Operational genebank manual of the

Crop Research Institute(CRI), Prague, Czech Republic

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The ISO Quality Management System according to ČSN EN ISO 9001:2009 for seed genebank activities was established in September 2011. All detailed descriptions of activities and related documents are available in Czech language on the genebank local network space and for external users it is available on request.
The contemporary certificate, issued by United Registrar of Systems, is valid until 2016 and every year is approved by the audits.

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).

Plant genetic resources within CRI are kept in different departments: Genebank (Prague) – seeds, Physiology and Cryobiology of Plants (Prague), Genetic Resources of Vegetables and Specialty Crops (Olomouc) – AEGIS collection of garlic and in Viticulture Research Station (Karlstejn) – collection of grapevine.
The Genebank in CRI operates as the central genebank for generatively propagated species maintained in Czech crop collections belonging to the National Programme for Conservation and Utilization of Plant Genetic Resources and Biodiversity. The National Programme was launched by the Czech Ministry of Agriculture in 1994 and the Genebank in CRI Prague coordinates the network of crop specialized institutions – collection holders. Responsibility for crop collections is divided among 12 institutes located in 15 workplaces. More than 80% of all collections are comprised of generatively propagated species. The target crops comprehend crops important for food and agriculture. The list of accessions is available on the website https://grinczech.vurv.cz/gringlobal/search.aspx

All activities with genetic resources are subjected to the law: The Act no. 148/2003 on Conservation and Utilization of Plant and Microbial Genetic Resources for Food and Agriculture.

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?*

The Czech genebank has concluded a formal agreement with the Slovak genebank 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?.

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

Seed samples acquisition is the responsibility of collection holders as legal bodies. Cooperating crop institutions and their responsibilities can be found at http://genbank.vurv.cz/genetic/resources/asp2/background_a.htm. Every cooperating institution makes a contract with CRI Prague, part of contract is a list of planned collecting/acquisition activities for the relevant year. The specification is related to increasing items in crop collections, mainly on a regional scale or accessions with important traits for breeders, but also their characterization and evaluation, regeneration, documentation, conservation and number of seed samples provided to the genebank storage.

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 verification of plant material as well as quality of accompanying information – including taxonomy. Collection curators are specialists with long-term experience in crop genetic resources. The 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
d) *use of a quality control system (e.g. ISO).*

Quality parameters for inclusion into genebank storage are based on the *Genebank Standards for Plant Genetic Resources for Food and Agriculture* (FAO 2014). Identification of samples is carried out according to acquisition protocols accompanying seed samples and compiled by responsible collection curators. Seed samples are identified by their

Accession numbers. Accession numbers and recorded passport information are necessary requirements for sample acquisition into genebank. Seed samples during the preparatory phase have provisional labels with the basic passport information. Provisional labels are replaced by the final barcoded labels just before putting accessions into the genebank storage. If the sample does not meet seed purity, germinability or health standards according to the ISO 9001:2009 used in our genebank, it is returned to the responsible collection curator together with the protocol describing the sample insufficiency.

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 2012 and it is applied not only to Annex 1 crops, but to all samples delivered from the genebank. Before 2012, the national version of Material Transfer Agreement had been used. All seed sample transfers from genebank storage are documented in the information system GRIN Czech. Accession transfers belonging to Annex 1 species are documented in the FAO reporting system.

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 organised jointly with 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, occurrence of targeted species in consultation with local specialists for botany, ecology, pomology, environment). The optimal period for collecting specific plant reproduction material (seeds, spikelet, fruits, infrutescence, 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 collecting number, collecting date, locality, description of the site, geographical coordinates, elevation, etc. The record structure follows MCPD descriptors related to collected material; it also includes the description of soil type, geological substrate, slope orientation, plant abundance, phenology phases, etc. Exact localization is provided by the GPS device. Samples should represent existing population variability and their size is dependent on abundance of plants (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.

SE2 – Provide any additional information on the germplasm collecting activities of your genebank, including the collaboration with others.

Collecting expeditions are organised with special attention to bordering areas, in cooperation with Slovak, 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 CRI Genebank has a formal agreement with the Slovak genebank in Piešťany 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 are gradually sent to Svalbard.

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

Safety duplication should follow the rules for the base collection: in this regime safety duplicates should be stored for all domestic material (originated in Czechoslovakia or in Czech Republic) and all foreign materials important for the relevant crop collection. 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 Genebank is situated in a safe location with very low probability of flooding or earthquakes. The building and refrigerating technology were completely reconstructed in 2010.

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*)

Security arrangements are using a combination of above-mentioned precautions: fences are used to protect the whole Institute's area and the area is security serviced. The genebank building is equipped with an alarm system and a standby generator. The air-conditioned area of the genebank storage is equipped with security doors and fire extinguishers. Regular technical service of the equipment is ensured.

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

Access to the genebank is only allowed to authorized genebank technical staff, genebank department Head, genebank storage manager, and technical service staff (specified in the ISO guideline text). All other persons or visitors can enter genebank storage rooms only with attendance 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 starts after 5 min of power supply cut-off. Regular servicing and maintenance is secured by 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 and relative humidity are monitored in the drying room. All storage rooms are kept at a temperature of -18ºC, this environment does not require humidity check. The corridors (manipulating space) are kept at +5ºC and 30-33% R.H. All rooms have two temperature sensors and all the data are stored via local network on the server. The data are in parallel regularly archived after 3-4 months on another PC.

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)*.

Financial means are provided partly from institutional budget and partly from the National Programme and it is stable (during the past 24 years) without major threat. Sustainable function of the genebank is legally supported by the Act No. 148/2003 Sb.

IPS2 – Describe how you secure adequate staffing of your genebank is?

Minimal staff for storage and documentation: 1 Head +2 technicians – 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.

We apply fire emergency rules valid in the institute; we do not have a particular contingency plan for disasters.

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

Genebank staff has yearly security training – together with other CRI staff. Genebank technicians are informed on the security situation – all doors are provided with an opening mechanism from "inside". Security is also one chapter in the ISO documentation.

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)

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.

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.).

Seed samples obtained from other cooperating institutes are maintained at +5ºC as long as space is not available in the drying chamber. Crops for which the genebank department has a responsibility within the National Programme as a curator (i.e. wheat, winter barley, triticale, buckwheat, sorghum, millet) are treated according to ISO documentation. After harvest the seed samples are cleaned and put into a dedicated space in the sideboard of the genebank's air-conditioned corridor (+5ºC and 30-33% R.H.) until further processing.

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

Dormancy is usually broken by the placement of sample at a temperature close to 0ºC for one or two weeks. One month of storage at +5ºC is sufficient for dormancy breaking. In case of hard seediness (particularly 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. No chemical agents are 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 14% R.H. and at +17ºC. High quality seeds are recommended for genebank storage – fully ripe, pure, healthy and viable (recommended standards are recorded in the Methodology and ISO documentation). If the seed sample does not meet the standards, it is returned to the collection curator together with the relevant error report.

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 recommended 5 years frequency of testing seems to be too short, as our conditions (-18ºC for both active and base collections) meet requirements of long-term storage. A 10-year period for viability monitoring is found to be sufficient. Moreover, the major part of stored accessions (about 50%) is cereals and their viability testing span of 20-25 years is sufficient. However, the actually used time period depends highly on the initial seed lot quality. Limits for 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 sample and replenish the stock in the genebank storage. Collection curators follow the current viability of their samples via our online database GRIN Czech, where samples with a viability below the requested level are being flagged. After regeneration seed samples belonging to different seed lots of the same accession are not mixed or replaced, all of them are kept in parallel in storage.

Currently we conduct systematic viability checks on seed samples that have been kept the longest in the genebank (i.e. 20-24 years).

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.

Species with short life expectation (vegetables, flowers, aromatic and medicinal plants) have been stored at -18ºC and the first testing of viability was carried out after 10-12 years

of storage (1999-2001).
The status of seed samples regarding their quality level is recorded in the GRIN Czech system and is available online to curators.

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.*

A germination level lower than the recommended standard is allowed in case of materials obtained from collecting expeditions or selected species, which by their nature do not reach 100% germination level. Expedition materials, which do not reach the recommended germination level are regenerated; in case that regeneration is not successful, materials are excluded from the active collection and transferred into the working collection. Self- and out-breeding species are handled in the same way.

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.

Seed samples are stored at a temperature of -18ºC in all storage rooms, whereas R.H. is not measured at this temperature. The drying chamber works on a regime of 14% R.H. and at +17ºC.

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 (370 ml or 220 ml jars) covered with twist sealing lids; a small bag with coloured dried silicagel is added to each sample. When the colour of silicagel changes it is known that a leakage has occurred.

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 moisture level of stored seeds differs for different species and varies from 3% to 9%.

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.

The total capacity of storage rooms after reconstruction in 2010 is 187 000 glass containers; this capacity is presently used at 60%.
Currently, there are nearly 45 000 accessions stored, that is about 95% of all generatively propagated crop collections within the National Programme.

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).

Currently, six of the ten storage chambers are in operation. The expected lifespan of freezing and drying equipment is 20-25 years. (The previous equipment was replaced completely after 22 years of running.).

A. In vitro Culture Collections

This part A applies only to the grapevine collection in CRI.

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).

During the winter cut (February to April), the buds are used for the production of new plants. We use only the visibly healthy plants from our field genebank collection. Protection of plants against pests and diseases is carried out as required and at least 4 times per growing season depending on the weather and the development of diseases.

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

As organ source we use one year old shoots, cut into nodal segments, put into water in plastic trays, at 15-20°C in greenhouse. After 3 weeks the shoots are completely defoliated, transferred to 30% SAVO (sodium hypochlorite 5% + sodium hydroxide 2%) with detergent Tween 20 for 25 minutes (laboratory shaker), than 3x washed with sterilized distilled water. The shoots are then planted into Erlenmeyer flasks with Quoirin and Lepoivere medium where agar, saccharose and phytohormones were added. Multiplication is made by multinodal segments which are planted into glass tubes with standard culture medium based on the Quoirin and Lepoivre formulation.

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

It is necessary to work quickly in the laminar flow box, because the explants are sensitive to dehydration injury. Also the temperature must be lower than 22°C.

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*).

Control checks are conducted regularly every week. These checks cover visual controls on fungal contamination.

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 is implemented. Experience and skill of staff are crucial for special decisions; this collective knowledge is efficiently shared between the expert team.

VM3 - Please provide information on non-specific thresholds that you might use for vigor 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?

Decision on multiplication regimes are taken according to personal experiences. Sub-culture duration is 6-8 weeks.

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.

Nodal segments are stored in greenhouse in trays with water, at 15-20°C; explants are in a temperature-controlled room (25°C), 16 h light, light intensity 40 µmol $m^{-2}.s^{-1}$.

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.

Shoots – Erlenmeyer flasks – 100 ml
Explants – cultivation tubes with plastic caps (16 x 1.7 cm)

Transfer procedure is done in laminar flow box, with usage of pre-sterilized instruments and glassed – scalpel, scissors, tweezers, forceps, aluminium foil, Petri dishes.
We use also steam sterilizer, heat sterilizer, analytical balance, pH meter, magnetic stirrer, refrigerator, microwave oven, laboratory shaker.

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

B. 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.

In vitro plants must be from plant material with good growing condition and free from contamination of microorganisms.

Cultivation media are plant-specific, based on MS medium.

In situ plants: the bulbils from *Allium* top-sets must be fully matured.
Woody species must be fully acclimated in eco-dormant stage.

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

According to our experience, removal of viruses from infected material does not significantly affect regeneration after cryopreservation in *Allium* plants.

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]

In preselected samples a random selection is carried out. Control samples of selected genotypes are evaluated for regeneration after 3 years and then again after 10 years.

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

A special system based on an Excel database is used. Species- and accession-specific decisions are based on Dussert's statistical model (Dussert et al. 2003). Experience and skill of the technical staff are crucial for special decision-making.

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;

The lower limit of regenerative capacity of plants, which can be considered as a successfully cryopreserved genotype, was set at 30 % in a group of minimum 40 control plant samples. In parallel, a minimum of 120 plant meristems are stored in liquid nitrogen.

b. any thresholds that you use [e.g. discard the material as not storable below a certain regeneration rate of the control],

Accessions with low regeneration (between 10 % and 30 %) could be cryopreserved in more runs to increase probability of regeneration of stored samples to desired level (on the basis of statistical calculation, Dussert et al. 2003).
If the regeneration of a control sample is less than 10%, it is necessary to apply a different method of cryopreservation or a modification of the currently applied method of cryopreservation for a given genotype to enhance the regeneration rate.

We do not support the policy of "non-storable" plant accession according to its low regeneration rate after cryopreservation. The cryoprotocol must be changed to increase the regeneration rate.

c. whether you apply different procedures for accessions with erratic regeneration rates of the control [e.g. increase the amount of explants stored]; etc. and

In the case of repeated low plant regeneration after cryopreservation it is necessary to increase the number of stored plant samples so that the level of regeneration has a statistically significant probability of successfully recovered genotype (Dussert et al. 2003).
If the regeneration of control plants is extremely low, the given genotype should be included in the group that requires further research of modification of a particular cryoprotocol or development of a completely new one so as to increase plant regeneration to required level.

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 vapor phase, automatic tank filling or filling by hand). In case they vary from tank to tank, please provide details for each.

In our cryobank all samples are stored in liquid nitrogen in Dewar flasks. Plant samples are stored in liquid nitrogen inside cryovials, cryotubes or plastic bags. The liquid nitrogen is filled manually. The liquid nitrogen in each Dewar flask is monitored (data stored) at two levels. The first is an operational level indicating us to fill the Dewar flask and the second level is signaling an accident.

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

Two types: LS750 and LS 4800 Taylor-Wharton.

SC3 - Do you treat different species differently?

Each species has a specific cryoprotocol; moreover for some genotypes of one species it is necessary to modify the species-specific cryoprotocol.

Different storage containers are used:
Pollen in straw (only for breeding purposes)
Shoot tips on aluminum strips, in 1.8ml cryovials, or in alginate beads in 2 ml cryovials.
Dormant buds in 50 ml centrifuge tubes or in plastic bags.

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

All our decisions are based on the calculation of probability of number of regenerated plants, according to Dussert et al. 2003 (see above).

We prefer cryoprotocols without DMSO to prevent potential mutagenesis of stored plants. Up to now, our cryobank has been using vitrification solutions without DMSO.

C. 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: for planting in order to use the healthy cloves, 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.

Grape: after the winter cut, the buds are used for the production of missing plant lines for the next year. The obtained material is used in the laboratory for preservation of individual cultivars under *in vitro* conditions. All accessions of the grape field collection are cultivated using standard techniques as standard conventional vineyard technology. The soil under plants is kept free of weeds as required, chemically and mechanically, three times per season at least. Protection of plants against pests and diseases is carried out as required but maximum four times per growing season, 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 once a month.

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.

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

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.*).

Garlic: the field collection is controlled twice a week by inspection. Occurrence of pests is monitored by yellow adhesive coated arrows, designed to catch harmful flying insects. It is important to abide strictly by crop rotation. Garlic has a very long vegetative period, therefore abiotic stress has a significant influence on garlic, e.g. black frost, period of drought in the spring and summer period, flooding.

Grape: 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 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.

Grape: multiplication has to be initiated if the amount of plants per accession in the field genebank collection is lower than 7.

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. Yellow adhesive coated arrows are used to catch harmful flying insects for monitoring pests. Insecticides are used as a 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 in the field drying room – this is a special plastic tunnel (greenhouse) with good air circulation. After 6–8 weeks the rest of the leaves and roots are cut and garlic is prepared for planting. Forty cloves per accession are planted in the field. The rest of the material is stored in a

storeroom with temperature ±5°C and humidity ±60% in netting bags hanging on a wire at eye level. This material is used both to distribute to requesters and as a reserve for field planting during spring period in case the field material is not in a good condition after the winter period.

Grape:
The same system as IV1 – Grape.

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

Garlic: not applicable.

Grape: 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 and for achieving the objectives of maintaining genetic integrity and should be briefly described. Please note that a distinction should be made between seed numbers for an accession and seed numbers for sub-samples per accession. The latter only applies if the seeds of a given accession are being stored and distributed as sub-samples. As genetically modified materials get more widely distributed and as it might have specific (legal, technical, administrative) requirements a separate box on this type of material is included.

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

Navigation 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.

Glass containers (see Box 3.1.3A) are used for seed sample storage. We do not create sub-samples for the active collection; glass containers are opened in case of seed distribution repeatedly, as necessary. For accessions of the AEGIS collection we use sub-samples in zipped plastic bags which are enclosed in glass jars with dried silicagel.

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

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.

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.)*

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 (which are available upon request).

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

During the vegetation period, 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*).

The major part of collections originated from central European countries, therefore the conditions for regeneration at our experimental fields are adequate. We do not distinguish for the regeneration between different types of 'biological status' in collections.
Of course, thermophilous materials have to be regenerated in specific areas. If such conditions are not available at the curator's location, the National Programme partners are asked for cooperation.
Several very sensitive materials have to be regenerated under greenhouse conditions. Selection of the proper regeneration method is a responsibility of the curators.
For regeneration matters, we do not collaborate with other European genebanks. Within the network of institutions participating in the National Programme, there are possibilities to ask for help of collection curators, who 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.

Seed samples intended for genebank storage should be orthodox when the actual water content is lower than 12-15%. All seed samples are dried before storage in the genebank drying chamber under one regime: +17ºC and 14% R.H. 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 +15ºC. The Genebank air-conditioned corridor (+5ºC) is used for temporary storage after harvest.

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).

Cleaned seed samples are put into dedicated sideboards in the genebank corridor at a temperature of +5ºC and 30-33% R.H. Samples are temporarily kept in paper bags or paper boxes.

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

After the reconstruction in 2010 we have all chambers running at -18°C; long-term storage regime is applied to all kind of collections.

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).

We do not store GMO seed samples, 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.

Not relevant.

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)?

We use more donor plants from accessions but they are true clones, *in vitro* cultures are treated as a whole clone.

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.

Shoots – Erlenmeyer flasks – 100 ml: medium Quoirin and Lepoivre, agar 6 $g.l^{-1}$, saccharose 3.4 $g.l^{-1}$, phytohormones – 0.7 $mg.l^{-1}$ BAP and 0.1 $mg.l^{-1}$ IAA.

Explants – cultivation tubes with plastic caps (16 x 1.7 cm) - medium Quoirin and Lepoivre, agar 6 $g.l^{-1}$, saccharose 3.4 $g.l^{-1}$, phytohormones 0.2 $mg.l^{-1}$ IAA.

Before, we used only 3.1 $g.l^{-1}$ saccharose, but higher concentration positively affects the growth of explants.

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

Minimum per accession is 35.

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)

Not defined.

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

6-8 weeks.

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

Absence of fungi formation, healthy looking, good vigour.

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).

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?

The minimum number of frozen shoot tips of each accession is 120. The minimum number of control plants to verify the success of cryopreservation is from 20 to 40 shoot tips or buds of each accession. In the case of low plant regeneration it is possible to increase the number of stored shoot tips, which guarantees safe regeneration of an accession owing to the increase of potentially regenerated plants.

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

Shoot tips are stored in 1.8 ml or 2 ml cryovials as a standard. Dormant buds are cryopreserved in 50 ml centrifuge tubes sealed by aluminum foil, or in plastic bags in paper cryobox.
Rack with paper or plastic cryobox.
We do not separate virus-free and virus-contaminated/non-tested plants, because the transfer of viruses during storage is unlikely. Otherwise we would use all the prevention against cross-contamination by microorganisms.

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

The minimum threshold number of stored shoot tips of an accession is 120.

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

Box 3.2.2.C Cryopreservation Procedures (as long as not crop specific)
SPP1 – Describe the protocol(s) that you use for preculture and pretreatment such as cold acclimation and dehydration.

Cold hardening at low temperatures – fruit trees and hop.
Frost dehydration – dormant buds of fruit trees.
Air dehydration – potato, hop, fruit trees from *in vitro.*
Osmotic dehydration – *Allium*, grape, potato.

SPP2 – Describe the protocol(s) that you use for cryopreservation proper (such as slow freezing, droplet freezing, vitrification, encapsulation etc.)

Vitrification – *Allium*, hop, potato, grapevine.
Encapsulation-dehydration – fruit trees from *in vitro.*
Slow freezing – fruit trees (dormant buds).

SPP3 – Describe the protocols that you use for regeneration (slow or fast rewarming, washing, dark periods etc.)

Fast warming in water at 40°C for *in vitro* shoot tips.
Dark first week of cultivation for all shoot tips.
Slow rehydration in moist peat – dormant buds of fruit trees.

SPP4 – Describe the time span and method(s) of survival and regeneration controls

2 weeks survival, 6-10 weeks regeneration for *in vitro* cultures.
3 months for chip budded dormant buds.

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

See VM3.

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).

Not applicable.

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?

Garlic: 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: 40.

Grape: 5, the same genotype.

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

Box 3.2.2.D Multiplication

PC1 - Please describe the multiplication procedures that you follow for your field genebank material (both, annual as well as 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.)*

Not relevant.

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 do you use, if any, etc.).

Garlic: the same as 3.1.3.D SC1.

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

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

Garlic: it is maintained as a field collection. The safety duplication is achieved through cryoconservation.

Grape: the limiting factor for the genebank is the available land.

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 GRIN Czech website. It is usually 100 seeds, only for inbreeding crops the amount is 30 seeds. We send seeds for breeding, research or education purposes under SMTA. The number of samples is limited to 30 samples per request; in case of larger requests for targeted research projects 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?

The recommended time from request to sending seeds is within 14 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.

Information is available on the GRIN Czech website – one can find full passport and available C&E data. Together with seed samples we send the list of accession numbers, completed by accession name, species and origin. This list is also 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 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/etc. 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. We do not use small bags for sub-sampling and distribution.
The exception are AEGIS samples, which are ready for distribution as sub-samples, 5 small marked plastic bags with 30-100 seeds in glass containers. The AEGIS collection has its own dedicated space (shelf) in the storage room.

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

Current availability is part of the information on PGR – see box 3.3.1A point AGP1. Due to the current regeneration status the material may be temporarily unavailable.

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

Seed samples of the AEGIS collection (European accessions) have to be readily available to users; the regeneration policy does take this into account.

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.

Stored seed samples are not treated. 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 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)*

Availability of accessions is visible on the GRIN Czech website. It is usually 5 tubes with explants. We send them for breeding, research or education purposes under SMTA.

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?

The recommended time is 14 days but it can be extended.

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.

Information is available on the GRIN Czech website – there one can find full passport and available C&E data. Together with samples we send the list of accession numbers, completed by accession name, species. This list is also part of the SMTA.

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.

5 plantlets per accession.

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).

Samples are distributed in glass tubes.

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.

The minimum number of plants per accession is 20: current availability is part of the information available on the GRIN Czech website.

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.

We store visibly healthy plantlets.

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

Germplasm should be exported with the phytocertificate.

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

See AGHA2.

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.

Usually 5 plantlets per accession.

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

Glass tubes with unrooted cuttings; curator must confirm the availability.

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*).

The cryobank acts as a duplicate (base collection) to other active crop collections of vegetatively propagated species (field, orchard, *in vitro*).
The requestor is in any case only the crop collection curator.
The purpose is to get the lost accession back into the active collection.

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?

Not relevant, we do not distribute material to users.

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.

Not relevant, we do not distribute material to users.

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

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

Not relevant.

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

Samples are returned to the collection curator depending on the type of plant (*in vitro* plants, rooted *ex vitro* plants or rehydrated dormant buds prepared for grafting).

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

Not relevant.

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

The *Allium* Core Collection is stored as safety duplicate in Germany and Poland, on a reciprocal basis.

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.

Our policy is to store plant material as germplasm safety back-up at first. We prefer to cryopreserve virus-free material if available. If not, we store also virus-infected plant germplasm.

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

Not relevant.

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.

Occasionally an accidental contamination by pathogens may occur during regeneration of control plants after cryopreservation. In the case of plant contamination during the cryopreservation procedure, it can be assumed that stored samples are contaminated too. Such a situation requires repetition of the cryopreservation procedure with healthy plant samples for long term storage or eradication of pathogens after regeneration from cryo-storage.

Box 3.3..C4 Germplasm Supply

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

Not relevant.

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.

When the number of cryopreserved regenerable plant samples decreases below the critical number we repeat the whole cryoprocedure.

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 work with genetic resources (GR) and the distribution of GR are realized on the basis of the legal frame of the National Programme on Conservation and Utilization of Plant Genetic Resources and Agro-biodiversity (NP PGR):
The Act no. 148/2003 on Conservation and Utilization of Plant and Microbial Genetic Resources for Food and Agriculture) and The Decree no. 458/2003 to the Act no. 148/2003 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.

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.

All data are available on the website:
https://grinczech.vurv.cz/gringlobal/search.aspx

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.

Grape: 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: we plant out 40 plants (cloves). It is enough material. The abiotic factors (flooding, black frost…) might have a negative influence on the availability of materials.

Grape: we plant 10-20 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: The same as 3.3.2.D AFSS2.

Grape: the 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".

See AGHA2.

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.

Grape: 5 cuttings per accession.

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.

In 2015 we moved all data from the Evigez system to the documentation system GRIN Czech (server Microsoft SQL 2008), the Czech version of GRIN Global which was developed by the USDA's Agricultural Research Service in cooperation with Bioversity International and the Global Crop Diversity Trust.
The system includes passport, characterization & evaluation data as well as genebank storage and distribution data. The network of institutions included in the National Programme uses the same system GRIN Czech online. The website with all data is open to the public with an online seed ordering tool.

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.

The user programme GRIN Czech allows handling all information, passport, C&E, storage and distribution data.

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

The web application of the National Crop Catalogue GRIN Czech is available at https://grinczech.vurv.cz/gringlobal/search.aspx and includes searchable database of passport and C&E data. Database manager and curators use their own online access to the GRIN Czech application.

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 and C&E data can be downloaded from the website.

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

The Genebank documentation specialist (permanent staff) is responsible for running of the PGR documentation system. Technical support by an external IT company is secured contractually.

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

The full database content is regularly backed-up every week and daily updates are stored every night as part of the central CRI back-up mechanism.
Beside this, a regular copy of all data is made several times per year and the copy is stored in another place than the institute.

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

The genebank documentation specialist is offering assistance to cooperating institutes within the National Programme network in order to install the user programme and provide guidance and a helpdesk function for usage of the programme.

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?).

Users can find information on the GRIN Czech website
https://grinczech.vurv.cz/gringlobal/search.aspx

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 available on our website are available for other web services. Passport data are published in EURISCO and subsequently in GENESYS.

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 the GRIN Czech website 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.*).

Accession number; accession name; taxon; origin.
Other relevant information is available on our website
https://grinczech.vurv.cz/gringlobal/search.aspx

References

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