Operational Genebank Manual of the "Mihai Cristea" Plant Genetic Resources Bank, Suceava, Romania (SVGB)



Banca de Resurse Genetice Vegetale Suceava



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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 second 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.

Suceava Genebank (SVGB) is a public agricultural research entity reporting to the "Gheorghe Ionescu Sisesti" Agricultural and Forestry Sciences Academy in Bucharest. Since its establishment in 1990, the Genebank was mandated to preserve under the long- and medium-term conditions all seed propagated species with relevance for Romanian agriculture. Besides, *ex situ* conservation activities, in the last years, the *in situ on farm* conservation strategy was addressed and supported. This was done mainly through providing, by free, small quantities of seeds belonging to local populations, especially vegetable and grain legume species to Romanians who agree to spread and grow in their fields or gardens, on a long term, those varieties.

Suceava Genebank keeps in its collections a total of 430 plant species, stored as seeds (classified into the following categories of crops: cereals, grain legumes, fodder grasses, forage legumes, vegetables, roots and tuber crops, industrial crops, medicinal and aromatic plants and ornamentals), field collections (potato, garlic and onion genotypes) and as "in vitro" seedlings, in slow growing conditions (local potato genotypes and garlic).

Approximatively 60% of the preserved material consists of local landraces, the result of more than 50 collecting missions, which geographically cover 1549 localities in all counties of the country.

All material is available under the terms and conditions of the SMTA of the International Treaty for Plant Genetic Resources for Food and Agriculture, including non-annex I material.

Genebank is a National Coordinator for Plant Genetic Resources related activities, as well as it is the National Focal Point for the implementation of the International Treaty for Plant Genetic Resources for Food and Agriculture.

Further information about SVGB and its mandate is provided on the SVGB website (www.svgenebank.ro).

To assure the quality of its operations Suceava Genebank has adopted a quality management system according to EN ISO 9001:2015.

GA2 – Specific agreements. Does your genebank have any specific formal agreements with other genebanks regarding the conservation of specified germplasm?

This should include:

- a) whether or not your genebank has any international agreements to conserve specified germplasm on behalf of other countries,
- b) a specific region, and/or
- c) the world, and
- d) which crops or genepools fall under these agreements?

No agreements for any plant species.

GA3 – In case your genebank has a germplasm acquisition policy, what does the policy entail?

Please specify which crops or which geographic area, if applicable.

The acquisition strategy has been changed over the years, according to the level of development of the Genebank and the main interests of the germplasm's users. Three ways are used to enlarge Genebank's collections, i.e., taking over breeding material from public domain breeding entities, collecting and exchanges with other stakeholders worldwide.

Landraces, obsolete cultivars and lastly crop wild relatives are prioritized for collecting actions due to the high threat of genetic erosion, followed by breeding materials with known and useful traits.

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.)?

For samples coming from other Gene banks or breeding and research entities, we rely on the information received from those donors.

Samples coming from our own collecting missions prior to their introduction into permanent conservation are checked by the curators during the first multiplication cycle in the Genebank's experimental field or greenhouses.

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).

The newly received biological material is visually examined by the curators to decide whether it meets the purity and health standards to enter the collections, following ISO Quality System of Genebank.

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 2007, all accessions being provided under the SMTA regulations. The SMTA is also used for non-Annex I species.

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.

Each collecting expedition is organized in accordance with the Annual Research and Development Plan, the ongoing projects and the budget allocated to this activity.

Priorities are established after analysing the information in the BIOGEN database, to cover gaps in the collections, in terms of species, varieties and geographical areas. Updated information on the main agricultural crops and as far as possible the traditional varieties grown in the area are obtained from local agricultural extension agencies. In general, the periods of collection missions are planned to be carry out in March - April, before sowing, respectively in autumn, during or after harvest. Sampling is done either from the farmers' fields/gardens, the farm or household store, as well as from local markets. In addition to passport descriptors, the collecting of biological material is accompanied by the recording of local knowledge, using a questionnaire dedicated to on-farm descriptors.

The wild and weedy forms, relatives of the cultivated plants, are collected, especially, from natural reserves, with the consent of the custodians of these protected areas and in compliance with the provisions contained in the International Code of Conduct for Germplasm Collecting and Transfer (FAO, 1994).

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

Collecting / exploration missions are also organized with mixed, multinational teams, based on bi- or multi-lateral agreements concluded and approved by responsible authorities from the participating countries.

2. Ensuring Security

This chapter refers to the security of the genebank structure itself (i.e. its physical security), the safety of its germplasm (i.e. the maintenance of viability) as well as the institutional and personnel security, aspects which together will ensure the long-term conservation of the entire collection.

2.1. Physical Security

To ensure the physical security of the collections, the following aspects are regarded as essential elements for achieving the objective:

Box 2.1.1. Safety Duplication (of long-term conserved germplasm)

SD1 – Please describe how your genebank implements the safety duplication of your germplasm material.

This description should include the following aspects:

- a) the type of safety duplication (e.g. black-box; no specific arrangement; other);
- b) the location(s) where you store your safety-duplicates (country; genebank);
- c) whether or not you are using a formal agreement with the genebank(s) that store your duplicates?
- d) whether the safety-duplicates are stored under conditions comparable to your own? Please provide details;
- e) do you maintain safety-duplicates from other genebanks at your genebank? If so, do you know any details of that material?

The unique accessions, collected from Romania and all the non-confidential material, with local or foreign origin will be sent for conservation to Svalbard Seed Vault. The first shipment was made at the beginning of 2020.

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

AEGIS accessions will be duplicated in the world collection in Svalbard Seed Vault.

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 located in Suceava town, at 325 m altitude, in a geological stable region of the North-Eastern part of Romania. The building was designed to withstand earthquakes of more than 7 on the Richter scale.

The temperate climate of the country does not generate extreme environmental conditions, such as: monsoon, typhoon, hurricane, etc. There is also a safe distance to the nearest running water, the Suceava River, about 3.4 km in a straight line, and the building is located at an upper-level difference of 87 m, which protects the building from 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).

The perimeter of the institute is delimited by the public area with fences. The building and access doors are monitored with a video system, which also covers the experimental field and greenhouses. Smoke detectors are located inside, while for fire interventions, each room is equipped with one or two fire extinguishers. Interior and exterior hydrants complement the fire protection system. The compressors for cold rooms have an electrical back-up system provided by a standby generator.

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

Box 2.1.3. Security Equipment

- **SE1** Provide details on the kind of emergency (back-up) equipment or arrangements that you have in place to ensure permanent electricity and cooling. *Aspects to consider are:*
 - a) "back-up" compressors for your cold rooms;
 - b) generator;
 - c) regular maintenance and trial runs;
 - d) other.

There is a spare for each type of compressor for the cold rooms. The main power is monitored by an automatic standby generator that starts when the main power is turned off or in case of outage. Every day, the compressors and cold rooms are monitored by the Genebank's specialist engineer. Regular maintenance is performed on the compressors in operation and periodic tests on those that serve the cold chambers that do not yet host samples, for the time being. Weekly, the operating status of the generator is checked and recorded.

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

Because the seed samples are stored in airtight containers, the relative humidity of the cold rooms is not controlled, while the temperature is strictly monitored, and the values are recorded by a computerized system.

The drying chambers have relative humidity and temperature monitored by dehumidifier sensors and independent devices, used to check the former ones. The independent recording devices have the possibility to record the evolution of the values of relative humidity and temperature.

Box 2.1.4. Institutional and Personnel Security

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

Aspects to consider are:

- a) timely transfer of funds from the "mother" organization to the genebank;
- b) do you have direct access to the "mother" organization that provides the budget?;
- c) internal "security" of accessing these funds;
- d) long-term security and stability of funding (compensation of inflation rates, avoiding variation in years)
- e) any other observations that are relevant in this context.

The Genebank is a public institution with finance provided from the state budget for basic activities and with the possibility of supplementation through extra-budgetary funds obtained from research projects. The budget construction is based on the annual proposals of the Genebank and approved by the "Gheorghe Ionescu Sisesti" Academy of Agricultural and Forestry Sciences, Bucharest.

There are no threats in terms of securing funding.

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

The institution operates based on the Government Decision no. 112/2018 and has in the organizational chart 25 positions, currently being occupied 24, all with a permanent individual employment contract.

Box 2.1.5. Contingency Plans

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

The Genebank has a contingency plan accredited by an authorized state institution. It includes administrative and organizational methods to minimize a broad range of risks. The plan consists of elements of information and property security, an evacuation component of the institute's staff and assets as well as a plan for the resilience of the institute's activities.

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.

The Genebank contracts specialized services for periodical training and briefing the staff about security risks, emergencies, and health risks.

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)

A. Seed Collections

Box 3.1.1.A. Initial seed viability

IV1 – Describe the procedures or practices that you have in place to ensure the highest possible initial viability of your seed, in particular during regeneration and post-harvest (e.g. cultivation practices, pollination aspects, use of specific equipment as shelters, storage of harvested seeds, cleaning, etc.).

During regeneration or multiplication activities, all procedures imposed by the biological requirements of the species are respected, starting with those related to the isolation of cross-pollinated genotypes, distances between plants, agrotechnical needs, or any other methods that may help to obtain an initial germination value, over 85%.

In the field genebank and during processing of the samples, the identity of the material is ensured by labelling and any possibility of contamination with other biological material or with various pathogens is avoided.

After receiving the seeds in the conservation laboratory, they are stored in a preservation cell, at $+4^{\circ}$ C, or in one of the drying chambers, if the space is available.

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

Various methods, such as scratching, pre-cooling/heating, wetting the seeds with gibberellin or potassium nitrate solution, are used to facilitate germination tests, based on ISTA procedures.

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

None.

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;

etc.

Considering that the seed lots destined for the two types of collections have a common origin, the viability testing is done from the seeds of the active collection, kept at +4°C and closed containers, every 5 years. When significant decreases are observed, the germination capacity of the seeds from the same accessions stored in the base collection is verified, maintained at -20°C.

The selection of samples for viability tests is made using specific filters in the database, BIOGEN. An adequate number of seeds, depending on species, quantity and size, are taken and delivered, on a list basis, to the viability assessment laboratory.

The viability test conditions are those provided by a growth chamber, Binder KBW / KBWF 240, in compliance with Gene Bank and ISTA standards.

The initial germination percentage must exceed 85% for most seeds of cultivated species, while for the wild species is around 60%.

Details regarding the germination testing procedures are described in the ISO Quality System of Genebank.

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

After the regeneration or multiplication phases and the processing of the seeds, the viability data are recorded, together with the number of regeneration cycles, in the BIOGEN database. If the seeds do not meet the required minimum germination standards, the material will remain in the attention of the curator of the collection.

For the monitoring of the seed samples included in collections, on request, depending on the reference year, files that indicate the accessions to be verified can be generated from BIOGEN.

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.*

At this moment there are no other rules regarding the testing of the viability for the biological material existing in the collections.

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.

The drying of the biological material, up to a threshold of 5 - 6% seed moisture content, is done with the help of dehumidifiers that ensure the reduction of the relative humidity of the air in the drying chambers to values ranging between 10 - 15%, and, also, they keep the ambient temperature below 20°C.

The seed storage rooms of the active collection have a thermostat set at $+4^{\circ}$ C, and those of the base collection are set at -20° C.

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

The samples for active collection are packed in glass jars covered with screw caps, and those in the base collection are packed in aluminium foil bags, with specific destinations (germination, regeneration, residual), which are mentioned on double labels (one is pasted on envelope, and another is placed inside, next to the seeds).

The packaging is performed in vacuum conditions using a Webomatic type sealing device.

SC3 – What is the range of seed moisture contents (smc) of your stored seeds of different species; what measures do you apply to keep and/or monitor the (low) moisture level? Do you treat different species differently?

The seeds are placed in trays, in a thin layer, together with the identity data written on a label that permanently accompanies the biological material, which is dried up to 5 - 6%, regardless the species.

Packaging is done as soon as possible after reaching these limits, and keeping this moisture, over the years, is based only on the tight closure of different types of containers.

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.

Suceava Genebank has as facilities, for the active collection (+4°C), four conservation rooms, with an area of 23 square meters each, equipped with a total of 15 racks with 12 shelves each, on which can be stored between 30,000 jars of 700 ml or 45,000 jars of 400 ml.

For the base collection (-20°C), there are 3 thermally insulated cells, with an area of 15 square meters, each equipped with three racks (two fixed and one moveable, in the middle) and about 120 drawers/cell. Depending on the size of the seeds, respectively the size of the aluminium foil bags, a drawer can contain between 200 and 1000 pieces of this packing type.

At this moment (December 2021), the storage capacity of Suceava Gene Bank is about 60%, for the active collection and 20%, for the base collection.

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).

There are no other data important for seed conservation.

B. In vitro Culture Collections

Box 3.1.1.B. Initial viability

IV1 – Describe the procedures or practices that you have in place to ensure the highest possible initial viability of your plant material, in particular during culture of donor plants (e.g. cultivation practices [field, greenhouse], phytosanitary pretreatments, like use of pesticides).

The main species for which the *in vitro* culture laboratory was created, in 1991, was *Solanum tuberosum*. The research plan included, also, *Allium sativum* in 2020.

The selection of both species was a consequence of the increasing number of varieties in the collections kept as living plants in the field genebank, as well as the mutually beneficial relationship of the two conservation types.

The selection of genotypes for introduction into *in vitro* collections is based on observations made in the field genebank or on the morphological characteristics of the vegetative organs brought from the exploration and collecting expeditions.

To reduce, as far as possible, the risk of infections during the *in vitro* culture initiation phase, the potato tubers are brought from the storage room, with controlled RH and temperature, to the laboratory where they are washed and left to generate shoots. No other methods to prevent infection, or disinfection of mother plants, are used.

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

Prelevation and inoculation of potato meristems is performed when the shoots are growing before the appearance of wilting and dehydration of the tubers. The work is done with the help of a binocular magnifier, in aseptic conditions, offered by the hood with laminar flow of sterile air.

Disinfection of the apex potato shoots, grown in the laboratory, is performed with 70% alcohol, for one minute.

Garlic bulbils are soaked in 96% alcohol, for 90 seconds.

In both cases, after the disinfection, the biological material is rinsed three times with sterile distilled water.

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

The selection of biological material, which is the basis of all inoculations, is made after the evaluation of its health and vigour, as it is detailed in ISO Quality System of Genebank.

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.

Infections, both in the initiation phase and in the late stage, occur quite rarely. The observations are made daily for the initiation phase and weekly or monthly for the multiplication and conservation phases, respectively.

Over the years, hyper-hydricity has occurred in very few cases in potatoes.

Research on micromultiplication and preservation of garlic varieties is ongoing, and hyper-hydricity was accidentally observed in an inoculum that had an abnormal morphological evolution.

VM2 – Describe the information "system" (i.e. an "expert system") that you might have in place that allows you to make more species - or even accession-specific decisions regarding when the next monitoring should take place.

There is no such system. The protocol is based only on the periodic examination of the plantlets and on the insertion of data, on the succession of subcultures, in an excel file.

VM3 – Please provide information on non-specific thresholds that you might use for vigour of *in vitro* cultures (i.e. multiplication rates, loss by weak growth) and for the amount of culture vessels (tubes, jars) left of an accession to initiate additional multiplication measures.

There is a great variability in the vigour of *in vitro* plantlets belonging to different local varieties, as there is in plants in the experimental field.

The morphological aspect, the size of the leaves, the diameter, the length, and the number of shoots, as well as the capacity to regenerate microtubers, can give information regarding the evolution of a variety on a certain culture medium.

To these morphological aspects, could be added the presence or absence of necrotic leaves, or microtubers that show signs of senescence, having consequences on the multiplication rate and regeneration capacity in the subculture.

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

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

In the growing room culture vessels are maintained at 20 - 22°C, with a photoperiod of 16 hours, in white fluorescent light, of about 2000 lux.

The temperature in the *in vitro* conservation cell, is about 6 - 10°C, (6-7°C, most of the time) and 10°C, in some hot summer days, during the photoperiod, which is of 12 hours, in white fluorescent light, of 1000 lux.

The humidity is not controlled.

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.

For the inoculation phase of the individual meristems, small glass vials with a diameter of 2 cm are used.

In the micromultiplication and preservation phases, larger glass jars, from the food industry, with a diameter of 5 - 6 cm and a capacity of 170 - 220 ml are utilized.

The *in vitro* culture laboratory has all the necessary facilities to carry out this activity located in spaces that ensure the technological flow from washing and sterilization, to the sterile room and growing or preservation conditions (laboratory glassware washing machine, oven, autoclave, water distillation and bidistillation devices, laboratory glassware and culture vessels, hood with sterile air flow, binocular magnifier with camera, tools for handling explants, air-conditioned growth and conservation rooms).

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

The presence of the conservation cell and the good functioning of the thermostatic installations, at $6 - 10^{\circ}$ C, is especially useful in prolonging the period between two subcultures, as well as in preventing the occurrence of senescence phenomena, even on culture media without growth inhibitors.

C. Cryopreserved Collections - NOT APPLICABLE

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* preculture 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];
- whether you apply different procedures for accessions with erratic regeneration rates of the control [e.g. increase the amount of explants stored]; and
- d. what is the threshold number of remaining explants of a given accession under which you initiate regeneration for multiplication.

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

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

D. Field Genebank Collections

Box 3.1.1.D. Initial viability

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

Conservation as living plants in the field genebank is used at the Suceava Genebank for potato, garlic, and onion collections.

Each accession received by the conservation laboratory will be accompanied by a registration number, which will be associated with a label mentioned in the field book, along with the available information.

Samples of plant material (tubers, bulbs, bulbils, etc.), regardless of their origin, are checked macroscopically to detect and eliminate the material affected by diseases or pests.

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

This type of conservation is used for the potato collection, starting from the tubers, the garlic collection, starting from bulbils and some onion varieties of *Allium cepa* var. *aggregatum*, which is based on bulb culture.

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

The area chosen, annually, for the regeneration/multiplication of accessions is suitable for the number of genotypes, and meets the crop rotation needs, to prevent or reduce the rate of disease and the attack of crop-specific pests.

The collection is planted in the field and respecting all the respective technological norms of the culture, the soil works, distances between rows and plants on the same row, as well as the optimal work period.

An accession consists of 10 to 15 plants for potato and onion crops.

For garlic, an accession has 20 - 60 plants, to cover the necessary material for distribution, to promote on-farm conservation.

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.

An identity label is used for each sample to be planted, according to the lists in the field register. The samples are checked individually, before planting, to remove biological material that shows signs of damage caused by disease or pests.

During the vegetation period, starting with the emergence phase of the plants, some morpho-physiological aspects are recorded, according to the specific descriptors of the culture and those regarding the resistance to diseases and the attack of pests.

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

There is no any system adapted to a certain species or variety.

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.

Not applicable.

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.

All works on weed removal, tillage, treatment of diseases and pests are carried out according to optimal cultivation practices to ensure the development of vigorous and healthy plants and quality planting material to perpetuate the collection and avoid degradation of biological material.

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

Potato samples are harvested when the material has reached physiological maturity, when weather conditions allow the selection of representative samples (15 - 20 medium size and healthy tubers).

They are packed in good quality paper bags, which ensure a proper winter conservation of the biological material, and also the resistance to transport and handling during storage, in a cold chamber with 85% RH and $4-5^{\circ}$ C temperature.

Garlic and onions are planted in the experimental field during the autumn, at the beginning of October. During summer the material is stored in a chamber set at 20°C temperature and under 70% RH.

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

3.2. Maintaining Genetic Integrity

Maintaining the genetic integrity of an accession can be achieved by minimizing genetic drift which may occur predominantly during the process of regeneration, due to too small numbers of individuals being planted, sub-optimal pollination and/or the introgression of alleles from other accessions or commercial crops or crop wild relatives. The following aspects are important for achieving the objectives of maintaining genetic integrity and should be briefly described. Please note that a distinction should be made between seed numbers for an accession and seed numbers for sub-samples per accession. The latter only applies if the seeds of a given accession are being stored and distributed as sub-samples. As genetically modified material gets more widely distributed and as it might have specific (legal, technical, administrative) requirements, a separate box on this type of material is included.

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

Navigation Box on Maintaining Genetic Integrity section

Seed – If applicable, please complete the section on Genetic Integrity for the activities related to seed genebanks (i.e. boxes 3.2.1.A – 3.2.5.A)

In vitro cultures – If applicable, please complete the section on Genetic Integrity for the activities related to *in vitro* culture (i.e. boxes 3.2.1.B - 3.2.3.B)

Cryopreservation – If applicable, please complete the section on Genetic Integrity for the activities related to cryopreserved collections (i.e. boxes 3.2.1.C - 3.2.3.C)

Field genebanks – If applicable, please complete the section on Genetic Integrity for the activities related to field genebanks (i.e. boxes 3.2.1.D - 3.2.3.D)

A. Seed Collections

Box 3.2.1.A. Seed Containers and Sample Size

SCSS1 – Do you document the initial number of seeds of individual accessions (either as received from collecting missions or through exchange)?

For the accessions coming from collecting missions, the initial stock is documented only if the accession meets all the conditions to go directly to conservation, without requiring a multiplication in the experimental field.

The material received from the breeding collections is documented regarding the quantity / number of seeds.

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.

Seeds of the accessions in the active collection are packed in glass jars covered with screw caps, and those from the base collection are packed in aluminium foil bags.

The determination of the number of seeds is done either directly by counting (seed counter device is used) or by using the mass value of 1000 grains. Usually, the amount of seeds is determined and recorded on each package.

The seeds of the same accession resulting from several regeneration / multiplication cycles are stored in separate containers, having specified the number of seeds, and viability in addition to other conservation descriptors.

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

The minimum number of conserved seeds is in accordance with the biological status and the reproductive system of the sample. Thus, in local populations, wild forms and all allogamous species, the number is in the range of 3000 - 15,000, depending on the size of the seeds.

Modern cultivars and autogamous species are represented by a lower number of seeds, placed in the range 1000 - 5000, also according to the size of the seeds.

The breeding lines, which are confidential, are kept only in the base collection, and the minimum number of seeds is 500. Regarding the genetic stocks from breeders, no limits are set for the number of seeds.

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

None.

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.

For regeneration/multiplication activities we use our own experimental field and 2 unheated greenhouses, with 18 compartments each, used especially for allogamous species.

For wind-pollinated species, pollination is facilitated by manually applied vibrations. In Cucurbitaceae and sunflower, artificial pollination and isolation are achieved using paper bags or textile bags.

Special attention is given to maize, which is numerically the most important and oldest crop in our collections, each year being included in the regeneration / multiplication plan. Pollination is controlled by various methods, either by spatial isolation with hemp curtains, or individual isolation of the female flower and manual pollination with a mixture of pollen. To avoid genetic drift, a minimum of 50 plants per accession are used.

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

None.

Box 3.2.3.A. Regeneration Environment and Procedures

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

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

Most of the species from the collections of the Gene Bank can be regenerated / multiplied in the pedo-climatic conditions of the area where the institution is located.

In general, few wild species are subject to regeneration / multiplication programs; instead, most varieties of medicinal, aromatic and fodder plants being recollected from their areas of origin.

Genetic stocks, most breeding lines are regenerated by donor breeding institutions, given that most of this type of collection is IPR protected.

In this field, we do not have collaborations with other genebanks in Europe, but at national level a regeneration / multiplication network has been created for local vegetable varieties, the financing being provided by the Ministry of Agriculture and Rural Development.

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

None.

Box 3.2.4.A. Seed Processing Procedures

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

Accessions harvested either from the experimental field or from greenhouses, as seeds or fruits, when they have reached physiological maturity, are packaged in labelled paper or raffia bags, and enter the seed processing facilities. This operation respects the physiological and technological needs of the species such as natural drying before 12 – 14% seed moisture content is reached, fruits post-maturation, etc.

The Genebank has several types of selectors, which ensure the best possible cleaning of the vegetal remains, but, before handing them over to the conservation laboratory, a manual cleaning is also done.

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

The drying of all seeds accessions is done in 2 drying chambers (one of 30 m², the other of 20 m²), equipped with professional dehumidifiers, which operate to achieve the following parameters: 10 - 15% relative humidity and temperatures below 20°C.

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

After harvesting or collecting, all seed accessions are processed (threshed, cleaned) as soon as possible, generally until the end of the year in which the regeneration / multiplication / collecting of the biological material was carried out. After that, the material is placed in the drying chamber for a period of 1 - 3 months, until the results of the germination tests performed in the Bank's laboratory for seed study are received.

Depending on the results of the germination tests and the quantity of seeds, the accessions are packaged and included in the two types of collections, active and base.

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.

One of the drying rooms serves to temporary store the material entered in the Bank, regardless of the species, from different sources (field genebank, collecting missions, donor institutions, etc.). The second is intended to complete the drying process, and the samples are grouped according to the size and chemical composition of the seeds.

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

Details are given at SCSS3.

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).

NOT APPLICABLE. Suceava Gene Bank does not own, or process organisms known to be genetically modified.

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).

Usually, several potato tubers are selected as a genetic source for the meristems taken from the shoot tips and the garlic bulbils come from several bulbs.

The number of meristems prelevated with the help of a binocular magnifier is about 10 -15 pieces for an accession and is noted in the Excel file dedicated to the laboratory work.

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

The type of culture vials was described in point 3.1.3.B.

The culture media are based on the MURASHIGE-SKOOG recipe (MS-1962). The main growth regulators used are kinetin, benzyl adenine, ∞ -naphthyl acetic acid, with or without the addition of daminozide.

The culture medium is poured in glass jars (20 ml / 170 ml vial), which are covered with aluminium foil and autoclaved for 20 min., at 121°C.

A number of 20 nodal segments of each variety, are distributed in 4 jars. After placing the inoculum in the medium, all types of vials are covered with double polyethylene foil, fixed with two rubber rings.

All operations regarding the inoculums and plantlets developed *in vitro* are performed in aseptic conditions using sterile instruments (scissors, tweezers, and scalpels). The callus is removed, given the need for clonal multiplication of the material in both, potatoes, and garlic.

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

Usually, in the multiplication and conservation phases, there are 40 plantlets, distributed on two different culture media, each with 20 specimens.

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).

There are no *in vitro* regenerated sub-clones.

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

The subculture is carried out at 2 - 3 months, in the case of *in vitro* plantlets developed on micromultilplication media, in the growth chamber (22°C) and at 6 - 8 months for those moved to the conservation cell (6 -10°C).

The plantlets in *slow-growth*, on conservation media with the addition of inhibitors and restrictive environmental conditions, from the conservation cell, reached a duration of 3.5 years, between two subcultures.

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

The general aspect of evolution, the presence of green leaves, the development of microtubers and rooting, are signs of a good evolution, while the appearance of necrosis, of etiolated leaves and devitalized shoots are signs of senescence that should be removed and eliminated by subculture, to save the biological material.

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).

NOT APPLICABLE

C. Cryopreserved Collections

Box 3.2.1.C. Cryopreservation Containers and Sample Size

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

NOT APPLICABLE

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

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

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

Box 3.2.2.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).

NOT APPLICABLE

D. Field Genebank Collections

Box 3.2.1.D. Accession Sample Size

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

The number of vegetative organs of each field genebank accession, received from outside or from the previous year's harvest, is mentioned in the list of crops preserved as live plants in the field genebank and is a parameter for comparing and analysing the evolution for later stages, respectively emergence, growth and harvest.

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

There are not sub-sampling procedures used for field collections of potato, garlic and onion.

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)?

An accession, conserved in the field, consist of 10 to15 plants for potato and onion crops.

For garlic, a sample has 20 - 60 plants, to meet the distribution needs.

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 and perennial species)

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

- a. any control measures to minimize or avoid cross-pollination between accessions (if applicable/relevant);
- b. the use of pollination cages for insect-pollinated species;
- c. the use of specific pollinators for insect-pollinated species;
- d. strategies to ensure that males and females participate equally in the reproduction);
- e. strategies to avoid any genetic drift (minimum number of plants, minimum number of plants at flowering stage before pollinators introduction, similar quantity of seeds harvested from each plant, etc.).

NOT APPLICABLE

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.

NOT APPLICABLE

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 APPLICABLE

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

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

The potato collection is stored, over the winter, in a cell provided with air conditioning that maintains temperatures around 4 - 5°C, and the relative humidity of the air varies from 70 to 85%.

The tuber samples are packed in paper bags, labelled according to the field list, and placed on metal racks.

After harvesting, the onion and garlic bulbs are stored in the post-ripening room at a temperature of about 20°C and a relative humidity of up to 70%.

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

NOT APPLICABLE

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 accessions from the active collection, which do not have an IPR protection, that have sufficient stocks and adequate germination capacity are distributed for the purpose of breeding/research/education, based on the Standard Material Transfer Agreement (SMTA) under the International Treaty for Plant Genetic Resources for Food and Agriculture.

To promote the on the farm / garden conservation of the Romanian crop landraces, the Genebank offers a maximum of 5 accessions, from its collections to each person interested in maintaining the agricultural tradition and the old varieties. A number of 15 - 50 seeds from autogamous, annual species are offered, based on a Suceava Genebank Transfer Protocol.

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?

For researchers and breeders, the response time is as short as possible, but does not exceed 10 days and is determined by the completion of the formalities for signing the SMTA.

For individuals, the shipment of samples begins in 10 - 14 days after the end of the established registration periods, in the chronological order of requests, so that the biological material is received in time for all geographical areas of the country.

Details on www.svgenebank.ro (Romanian language).

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.

Material for conservation, breeding or research is accompanied by passport descriptors and characterization / evaluation descriptors when available.

The label that accompanies the samples sent to individuals contains the following information: accession number, the location of origin, scientific and local name.

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).

For research / breeding purposes there is no set limit for reasonably requests, and the number of seeds being correlated with the requests and the availability of stocks. For the distribution of local varieties to small growers in the country, the number of seeds varies from 15 to 50, depending on the size of the seed. For most varieties, 25 seeds are offered.

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

The Genebank distributes material only from the active collection and does not have sub-samples of conserved accessions specially dedicated to this purpose.

The varieties provided to individuals come from the stocks obtained by annual multiplication, which are separated from the stored collections.

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

By querying the Database, BIOGEN, regarding the total stock of seeds and the germination capacity, lists of priorities are made in order to perform the multiplication / regeneration.

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

None.

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.

All seed accessions regenerated / multiplied by the Genebank are monitored for their health, the presence of diseases or pests, both during their growth and development in the field genebank and in the phases of preparation of biological material either for storage or for distribution.

After extracting the seeds, all the samples are checked and get an internal health certificate from the Genebank's phytosanitary specialist.

Only seed samples that have received this certificate can be stored or distributed, so they are declared "disease free".

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

Suceava Genebank complies with the norms for preventing the spread of quarantine diseases and collaborates, in this sense, with the Plant Protection Agency which verifies the material and issues phytosanitary certificates.

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

All the necessary steps to obtain phytosanitary certificates issued by Plant Protection Agency are followed in order to dispatch the seed, in accordance with European rules and the legislation of other states, which require genetic material.

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

Not applicable.

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.

Answer is given at AGSS1.

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

If the germination is lower than the recommended standard, we always increase the number of seeds and inform the beneficiary about this.

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.

NOT APPLICABLE.

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

AGP3 – Describe how you treat "related information" about the requested accessions that you make available to the requestor, i.e. provide details on the typical information you send out with the germplasm.

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

AGSS1 – Please provide details on the maximum amount of *in vitro* samples that you distribute.

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

AGSS3 – Describe how you manage the availability of adequate plants per accession, including the use of an absolute lowest minimum of plants per accession as the threshold to decide to regenerate.

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

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

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

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

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

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

Box 3.3.4.B. Germplasm Supply

- **GS1** Describe the policy of your genebank with respect to the sample size that you use for distribution purposes.
- **GS2** Please provide details of your routine methodology of containers etc. that you use to distribute *in vitro* cultures.
- **GS3** Please provide information on any other aspects related to *in vitro* plant supply.

C. Cryopreserved Collections

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

AGP1 – Describe the germplasm distribution policy that you follow at your genebank. Cryopreserved material is for distribution in exclusive cases only – e.g. for special research, please describe your policy; who the requestor is; what the purpose of the germplasm request is; any restrictive conditions and/or the total amount of accessions sent per request for distribution of germplasm; use of a formal agreement to distribute the germplasm.

NOT APPLICABLE

- **AGP2** Indicate if you have as part of your service-rendering policy aspects such as a "regular or maximum time" between receiving a germplasm request and distribution of the germplasm.
- **AGP3** Describe how you treat "related information" about the requested accessions that you make available to the requestor, i.e. provide details on the typical information you send out with the germplasm.

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

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

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

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

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

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

AGHA1 – Describe how you store seed/other germplasm with respect to germplasm health considerations, including whether you have a "policy" of storing only "disease-free" (as far as you can see or determine) accessions, at least for the quarantine pests and diseases. You could also add data on separation of differently infested material in separate cryotanks, etc.

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

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

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

Box 3.3.4.C. Germplasm Supply

- **GS1** Describe the policy of your genebank with respect to the sample size that you use for distribution purposes.
- **GS2** Please provide details of your routine methodology of containers etc. that you use to distribute cryopreserved material.
- **GS3** Please provide information on any other aspects related to cryopreserved material supply.

D. Field Genebank Collections

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

AGP1 – Describe the germplasm distribution policy that you follow at your genebank. You might want to consider in your response the following aspects: crop/species specificity; whether or not sufficient seed stock is available; who the requestor is; what the purpose of the germplasm request is; any restrictive conditions and/or the total amount of accessions sent per request for distribution of germplasm; use of a formal agreement to distribute the germplasm.

Only samples of garlic local varieties, included in the Genebank's distribution program for the autumn campaign, could be considered for this category of genetic resources.

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.

The distribution protocol considers complying with the optimal planting period on the side of the requestors.

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.

Answer is given at AGP3.

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).

Usually, the samples for garlic distribution consist of about 10 bulbils per variety.

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.

The entire garlic field collection is annually cultivated. Details are given at IV3 and SCSS3.

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

None.

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.

All distributed biological material is checked macroscopically and all bulbils with signs of disease or pest attack are removed. Only bulbils that, as far as we know, are free of disease, receive approval for shipment to the users.

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

NOT APPLICABLE.

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

NOT APPLICABLE.

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

None.

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.

The answer is given at AGSS1.

GS2 – Please provide information on any other aspects related to seed supply.

None.

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.

The Genebank's information system, BIOGEN is based on the Visual Fox Prodevelopment environment.

It does not use information management manual, but only internal procedures of activities.

The computer system of the Genebank contains two databases, one for the collections kept in the Bank, called BIOGEN and another for the national inventory of plant genetic resources. Public access is only allowed to a minimum set of passport descriptors. For accessions under IPR protection, public access is strictly forbidden to any kind of data.

The activities covered by BIOGEN database system are collecting of crop wild relatives and local varieties, multiplication and regeneration, characterization and evaluation, seed conservation, and distribution. All activities are connected through a unique key represented by the accession number. The system allows access to additional information related to an accession.

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.

BIOGEN contains passport data, local knowledges data, herbarium data, management data, viability data, characterization & evaluation data, taxonomy, storage, and distribution data.

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

The answer is given at GD1 c.

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).

If requested, available data can be provided either as hard copy or electronically as Excel file.

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

Technical support is provided by the IT compartment of the Genebank.

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

Database backup is performed weekly in electronic format, on different stations, and annually on external memory devices, by the IT compartment of the Genebank.

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

None.

Box 4.2. Information Exchange

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

Passport data (BIOGEN database and national inventory) with minimum descriptors is available publicly on SVGB website. All passport descriptors of national inventory database are available via the European Search Catalogue for Plant Genetic Resources (EURISCO) website.

- **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.)
 - b. which web-service interfaces are available (i.e. GBIF IPT, BioCase, TapirLink).

NOT APPLICABLE.

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

Passport data (BIOGEN and national inventory) is published to EURISCO and updated annually. Characterization and evaluation data for AEGIS accessions are also published on EURISCO.

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

None.

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.

The answer is given at AGP3.