Genebank Quality Manual

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Operational Genebank Manual of the JKI Institute for Grapevine Breeding Geilweilerhof

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1 Germplasm Acquisition and Accessioning

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

Box 1.1 Germplasm Acquisition and Accessioning

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

(This description should include details on:

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

The Julius Kühn Institute (JKI) is the Federal Research Centre for Cultivated Plants in Germany and an autonomous superior federal authority in the portfolio of the Federal Ministry of Food and Agriculture. The Institute for Grapevine Breeding (IRZ) Geilweilerhof is one of the 17 research institutes of the Julius Kühn-Institute. It is committed to grapevine breeding, and has the national mandate to preserve grapevine genetic resources, given by the Ministry.

The Institute maintains more than 3500 accessions of 34 *Vitis* species, cultivars and breeding lines of the European species *Vitis vinifera* and fungus resistant breeding lines for breeding purposes. The Institute

preserves, characterizes, evaluates and documents the diversity maintained in the collection. Safeguarding and description of old and neglected German cultivars with a socio-cultural, local and historical relation to Germany are in the focus of research.
The germplasm repository of the institute contributes to the implementation of the 'National Program for Conservation and Sustainable Utilization of Genetic Resources of Agricultural and Horticultural Crops'. The Institute is the manager of the CCDB Vitis (European Vitis Database) and a member of the 'Working Group Vitis' of the European Cooperative Program for Plant Genetic Resources (ECPGR). Furthermore, the institute is coordinator of the decentralized national network "Deutsche Genbank Reben".

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?

The German Grapevine Genebank (http://www.deutsche-genbank-reben.julius-kuehn.de/) was founded as a decentralized network in 2010. It includes six grapevine collections. The IRZ is the national coordinator. The institute manages the European Vitis Database. It has a representative in the ECPGR Vitis Working Group.

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

a) please specify which crops or which geographic area, if applicable.

The genebank has no strict acquisition policy.

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.)?
We are in close contact with Vitis genebank curators of countries all over the world, in particular Europe to clarify identity and description of accessions.
A SSR-marker-database was established at IRZ, encompassing fingerprints from about 6000 distinct cultivars. Numerous bibliographical sources and Grape Databases are available providing descriptions, drawings, photos and herbarized leaves from grape cultivars. Both tools in combination are applied for trueness to type assessment of accessions in the IRZ grapevine repository. SNP data for variety identification is a further tool being developed. SSR and SNP data are also accessible via www.vivc.de.

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).

Trueness to type control of newly introduced accessions is conducted after planting. Some morphological features, like bunch characteristics, can be recorded first in the 2nd or 3rd year of growth. To prevent Phylloxera infestation the accessions are grafted on tolerant rootstocks. Every year a fraction of the genebank is tested for the most important viral diseases of grapevine. Virus infection will be recorded. In case of replanting, virus-free plants will be requested if they are available. Material from outside the European Union is observed in the quarantine station according to the legal requirements.

GA6 – Describe whether and how the SMTA is being implemented

a) Extent of materials covered by SMTA (crops, numbers of accessions)
b) Ways of SMTA implementation and documentation of transfers of PGR
c) Other aspects (e.g. monitoring, supervision)

SMTA is implemented since 2011, despite the fact that the genus *Vitis* is a non-Annex1 crop. All distribution steps are documented. Every two years, the number of accessions distributed and the number of samples distributed to the different categories of recipients are transmitted to the National Focal Point.

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*).

Collecting missions in the United States have been performed to acquire *Vitis* species carrying genes for fungus (*Erysiphe necator*, *Plasmopara viticola* and *other diseases*) and Phylloxera resistance.

Planning and implementing collecting missions for *Vitis* wild relatives were realized in close cooperation with specialists in the host country. They are familiar with the wild habitats and the ecogeographical requirements of the desired Vitis species.

During the expedition, the original samples were documented via GPS-data. Fruits were collected and later seedlings raised from seeds. The young seedlings are transferred to the field to be evaluated for various agronomic traits, in particular fungus resistance. Marker analysis is performed in order to establish a species-specific core collection.

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

none

2 Ensuring Security

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

2.1 Physical Security

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

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

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

(*This description should include the following aspects:*

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

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

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

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

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

The agreement of the German genebanks envisages safety duplication. Trueness to type in these collections was stated in the scope of a three years project (2014 – 2016) using SSR-markers and ampelography. As a result, misnomers, synonyms and homonyms were identified, as well as unique and thus endangered accessions. Unique accessions in the five federal state collections were duplicated at IRZ. Conditions under which the material has to be preserved were agreed.

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 Vitis genebank consists of *ex situ* collections in the field. No earthquake area; in general, no high wind/storm exposure; 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*).
Field genebank. No security arrangements are needed.
SS3 – Please provide information on any other structural security aspects that you might have in place.

Box 2.1.3 Security Equipment
SE1 - Provide details on the kind of emergency (back-up) equipment or arrangements that you have in place to ensure permanent electricity and cooling.
(*Aspects to consider are:*
a) *"back-up" compressors for your cold rooms;*
b) *generator;*
c) *regular maintenance and trial runs;*
d) *other*).

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

Box 2.1.4 Institutional and Personnel Security
IPS1 – Provide details on the "institutional security", in particular with respect to the provision of financial means to operate the genebank
(*Aspects to consider are:*
a) *timely transfer of funds from the "mother" organization to the genebank;*
b) *do you have direct access to the "mother" organization that provides the budget?;*
c) *internal "security" of accessing these funds;*
d) *long-term security and stability of funding (compensation of inflation rates, avoiding variation in years)*
e) *any other observations that are relevant in this context)*.
Foundation under public law, funding provided annually by Federal Ministry of Food and Agriculture.
IPS2 – Describe how you secure adequate staffing of your genebank is?
Staff is secured by 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.

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.

3 Germplasm Maintenance

This chapter deals with key aspects of managing germplasm in a genebank, i.e. the maintenance of the viability, the genetic integrity, the availability of the conserved germplasm as well as the management of the corresponding information. Given the fact we are covering seed, in vitro cultures and entire plants it might well be that not all aspects are covered by one and the same genebank. In those cases it is suggested that only the applicable sections are completed. Accordingly, at the beginning of each section of this chapter you will find a "navigation box" (highlighted in yellow) that will help you as user of the template to complete the correct section(s).

3.1 Maintenance of Viability

This section refers to the maintenance of the longevity of the seeds or of tissue cultures or living plants in storage. A high initial viability is the most important pre-condition for achieving the longest lifespan of seed accessions in storage, hence maximum efforts need to be taken to ensure that seeds to be stored have the highest possible viability. Optimum growing conditions when multiplying/regenerating the accessions, efficient management of the preparatory steps before storing the germplasm, adequate storage conditions as well as proper monitoring of the viability are critically important.

Navigation Box on Maintaining Viability section

Seed – If applicable, please complete the section on Maintaining Viability for the activities related to seed genebanks (i.e. boxes 3.1.1.A – 3.1.3.A)

In vitro cultures – If applicable, please complete the section on Maintaining Viability for the activities related to in vitro culture (i.e. boxes 3.1.1.B – 3.1.3.B

Cryopreservation – If applicable, please complete the section on Maintaining Viability for the activities related to cryopreserved collections (i.e. boxes 3.1.1.C – 3.1.3.C)

Field genebanks – If applicable, please complete the section on Maintaining Viability for the activities related to field genebanks (i.e. boxes 3.1.1.D – 3.1.3.D.
Maintaining the genetic diversity of vegetative propagated crops like grapevine is more demanding than in the case of most seed-producing plants, because the specific genotype must be preserved.

Seed Collections not relevant

Box 3.1.1.A Initial seed viability
IV1 - Describe the procedures or practices that you have in place to ensure the highest possible initial viability of your seed, in particular during regeneration and post-harvest (e.g. cultivation practices, pollination aspects, use of specific equipment as shelters, storage of harvested seeds, cleaning, etc.).

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

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

Box 3.1.2.A Seed Viability Monitoring
VM1 - Describe the routine seed viability monitoring system that you use.
(*The monitoring system should include the following aspects:*

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

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

VM3 - Please provide information on non-specific thresholds that you might use for viability of seeds (i.e. percentage of germination) and for the amount of seeds left of an accession to initiate regeneration? *In case you differentiate between self- and outbreeding species, please answer for each category separately.*

Box 3.1.3.A Seed Storage Conditions (for the different types of collections, i.e. short/medium- or long-term storage)
SC1 - Please provide details on temperature and relative humidity conditions of your storage and drying rooms. In case they vary from room to room, please provide details for each.

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

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

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

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

A. In vitro Culture Collections not relevant

Box 3.1.1.B Initial viability

IV1 - Describe the procedures or practices that you have in place to ensure the highest possible initial viability of your plant material, in particular during culture of donor plants (e.g. cultivation practices [field, greenhouse], phytosanitary pre-treatments, like use of pesticides).
Cultivation in quarantine/greenhouse. Evaluation of vigor of potted plants.

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

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

Box 3.1.2 .B Viability Monitoring

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

a) *regular control of contamination events,*
b) *control of hyper-hydricity,*
c) *control of health state (if different from a above),*
d) *etc*).

VM2 - Describe the information "system" (i.e. an "expert system") that you might have in place that allows you to make more species or even accession-specific decisions when the next monitoring should take place.
The genebank is regularly checked for missing plants. Gaps are filled upon propagation to keep the minimum number of (grafted) plants.

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

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

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

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

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

B. Cryopreserved Collections not relevant

Box 3.1.1.C Initial viability

IV1 - Describe the procedures or practices that you have in place to ensure the highest possible initial viability of your cryopreservation explant (source: in vitro pre-culture or directly from in situ explants), sterilization and explant isolation.

IV2 – Please provide any other information on procedures that you follow to ensure highest possible initial viability (e.g. elimination of virus diseases).
We have no standard procedure for virus elimination. Virus is becoming a major problem without a solution.

Box 3.1.2.C Viability Monitoring

VM1 – Please indicate whether (and if so when and how) you perform random viability tests after the initial viability test? [see also VM3 below]

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

VM3 – Indicate for the initial regeneration control,

a. what is the percentage of regenerated control explants relative to the total number of explants per accession;
b. any thresholds that you use [e.g. discard the material as not storable below a certain regeneration rate of the control],
c. whether you apply different procedures for accessions with erratic regeneration rates of the control [e.g. increase the amount of explants stored]; etc. and

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

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

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

SC3 - Do you treat different species differently?

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

C. Field Genebank Collections

Box 3.1.1.D Initial viability

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

At IRZ replanting of accessions maintained in the field genebank is carried out about every 25 years.

For accessions to be replanted, sampling of cuttings is carried out during the winter, based on visual screening (declining vines, decrease of vigour, ESCA-disease, grapevine leaf roll virus and grapevine fanleaf degeneration) during vegetation period and on subsequent virus tests. If accessions are virus infected, it is attempted to acquire the same genotype virus free tested from another national collection in Germany or from a genebank within the EU.

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

In April buds of cuttings are grafted on phylloxera tolerant rootstocks.

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

Cuttings from weak accessions are taken and planted in greenhouse until they reach appropriate vigour for grafting.

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*).

Treatments against fungal diseases are conducted in a 10-14 days' rhythm; pheromone capsules are hung out against moths' attack. Plant health is checked regularly by visual screening, see IV1. Grafting is done to control phylloxera.

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.

The genebank is managed via a local database. Every year, after harvest and before leaf drop, a field inspection is carried out to record loss of vines and accessions, in order to restore them and keep the number of 3 vines / accession stable. From accessions with missing individual vines, propagation by buds from cuttings (during the winter) takes place. In case of a complete loss, it is tried to obtain the same genotype from elsewhere to restore the lost accession.

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?

As a general rule, multiplication is carried out whenever a vine is missing. Multiplication is done by cuttings if the shoots from remaining vines of the accession are thick enough for grafting. If all the plants from one accession are weak (with generally thin shoots, respectively canes) and thus threatened

by loss, propagation by rooted cuttings and culture in greenhouse is carried out until the canes reach the diameter of about 10 mm, the prerequisite for grafting.

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.

Head pruning with one cane (= shoot) is conducted in winter. In the next year one spur (spur is a two buds' shoot) will be taken as cane for the next season. Treatments against fungal diseases are performed in a 10-14 days' rhythm during the growing season; pheromone capsules are hung out against moths attack. The cultivation practices (fertilization, weed control, trellising and summer pruning) follow the routine operations of a commercial grapevine grower.

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

Muscadinia rotundifolia is frost susceptible. A few varieties of *Muscadinia* are thus maintained the whole year around in the greenhouse.

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

3.2 Maintaining Genetic Integrity

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

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

Navigation Box on Maintaining Genetic Integrity section

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

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

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

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

A. Seed Collections

Box 3.2.1.A Seed Containers and Sample Size

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

SCSS2 – Please describe what kind of containers (and equipment) you use, the procedure you follow with respect to sub-sampling, seed numbers per container, etc.

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

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

Box 3.2.2.A Pollination Control

PC1 - Please describe the regeneration procedures that you follow for self- and outbreeding species.

(*Please include in your description the following aspects:*

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

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

Box 3.2.3.A Regeneration Environment and Procedures

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

(*Consider the following aspects:*

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

b) *Do you use controlled environments?;*

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

d) *others*).

With the listed exception of *Muscadinia* all other accessions can be cultivated under the institutional conditions without any additional cultivation requirements.

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

Box 3.2.4.A Seed Processing Procedures

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

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

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

SPP4 – Please describe how and where you store (in a temporary manner) newly harvested seeds.
(Please provide details on the temperature and relative humidity of the storage room/space; what type of containers do you use, if any).

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

Box 3.2.5.A Genetically Modified Material

GMM1 – In case you treat GMO material differently from “normal germplasm”, please provide here the details for each of the deviating procedures (and equipment).

No GMO grapevine cultivars exist in the JKI grapevine genebank.

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

B. In vitro Culture Collections

Box 3.2.1.B In vitro Culture Vessels and Sample Size
SCSS1 – Indicate if you document the initial number of explants of individual accessions when culture is initiated (from one or from more clonal donor plants)?
No in vitro culture is used.
SCSS2 – Please describe in general terms the type of culture vessels (as far not already done in section SC2 in Box 3.1.3.B), media and phytohormones you use as well as the procedures you follow with respect to cutting technique, callus exclusion, etc.

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

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

Box 3.2.2.B In vitro Culture Procedures
SPP1 – Describe the numbers of sub-clones you may cultivate per accession (assuming that this is not crop specific)

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

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

Box 3.2.3.B Genetically Modified Material
GMM1 – In case you treat GMO material differently from "normal germplasm", please provide here the details for each of the deviating procedures (and equipment).

C. Cryopreserved Collections

Box 3.2.1.C Cryopreservation Containers and Sample Size
SCSS1 – Indicate if you document the initial number of explants of individual accessions?
No cryopreservation is used.
SCSS2 – Please describe what kind of cryopreservation vessels (and equipment) you use (only if they differ from the corresponding answers in previous boxes), the procedure you follow with respect to separate material containing viruses or bacteria from healthy material

SCSS3 - What is the number of explants that you use as the minimum threshold per accession?
SCSS4 – Please provide details on other aspects that are important in this context.

Box 3.2.2.C Cryopreservation Procedures (as long as not crop specific)

SPP1 – Describe the protocol(s) that you use for preculture and pretreatment such as cold acclimation and dehydration.

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

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

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

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

Box 3.2.3.C Genetically Modified Material
GMM1 – In case you treat GMO material differently from "normal germplasm", please provide here the details for each of the deviating procedures (and equipment).

D. Field Genebank Collections

Box 3.2.1.D Accession Sample Size
SCSS1 – Indicate if you document the initial number of plants of individual accessions (either as received from collecting missions or through exchange)?
From each introduced accession three grafted plants are produced and planted in the field genebank.
SCSS2 – Please describe what kind of procedures you follow, if any, with respect to sub-sampling and subsequent place/container/etc. of maintenance?
Misnomers are pulled out and replaced by true to type material. Same procedure is applied: cuttings of the true to type vines are sampled in winter, grafted on rootstocks in spring and planted in the collection the following year. In case of wild relatives each collected plant becomes an accession.
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)?
For each accession three grafted plants are planted in the germplasm repository.
SCSS4 – Please provide details on other aspects that are important in this context.

Box 3.2.2.D Multiplication not relevant
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 not relevant

SPP1 – Describe the protocol(s) that you use for threshing and seed cleaning, if used as an intermediate step for the management/multiplication of your field genebank accessions.

SPP2 – Please describe how and where you store (in a temporary manner) newly harvested planting material.
(Please provide details on the temperature and relative humidity of the storage room/space; what type of containers do you use, if any, etc.).

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

3.3 Ensuring Availability

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

Navigation Box on Ensuring Availability

Seed – If applicable, please complete the section on Ensuring Availability for the activities related to seed genebanks (i.e. boxes 3.3.1.A – 3.3.4.A)

In vitro cultures – If applicable, please complete the section on Ensuring Availability for the activities related to in vitro culture (i.e. boxes 3.3.1.B – 3.3.4.B

Cryopreservation – If applicable, please complete the section on Ensuring Availability for the activities related to cryopreserved collections (i.e. boxes 3.3.1.C – 3.3.4.C)

Field genebanks – If applicable, please complete the section on Ensuring Availability for the activities related to field genebanks (i.e.boxes 3.3.1.D – 3.3.4.D

A. Seed Collections

Box 3.3.1.A Ensuring Availability of Germplasm – Policy Aspects
AGP1 – Describe the germplasm distribution policy that you follow at your genebank.
(*You might want to consider in your response the following aspects:*
a)*crop/species specificity;*
b) *whether or not sufficient seed stock is available; who the requestor is;*
c) *what the purpose of the germplasm request is;*
d) *any restrictive conditions and/or*
e) *the total amount of accessions sent per request for distribution of germplasm;*
f) *use of a formal agreement to distribute the germplasm*).

AGP2 - Do you have as part of your service rendering policy aspects such as a "maximum time" between receiving a germplasm request and distribution of the germplasm?

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

Box 3.3.2.A Ensuring Availability of Germplasm – Seed/Germplasm Stock Aspects
AGSS1 - Please provide details on the minimum/maximum amount of seed, plant, in vitro samples that you distribute (where relevant, differentiated by species groups, i.e. self-pollinating, cross-pollinating and/or whether an accession is homo- or heterogeneous).

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

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

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

Box 3.3.3.A Ensuring Availability of Germplasm – Health Aspects
AGHA1 – Describe how you store seed/other germplasm with respect to germplasm health considerations, including whether you have a "policy" of

storing only “disease free” (as far as you can see or determine) accessions, at least for the quarantine pests and diseases.

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

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

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

Box 3.3.4.A Germplasm Supply
GS1 – Describe the policy of your genebank with respect to the sample size that you use for distribution purposes, including whether you differentiate between germplasm from self- or outbreeding species, heterogeneous accessions, and possibly other aspects.

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

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

B. In vitro Culture Collections

Box 3.3.1.B Ensuring Availability of Germplasm – Policy Aspects
AGP1 – Describe the germplasm distribution policy that you follow at your genebank.
(*You might want to consider in your response the following aspects: is the user informed about the option to get provided with in vitro cultures and whether they are available all the time of the year, are in vitro samples an option or the only way to get material; who the requestor is; what the purpose of the germplasm request is; any restrictive conditions and/or the total amount of accessions sent per request for distribution of germplasm; use of a formal agreement to distribute the germplasm)*

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

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

Box 3.3.2.B Ensuring Availability of Germplasm – Germplasm Stock Aspects
AGSS1 - Please provide details on the maximum amount of in vitro samples that you distribute.

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

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

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

Box 3.3.3.B Ensuring Availability of Germplasm – Health Aspects
AGHA1 – Describe how you store germplasm with respect to germplasm health considerations, including whether you have a "policy" of storing only "disease free" (as far as you can see or determine) accessions, at least for the quarantine pests and diseases.

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

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

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

Box 3.3.4.B Germplasm Supply
GS1 – Describe the policy of your genebank with respect to the sample size that you use for distribution purposes.

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

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

C. Cryopreserved Collections Not applicable

Box 3.3.1.C Ensuring Availability of Germplasm – Policy Aspects
AGP1 – Describe the germplasm distribution policy that you follow at your genebank.
(*Cryopreserved material is for distribution in exclusive cases only – e.g. for special research, please describe your policy*; *who the requestor is; what the purpose of the germplasm request is; any restrictive conditions and/or the total amount of accessions sent per request for distribution of germplasm; use of a formal agreement to distribute the germplasm*).

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

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

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

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

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

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

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

Box 3.3.3.C Ensuring Availability of Germplasm – Health Aspects

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

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

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

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

Box 3.3..C4 Germplasm Supply

GS1 – Describe the policy of your genebank with respect to the sample size that you use for distribution purposes.

GS2 – Please provide details of your routine methodology of containers etc. that you use to distribute cryopreserved material.

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

D. Field Genebank Collections

Box 3.3.1.D Ensuring Availability of Germplasm – Policy Aspects

AGP1 – Describe the germplasm distribution policy that you follow at your genebank.

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

Germplasm registered in the German Grapevine Genebank (http://www.deutsche-genbank-reben.julius-kuehn.de/) is distributed only during the first months of the year as long as sufficient material is available.

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?

No

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.

The accession name/cultivar name and the accession number are provided together with a link to the Vitis Internation Variety Catalogue.

Virus status is transmitted, if known.

Box 3.3.2.D Ensuring Availability of Germplasm – Seed/Germplasm Stock Aspects

AGSS1 - Please provide details on the minimum/maximum amount of plants or organs (cuttings, bulbs, tubers, etc.) per plant that you distribute per accession (where relevant, differentiated by species groups, i.e. annual or perennial; woody or herbaceous; other) and/or whether an accession is clonally or sexually propagated).

Usually, budwood is provided. The limit is determined by the number of plants (usually 3) per accession available in the field genebank.

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.

Availability is ensured because with three individuals per accession around 50 plants can be produced.

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.

We inform recipients about the virus status as far as it is known, without any warranty.

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

Phytosanitary certificates are issued by the responsible authority.

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

Budwood is placed in a plastic bag in a package, distributed by a parcel service along with the pertinent information.

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.

Distribution of germplasm is depending on availability only.

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?).

MySQL and Delphi, Yii-frame

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,

The internal DB is the source of what is visible from outside.

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

Year of acquisition, donor, year of planting, year of uprooting, location in the collection.

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, characterization and evaluation data, genotypic data, photos of shoots tips, leaves and bunches, herbarium specimen (leaves and shoot tips), virus status, and distribution of cuttings.

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

Only some information on the history of an accession (e.g. replanting, removal etc.) is stored as internal information.

GD4 – Describe in which form you send accession specific data (e.g. as hard copy, electronically – if the latter, please specify (in plain text) which file format, i.e. Excel, Access, others is used).

If requested, available data can be provided as Excel file.

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

Technical support is provided by a programmer at IRZ and in cooperation with the IT-group of JKI.

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

There is a permanent institutional backup to the contents.

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?).

Passport data are publicly available via the European Vitis Database (www.eu-vitis.de) and the German Grape Genebank (http://www.deutsche-genbank-reben.julius-kuehn.de/).

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).

Passport data, genotypic data, photos of shoots tips, leaves and bunches and virus status are available via the German Grape Genebank database.

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

The passport data are published by EURISCO. An update is carried out annually.

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.*).

The accession name/cultivar name and the accession number are provided. Virus status is transmitted if known.

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