Quality Manual

I P K

Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung

Contact:

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)
Corrensstrasse 3
06466 Gatersleben
Germany
Phone: 0049-39482-5-0
Fax: 0049-39482-5-139
Internet: www.ipk-gatersleben.de
Email: info@ipk-gatersleben.de

Quality Manager
Dr. Ulrike Lohwasser
Phone: 0049-39482-5-282
Fax: 0049-39482-5-155
Email: lohwasse@ipk-gatersleben.de

Operational genebank manual of IPK

0 Date of compilation

Day/month/year: 15.03.2011

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 Federal ex situ Genebank is organized as a department at the Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben. It is overseen by its two funding bodies, the Federal Ministry of Education and Research (BMBF) and the State Ministry of Education and Cultural Affairs of Saxony Anhalt.

The national mandate of the Federal ex situ Genebank at IPK is mentioned in the statutes of IPK and details of the ensuing obligations and responsibilities are described in the standing rules of the Genebank. These include the range of crop species to be maintained along with the terms of reference regarding its acquisition, conservation and distribution. Moreover the standing rules address the research fields to be addressed (conservation and utilization of PGR). The target crops comprise agricultural and horticultural crop species from temperate regions except along with their wild relatives, excluding ornamentals and (fruit)trees.

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?*)
Based on a formal agreement of 1994 between the Federal ex situ Genebank of Germany and The Center for Genetic Resources (CGN), The Netherlands Wageningen (NL), the two institutions share the responsibilities for a defined set of germplasm (potatoes, sugar beet).

GA3 -In case your genebank has a germplasm acquisition policy, what does the policy entail?
a) please specify which crops or which geographic area, if applicable.
The genebank has no acquisition policy, which is written in stone. In the past selected geographic regions have been visited to collect a broad range of different crop species. The goal is to capture and safeguard the genetic diversity on a region scale. In addition to these efforts, crop specific collections have been performed for apple, forage grasses and potato.

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.)?
Each accession that is supposed to be included into the collection is firstly grown in a field nursery or in the greenhouse to determine its taxonomic status and to compare the material with other accessions. As to the remaining passport data, we rely on 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).*
Visual control of plant material followed by initial cultivation.
Potatoes: regarding wild species, seeds are obtained most of the time in quantities too low to conduct germination tests; therefore initial cultivation is conducted under quarantine conditions; tubers are first introduced to tissue culture, then – as well as newly received tissue cultures – checked for viral diseases internally and for quarantine diseases by responsible authorities; After all checks are positive the accession will be included in the active collection.
IPK genebank is certified according ISO 9001:2008. All processes are defined by procedure and working instructions.

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)*

SMTA is implemented since November 2009; all accessions are provided under the SMTA regulations, for non-annex1 crops an additional agreement is necessary. All documentation is recorded in our genebank information 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*).

Preferential planning and implementing multispecies missions for crops and their wild relatives on the basis of available ecogeographical and ethnobotanical data in close cooperation with specialists in the host countries and consulting local experts in the collecting areas. Germplasm collecting activities are generally based on written contracts between IPK and host institution/country.

Documentation of the original accessions by herbarium material during the mission and by herbarium material resp. material for seed, fruit and spike reference collections from the first multiplication in the genebank field.

Preparation of collection lists with preliminary botanical characterization before including the material into the genebank.

Verification of the botanical determination by the collectors/taxonomists during first multiplication in the genebank fields.

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 black-box(e.g. black-box; no specific arrangement; other);*

SEEDS: Specific arrangement with NordGen. Safety duplicates from recent multiplications (not older than two years) are sent to Svalbard.

In vitro and Cryo material: black box system for potato and mint installed, for garlic establishment of safety duplication planned within EURALLIVEG

b) *The location(s) where you store your safety duplicates (country; genebank*

SEEDS: Norway, SGSV

In vitro and Cryo Material: potato and mint – DSMZ Braunschweig, Germany; garlic: CRI Prague, Czech Republic, RIVC Skierniewice, Poland (genebanks)

c) *Whether or not you are using a formal agreement with the genebank(s) that store your duplicates*

SEEDS: Yes a formal agreement

In vitro and Cryo Material: potato and mint: Formal agreement with DSMZ Braunschweig implemented

d) *Whether the safety duplicates are stored under conditions comparable to your own? Please provide details*

SEEDS: in freezing room, at –18°C at SGSV and -18°C at IPK;
Cryopreservation in liquid nitrogen same as IPK's conditions

e) *Do you maintain safety duplicates from other genebanks at your genebank?*

Yes, from CGN, The Netherlands and FAL-Agroscope Reckenholz-Tänikon, Switzerland and the German Collection of Microorganisms and Cell Cultures (DSMZ)

If so, do you know any details of that material?)

In some cases we have a list with all accession numbers and species, in others black box material

SD2 – Do you have a safety duplication policy? If so, please provide essential details.
Yes, only fresh material after regeneration having adequate germinability qualifies as safety duplicate. Seed samples are divided by separately storing species with either short, medium or long term storability.

Box 2.1.2 Structure
SS1 - Please provide details on how your genebank building has been designed to resist natural disasters (e.g. earthquakes; flood; storm).
No earthquake area; no high wind/storm exposure; standard construction practices were followed; Building holding cold stores has been raised 1.5 m above ground to prevent flooding;

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*).
Genebank is located in a fenced territory which at night is supervised by a security service; Locked doors; Alarm system for temperature deviations in cold stores, Fire detectors; In case of blackouts standby generators will automatically kick in.

SS3 – Please provide information on any other structural security aspects that you might have in place.
Automatic CO_2 fire extinguisher for herbarium collections, admission of authorised staff only

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*).
There is a stand-by duty for the entire institute consisting of a technical engineer, an electrician and a mechanic. Based on a facility management system an alarm system for temperature deviations in cold stores exists. In case of blackouts standby generators will automatically kick in.

SE2 – Describe how you monitor temperature and relative humidity in your cold stores and drying room?
Headquarters: Computer controlled facility management system. Branch stations: daily control by staff

Box 2.1.4 Institutional and Personnel Security

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

(*Aspects to consider are:*

a) *timely transfer of funds from the "mother" organization to the genebank;*

b) *do you have direct access to the "mother" organization that provides the budget?;*

c) *internal "security" of accessing these funds;*

d) *long-term security and stability of funding (compensation of inflation rates, avoiding variation in years)*

e) *any other observations that are relevant in this context)*.

Foundation under public law, funding provided annually by Federal and State Ministry

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

Staff secured by permanent work contracts. Adequacy of staffing monitored by regular external reviews

Box 2.1.5 Contingency Plans:

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

The genebank has contingency plan to cover all conceivable risks.

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

Staff informed and trained regularly about emergency situations like fire and health hazards

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" 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.).

Reproduction protocols (cultivation, pollination, pre- and post harvest treatments, etc.) for all crops are internally available (only in German) available.

IV2 – Describe procedures how you deal with a) dormancy and b) hard seeds?
Protocols for seed pre-treatment (gibberellic acid, cold treatment scratching) internally (only in German) available

IV3 – Please provide any other information on procedures that you follow to ensure highest possible initial viability.
Harvest and post harvest treatment as gentle as possible (crop specific protocols only in German)

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*).
Crop specific protocols for germination testing internally (only in German) available following ISTA standards adopted for IPK

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
Following a species-specific schedule that takes into account empirical expertise on the decay of germination rates, germination tests are performed.

VM3 - Please provide information on non-specific thresholds that you might use for viability of seeds (i.e. percentage of germination) and for the amount of seeds left of an accession to initiate regeneration? ?: less than 50 % germ rate and/or less than 2 grams seed amount *In case you differentiate between self- and outbreeding species, please answer for each category separately.*
Generally, for self- and outbreeding species regeneration takes place, if 1. germination rate falls below 70%, or 2. remaining seed stock has been depleted below amount needed for two regeneration cycles

Box 3.1.3.A Seed Storage Conditions (for the different types of collections, i.e. short/medium- or long-term storage)
SC1 - Please provide details on temperature and relative humidity conditions of your storage and drying rooms. In case they vary from room to room, please provide details for each.
Short and long term storage at -18 °C (part of the short term storage collection at +4 °C (potatoes))
Pre drying 18 °C, 20%RH
Final drying 18 °C, 10%RH

SC2 – Provide details on the type of containers and the packaging procedures (and the corresponding equipment, if any) that you use.
Active collection: air-tight glass jars with silica gel as humidity control agent

Base collection and safety duplicates: laminated aluminium bags sealed under vacuum

SC3 – What is the range of seed moisture contents (smc) of your stored seeds of different species; what measures do you apply and/or monitor the (low) moisture level? Do you treat different species differently?
<8% for all species (no species-specific differentiation); humidity is controlled by a computer system

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
Total storage capacity for 180,000 glass jars in Gatersleben and the two branches (number of accessions depends on seed size), 90% filled.

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

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).
Donor material is recommended to be pre-potted in greenhouse for an appropriate time prior to use. Plant protection measures should be performed to prevent diseases. In garlic, bulbils (if present) are preferred to be used instead of cloves.

IV2 – Describe procedures of explant isolation (organ source in the plant, manipulations) and sterilization (chemical and handling) of the explants.
Potatoes: organ source: vigorous sprouts, size appr. 1 cm. completely cut, transferred to sodium hypochlorite (5%) for 30 sec, 2x washed with distilled water for 30 sec each, transferred to Murashige & Skoog medium
In garlic, shallot and other *Allium* crops either shoot tips on the basal plate, unripe inflorescences or bulbils are used for primary explants. In mint and other Lamiaceae species, shoot tips and sometimes nodal cuttings are used as primary explants. They are sterilized shortly by alcohol and then by sodium hypochlorite (3 % active chlorine). For culture standard media based on the formulation of Murashige & Skoog (1962) are used with modifications specific for the cultivated species.

IV3 – Please provide any other information on procedures that you follow to ensure highest possible initial viability
As source for explants only healthy material is used.

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

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.

No specific system implemented. Experience and skill of the technical staff is crucial for special decision.

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

Decisions on the cultivation temperature and multiplication regimes are taken according to the personal experience of the responsible staff members.
Decisions are made accession-specific and will be recorded in the running laboratory protocols. They cannot be published as standardized recommendations.

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

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

Potatoes: standard in vitro culture room: 20 °C, 16 h light, 70 % humidity;
Induction of microtuber formation: 10 °C, 10h light, 80 % humidity;
microtuber storage: 4 °C, no light, 80 % humidity;

Others: Temperature controlled culture rooms (25 °C and 20 °C) with mean light intensity of 60-80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with 16 h light and relative humidity of 35 – 38 %. Cold-storage rooms have 4 - 7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (at 10 °C) and 2 - 4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (at 2 °C) with 12 h light and relative humidity of 85 – 88 %.

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 tubes, inner diameter 1,6 cm , length 15,6 cm; scissors, forceps, scalpel, heat sterilizer (250 °C), Bunsen burner, glass cutting plate, sterile bench, alcohol

Others: depending on the species and step of the procedure, we use various types of glass vessels: tubes of ø 3 cm and 9.5 cm length for *Allium* of ø 2.6 and 15 cm length for yams *in vitro* storage, conserve jars of ø 7 cm and 10 cm height for all other cultures (*Allium*, mint and minor crops). Tubes are covered by aluminium caps and jars covered by glass lids fixed by a metal clip and equipped by a felt ring for gas exchange. Transfer is done under the laminar flow box by means of instruments pre-sterilized in an oven and permanently re-sterilized by glass bead sterilizers. Gas safety burners are used to flame the vessels.

SC3 – Please include any other aspects regarding in vitro culture and storage conditions at your genebank that you regard as important.
All *in vitro* material is endangered by endophytes, which cannot be removed completely from the explants by sterilization. Two factors may be serious obstacles for *in vitro* storage in the long term: 1) sudden outbreaks of bacteria after changes of culture conditions, conditions of high stress or even spontaneously, 2) gradual vigor decline of the material until total dying off. The effect of endophytes depends on the genotype, the history of the plants prior to *in vitro* culture and the conditions within culture.

Hyperhydricity can be reduced by cooling the shelves by about 1 - 2 °C in comparison to the surrounding air. This causes retention of water within the agar at the bottom of the vessel and lower humidity of the culture vessel air. The reduction of air humidity is favorable to reduce hyperhydricity. One third of our culture room shelves are equipped by a cooling system.

Since hyperhydricity is not a problem in warm culture under our conditions, we use cooling shelves and their favorable effects mainly for special stages in the recovery phase of cryopreservation. In cold storage hyperhydricity is a reason for shortening the storage time. This will be decided in each individual case by visual monitoring (see above VM1).

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.
When a given protocol is exactly followed, three factors are most important for highest initial viability: 1) quality of explant preparation, 2) absence of endophytes 3) genotype.

1) requires qualification and training of the staff with respect to all tissue culture procedures needed and knowledge about the morphogenetic patterns and special reaction of target species and exact observance of the suitable developmental stages of the donor material,

2) requires bacteria tests of the material, exclusion of the obviously heavily infested material and monitoring of the material not so clearly infested.

3) This is an intrinsic factor, which cannot be influenced. In cases of very weak material it may happen that is must be declared “non-storable with the given protocol”.

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]
There is no policy for random viability tests. We monitor viability in all cases of requests of material and when scientific experiments on material are performed. As testing is laborious, we used a situation of additional temporary staff for a general test of all accessions present in cryostorage at a given date. The criterion of the suitability of the method used is the regeneration rate, which did not significantly change in comparison to the initial rate. The conclusion was to continue using this method.

VM2 - Please describe the information “system” that you might have in place that allows you to make more species or even accession-specific decisions.
No special system is present. Experience and skill of the technical staff is crucial for special decision.

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 regeneration control is one third of the total cooled sample (e.g. total cooled sample 300 explants = 200 stored explants + 100 explants for regeneration control)
b. any thresholds that you use [e.g. discard the material as not storable below a certain regeneration rate of the control],
   Under our conditions, the lowest acceptable limit for regeneration in the control is 10 %. Weaker material is declared as non-storable by the given method.
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 case of *Allium*, we store 100 explants regularly. When regeneration rate is between 10 and 30 %, we store a second sample. When regeneration is below 10 %, we declare the sample as non-storable by the given method.
   If an accession is lower than 10 %, we request new source plant material and repeat the procedure in order to find out, whether it might possible that the weak reaction is especially caused by the situation of the former sample (e.g. strong endophytic concentration).
d. what is the threshold number of remaining explants of a given

accession under which you initiate regeneration for multiplication?
Samples originally consisting of 200 explants need regeneration when explant number falls below 100. Samples of 100 explants (*Allium*) will be regenerated when the explant number falls below 60.

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.
Liquid nitrogen is conducted by supra-isolated pipe system from the bulk nitrogen tank to the storage containers. Individual containers are filled by manually operating valves.

SC2 – Provide details on the type of cryopreservation tanks and storage system within the tank that you use.
We use cryotanks 4800 RS (Harsco) and CryoSystem 6000 (MVE) provided with standard rack systems consisting of 6 racks with 8 boxes (4800 RS) or 10 boxes (CryoSystem 6000) and 100 ampoules per box.

SC3 - Do you treat different species differently?
Different protocols are used for cryopreservation: potato is cryopreserved by the DMSO droplet method, garlic by vitrification and mint by droplet vitrification. All material is then stored in the same way.
All these protocols are published. In case of modifications, an exact record of the date of their introduction is kept in the cryopreservation protocol in a separate Excel worksheet.

SC4 – Please include any other aspects regarding storage conditions at your genebank that you regard as important.
The choice of the cryopreservation method is mainly justified by empiric experience. Evidence exists also in our laboratory, that other methods are suitable, too. It is, however, advisable to use always the same method in routine processes in order to be better aware of emerging problems like infection, weak genotypes or accidents.
The used methods are published either in Barbara Reed's book "Plant Cryopreservation A Practical Guide", Springer, 2008, or in publications in CryoLetters. For *Allium*, it is also on the ECPGR Website as a result of the Model Crops Meeting in Skierniewice.

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.).
Procedures are given for potatoes, protocols for other vegetatively maintained

crops are internally (only in German) available.

Potatoes: after harvest, potato tubers are screened visually and deformed, damaged or rotten tubers are discarded. Only visually healthy material is stored at 4 °C. In the following spring the potato tubers are taken out of the refrigeration and treated with Monceren (800 ml/40 l water) to reduce the infection with *Rhizoctonia solani*, to obtain an even crop emergence and to support a healthy plant development. After planting, measures such as fertilization and pest management are carried out according to agricultural standards.

IV2 – Describe any particular procedures you use (e.g. which organ of the donor plant you use to reproduce the planting material).
field material reproduced either from field or greenhouse tubers

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

Box 3.1.2 .D Viability Monitoring
VM1 - Describe the routine field genebank monitoring system that you use. (*The monitoring system could include the following aspects: regular control of disease or pest contamination, other types of damages to the plants, etc*).
Plant health is checked every second or third day and visually diseased plants are removed weekly; pest control measures are taken 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.
monitoring takes place frequently during growing season by permanent staff. Data included in the genebank information system.

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.
Cultivation and pest/disease management according to good agricultural practice (well trained staff); specialists for pest and disease control available; no irrigation; fences against wild boars or deer

SC2 – In the case of annual or sub-perennial species that cannot over-winter in the field genebank, what measures do you take?
potatoes harvested every fall, harvested tubers planted next spring

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 – 3.2.5.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 – 3.2.5.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)?

Yes, number of seeds or weight of sample

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.
seed storage containers: see above; number of seeds per container not fixed but traceable via database

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)?
Species (accession) specific thresholds (amount of at least two sowings) depending on reproduction biology, heterogeneity of accession and biostatus

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;*
Crop specific distance (wind pollinated cross pollinators > 100m, avoiding downstream planting in major wind direction) and isolation methods (isolation cabins, tents, bags, glass-houses)

b. *The use of pollination cages for insect pollinated species;*
Yes, nets and cabins of different sizes.

c. *The use of specific pollinators for insect pollinated species;*
Solitary bees (Osmia rufa) for all species, bumble bees especially for red clover and alfalfa , flies for carrot

d. *Strategies to ensure that males and females participate equally in the reproduction*).
No strategy as yet

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.)*

For outbreeding species the number of individuals is kept as large as possible to reduce the effects of genetic drift, depending on regeneration capacities. Species-specific data available as internal (only in German) protocols.

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?;*
German field conditions and standard green-house conditions for regeneration, no special environmental conditions.

b) *Do you use controlled environments?;*
In the greenhouse the conditions are controlled and adaptable.

c) *Do you collaborate with other genebanks in Europe?;*
no explicit collaborations, just exchange of experience

d) *others*).

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

Box 3.2.4.A Seed Processing Procedures

SPP1 – Describe the protocol(s) that you use for threshing and seed cleaning.
Threshing and cleaning by machines and by hand, sieving, hand cleaning and hand sorting, partly enzyme treatment (e.g. tomato seeds)

SPP2 – Describe the protocol(s) that you use for seed drying, including whether you use different drying procedures for different types of species.
Pre-drying of fresh harvested material at 18°C, 20%RH (around 10 days for all species depending on the weather conditions during harvest)
Final drying of cleaned seeds 18°C, 10-15%RH (around 10 days for all species depending on the weather conditions). Seed moisture content around 3-7%.

SPP3 – Please describe how you keep the time between harvesting and the actual (long-term) storage of seeds as short as possible.
Finish post harvest treatments as fast as possible, depending on staff capacity

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).
Drying chambers, after drying at room temp and ambient humidity until completion of germination tests.

SPP5 – Describe the criteria you use to decide on seed quantity per accession for the long-term storage.
As much as possible. Upper limit is given by size of storage container (glass jars, volume 1 liter). Large seed species (*Vicia faba*, *Phaseolus coccineus*) get two containers.

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).
No GMOs in the genebank

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

B. In vitro Culture Collections

Box 3.2.1.B In vitro Culture Vessels and Sample Size
SCSS1 – Indicate if you document the initial number of explants of individual accessions when culture is initiated (from one or from more clonal donor plants)?
When it is clear, that the accessions are true clones, the *in vitro* cultures are treated as a whole clone (generally in mint, yams and others). In unclear cases, such as in many garlic accessions, a special suffix was added to the clone number to be sure that we produce true clones. The number of initial explants is documented in our system. In populations, such as in wild *Allium* species, the clonality is always ensured by separate treatment and addition of suffixes to the accession numbers.

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.
Medium is usually based on Murashige and Skoog's (1962) formulation. As a rule, phytohormones are not used for storage. In *Allium,* sometimes 0.5 mg/l 2-iP and 0.1 mg/l NAA are used for limited increase of multiplication. In *Dioscorea* one part of the clones is cultivated on medium with 2 mg/l BAP and 0.1 mg/l NAA. There, activated charcoal is used to reduce self-poisoning by phenolic exudates. Cutting of bunches in *Allium* and nodes in the other plants is usually done in order to release already existing buds from apical dormancy. A certain extent of adventitious bud formation is present in *Allium*, especially in the primary phases. Callus is excluded. In special cases callus is formed secondarily at the bottom of explants (cut surface). It is usually cut off from the explants during transfer to the next subculture.

SCSS3 – Please indicate whether or not you use a minimum number of in vitro plantlets per accession?
The minimum number of *in vitro* plantlets per accession is 40 (*Artemisia, Antirrhinum, Mentha*), 36 (*Brassica, Orthosiphon*), 10-50 (*Allium*), 18 (*Dioscorea*).

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)
Potatoes: 2 lines with at least 3 plantlets per line permanently, 6 plantlets per

line for microtuber induction, 5 plantlets per line for microtuber storage

OTHERS: We cultivate regularly two subclones independently of each other with respect to the time of transfers and most often in separate chambers.

SPP2 – Describe the sub-culture duration (if not crop specific)
Potatoes: 4 to 6 weeks

OTHERS: Sub-culture duration is 2-5 months in *Artemisia*, *Mentha* and *Orthosiphon, Dioscorea* is cultivated in subcultures of 6-8 months.

SPP3 – Describe the criteria you use to decide on in vitro plant quality (if not crop specific).
Potatoes: No bacteria/fungi/callus formation, healthy looking
OTHERS: Criteria are good vigor and no hyperhydricity, all other is crop-specific.

Box 3.2.3.B Genetically Modified Material
GMM1 – In case you treat GMO material differently from "normal germplasm", please provide here the details for each of the deviating procedures (and equipment).
We do not store genetically modified material.

C. Cryopreserved Collections

Box 3.2.1.C Cryopreservation Containers and Sample Size
SCSS1 – Indicate if you document the initial number of explants of individual accessions?
The initial number of explants and the regeneration control explants are documented in our system.

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.
The material is stored in cryotanks. We use cryotanks 4800 RS (Harsco) and CryoSystem 6000 (MVE) provided with standard rack systems consisting of 6 racks with 8 boxes (4800 RS) or 10 boxes (CryoSystem 6000) and 100 ampoules per box. Cryoboxes are from Nunc, ampoules (tubes) are also from Nunc. They are equipped with special ribs on the bottom and inner thread to ensure opening by one hand and color caps for quick recognition of the right samples.

SCSS3 - What is the number of explants that you use as the minimum threshold per accession?
Minimum threshold is 200, in case of *Allium* only 100 (see above the discussion about safely limits).

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 acclimation is used for most species: 22 / 8 °C – day/night (potato) or 25 / -1 °C – day/night (*Allium*, mint)

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

SPP3 – Describe the protocols that you use for regeneration (slow or fast rewarming, washing, dark periods etc.)
Potato: Fast rewarming by plunging aluminium foil strips in liquid medium at room temperature. All other crops: fast rewarming in water bath 40 °C, washing with liquid medium containing 1.2 M sucrose, dark periods of 1 week in *Allium* only. Mint is kept 24 h in dark.

SPP4 – Describe the time span and method(s) of survival and regeneration controls.
Survival is counted 2 weeks (*Allium*, *Mentha*) or 3-4 weeks (potato) after rewarming. Regeneration is counted 4-6 weeks (*Mentha*), 6-8 weeks (potato) or 7-10 weeks (*Allium*) after rewarming.

SPP5 – Describe the criteria you use to decide on explant quantity per accession for the long-term storage.
The numbers are standardized for all material. Since preparation is much more laborious for *Allium* than for the other crops, reduction to 100 explants was decided with the option to store more in case of low regeneration (see above).

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).
We do not store genetically modified material.

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)?
Procedures are given for potatoes, protocols for other vegetatively propagated crops are internally available
Initial number of plants is documented. However, but as newly introduced

material passes in vitro culture first, we generally start with two initial tubers

SCSS2 – Please describe what kind of procedures you follow, if any, with respect to sub-sampling and subsequent place/container/etc. of maintenance?
none

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)?
10 plants, all 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);*
not relevant

b. *The use of pollination cages for insect pollinated species;*
not relevant

c. *The use of specific pollinators for insect pollinated species;*
not relevant

d. *Strategies to ensure that males and females participate equally in the reproduction*).
not relevant

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, but tuber harvest, if possible, not at high soil moisture
.

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.).
storage in potato boxes at room temperature for drying, after three days transferred to cold storage at 4°C with appr. 80% humidity

SPP3 – Describe the criteria you use to decide on the number of plants per accession intended for the long-term conservation.
due to clonal nature, only few tubers already sufficient; 20 tubers stored until planting next spring

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*).

All requests are processed based on SMTA (no restrictions). For inbreeding crops 30 seeds are shipped. In case of outbreeding and heterogeneous crops/accessions 100 seeds are shipped. In specific cases (e.g. research projects) more seeds will be available based on case by case decisions.

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?

Max handling time is 4 weeks after receiving signed SMTA, special regulations for potatoes:
health tested accession see above; untested accession: depending, at least three months

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.

Passport data, Other information only on request and availability

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).

Crop specific; as a rule of thumb: 30 seeds for inbreeding species, 100 seeds for outbreeding species

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.

Each harvest from a given accession will be placed in a different bag/container.

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.

The available amount of seed and the corresponding germination data can be retrieved for each accession from the genebank information system. The absolute lowest minimum is the amount of seeds necessary for two sowings

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.
Potatoes: only seed transmitted diseases are tested (PSTVd, Andean viruses), other diseases irrelevant, tests conducted once per accession
Others: No crop specific tests

AGHA2 – Describe how you follow plant quarantine rules and regulations when exporting germplasm abroad (especially to countries at another continent).
within EU provision of plant passports (potatoes only), outside EU phytosanitary certificate (valid health tests and mostly import permits) required.

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.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.
Depending on availability:
Selfbreeding crops 30-50 seeds
Outbreeding and heterogeneous crops/accessions around 100 seeds

GS2 – As GS1 above, but in case your germplasm samples do not possess the minimum viability, would you increase the number of seeds?
only if sufficient seeds are 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)*
Potatoes: material options indicated in online information system; availability year round, but depending on status in maintenance cycle (dormant microtubers usually not distributed); *in vitro* samples one of two options,

usually not distributed to private individuals; no restrictive conditions/limitations in total amount of accessions; SMTA as formal agreement to distribute the germplasm

Mint: In vitro cultures are regularly distributed, where *in vitro* material exists.

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?
due to maintenance cycle requirements no time period fixable, additional factors: health testing at outside-IPK authorities

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.
typical information: acc. name and number, numb. of iv line/plantlet, country of origin and year of varietal listing, partially pedigree and donor; more information via GBIS or on request from curator

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.
no distinct limits fixed, but usually 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).
Potatoes: culture and distribution in glass tube

Others: distributed in plastic bags with wet paper inside.

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.
generally between 6 and 12

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).
if explicitly requested, transfer of material to greenhouse for mini-tuber production or vegetative clones is offered

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.
Potatoes: iv material tested for six viruses (PLRV, PVA; PVM; PVS; PVX; PVY) internally and virus removed either by chemical (ribavirine) or heat

treatment; freedom of quarantine disease tested in by official authorities, at least after iv establishment and before distribution/transfer to greenhouse/field
Others: No further policy is followed on disease-free material

AGHA2 – Describe how you follow plant quarantine rules and regulations when exporting germplasm abroad (especially to countries at another continent).
within EU provision of plant passports (potatoes only), phytosanitary certificate for outside EU provision (valid health tests and mostly import permits required)

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.
Potatoes: standard cultivation glass tube

Others: In plastic bags with wet paper inside.

GS3 – Please provide information on any other aspects related to in vitro plant supply.
not shipped in periods of frost; express delivery for remote countries, even within Europe (periods without light as short as possible)

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*).
There is no distribution of cryopreserved material until now. If so, we will follow the general distribution policy.

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?

Due to maintenance cycle requirements no time period fixable

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.
Typical information: acc. name and number, no. of cryo line/plantlet, country of origin and year of varietal listing, partially pedigree and donor; more information via GBIS or by request from curator

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.

There is no direct distribution of cryopreserved material. In potato, so far cryopreservation is the safety backup and will be sent to the *in vitro* collection in case of exhausting the *in vitro* clone. This is done as *in vitro* cultures.

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.
Virus-free material of *Allium* was produced in a project some years ago and was transferred in cryopreservation to some extent. There is no further policy on disease-free material.
We store virus-free and virus-containing material in separate tanks.

AGHA2 – Describe how you follow plant quarantine rules and regulations when exporting germplasm abroad (especially to countries at another continent).
Quarantine rules are the same as in the whole genebank.

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

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

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.
In case of potato re-transfer to the *in vitro* collection the number of re-warmed explants is depending on the regeneration rates of the control (regularly 1-2 tubes – 10-20 explants)

GS2 – Please provide details of your routine methodology of containers etc. that you use to distribute cryopreserved material.
We do not send material in cryopreservation. Material from cryopreservation is sent in the *in vitro* phase.

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*).
No species specificity, no immediate distribution, if no tubers or other vegetative clones are available (but feasible after regeneration; delay communicated to requestor); SMTA as 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?
due to availability only after field harvest and in case of potato only with existence of valid health tests, tuber distribution only from fall to spring, due to frost sensitivity usually not during winter

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.
typical information: acc. name and number, country of origin and year of varietal listing, brief description of accession, partially pedigree and donor; more information via Genebank Information System (GBIS) or by request from curator

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).
3 – 5 clones per accession, depending on availability

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.
For potatoes every year 10 plants for multiplication in the field, at lower yield all tubers stored over winter, at higher yields 150 tubers maximum; due to annual need for recultivation constant necessity for multiplication. Other crops are maintained permanently in the field

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.
Potatoes: for introduction of new material see in vitro section, thus only healthy material in the field; field maintained material screened at least every third year for PSTVd and *Ralstonia* plus *Clavibacter*, screening for Andean viruses only once and only if material originates from this area
Others: no further policy is followed on disease-free material

AGHA2 – Describe how you follow plant quarantine rules and regulations when exporting germplasm abroad (especially to countries at another continent).
within EU provision of plant passports (potatoes), phytosanitary certificate for outside EU provision (valid health tests and mostly import permits required)

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

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.
Only depending on availability

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.

IPK has its own genebank information system (Oracle database) consisting of:
(I) internal management system which includes passport data, botanical determination, handling of botanical names, seed storage data, germination data, regeneration information etc.
(II) Mobile data management for agronomical and morphological data in order to do online data acquisition directly during regeneration without using paper sheets
(III) Internet system public available with the possibility of on-line 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.
passport data, botanical determination, handling of botanical names (taxonomy), seed storage data, germination data, regeneration information, characterization and evaluation data etc.

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

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).
with short excerpt of passport data; if requested and available additional data can be provided as Excel file

GD5 - Provide information on how technical support for development and maintenance of the documentation system is arranged
One person (permanent staff) is responsible for technical support

GD6 – Describe your genebank policy with respect to backing-up of the

database contents, including with which frequency?
Automatic permanent back-up system

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

Box 4.2 Information Exchange

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

IE2 - Please indicate if your data is available as machine to machine webservices. 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 which are available in our internet database are also available for GBIF and other web-services

IE3 - Please indicate if your data is published to EURISCO. Describe which data is published to EURISCO and at which intervals.
Our data are published to EURISCO and updated regularly.

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
Botanical name
Country of origin
Life form