INSTITUTE OF PLANT GENETIC RESOURCES "KONSTANTIN MALKOV"

OPERATIONAL GENEBANK MANUALS OF THE NATIONAL GENEBANK OF BULGARIA

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1 Germplasm Acquisition and Accessioning

Genebanks can obtain the germplasm they want to conserve through a number of different ways. Conducting collecting missions is possibly the best way of acquiring germplasm material in the most reliable manner. Germplasm exchange with other genebanks is a third route to add genetic diversity to the collection. Obtaining and storing germplasm from researchers and plant breeders is another route to acquire genetic material. Such acquisitions should be guided by a formal mandate that the genebank concludes with its host organization or government and that provides the basis for a genebank acquisition policy. The actual accessioning of acquired germplasm samples, i.e. formally including it into the collection with its unique accession number, is a complex process during which the curator has to check a number of aspects such as the verification of the identity of the material, the health status, the availability of pertinent information, etc. It is further understood that also legal aspects form part of this activity, e.g. was the material collected/obtained in legal manner, are there any restrictions on its use, etc.

Box 1.1 Germplasm Acquisition and Accessioning

GA1 - Briefly describe any formal mandate that your genebank might have concluded with or received from your "mother organization" (e.g. institute, governmental body).

(*This description should include details on:*

a) which species you conserve and make available;

In the genebank of Bulgaria are preserved above 60 000 seed samples, presented from 33 families, 150 genera and 600 plant species.
The conservation of the diversity of cultivated plant species and their wild relatives is achieved by maintaining three collections: base, working and exchange collections.
The crop groups of ex situ collections in the genebank include: cereals, grain legumes, oil and industrial crops, forage grasses, vegetables, ornamental and medicinal species.
The collections provide good opportunity of utilization based on existing large scale genetic diversity: wild species, local populations, primitive varieties, breeding materials and modern varieties with different origin.
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.
The exchange collection contains 2 452 accessions from 104 plant species. All materials are 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.

b) who decides on what your mandate is and, if different,

Since its establishment in 1984, The National genebank is a part of Institute of Plant Genetic Resources "Konstantin Malkov"-Sadovo (IPGR-Sadovo).
IPGR-Sadovo is a member of the Agricultural Academy.
The Agricultural Academy is a National Autonomous Budget Organization under the Minister of Agriculture.
With the law adopted on February 28, 2018, the Agricultural Academy became a national autonomous budget organization under the Minister of Agriculture, which deals with issues related to research, applied research, innovation and educational activities in the field of agriculture and food.

c) *from whom do you received the mandate;*

From the Institute of Plant Genetic Resources "Konstantin Malkov"-Sadovo.

d) *the main aspects of the mandate; and*
e) *legal considerations on PGR as foreseen in national legislation.*

In the Strategy for the Biological Diversity in the Republic of Bulgaria the Institute of Plant Genetic Resources – Sadovo is given as a competent authority for strategy application.

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 specific formal agreements.

GA3 -In case your genebank has a germplasm acquisition policy, what does the policy entail?
a) please specify which crops or which geographic area, if applicable.

Since its establishment in 1984, the GenBank's main policy has been to acquire germplasm from different geographical origins:
- needed for breeding programmes in the country and for research;
- to fill gaps in the collections whose diversity is lacking or under-represented;
-preventing genetic erosion by collecting seed material of threatened species.
Recently the main priority for inclusion in the genebank collections is given to species with Bulgarian origin - landraces, primitive cultivars and crop wild relatives, as well as breeding lines and varieties deposited by other scientific organizations in Bulgaria.

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 materials received from other genebanks and international centers we rely on the donor's passport information. For materials collected from gardeners and farmers we rely on the information provided by them. For crop wild relatives collected in our missions we rely on our own experience. In all cases the first step is multiplication of the materials to confirm the taxonomic status and make characterization. The materials are also compared with other accessions.

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.
When accessions are collected in country missions or are presented as original breeding material they are multiplied directly in the experimental field and are comprehensive evaluated in the relevant working collections.
Assessment of the quality of the seeds before storage we do through visual control of seeds, estimation of viability, purity, seed weight, and moisture content. Seed testing is done in accordance with ISTA Standard.

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 has been implemented since 2010. All accessions including non-annex 1 cops are provided under the SMTA regulations.

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*).

Due to lack of funding from the budget for that purpose, collecting missions are carried out only within national and international projects. Priority is given to collecting of old local varieties and populations and crop wild relatives.
Planning and implementing of collecting missions for crops and their wild relatives is based on the available ecogeographical, ethnobotanical and bibliographical data.
Preparation of collection lists with preliminary botanical characterization before including the material into the genebank. Verification of the botanical determination by the curators during first multiplication in the fields.
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.

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

2 Ensuring Security

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

2.1 Physical Security

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

Box 2.1.1 Safety Duplication (of long-term conserved germplasm)
SD1 - Please describe how your genebank implements the safety duplication of your germplasm material.
(*This description should include the following aspects:*

a) The type of safety duplication (e.g. black-box; no specific arrangement; other);

1. *Seeds: black-box agreement. Safety duplicates of 762 accessions from 4 crops (melons-110, pumpkins-490, cucumbers-54, watermelons-109) are sent to Centre for Genetic Resources (CGN)*
2. *Seeds: black-box agreement .Safety duplicates of 2119 accessions from 27 plant species are sent to Svalbard.*

Sending to Seed Vault in Svalbard will continue.

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

Netherlands, Centre for Genetic Resources (CGN).
Norway, Svalbard Global Seed Vault (SGSV).

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

Memorandum of understanding between IPGR-Sadovo and CGN are used.

Standard agreement between the IPGR-Sadovo and the Royal Norwegian Ministry of Agriculture and Food are used.

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

The base collection is for long-term conservation where the seeds are stored at 3–7% moisture (depending on the species) and at sub-zero temperatures (−18 °C) in hermetically closed containers (glass jar or three-layer laminated foil packets (PET 12μm+Al 9μm+PE=113 μm)).

In SGSV and CGN the accessions are maintained at –18°C in three-layer laminated aluminium foil packets.

e) *Do you maintain safety duplicates from other genebanks at your genebank? If so, do you know any details of that material?*)

No, we don't maintain safety duplicate from other genebanks.

SD2 – Do have a safety duplication policy? If so, please provide essential details.
No.

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 building was constructed in 1984 and was designed to withstand earthquakes of more than 7 on the Richter scale. Hurricanes are not typical for our geographical area.

The collection is maintained in the cold rooms located on the second floor of the building, therefore there is no risk of flooding. Precautions against fires are taken by means of a fire protection system. Annual checks are carried out by a licensed fire installation service company. Refrigerant (Freon) is used for the refrigeration system, which does not fall into the category of flammable and explosive refrigerants in case of fire.

The building is protected against lightning by the lightning protection installation. The State Health Inspectorate performs periodic inspections to ensure that the building is in good working order and that the environment is suitable and safe for staff to work in.

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. There is 24-hour security provided for the entire institute, which includes a physical presence of a security guard and an alarm system at the General Bank building. The doors of the genebank building are locked permanently and there is regulated access. Access to the genebank is restricted to staff employed on permanent contracts.
Fire extinguishers and pumps are provided on each floor of the building, as well as fire hydrants on the first and top floors of the building.
Personal protective clothing is provided for working in freezing temperatures, hazardous and noxious environments.
There is no back-up generator, but there is a back-up power line to the substation.
There is a back-up compressor provided for the two cold rooms.

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

Access is provided only to staff secured by permanent work contracts.

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 staff at the National genebank - refrigeration engineer, and an electrician. The compressors and cold rooms are monitored daily by the genebank specialist engineer. Regular maintenance of the compressors and periodic trial runs are performed.

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

Monitoring of temperature and relative humidity in our cold stores and drying room are controlled manually by the staff every day. The minimum and maximum temperatures are recorded 3 times a week as well as the current temperature at the time of the reading. In case of deviations of temperature from the norms, measures are taken to remedy the problem by the refrigeration engineer and the electrical technician. In case of a more serious

problem, a specialized repair company is hired. A record of the temperature in the chambers is maintained as well as a log of repairs and manipulations carried out on the refrigeration system.

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 management of the Genebank is assured by a budget of the Institute of Plant Genetic Resources-Sadovo, part of which is the genebank. The Institute is a public institution with finance provided from the Agricultural Academy for basic activities and with the possibility of supplementation through extra-budgetary funds obtained from research projects.

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

The staff has permanent work contracts.

Box 2.1.5 Contingency Plans:

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

There is a contingency and emergency plan.

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 staff is informed and trained regularly (at least annually) about emergency situations-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” (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.

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 processes in different cultures the specific requirement of each plant genera is observed in order to obtain seeds with high quality. The specific agro technical activities are applied according to plants requirements. Specialist in plant protection makes regular observations and gives recommendations for plant protection activities. Each curator keeps record for the plant protection activities. All post-harvest activities are also done according to the plant's specifications.

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

In the cases where the viability-tests are hampered by dormancy or hardness, appropriate measures are taken, as far as these are known- treatment with Gibberellic acid, KNO_3, cold and hot treatments or scarification etc.

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

Box 3.1.2.A Seed Viability Monitoring

VM1 - Describe the routine seed viability monitoring system that you use.
(*The monitoring system should include the following aspects:*

a) *frequency of testing;*
b) *sampling method applied;*
c) *any thresholds that you use;*
d) *whether you apply different procedures for crops/species with erratic*

initial viability or irregular viability lifespan;
etc).

In the Genebank of Bulgaria seed accessions in long-term storage are monitored 10 years after adoption in the genebank. Subsequent control tests are carried out at intervals of 10-20 years based on the results of previous tests, with priority given to species with short seed life. Seed viability monitoring is scheduled at the beginning of each year. Control tests in time of storage are implemented after pre-conditioning of seeds: 24 h equilibration of seed containers at ± 2°C, followed by 24 h equilibration of seed containers at 12±2°C and 24 h equilibration of seed containers at room temperature. Re-humidification of seeds in a chamber (48 h at relative humidity 90%) before the seeds is set to germinate. The specific requirements for each crop according to the ISTA Standard and other sources are applied when testing the seeds.

The viability tests from the seeds of the exchange collection kept at +6°C and closed containers are made every 5-10 years.

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

Seed storage database is maintained in EXCEL format. The planning of the germination tests is done on the basis of the results in this database.

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.*

The initial germination value must exceed 80 percent for the cultivated species. For wild species a lower percentage is accepted.
The decision to regenerate accessions depends both on the viability and quantity of seeds held in store. We regenerate an accession when seed viability drops below 20 % of initial viability. Regarding the quantity of stored seeds, for the base collection, regeneration is done when number of seeds fall below 1000. In exchange collection, regeneration is conducted when the amount of seeds falls below the amount needed to perform three 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.

The base collection is for long-term conservation where the seeds are stored at 3–7% moisture (depending upon species) and at subzero temperatures (−18 °C) in hermetically closed containers.
The working collection is for short-term conservation where seeds are stored at 6-7 °C, air relative humidity 40-45%, and free access of air.
The collection for free exchange is for medium-term conservation where seeds are stored at +6 °C, air relative humidity 40-45%, 3–7% seed moisture content (depending upon species) in hermetically closed containers.

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

Base collection in glass jars or three-layer laminated foil packets (PET 12μm+Al 9μm+PE=113 μm).
Working collection is in paper bags.
The collection for free exchange is in three-layer laminated foil packets.

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 dried in a sorption drying cabin supplied with an air dehumidifier Munters MD300. The level of equilibrium seed moisture (3-7%) is achieved at 12-15% air RH and 20±2 °C room temperature.
All species are treated the same way.

SC4- Provide data on the total storage capacity (number of containers, number of accessions) and an estimated percentage to which extent this capacity has been filled.

The National genebank of Bulgaria uses two refrigeration rooms (for base collection at -18° C, for working collection and collection for free exchange at +6°C) with 1040 m^3 totally area.
The total storage capacity is filled at 98%.

SC4 – Please include any other aspects regarding storage conditions at your genebank that you regard as important (e.g. anticipated lifespan of freezing and drying equipment and related prudent financial management).

B. 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.).

In field collections we preserve potatoes, medicinal and aromatic plants and perennial forage plants. Potato collection comprises old varieties and is

located in the experimental field of the Institute in town of Koprivstitza at 1060 m above sea level. We follow the typical for each plant type cultivation practices.

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

For potato collection are used the tubers, for the medicinal and aromatic plants – rhizome and seeds and for the forage plants only seeds.

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

For potato collection crop rotation is applied. All field collections are planted in the field, respecting the required agro technological norms of the culture, the soil works, distances between rows and plants on the same row, as well as the optimal work period.

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*).

For the new materials we make visual evaluation of the health status of the tubers and rhizomes. For the seeds germination tests are made. During the vegetation period is made regular control of the disease or pest contamination.

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.

We do not have such 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?

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 activities related to the control of weeds, diseases and pests, as well as soil tillage and watering are carried out in accordance with the best practices for growing of each crop.

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 and are stored in dedicated potato-storage.

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

A. Seed Collections

Box 3.2.1.A Seed Containers and Sample Size

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

We do not record the seed number form seeds received from collecting missions or from exchange before multiplication and evaluation.

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.

Paper bags for short-term storage, three-layer laminated foil packets (PET

12µm+Al 9µm+PE=113 µm) for medium-storage and three-layer laminated foil packets (PET 12µm+Al 9µm+PE=113 µm) and glass jars for the long-term storage are used.
The number of seeds is determined by using the total weight and mass value of 1000 grains.

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

Species specific thresholds depending of reproduction biology, heterogeneous samples and biological status. For the new accessions in the base collection the minimum threshold per accession is 2500-6000 seeds for self-pollinated and cross pollinated species, respectively. We conserve accessions even if the number of seeds is very low. However, we strive to maintain a minimum of 1000 seeds per specimen. If the number of the seeds is under 1000, the accession is given for regeneration.

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

Box 3.2.2.A Pollination Control

PC1 - Please describe the regeneration procedures that you follow for self- and outbreeding species.
(*Please include in your description the following aspects:*

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

Accessions are regenerated in the experimental fields of IPGR-Sadovo, by the curators of the respective crops of the PGR department. In order to maintain the genetic integrity of accessions for regeneration are used seeds from the most-original-sample. The curators follow the specific requirements of ECPGR WG for regeneration of accessions. The number of plants depends on the multiplication rate and available saved seeds but to avoid genetic drift, a minimum of 50-100 plants per accession are reproduced.
In sunflower, artificial pollination and isolation are achieved using textile bags. In maize pollination is controlled by individual isolation of the female flower and manual pollination with a mixture of pollen.

PC2 – Provide any other relevant information on procedures that you apply to control pollination of your germplasm.
During the vegetation period, any visible admixtures are removed from the

plot.

Box 3.2.3.A Regeneration Environment and Procedures

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

(*Consider the following aspects:*

a) *In how far are the environmental conditions of the current regeneration of individual germplasm accessions comparable to the environmental conditions that existed at the original collecting or breeding site?;*

The specific environmental conditions in Sadovo allow most of the species, maintained in the genebank to be regenerated successfully in field conditions.

b) *Do you use controlled environments?*

No.

c) *Do you collaborate with other genebanks in Europe?*

No.

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 seed cleaning by machine and by hand.

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

Drying of cleaned seeds at 12-15% RH and 20±2 room temperature to achieve 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.

Seed accessions are processed (threshed, cleaned) as soon as possible after harvesting. After that germination tests are made. Seeds with sufficient germination rate and quantity are placed in the drying chamber for a period of 1 - 3 months until the necessary moisture level is reached. Then accessions are packed and included in exchange or base collection.

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).

The newly harvested seeds we keep in paper bags in storage room at room temperature and ambient humidity until finish germination test.

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

The criteria to decide the number of seeds per accession are based on the specific need: to monitor the viability over time; to regenerate the sample. The number of seeds is from 2500 to 6 000 seeds per accession for self pollinated species and cross pollinated species, respectively. For the rare and threatened wild species, where production of seeds is limited, preservation of 1000 and even less number of seeds.

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.

C. 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)?

In case of receiving vegetative organs from abroad or from collecting missions in country the accessions are included in the list with temporary number and passport information for later evaluation.

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 no sub-sampling procedures.

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)?

In field collection we usually maintain between 10-15 plants from each accession.

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

Box 3.2.2.D Multiplication

PC1 - Please describe the multiplication procedures that you follow for your field genebank material (both, annual as well as perennial species)?
(*Please include in your description the following aspects if they would apply to your field genebank management procedures):*

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

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

Box 3.2.3.D Planting Material Processing Procedures
SPP1 – Describe the protocol(s) that you use for threshing and seed cleaning, if used as an intermediate step for the management/multiplication of your field genebank accessions
.
SPP2 – Please describe how and where you store (in a temporary manner) newly harvested planting material.
(Please provide details on the temperature and relative humidity of the storage room/space; what type of containers do you use, if any, etc.).

Bulbs are stored in paper bags in 4-5o. Potato bulbs are stored in dedicated underground potato-storage.

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

3.3 Ensuring Availability

An important objective of conservation efforts is to facilitate the effective utilization of germplasm accessions by researchers, breeders and farmers. Thus, ensuring the ready availability of stored germplasm is an important principle. It refers to the ability of genebanks to supply and distribute the stored germplasm, together with any associated information, in an adequate way to users. Aspects that can affect the availability include: (a) policies, (b) seed stock, (c) health status of accessions, and (d) distribution quantity. Although most of the questions are not relevant in the ECPGR/AEGIS context, it was decided to keep the questions and to allow for a comprehensive genebank manual that can be used “globally”.

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 materials are distributed based on SMTA of the International Treaty for Plant Genetic Resources for Food and Agriculture.
The total amount of accessions per request is maximum 100 and usually 25-50 seeds per accession are shipped.

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?

A "maximum time" is 1 week after receiving signed SMTA for the European country and 4 week for non-European country.

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.

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).

Minimum -25 seeds, maximum-50 seeds.

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.

The accessions for free exchange are stored in three laminated foil packets at +6◦C after drying to 3-7% moisture content.

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 availability number of seeds and seed storage database is maintained in the database. The absolute lowest minimum is the number of seeds

necessary for three 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.

No crop specific tests.

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

Outside EU - phytosanitary certificate.

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

Outside EU - phytosanitary certificate received from Bulgarian Food Safety Agency.

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: 25-50 seeds per accession.

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

Yes, if we have enough seeds.
GS3 – Please provide information on any other aspects related to seed supply.

B. 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).

We distribute only seed material, 25-50 seeds per accession. Not vegetative.

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?

One week for EU and 4 weeks for outside EU.

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 information.

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).

We distribute only seed material, 25-50 seeds per accession. Not vegetative.

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

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

Box 3.3.3.D Ensuring Availability of Germplasm – Health Aspects

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

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

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

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

Box 3.3.4.D Germplasm Supply

GS1 – Describe the policy of your genebank with respect to the sample size that you use for distribution purposes, including whether you differentiate between germplasm from annual or perennial species, clonally or sexually propagated accessions, and possibly other aspects.

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

4 Providing Information

The lack of adequate information on a given accession may well decrease the value of that accession to the user. The information on individual accessions should be as complete as possible in order to facilitate the identification of duplicates and/or to select accessions with desirable characteristics. A genebank should have a documentation system in place that allows to optimize management of the collections as well as to provide access to information about the collection to users.

Box 4.1 Genebank Documentation System

GD1 - Please provide details on the technical aspects of the genebank information management system(s) that you use.

a) On which software is the system based (i.e. Oracle, Fox Pro, MS Access, MS excel, MS Word, other?).

MS Access and EXCEL databases are use for documentation of seed accessions in Genebank – Bulgaria.

b) In case you use a manual information management system, please provide details.

IPGR – Sadovo maintains an electronic register Phyto 2000 in Microsoft ACCESS format. It contains all the information about the seed accessions kept in the Genebank from 1982. Each accession has a unique catalogue number and passport characterization according to the Multi-Crop Passport Descriptor of EURISCO (FAO/Biodiversity, 2017). Characterization and evaluation databases according to ECPGR descriptors are maintained in EXCEL format from every curator by crop groups. Seed storage database is maintained in EXCEL format. The planning of the germination tests is done on the basis of the results in this database. The passport information of ex situ collections are visible through EURISCO.

c) In case your “internal” database(s) is/are different from the publicly available database(s), please provide details on both,

IPGR - Sadovo conducts a project, funded by National Science Fund, for creation an intelligent, integral information system for plant genetic resources

in Bulgaria for management of all current MS Access and EXCEL databases with specialized software for the purposes of the Genebank, the information centre and all curators, according to the international standards. The Genebank information management system BGPLANTNET is in a process of establishment and has to be implemented in practice up to the end of 2022. The Genebank information management system will allow free access to the conserved gene pool by establishment of a National Information Portal. The Information management system will significantly improve the work of the Genebank, to ensure documentation of the accessions and the possibility of inclusion of all plant genetic resources collections held by various scientific organizations in Bulgaria.

d) Describe which activities of the genebank are covered by the system.

Passport data, characterization and evaluation data, seed storage data, germination data and regeneration information.

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 names (taxonomy), characterization & evaluation data, seed storage data, germination data and regeneration information.

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

Characterization and evaluation information is not publicly available. Only characterization and evaluation data for AEGIS accessions is published on EURISCO.
Germination information is not publicly available.
Landraces are a priority for collecting in recent years. Their documentation includes additional information such as the name of the farmer who donated the seeds, a brief description by the farmer, the local variety usage, traditional applications and others. The information is not publicly available.

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).

Hard copy and EXCEL file with passport data.

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

One technical assistant is responsible for passport documentation. One technical assistant is responsible for seed storage database. A senior researcher is EURISCO focal point.

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

database contents, including with which frequency?

Periodically 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?).

*Hard copy, internet***.**

IE2 - Please indicate if your data is available as machine to machine web-services. In case it is, describe

a. what types of data (passport data, characterization & evaluation data etc) and

Passport data according to the Multi-Crop Passport Descriptor of EURISCO (FAO/Biodiversity, 2017).

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

Through EURISCO, through establishment of a National Information Portal BGPLANTNET.

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

Passport data is published on EURISCO and is updated annually. Characterization and evaluation data for AEGIS accessions is also published on EURISCO.

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.*).

Passport data: accession number, accession name, botanical name, biological status, country of origin.

Thank you for the efforts you have made to answer all the questions. This information will be important to you and your colleagues at the genebank

as well as to the Working Groups and other bodies in ECPGR for the establishment of a quality genebank management system!

The ECPGR Secretariat