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CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

INIA
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

Spanish Plant Genetic Resources Centre (CRF)

OPERATION MANUAL

Contact:
Spanish Plant Genetic Resources Centre (CRF)
National Institute for Agricultural and Food Research and Technology (INIA-CSIC)
Autovía de Aragón Km 36,
28805, Alcalá de Henares, Madrid, Spain
Phone: (+34) 915026666
Fax: (+34) 918819287
Website:
https://www.inia.es/en-en/Pages/Home.aspx
Email: seccrf@inia.csic.es

Head:
Dr. Luis Guasch
Phone: (+34) 915026688
Email: luis.guasch@inia.csic.es
Curator:
Dr. Isaura Martin
Phone: (+34) 915026682
Email: martin@inia.csic.es
Documentation manager:
Dr. Lucía de la Rosa
Email: lucia.delarosa@inia.csic.es
Phone: (+34) 915026670

Acronyms

Acronym	Meaning
CBD	Convention for Biological Diversity
CRF	Plant Genetic Resources Centre
CWR	Crop Wild Relatives
CSIC	Spanish National Research Council
FAO	Food and Agriculture Organization of the United Nations
EU	European Union
INIA	National Institute for Agricultural and Food Research and Technology
ISTA	International Seed Testing Association
IT (TIRFAA)	International Treaty (International Treaty on Plant Genetic Resources for Food and Agriculture)
MAPA	Ministry of Agriculture, Fisheries and Food
PNRF	National Programme for Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture
PGR	Plant Genetic Resources
PGRFA	Plant Genetic Resources for Food and Agriculture
SID	Seed Information Database
SMTA	Standard Material Transfer Agreement

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1. Germplasm Acquisition and Accessioning

Genebanks can acquire germplasm to conserve through a number of different ways. Conducting collecting missions is the most important way of acquiring germplasm material in a reliable manner. Germplasm exchange with other genebanks is a second route to add genetic diversity to the collection and donations coming from other institutions/particular, including researchers and plant breeders is a third manner. Such acquisitions should be guided by a formal mandate that the genebank concludes with its host organization or government and that provides the basis for a genebank acquisition policy. The actual accessioning of acquired germplasm samples, i.e. formally including it into the collection with its unique accession number, is a complex process during which the curator has to check a number of aspects

such as the verification of the identity of the material, the health status, the availability of pertinent information, etc. It is further understood that also legal aspects form part of this activity, e.g. was the material collected/obtained in legal manner, are there any restrictions on its use, etc.

Box 1.1. Germplasm Acquisition and Accessioning

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

This description should include details on:

a) *which species you conserve and make available;*
b) *who decides on what your mandate is and, if different,*
c) *from whom do you received the mandate;*
d) *the main aspects of the mandate; and*
e) *legal considerations on PGR as foreseen in national legislation.*

The Spanish Plant Genetic Resources Centre (CRF) belongs to the National Institute for Agricultural and Food Research and Technology (INIA-CSIC) of Spain. INIA has been recently assigned to the Spanish National Research Council (CSIC), which is the largest public research institution in Spain, affiliated to the Science and Innovation Ministry.

The INIA started PGR conservation activities in 1977, thanks to the funds from FAO and The World Bank to collect grain legumes from the west of the country. Relevant breaders' collections from regional institutes, mainly of cereal species, were also incorporated to the centralized collections of INIA in Alcalá de Henares (Madrid).

The CRF was created in 1981 to fulfil the legal obligations of the Spanish government regarding plant genetic resources conservation and research, as well as to provide technical assistance for the Spanish representation in international fora in this field. At that time, INIA belonged to of the Ministry of Agriculture.

In 2006, the law on Seeds and Nursery Plants and Plant Genetic Resources (Law 30/2006, 26th July 2006, https://www.boe.es/eli/es/l/2006/07/26/30) provided to the CRF a 'national' status and defined its main functions:

- Management of the National Base Genebank and storage of safety duplicates from all the seed collections from the Spanish Network of genebanks.
- Management of the National Inventory of PGR from the Spanish Network of genebanks.

The last Regulation of the Spanish National Programme for Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture (PNRF) was approved in 2017 (Royal Decree 119/2017, https://www.boe.es/eli/es/rd/2017/03/03/199), involving both the Ministry of Agriculture and the Ministry of Science and Innovation. This National Program aims

to prevent the loss of genetic diversity of species, traditional varieties and native ecotypes, and to guarantee the availability of the necessary genotypes for genetic improvement, research and direct use. This regulation establishes the legal basis of the Spanish Network of genebanks and the procedure to fund this Network. It also assigns to the CRF the function of conserving, regenerating and characterising the active collections under its care, and the coordination of the Spanish Network of genebanks, which are allocated all over the country.

Currently (March 2022), the **security or base collection** of the CRF preserves around 45,000 accessions, 79% of Spanish origin. Accessions are grouped in winter cereals (18.5%), spring cereals (5.8%), grain legumes (21.5%), horticultural crops (32.3%), forages (15.2%), industrial crops (2.3%), aromatic and medicinal species (2.7%) and other plants (1.8%). Around 65 % and 25% of these materials correspond to landraces and wild materials, respectively.

CRF also conserve important **active collections** for utilization purposes, under less stringent temperature conditions than the base collection. Currently (March 2022), the CRF active collections comprise around 22,600 accessions, 71% of Spanish origin. Active collections are mainly composed by cereals (49.6%) and grain legumes (44.2%). Industrial crops and other species of interest for food and agriculture are represented in the CRF active collections, to a lesser extent. Most of the active collection materials corresponds to landraces (73.3 %). Eighty percent of the active collection samples have a duplicate in the base collection. Accessions in active collection are duplicated in the base collection when sufficient material is available.

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?*

CRF-INIA was designated for the conservation of the safety duplicates of the seed collections from the Spanish Network of genebanks and for the management of the National Inventory of PGR. The species conserved by the Spanish Network of genebanks comprise about 77.000 accessions of agricultural, horticultural, fruit and tuber species of the main crops in Spain.

Recently, CSIC has signed a formal agreement with Svalbard Global Seed Vault conservation facility in order to assure the conservation of a black box duplicate for the more relevant Spanish accessions of the security collection. First thousand samples from CRF genebank were sent in June 2022.

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.

General criteria for the acquisition of germplasm are established by the 'National Committee' of the PNRF. In general, collections should contain at least 60% of Spanish local landraces and crop wild relatives (CWR), germplasm collected under public research projects and obsolete commercial varieties. Exceptionally, foreign germplasm coming from international exchanges with other collections is also conserved in the CRF-INIA genebank.

The CRF base collection also receives seed samples from all the active collections of the Spanish Network of genebanks (including CRF active collection), for the conservation of security duplicates.

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.)?

Genebank curator and crop specialists are in charge of the identity verification of the plant material received.

Firstly, identification codes are carefully checked, especially for material coming from other members of the Spanish network, or from internal or external multiplications. The identity of the samples is evaluated by a technician by visual analysis, relying on provider's information, or by comparison with other accessions stored. For species that have seeds with distinctive characteristics (e.g. maize and most grain legumes) the photo collection of the accessions is used. In some cases, biochemical testing (protein electrophoresis) and chromosome count are also applied, especially in cereal species, in case of doubt about the identity of the sample.

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).*

According to the species and to current phytosanitary requirements, the available phytosanitary certificate or other documents and relevant information are checked and recorded. The genebank has set up an exchange database where the documentation related to the acquisitions and distribution of plant material are recorded.

Seed quality, including seed health, is visually evaluated. In the case that a high

proportion of empty seeds in the sample is suspected, an X-ray examination with a Faxitron equipment is carried out for confirmation.

If the requirements are met, samples are prepared for subsequent conservation procedures (cleaning, drying, viability testing, storage).

GA6 – Describe whether and how the SMTA is being implemented:

a) *extent of materials covered by SMTA (crops, numbers of accessions)*
b) *ways of SMTA implementation and documentation of transfers of PGR*
c) *other aspects (e.g. monitoring, supervision).*

The access to PGRFA is regulated in Spain by the Royal Decree 429/2020, 3th March 2020, https://www.boe.es/boe/dias/2020/05/11/pdfs/BOE-A-2020-4915.pdf.

According to this regulation, CRF has decided that all material of the CRF active collection is distributed under SMTA, also including non-Annex I species, when the intended use is research, breeding or training for Food and Agriculture. Materials for other uses (pharmaceutical, cosmetic, etc.) are distributed following the procedure implemented by the Ministry of Agriculture under Nagoya Protocol regulation.

Since 2020 all SMTAs are managed with the "Easy SMTA" application, usually with the Shrink-Wrap option. The Ministry of Agriculture (MAPA) that has the political responsibility for the PGRFA, is authorized to see the notified SMTAs.

A simplified document is used for the distribution of the material to farmers or hobbyists for their direct use. In this case, the recipient undertakes to use the material only for that purpose.

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*.

CRF collecting missions

From the early 1980s to 2014, the CRF has conducted numerous multi-crop collecting expeditions across Spain. Currently, collecting missions are not a priority for the CRF. Notwithstanding that, some of them can take place under specific research projects.

CRF collecting missions prioritize local landraces, minor or underutilized species and CWR. Collecting missions are exhaustively planned, considering the material already conserved in the genebank, the historical evolution of crops in the prospected areas and the information obtained from local agricultural authorities or other local contacts. For wild material, information is gathered from specialised databases, available bibliography or local experts. Legal requirements are studied carefully and appropriate permits are sought from local and national authorities if necessary.

Materials and instruments required for sampling and storage of material, travel routes and accommodation are carefully prepared prior to the trip. Collecting trips for cultivated species are usually made in autumn, when farmers have already harvested their seeds. For wild species, trips are normally carried out during the flowering period to identify the populations and during the ripening period to harvest the seeds.

During collecting missions, CRF staff follow specific sampling procedures in order to have a good representation of the genetic diversity of the sample and to assure a good seed quality. Acquisition of duplicates is prevented as much as possible. Where feasible, enough quantity of seed is collected to reduce multiplication requirements, but depletion of wild plant populations or farmer stocks' is always avoided. Relevant passport data are recorded using pre-designed forms, and pictures are also taken. All these data are incorporated to the National Inventory when a new code is assigned to the new materials.

Other collecting missions

According to the RD429/2020, the collecting missions of RFGFA in Spain for utilization purposes must be authorized by the Ministry of Agriculture. One replicate of the collected material should be deposited in an active genebank of the Spanish Network, whenever it is considered appropriate.

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

Recent collecting missions have been performed by CRF staff under the scope of research projects regarding *ex situ* conservation of CWR under-represented in the collections of the Spanish Network.

A specific methodology based on an ecogeographic approach was used in the project funded by the Crop Trust: GS16001 (2016-2018). *Towards a more complete coverage of the diversity of crop wild relatives in ex situ collections. Collection of cereals and grain legumes crop genepools in Spain.*

García, R. M., Parra-Quijano, M., & Iriondo, J. M. (2017). A multispecies collecting strategy for crop wild relatives based on complementary areas with a high density of ecogeographical gaps. Crop Science, 57(3), 1059-1069.

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?*

According to Spanish legislation, CRF keeps the safety duplicates of seed samples from the Spanish Network of Plant Genebanks in the base collection under freezing conditions at -18 °C, at its facilities in Alcalá de Henares (see SD2).

Additionally, CSIC has recently signed a formal agreement with the Svalbard Global Seed Vault in order to assure a black box triplicate conservation for the more relevant Spanish accessions. The first thousand samples were sent to Svalbard in June 2022.

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

The safety duplication policy for CRF active collections, as part of the Spanish Network of genebanks, was established in Spanish legislation and it consists on the mandatory duplication of the seed active collections in the CRF base collection.

In addition, the PNRF envisages actions directed to achieve the safety duplication of seed and field collections conserved in the genebanks of the Spanish Network.

CRF-INIA initiated last June the delivery of accessions to Svalbard Global Seed Vault facilities.

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). CRF is located in Alcalá de Henares (Madrid), in the centre of Spain, which is a low risk area regarding seismicity, flooding or high intensity winds. Although CRF is placed in a river terrace, the dams built upstream make flooding difficult. Furthermore, the flooding area for a return period T=100 years is out of the building area. Therefore, standard construction practices were followed.
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 main building of the CRF where conservation chambers are located has locked doors and is located in a fenced area. A specific security service is provided for the whole farm outside working hours. Regarding fire protection, there are several smoke detectors and fire alarms distributed along the building. Fire extinguishers and a specific emergency plan are available. Autonomous power generators are available to ensure the supply of electricity in case of failure of the general grid. Additionally, the installation of a 50 kWp solar photovoltaic plant is planned to cover the power supply of the facility. **SS3** – Please provide information on any other structural security aspects that you might have in place. The place where the genebank is located belongs to a larger complex of research and farm facilities that have a restricted common access.

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*.

An autonomous power generator is installed in order to prevent eventual power cuts. Preventive surveillance and maintenance plans are in place to assure the adequate functioning of key equipment and facilities. Regular maintenance service is provided by an external company under contractual agreement.

A new cold chamber for the base collection is currently under construction. Back-up compressors and double cooling circuit are foreseen is this new facility.

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

Cool chambers and desiccation rooms have temperature and humidity indicators that are under supervision of genebank staff. Security service performs periodic rounds in cold chambers outside working hours.

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*.

According to the Spanish Royal Decree 199/2017, INIA, as an organisation of the Ministry of Science, is responsible for funding the permanent conservation activities of the Spanish network of genebank of the PNRF. However, changes in INIA's competencies in the last few years have complicated and delayed the funding of the Spanish network of genebanks. Therefore, there is currently a certain degree of uncertainty regarding the genebank funding.

CRF-INIA also gets funding for some of the activities of the genebank from external and internal research projects.

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

As INIA is a public organization, the staff is provided through scheduled administrative procedures that guarantee merit and ability and also transparency and fair evaluation of the qualification according to the tasks to be performed. Notwithstanding that, some difficulties are encountered due to the bureaucracy inherent to public administration. Staffing for field workers is especially complicated because this type of personnel is quite exceptional for public administration.

In the past years, there has been a general tendency to reduce staff in the public administration, due to the economic crisis. This has also led to a decrease in CRF staff over the last decade.

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.

Our Institute has a Prevention Service, which provide training in emergency actions and preventive measures. An Emergency Plan for our centre was elaborated and disseminated amongst staff and an emergency team has been created to act in accordance to the plan. The plan refers to different kinds of emergencies such as fires and other hazards.

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.

INIA provides periodically several training plans that include training in firefighting, first aid and emergency response.

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.

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.).

When collecting missions are carried out, they are organised and planned in order to get seeds of good quality, as close to maturity as possible. Adequate conservation and transportation conditions are observed to avoid seed damage. Seeds from other genebanks or from other donors are visually inspected for quality and health.

Regeneration and multiplication activities are performed following internal procedures and specific cultivation practices in order to get the maximum quantity of healthy and good quality seeds. Seeds or fruits resulting from multiplication or regeneration activities are harvested from healthy plants after seed maturity. Harvested material is stored in warehouses or in the cleaning room to be processed as soon as possible. Seeds are extracted and cleaned when necessary following specific procedures that can also include X-Ray analysis (see SPP1, IV3).

After cleaning and documenting, accepted material is desiccated in a drying chamber (20°C, 13-15% relative humidity) until seed equilibrium humidity. Initial viability testing is performed after drying and before packaging.

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

Procedures based on ISTA rules, Seed Information Database (SID) - Kew Garden or

specialized bibliography are followed to break seed dormancy and hardseededness. Mechanical scarification, gibberellic acid application and cold stratification are procedures that are routinely used for some taxa.

In some cases, specific assays are carried out to improve seed dormancy removal protocols. Some of these studies has been published as scientific papers:

Martin, I., & De la Cuadra, C. (2004). Evaluation of different scarification methods to remove hardseededness in *Trifolium subterraneum* and *Medicago polymorpha* accessions of the Spanish base genebank. Seed Science and Technology, 32(3), 671-681.

Martín, I., & Guerrero, M. (2014). Effect of sulphuric acid scarification on seed accessions of cluster clover (*Trifolium glomeratum*) stored in a genebank. Seed Science and Technology, 42(2), 293-299.

Vivanco, P., Oliveira, J. A., & Martín, I. (2021). Optimal germination conditions for monitoring seed viability in wild populations of fescues. Spanish Journal of Agricultural Research, 19(3), e0804-e0804.

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

Special procedures for specific taxa may include:

- Freezing legume seeds (-20ºC for one week) as soon as possible after harvest to kill bruchid larvae.

- Allowing seeds to ripen inside the fruit after harvest: eggplant, some cucurbits.

- Fermentation of tomato seeds for two days to improve seed cleaning and health.

- X-ray inspection to assist seed cleaning of seed lots that are suspected of having a high proportion of empty seeds.

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 diferent procedures for crops/species with erratic initial viability or irregular viability lifespan;*
e) *etc*.

Seed viability monitoring is carried out through germination tests. Germination protocols have been set up for the different species, based on ISTA standards. For species not covered by the ISTA rules, information is sought in the scientific literature.

The most common methods used for germination tests are top of paper and between

paper methods. Sand is used as germination subtract in large seeds (e.g. bean, faba bean, castor bean) to reduce fungal spreading and improve seedling evaluation. Prior to germination tests, all seed samples are kept at room conditions for at least one week, to increase their moisture content and reduce damage caused by rapid imbibition. Seeds that are highly susceptible to imbibition damage (e.g.some legumes), are pre-hydrated in a humid environment before the germination test. Germination percentages are estimated on the basis of normal seedlings. Percentages of abnormal, hard, empty and 'fresh' seeds are also scored and recorded.

Initial viability testing is performed after seed drying with a maximum of 200 seeds. Subsequent tests are carried out with 50-100 seeds. Germination monitoring is not conducted when the number of available seeds is less than 500.

In previous years, the general rule was to conduct germination tests every 10 years. Currently, on the basis of historical data obtained over time, first interval for viability re-testing have been shifted from 10 to 20 years, with the exception of samples with initial seed viability below 85%.

At the beginning of each year, seed viability monitoring is scheduled. When the number of tests exceeds the capacity of the germination laboratory, priority is given to short-lived seed species, species with little historical data available and samples preserved in the active collection. Although the selection of samples to be monitored follows a common sense rule, there is no written standard that systematises this procedure. A general rule should be established.

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.

Results from all germination tests performed on genebank accessions are stored in a MS Access file, included in the information system of the CRF genebank. MS Access queries have been set up to analyse seed germinability over time. Currently (March 2022) more than 125,000 germination data are recorded in the germination table of the 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.*

Criteria for regeneration is based on viability data and number of seeds conserved. In general, a germination percentage threshold of 85% is considered for initial samples of crops species. In case of wild material or species in which high levels of germination are difficult to achieve, a lower value can be accepted (70% or less). During storage, the viability threshold for regeneration is set according to FAO

Genebank Standards at 85 percent of initial viability.

Regarding the quantity of seeds stored, for the base collection, regeneration will be required according to the following general criteria:

< 1.500 seeds for self-pollinated species,

< 3.000 seeds for cross-pollinated species.

< 1000 seeds or less for problematic accessions,

In active collections, 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.

CRF long-term (base) collections are stored at -18°C. Relative humidity is not controlled in the cold chamber. Base collection containers (cans) proved to be completely tight in all test carried out (see SC3).

CRF medium-term (active) collections are stored at -4°C. Relative humidity is not controlled in the cold chamber. Humidity indicators are used in the active collection containers (jars) to detect occasional sealing failures (see SC3).

Drying chambers are maintained at a temperature of 20°C and 13-15% of relative humidity.

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

In active collections, seeds are stored in 720 cm^3 twist-off glass jars with metal lids. Currently glass jar and lids with six thread-points are used. Old jars and lids with four thread-points are been replaced due to their worse performance.

Sealed 750-400 cm^3 cans are used for base collections (see also SCSS2). For small seeds (lettuce, cabbage, tomato…..) several samples are stored in one can, individually packed in sealed foil bags.

Vacuum sealed foil bags (PET/AL/PE, 12/12/75 microns) are used for Svalbard duplicates.

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?

Dessication chambers are maintained at 20°C temperature and 13-15% relative humidity. Seeds are placed in plastic trays and left to dry until they reach the equilibrium moisture content. Time required to equilibrate may vary from 1-6 weeks depending on the size of the seeds, although seeds are usually kept in the drying chamber for longer than necessary. In those conditions seed moisture content is around 3-7% depending on the oil content of the seeds.

Seed moisture level is not routinely measured in seed samples but water activity measuments (with a Rotronic equipment) or gravimetric determinations of seed moisture content can be performed in a random selection of samples to check the good performance of the procedure.

Humidity indicator cards are placed in the glass jars of the active collection to detect any increase in humidity along the storage period. No additional measures are taken in the base collection because leakage failures have never been detected in cans in all checks carried out.

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.

Currently, around 44,700 and 22,600 accessions are preserved in CRF base and active collections respectively (March 2022). It has been calculated that there is enough available place in the -18°C cold chambers until 2024, according to the current rhythm of operation of the genebank (see SC5).

Now, there are no space problems in the active collection chamber. The entry of new samples in this collection is much lower than in 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).

A new chamber for the base collection with a mobile storage system, designed to double the capacity or the current chamber, is under construction, and it is expected to be ready in a few months.

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.

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)?

Total weight and 100-seeds weight are recorded in the management database for all samples, to calculate the number of seeds stored.

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.

Twist-off glass jars (720 cm3) with metal lids are used for the active collection and cans of two different sizes (750 and 400 cm3) for the base collection. For small seeds several samples are stored in the same container, individually packed in sealed foil bags.

No sub-samples of material are conserved. When a sample is derived for an original sample (e.g. separation of types or single seed descent), it is treated as a new accession with a new accession number.

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.

Ideally, the quantity of seeds should be ≥ 3000 for cross pollinated species and ≥ 1500 for self-pollinated species, both in base and active collections. For problematic species (e.g. wild species, very large seeds…) less number of seeds can be accepted. In general, 1000 seeds is considered the strict minimum threshold for the base collection. 500 seeds is the threshold for viability monitoring.

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

Minimum quantity of seeds that is accepted is flexible depending on the type of material and species.

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 small-medium sized plans (e.g. winter cereals and most of the winter grain-legumes) regeneration plots are usually 1.2 m x 2 m, with a spacing of at least 60 cm between them. In these cases, the resulting number of plants is far higher than 200 plants.

For larger plants (e.g. *Vigna*, sunflower, castor bean…), plot size is adapted to have at least 50 and 30 plants/accession in self- and cross-pollinated species, respectively.

In the case of small-medium sized plants, the whole plot is generally harvested and threshed. When fruits/seeds are harvested by hand, a similar number of fruits/seeds is collected from each plant.

For wild species with high degree of seed dehiscence, inflorescences may be bagged and legume pods are usually collected sequentially, as soon as they are dry. Wild legumes with a high proportion of hard seeds are scarified before sowing.

In self-pollinated species, different species are alternated in neighbouring plots, if possible, in order to avoid any sporadic outcrossing.

In cross-pollinated species, accessions are isolated by spatial separation (500 m, or

less if there are physical barriers) or by using anti-pollen/anti-insect cages. Anti-pollen cages are made of textile material and are used for wind-pollinated plants (e.g. rye). These cages are opened alternatively to facilitate pollination and decrease humidity inside them. For insect-pollinated species, anti-insect screen structures are used. Pollinating insects (*Bombus*), provided by specialised companies, are placed into these cages if necessary. Several species can be grown in the same anti-insect cage, with one accession per species.

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

Box 3.2.3.A. Regeneration Environment and Procedures

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

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

In our Centre, all regeneration work is carried out under field conditions. We do not use controlled environments. When our environmental conditions are not suitable for a specific crop regeneration, those activities are commissioned to another Spanish genebank or institute as an external service (e.g. beans).

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.

At harvest, plants are collected in textile sacks and labelled outside and inside. Small grains are normally threshed outdoors with a small threshing machine. Different species are alternated during threshing to minimize admixtures. Large pods and

other dry-fruits are threshed and cleaned manually by well-trained personnel.

Devices like sieves with different mesh sizes, seed blowers, airsoft gun and an air extraction cabin are used when needed to get a more efficient and safe cleaning.

In case of fresh fruits a wet cleaning is performed consisting on removing the flesh and rinsing thoroughly with running water. In seeds containing mucilage (e.g. tomato), it is eliminated by fermentation in glass jars.

Legume seeds are freezed (-20ºC for one week) as soon as possible, to prevent insect (bruchid) development. X-ray inspection may be used to assist seed cleaning of seed lots suspected of having a high proportion of empty seeds.

Last cleaning step is normally carried out by hand to remove broken, damaged or foreign seeds. Care is taken during the whole cleaning process to avoid seed damage.

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

Seed drying is performed by placing the seeds on trays in a drying room which provides a temperature of 20°C and a humidity of 13-15%, they are left inside until they reach the equilibrium moisture content. Time required to equilibrate may vary from 1-6 weeks depending on the size of the seeds, but normally all seeds are kept in the drying chamber for more than a month.

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

Harvesting and cleaning activities are planned in order to get the material into in the drying chamber within 2 months since harvesting.

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.

Harvested plants or fruits are temporary stored in warehouses or in the cleaning room. Our climate is semi-arid, thus relative humidity is usually low (around 30%), especially in the harvest time (summer).

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

The criteria to decide on the minimum quantity of seeds per accession to be stored in

long-term storage collections are mainly based on the reproduction type of the species and the type of material. Whenever possible, the number of seeds per accession in the long-term storage is at least 1500 seeds for self-pollinated species and 3000 for cross-pollinated species. If enough seeds are available, the container is completely filled to reduce the air content.

One thousand seeds is considered the strict minimum threshold for the base collection.

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).

CRF genebank does not conserve GMO.

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

CRF does not have policy or procedures related with GMOs distribution. In any case, our regeneration practices prevent GMO contamination.

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.

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; use of a formal agreement to distribute the germplasm.

CRF active collections are available to any user for research, breeding, training and cultivation purposes. In the period 2016-2020, an annual average of 1,600 samples were distributed in 85 shipments. CRF-INIA security collections are not available for distribution, as they are safety back-ups from the active collections of the Spanish Network of genebanks.

As a global approach to determining seed availability, an accession is considered as 'not available' for distribution when less than 300 seeds are conserved in the active collection. An accession is considered to be of 'limited availability' when it is not duplicated in the base collection. In this case, distribution depends on seed stocks, type of petitioner and intended use, at the curator's criteria.

In general, the germplasm distribution policy of the CRF-INIA for all crops stored in active collections follows the conditions of the International Treaty, regardless of whether or not they are listed in the Annex I of the Treaty, based on the provisions of Royal Decree 429/2020 (see GA6).

All materials requested for research, breeding or training purposes for food and agriculture are distributed under SMTA. Since 2020, SMTAs are managed with the Easy SMTA application, normally with the Shrink-Wrap option. Material for other uses (pharmaceutical, cosmetic…..) must be requested through the Agriculture Ministry, according to the procedures established under the Nagoya Protocol, for commercial or non-commercial uses (Spanish Royal Decree 429/2020).

A simplified document is used for material distributed to farmers (including hobbyists/home gardeners) for direct use. In this case, the recipient undertakes to use the material only for that purpose.

The number of accessions per request is not limited, but requests for large number of samples are considered on a case-by-case basis.

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?

Every request is managed as soon as possible. There has not been set any 'maximum time' commitment for germplasm distribution in response to the requests. In general, processing the seed requests usually takes less than two weeks.

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 of each accession are provided together with seed material. When available, other specific information requested by the user is also provided.

Other details, such as special requirements for seed germination (e.g. methods to eliminate seed dormancy) or the need for isolation in cross-pollinated species is often included in the exchanged e-mails.

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).

The quantity of seeds for distribution depends on both the species and the available stock. In general, an amount of 50-100 seeds is provided unless the requestor asks for a lower seed quantity.

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.

In the active collection, each CRF-INIA accession is stored in a unique container. In the base collection there may be up to two containers (cans) for a given accession when the seeds are very large, to enable the storage of at least 1000 seeds. After each removal, the left seed weight is updated in the management database.

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.

As specified in Box 3.3.1.A, in the active collection, 300 seeds is an absolute minimum threshold, below which an accession is considered as ‘not available’. An accession is considered to be of ‘limited availability’ when it is not duplicated in the base collection. In this case, distribution depends on seed stocks, type of petitioner and intended use, at the curator's criteria.

In any case, regeneration should be carried out when the amount of seeds falls below the amount needed to perform three regeneration cycles.

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

In active collections, measures are taken to regenerate from seeds coming from no more than 5 cycles of the original seed.

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.

For its active collections, CRF only admit apparently healthy seeds, but no specific health analysis are performed on seeds so far.

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

Currently we cannot provide phytosanitary certificates. Petitioners from non-EU countries are always warned on this issue.

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

See AGHA2 above.

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

In previous years, a seed pathologist was temporarily working at the CRF. During that time, numerous analyses of seed-borne fungi were carried out on the germination tests of the genebank samples. Furthermore, some CRF staff were trained in the morphological identification of the most frequent seed-borne fungal genera.

We expect a full-time pathologist to join the CRF shortly, which will facilitate the development of seed health activities in the future.

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.

As indicated in AGSS1 above, the minimum quantity of seed to be distributed depends on the species and the stock available. In general, a quantity of 50-100 seeds is provided unless the user indicates a lower seed requirement.

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

The number of seed delivered is usually increased in case of low seed viability, if there is sufficient seed stock.

GS3 – Please provide information on any other aspects related to seed supply.

4. Providing Information

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

Box 4.1. Genebank Documentation System

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

a) On which software is the system based (i.e. Oracle, Fox Pro, MS Access, MS excel, MS Word, other?).
b) In case you use a manual information management system, please provide details.
c) In case your "internal" database(s) is/are different from the publicly available database(s), please provide details on both,
d) Describe which activities of the genebank are covered by the system.

CRF-INIA is responsible for the management and publication of the National Inventory of PGR, which includes the passport information of all the accessions conserved in the Spanish Network of genebanks.

Software used at CRF is MS- Access, both for information related with the Spanish National Inventory and for all the internal information of CRF genebank.

The Spanish National Inventory of PGR is available on-line through the INIA webpage, https://bancocrf.inia.es/en/. The web database is an adapted version, periodically updated from the raw passport database held at CRF-INIA.

CRF internal databases cover all the main activities developed in the Centre: material acquisition, accession management, viability monitoring, characterisation and material distribution. Data generated by these activities are included in a relational system with different tables and files.

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 descriptors of the Spanish National Inventory are based on FAO/ Bioversity Multicrop Passport Descriptors (MCPD V2.1) modified to cover special needs of the Spanish system.

A unique Inventory number (NCXXXXXX) is assigned to each genetic material conserved in the Spanish network, but each genebank also uses their own accession

numbers for internal identification. When the same material is stored in different genebanks, the accession numbers used by each of them are included in the passport table.

MS-Access files of the CRF information system are: Inventory (with passport, CRF management and auxiliary tables), Germination, Exchange (tables for material distributed and received), and CRF Characterization for each species. Most of the tables can be related by accession or Inventory numbers.

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

The web database is an adapted version, periodically updated, from the raw passport database held in CRF-INIA.

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).

Both hard copy data and Excel files are used to fill the internal Access tables.The web database is loaded through a specific procedure that includes the use of Excel templates.

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

The internal information system is mainly maintained by advanced MS-Access users of the CRF staff. The CRF does not have experts in software development. INIA services provide computer and informatics technical support when necessary. The current web version of the National Inventory was developed by external experts. The lack of maintenance of this web database is now a major constraint that must be addressed.

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

CRF-INIA databases are hosted on the SQL server located at CRF facilities, as well as on the central server at INIA headquarters, both with daily updating of information. An external back-up copy of the database is made monthly.

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?).

As mentioned in GD1, the passport data are available through the INIA website. When required, data excel files are sent to users.

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

a. what types of data (passport data, characterization & evaluation data etc.) and
b. which web-service interfaces are available (i.e. GBIF IPT, BioCase, TapirLink).

For the time being, passport and characterisation data are periodically uploaded to the web database through procedures implemented for this purpose, at least twice a year.

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

Passport data of the Spanish National Inventory are published to EURISCO, and updated at least once a year.

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

IE5 – Describe the kind of information you distribute together with the germplasm to persons that request germplasm.
Please consider the following data types: Passport, Characterization; Evaluation, and/or Germplasm management data (e.g. viability percentage; protocols followed for routine operations; etc.

As mentioned in AGP3, passport data of each accession are provided with seed material. When available, other information requested by the user, such as characterisation data, is provided.
Other details, such as special requirements for seed germination (e.g. methods to eliminate seed dormancy) or the need for isolation in cross-pollinated species is often included in the exchanged e-mails.