



**Operational genebank manual of the  
Potato Research Institute(PRI),  
Havlíčkův Brod, Czech Republic**

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Contact:  
Potato Research Institute Havlíčkův Brod, Ltd.  
Dobrovského 2366  
Havlíčkův Brod  
CZ-580 01

Phone: +420 569 466 200  
Secretary:+420 569 466 213  
E-mail (Secretary): [vubhb@vubhb.cz](mailto:vubhb@vubhb.cz)  
[www.vubhb.cz](http://www.vubhb.cz)

Head of Department of Genetic Resources  
Collection curator  
Ing. Jaroslava Domkářová, Ph.D.  
email: [domkarova@vubhb.cz](mailto:domkarova@vubhb.cz)  
phone: +420 569 466 227

Head of Laboratory of Tissue Cultures  
Head Genebank  
Ing. Vendulka Horáčková, CSc.  
email: [horackova@vubhb.cz](mailto:horackova@vubhb.cz)  
phone: +420 569 466 220

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## 1 Germplasm Acquisition and Accessioning

Genebanks can obtain the germplasm they want to conserve through a number of different ways. Conducting collecting missions is possibly the best way of acquiring germplasm material in the most reliable manner. Germplasm exchange with other genebanks is a 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 Potato genebank in the Potato Research Institute Havlíčkův Brod, Ltd. is responsible for the maintenance of the potato collection in the Czech Republic within 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 is coordinated by the Genebank in the Crop Research Institute (CRI) Prague.

The list of accessions is available on the website

<http://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 gene pools fall under these agreements?*

Our genebank has no specific agreements.

**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.*

PRI has concluded an agreement with CRI Prague; part of the agreement is a list of planned 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 storage of samples in the genebank of PRI.

Data about cooperation with CRI can be found on the website [http://genbank.vurv.cz/genetic/resources/asp2/default\\_a.htm](http://genbank.vurv.cz/genetic/resources/asp2/default_a.htm)

**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.)?

When incorporating the germplasm material into the genebank we rely on the donor's information.

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

(*This description includes:*

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

Potatoes: identification of samples is carried out according to accompanying protocols; samples are identified by their accession numbers and recorded passport information. Health status is checked, although it is a part of passport data.

**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 sample transfers from the genebank are documented.

### **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).*

We do not organize collecting missions.

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

## **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?)*

For safety duplication we use the cryobank in CRI Prague Ruzyně.

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

Safety duplication should follow the standard rules for the base collection: in this regime all domestic material (originated in Czechoslovakia or in Czech Republic) and all foreign materials important for the relevant crop collection should be safety duplicated. All AEGIS accessions should also be safety duplicated.

### **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.

**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. The air-conditioned area of the genebank is equipped with security doors. 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 and collection curator. 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).*

All devices (compressors and evaporators) are regularly serviced by an authorized company.

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

Potatoes – *in vitro* collection. In the culture room for standard *in vitro* plantlet growing the temperatures is kept at +20°C and relative humidity is 70%; in the culture room for tuberization induction the temperature is kept at +10°C and relative humidity is

80%. Values are monitored using sensors and can be visually checked on a panel outside the culture rooms.

#### **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 Coll.

**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 regular security training – together with other PRI staff. Genebank technicians are informed on the security situation – all doors are provided with an opening mechanism from “inside”.

### 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.).

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

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

### **Box 3.1.2.A Seed Viability Monitoring**

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

*(The monitoring system should include the following aspects:*

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

**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.

**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.*

### **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.

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

**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?

**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.

**SC4** – 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).



## A. 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).

Above-ground and below-ground stem parts (foliage, tubers) are donor materials in potato. To ensure high initial viability, planting into pots in the greenhouse and ELISA test for six usual potato viruses are recommended. For tubers a selection of variety-specific tubers, without injury and visible disease symptoms. Use of fully ripe true seeds is limited. For greenhouse materials plant protection measures are performed to prevent diseases (isolated planting, chemical and biological protection).

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

Organ source for the establishment of an aseptic *in vitro* culture for potato involves stem segments consisting of one node with axillary bud, cut sprouts of ca. 10 mm or true seeds. Surface sterilization into 2% Chloramine T solution (tosylchloramide sodium, active chlorine content is 25%) for 12-15 min, subsequently into 70% ethanol solution for 60 seconds. After that thorough rinsing with sterile water and putting on agar MS medium supplemented with ATB, in case of seeds with addition of dichlorophenoxyacetic acid – 2,4D.

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

Healthy materials are used for establishment of an explant culture.

### 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 monitoring is done during every regeneration subculture onto new media, simultaneously hyper-hydricity is excluded. During culture in cold chambers samples are visually checked every month, especially for occurrence of bacterial and fungal contaminations. During culture at usual temperature check is done after 2 weeks.

**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 for timing of monitoring is implemented. Practical experience and discretion of the technical staff are crucial for specific decisions.

**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?

Culture conditions correspond to the purpose of the performed multiplication. Application of specific culture conditions results from personal experience of responsible staff and is mentioned in running protocols. There are no standardized procedures in the laboratory.

**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.

Storage conditions for potato under *in vitro* culture: air-conditioned culture rooms with controlled temperature and photoperiod.

Standard culture room: +20°C, 16 h light, 70% humidity.

Induction of microtuber formation: +10°C, 10 h light, 80% humidity.

**SC2** – Provide details on the type of cultivation vessels (tubes, jars plastic vessels etc.) and the transfer procedures (including the corresponding equipment, if any) that you use.

Potatoes:

Cultivation vessels: glass microbiological tubes with a metal cap, 17 x 120 mm, plastic cultivation vessels 8 x 9 x 11 mm.

Tools and equipment: scalpel, forceps, scissors, ethanol burner, glass plates for cutting, glass beakers, erlenmeyer flasks, disposable sterilization micro-filters 0,22 µm, stand for tubes, plastic volumetric vessels, bottle with thread GL 45, analytical scales, precision balance, pH meter, ethanol, hot air sterilizer up to 250°C, steam sterilizer – tabletop autoclave, sterile bench, demineralization device.

Surface sterilization of source segments and transfer into *in vitro* are done in laminar flow-box, using sterile tools repeatedly sterilized in hot-air dryer.

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

Continuous observation of occurrence of bacterial contaminations, caused by endophytes during long-term storage, which could not be eliminated during transfer into *in vitro* using surface sterilization of source segments, is being carried out in order to allow early capture of vitality changes and bacterial contamination occurrence.

In samples held under cold conditions occurrence of hyper-hydricity is negligible, during usual culture the occurrence is monitored through regular check of samples.

## 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.

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

### 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]

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

**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]; etc. and
- d.

### 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.

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

**SC3** - Do you treat different species differently?

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

## 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.).

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

**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).*)

**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.

**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?

### 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.

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

**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)?

**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.

**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

**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.)*

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

### **Box 3.2.3.A Regeneration Environment and Procedures**

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

*(Consider the following aspects:*

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

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

### **Box 3.2.4.A Seed Processing Procedures**

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

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

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

**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).*

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

**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).

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

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

**SCSS1** – Indicate if you document the initial number of explants of individual accessions when culture is initiated (from one or from more clonal donor plants)?

True accessions of potato genotypes are maintained as one clone; clones derived from active elimination of viral diseases are maintained as such. During transfer of seed populations the number of clonal plants is documented.

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

For long-term potato storage and induction of *in vitro* tuberization Murashige and Skoog (1962) medium is used, supplemented with an increased rate of sucrose (60 g/l) and/or growth regulator Alar 85 – daminozid (3-5 mg/l). Basic medium according to Murashige and Skoog (1962), without phytohormones, is used for subculture and usual multiplication of accessions.

Regeneration subculture is usually done after 14-18 months of culture. For subculture microtubers after dormancy period and their shoots, and/or surviving parts of initial plants are used. Before putting microtubers on basic medium, they must be cut at the stem end. The stem end is thrown away and the rose end is put on the medium. The required number of subcultures on basic medium is ensured using stem cuttings; interval between subcultures is 3-4 weeks. Then, repeated subcultures onto nutrient media are done for long-term culture.

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

Minimum number of *in vitro* plantlets per accession is 6 (Appendix 2 of the Decree No. 458/2003 Coll.).

**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)

In potato 6 plantlets per accession are cultured for microtuber induction. For regeneration only part of the maintained accession is used.

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

Subculture duration of long-term maintained *in vitro* potato plantlets under cold conditions is 14-18 months.

Subculture duration of *in vitro* potato plantlets under usual culture regime is 3-4 weeks.

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

Potato plantlets with well-developed leaves, rooted, without visual signs of bacterial and fungal contaminations. In long-term culture the morphological state must correspond to the phenotypic appearance according to the time phase of culture.

### **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).

No GMO material is included in the National Programme of the Czech Republic.

## **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?

**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 3.2.2.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.

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

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

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

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

**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)?

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

**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)?

**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.)*

**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

**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.).

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

### **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).*

**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?

**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.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).

**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.

**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.

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

### **Box 3.3.3.A Ensuring Availability of Germplasm – Health Aspects**

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

**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.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.

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

**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*)

The accessions from the *in vitro* potato collection are available year-round. The speed of request fulfillment depends on the status in maintenance cycle; accessions are distributed as *in vitro* plantlets transferred from cold conditions. They are delivered to breeding workplaces, universities, research workplaces and other users, exceptionally to private persons (max. 10 accessions based on the Decree No. 458/2003 Coll.). Official rules set by the SMTA for germplasm distribution are followed. A list of maintained accessions is available in the online information system <http://grinczech.vurv.cz/gringlobal/search.aspx>.

**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 time of distribution only depends on the status in maintenance cycle of the given accession, and/or ensuring the testing for health state in accordance with the user’s request.

**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.

Delivered information: accession evidence number, (ECN – national evidence number) name, line evidence number. Detailed data are available in respective datasets.

### **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.

In the Decree No. 458/2003 Coll. the limit is set to a maximum of 10 accessions per user annually. We usually provide 5 plants 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 or plastic bags).

Culture and distribution of *in vitro* potato accessions are done 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.

For preparation of the requested accession 2 plantlets are used for cutting and multiplying to 5 plants, enabling distribution to the requestor and simultaneously regeneration subculture of the accession.

**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).

Upon request, it is possible to perform, in a feasible way, planting in the greenhouse for minituber production.

### **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.

Materials maintained in the *in vitro* potato collection are tested for six usual potato viruses (PLRV, PVA, PVM, PVS, PVX, PVY) in the workplace; virus elimination is done using removal of meristems after thermotherapy and chemotherapy (Ribavirin), testing for quarantine diseases is performed in the accredited workplace after *in vitro* culture establishment.

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

For delivery of accessions within EU a phytosanitary passport is issued (based on the EU directive) upon request of the user, for delivery outside EU valid regulations are followed.

**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, less than 10 *in vitro* potato plantlets per accession are distributed.

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

*In vitro* potato accessions are delivered in standard glass tubes.

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

*In vitro* potato accessions are continuously supplied during the year, except for winter period, using express mail-order.

### **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*).

**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 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/etc. 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*).

**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?

**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.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).

**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.

**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.

**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.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.

**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.

We use the documentation system GRIN Czech (Microsoft SQL) based on GRIN Global 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. We have been using the same system online as all institutions included in the National Programme. The website with all data is open to public with online seed ordering.

**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 data, storage data including 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 to be able to work with records.

**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 (in the coordinating centre of the National Programme network – Crop Research Institute in Prague) is responsible for running of the PGR documentation system GRIN Czech.

**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 twice a 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 curator of the collection uses assistance of the genebank documentation specialist from CRI to cooperating institutes within the National Programme network in order to install the user programme and uses 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.

**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

Other relevant information is available on the website.

### Reference

Murashige T, Skoog F. 1962. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarum* 15(3):473–497.