procedure you follow with respect to separate material containing viruses or bacteria from healthy material.

SCSS3 – What is the number of explants that you use as the minimum threshold per accession?

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

Box 3.2.3.C. 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).

D. Field Genebank Collections

Box 3.2.1.D. Accession Sample Size

SCSS1 – Indicate if you document the initial number of plants of individual accessions (either as received from collecting missions or through exchange).

The number of plants of individual accessions (either as received from collecting missions or through exchange) is documented.

SCSS2 – Please describe what kind of procedures you follow, if any, with respect to sub-sampling and subsequent place/container/etc. of maintenance.

Not applicable.

SCSS3 – What is the number of plants that you use as the minimum threshold per accession? Are these plant numbers of a given accession based on genetic parameters (such as reproduction biology; heterogeneous samples)?

Garlic: 20 plants/cloves.

Grapevine: 1–6 plants for the same genotype

Fruit trees: 1–3 trees for the same genotype

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

PC1 – Please describe the multiplication procedures that you follow for your field genebank material (both annual and perennial species)

Please include in your description the following aspects if they would apply to your field genebank management procedures):

- a. any control measures to minimize or avoid cross-pollination between accessions (if applicable/relevant);*
- 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.).*

When propagating cross-pollinating plant species, isolation distances are observed for individual plant species on the basis of UKSUP methodologies.

The use of pollination cages for insect-pollinated species is significant in terms of propagation of all cross-pollinating plant species with insufficient capacity of propagation areas and inability to maintain the required isolation distances for individual plant species. This method of propagation significantly expands the capacity to propagate multiple genotypes of one species in one year, or combine multiple species in one isolator under strict compliance with the number of individuals of each species to avoid genetic drift of the propagated species.

The use of specific pollinators for insect-pollinated species is important in terms of forage production, species with a long flowering tube – clover and alfalfa with the help of bumblebees.

PC2 – Provide any other relevant information on procedures that you apply to control pollination of your germplasm in case of harvesting planting material from your field genebank material.

Box 3.2.3.D. Planting Material Processing Procedures

SPP1 – Describe the protocol(s) that you use for threshing and seed cleaning, if used as an intermediate step for the management/multiplication of your field genebank accessions.

Not relevant.

SPP2 – Please describe how and where you store (in a temporary manner) newly harvested planting material.

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

Fruit trees and grapevine: we outsource this step and the grafted material is returned to the field next season.

Garlic: Harvested plants are dried. After 6–8 weeks the rest of the leaves and roots are cut and garlic is prepared for planting. They are stored in perforated plastic boxes on top of each other in a dark room with constant air circulation.

Temperature: -1 – -3°C

Humidity: 70 – 90 %

Sugar and fodder beet: healthy roots without leaves are collected, smaller (10–15 cm with a root diameter of 8–10 cm) are placed in perforated plastic boxes on top of each other in a dark room with constant air circulation

Temperature above 0 – +2 °C;

Humidity: minimum 90 %

Cabbage: we collect a healthy stump with root, which is then stored in a perforated plastic or paper box, it can be sprinkled with moist sand or sawdust, Temperature: 0 °C

Humidity: 90 %

SPP3 – Describe the criteria you use to decide on the number of plants per accession intended for the long-term conservation.

It depends on whether the plant species is self-pollinating or cross-pollinating, homo- or heterozygous. The aim is to preserve the complete genetic information as a population in future generations.

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 Box 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)

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.*

Availability of seeds and the distribution amount for the different crops are visible on the web site griss.vurv.sk (GRISS). We send usually 10–200 seeds for breeding, research or education purposes under SMTA. The number of samples is limited to 30 samples per request, twice a year.

In case of larger requests for samples or amount of seeds, we communicate with collection curators to obtain their agreement.

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?

Estimated time from request to sending seeds is within 21 days.

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.

Related information is available on our website griss.vurv.sk (GRISS). Passports and available C&E data can be found there. Together with seed samples, we send the list of accession numbers, completed by accession name, species, origin, form and amount of seeds. This list is also a part of the SMTA.

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).

A maximum of 100 seeds are distributed in case of cultivated cereals, grasses, fodder crops or oil crops. Large-seeded legumes and the majority of vegetables, flowers, medicinal and aromatic plants are distributed in amounts of 30–50 seeds. The actual purpose of the utilization is also crucial – e.g. for molecular characteristics or genetic markers

determination the amount of 5–10 seeds is fully sufficient. In case of regeneration, we follow collection curators' demands (250 seeds for legumes, 500–1000 seeds for cereals, etc.) .

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

Seed samples are stored in glass containers with sealed cover with label. Multiple containers per accession item differ only by the new accession number. We do not use small bags for sub-sampling and distribution.

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.

Each plant species has a set minimum amount of seed supply per sample / item. This is the minimum seed balance below which the seed stock behind the sample must not fall in order to be still regenerable. The lower limit of seed supply represents the sum of seeds in all containers per item. When the seed condition drops below 1x TSW from the critical minimum, the sample is sent for regeneration. A sample that falls below this threshold will not be provided to the applicant and is sent for regeneration. At the time of regeneration, the sample is not provided to the applicant. The sample is available only after its regeneration.

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

Seed samples of the AEGIS collection (European accessions) have to be readily available to users; the regeneration policy does take this into account. At the time of regeneration, the sample is not provided to the applicant. The sample is available only after its regeneration.

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.

We store visibly healthy seed samples and declare them as disease-free. Samples not matching health standards are returned to the collection curator together with the relevant health status report.

The collection curator is responsible for the good health of seeds. New acquisitions to the collection are sown first by the curators in quarantine nurseries and checked for their health status before accessing the material.

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

Plant material is accompanied by a phytosanitary certificate for countries outside the EU. Some countries (USA, Australia) require also an import permit for imported plant material, thus it is necessary to verify whether an import permit is necessary.

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

The State Phytosanitary Administration (SPA) office is contacted for issuing the phytosanitary certificate. SPA office staff visits the genebank or genebank staff brings samples to SPA office for health check/approval. After that, a phytosanitary certificate is issued, the original document is part of the distributed package and a copy is archived together with all documents related to the request number.

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.

See box 3.3.2A.

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

In case of lower germinability, we distribute a higher number of seeds to keep the most preferred rule: 100 germinating seeds per accession but only if sufficient amount of seeds is available.

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.

We do not provide *In vitro* samples.

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.

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.

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.

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 or plastic bags).

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.

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.

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

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

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.

GS2 – Please provide details of your routine methodology of containers etc. that you use to distribute *in vitro* cultures.

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

C. Cryopreserved Collections

Box 3.3.1.C. Ensuring Availability of Germplasm – Policy Aspects

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

Cryopreserved material is for distribution in exclusive cases only – e.g. for special research, please describe your policy; 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.

Not applicable.

AGP2 – Indicate if you have as part of your service-rendering policy aspects such as a “regular or maximum time” between receiving a germplasm request and distribution of the germplasm.

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.

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

AGSS1 - Please provide details on samples that you distribute (where relevant).

AGSS2 – Describe how you store, for distribution, the cryopreserved material of a given accession with respect to the use of special equipment such as dry-shippers etc.

AGSS3 – Describe how you manage the availability of adequate cryopreserved material.

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

Box 3.3.3.C. 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. You could also add data on separation of differently infested material in separate cryotanks, etc.

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

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

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

Box 3.3.4.C. Germplasm Supply

GS1 – Describe the policy of your genebank with respect to the sample size that you use for distribution purposes.

GS2 – Please provide details of your routine methodology of containers etc. that you use to distribute cryopreserved material.

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

D. Field Genebank Collections

Box 3.3.1.D. 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: crop/species specificity; whether or not sufficient seed stock is available; 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.

The maintenance of genetic resources and their distribution are realized based on the legal framework of the National Programme of Conservation of Plant Genetic Resources for Food and Agriculture, the Act No. 215/2001 Coll. On Conservation of Plant Genetic Resources for Food and Agriculture and Standard Material Transfer Agreement.

AGP2 – Indicate if 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.

Depending on the availability of samples, within 3 weeks.

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.

Related information is available in GRISS (griss.vurv.sk).

Box 3.3.2.D. Ensuring Availability of Germplasm – Seed/Germplasm Stock Aspects

AGSS1 - Please provide details on the minimum/maximum amount of plants or organs (cuttings, bulbs, tubers, etc.) per plant that you distribute per accession (where relevant, differentiated by species groups, i.e. annual or perennial; woody or herbaceous; other) and/or whether an accession is clonally or sexually propagated).

Garlic: 2–10 bulbs

Fruit trees and grapevine: 5 cuttings

AGSS2 – Describe how you manage the availability of adequate organs per accession, including the use of an absolute lower minimum of plants per accession as the threshold to decide to multiply.

Garlic: 20 plants/cloves

Fruit trees: 1–3 plant per accession

Grapevine: 1–6 plants per accession

AGSS3 – Provide here information on any other aspects that are relevant to manage plant material stocks.

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

AGHA1 – Describe how you maintain field genebank (and any intermediate storage step) accessions with respect to health considerations, including whether you have a “policy” on accepting/planting only “disease-free” planting material (as far as you can see or determine) accessions, at least for the quarantine pests and diseases.

Garlic: cloves for multiplication are only from visually healthy plants

Fruit trees and grapevine: buds for multiplication are only from visually healthy plants. We outsource this step and the grafted material is returned to the fields next season. Cultivation and pest/disease management are done according to good agricultural practice.

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

Samples are sent in good condition without mechanical damage, pests and diseases. We use the phytosanitary certificate issued by the Central Institute for Supervising and Testing in Agriculture for countries outside the EU. All material is sent under a SMTA.

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

We use the phytosanitary certificate issued by the Central Institute for Supervising and Testing in Agriculture for countries outside the EU. All material is sent under a SMTA.

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

Box 3.3.4.D. 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 annual or perennial species, clonally or sexually propagated accessions, and possibly other aspects.

Garlic: 2–10 regenerated parts; 10 samples can be sent per applicant during one calendar year.

Fruit trees and grapevine: 5 cuttings per accession. 10 samples can be sent per applicant during one calendar year.

GS2 – Please provide information on any other aspects related to seed 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?).
- 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.
- e)

NPPC – Gene Bank of the Slovak Republic has its own genebank information system - GRISS (<http://griss.vurv.sk>) The IS GRISS consists of an internal – non-public and external – public part. The internal part of the system includes passport data, C&E data, seed storage data, as well as data containing the complete data flow from the germination test, storage process, regular monitoring, and material regeneration to the provision of samples to users. The external part of the system includes the publication of accessions data as well as online seed ordering. The system is built on a MS SQL Server running on MS Windows Server 2012 R2 6.3 in a virtualized environment on VMvare vSphere 6.5.

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, C&E data, storage data, monitoring and material regeneration data, input and output protocols, material distribution data including SMTA.

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

Not Relevant.

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).

Passport data are available on the website of IS GRISS and data can be provided as Excel or CSV file, if requested.

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

All technical support is provided by the Ministry of Agriculture and Rural Development SR.

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

The backup of the database content is updated daily at 0:00 a clock and manually in the event of major changes.

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

The genebank documentation specialist provides helpdesk function for usage of IS GRISS.

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?).

All information is made available to users via the Internet. Users can find information at the IS GRISS website <http://griss.vurv.sk>. Passport data download is supported to Excel or CSV files.

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).

All data are available online in our IS GRISS. No other services are currently available.

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

The passport data set included in IS GRISS is also published in EURISCO. Data sets are updated usually once a year.

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.

ACCENUMB, ACCENAME, GENUS, SPECIES, ORIGCTY, FORM and quantity of seeds.

MultiCropPassportData + EURISCO DESCRIPTORS, if available