DRAFT GENEBANK STANDARDS FOR THE CONSERVATION OF NON-ORTHODOX SEEDS AND CLONALLY-PROPAGATED PLANTS

The *Draft Genebank Standards for the Conservation of Non-Orthodox Seeds and Clonally-Propagated Plants* has been prepared in cooperation with the International Treaty, Consultative Group on International Agricultural Research centers, Global Crop Diversity Trust and relevant international institutions. It may be downloaded from the FAO website: http://www.fao.org/agriculture/crops/core-themes/theme/seeds-pgr/en/

All inputs and suggestions should be sent by May 14th 2012 to ITWG-PGRFA@fao.org or by fax: (+39) 06570-56499

Contents

I. Introduction

1. Plant genetic resources are the raw materials utilized in crop improvement and ensuring global food and nutrition and nutrition security. Their sustainable conservation and use depends on an effective and efficient management of genebanks, applying standards and procedures to ensure the continued survival and availability of plant genetic resources. Genebanks are also crucial in facilitating availability of germplasm to plant breeders, farmers, researchers and other users.

2. Plants which produce non-orthodox seeds, also known as recalcitrant or intermediate seeds, as well as those which are vegetatively propagated, cannot be conserved in the same way as orthodox seeds. Orthodox seeds are desiccation-tolerant and at maturity have, or can be dehydrated to low water contents and are conserved at low temperature and moisture content. Non-orthodox seeded plants and clonally propagated plants require other methods of *ex situ* conservation and can either be conserved: (i) as live plants in field genebanks or (ii) in the laboratory using different plant parts (seeds, apical meristems, shoot tips, cell suspension) *in vitro* (slow growth) or in liquid nitrogen (cryopreservation).

3. There is a need for these standards as, due to the difficulty and specific requirements of these plants, no general standards for their conservation *ex situ* exist. At the request of the Commission on Genetic Resources for Food and Agriculture, standards were developed for those plants not covered by the Draft Revised Genebank Standards for the Conservation of Orthodox Seeds[1]. The standards here discussed have been prepared for the conservation of non-orthodox seeds and clonally-propagated plants using *in vitro* culture and cryopreservation and field genebanks. The genebank standards were developed based on a series of consultations with a large number of experts in cryopreservation, *in vitro* conservation and field genebanks worldwide. These consultations were instrumental in shaping the standards as presented in this document.

4. While genebanks should aim at using the Standards as a basis for conserving the germplasm of non-orthodox-seeded species and vegetatively-propagated crops, it must be noted that this is not a manual. It should be used in conjunction with other more technical publications and manuals that provide practical details, guidelines and protocols. Further to this, ongoing advances will continue to build on, and refine, the recommendations. Thus, it is important that germplasm conservation managers and practitioners should remain familiar with updated and relevant literature.

1.1. Standards – Structure and Definitions

5. The Standards as described in this document, define the level of performance of a routine genebank operation below which there is a high risk of losing genetic integrity.
Each section is divided into:

A. Standards

B. Context

C. Technical aspects

D. Contingencies

[1] http://www.fao.org/fileadmin/templates/agphome/documents/PGR/genebank/Genebankstandards-Nov2011english_FINAL.pdf

E. Selected references

The **Standards** are detailed in:

(i) Eight sections for the *Draft Genebank Standards for* In Vitro *Culture and Cryopreservation*: acquisition and initial handling, testing for non-orthodox behaviour and assessment of water content, vigour and viability, hydrated storage for recalcitrant seeds, i*n vitro* culture and slow growth storage, cryopreservation, documentation, distribution and exchange, security and safety duplication;

(ii) Ten sections for the *Draft Field Genebank Standards*: choice of location, acquisition of germplasm, establishment of field collections, field management, regeneration and propagation, characterization, evaluation, documentation, distribution, security and safety duplication.

The **Context** provides the basic necessary information in which the standards apply. It provides a brief description of the routine genebank operation for which the standards are defined and the underlying principles for them.

The **Technical Aspects** explain technical and scientific principles important to understand and underpin the standards.

The **Contingencies** provide recommendations in the case that standards cannot be applied to a given species, for example exceptions, alternative routes, and risk management options.
Selected sources of information and references are provided in all sections.

II. Underlying Principles

6. Genebanks across the globe share many of the same basic goals, but their missions, resources, and the systems they operate within, often differ. As a result, curators have to optimize their own genebank system and this requires management solutions which may differ substantially across institutions while achieving the same objectives. Underlying principles explain why and for what purpose plant genetic resources are being conserved. These principles provide the basis for establishing the norms and standards essential for the smooth operation of a genebank. The major underlying principles for conservation are described in the section below.

Identity of accessions

7. Care should be taken to ensure that the identity of sample accessions conserved in genebanks is maintained throughout the various processes, beginning with acquisition through to planting in the field, propagation and distribution. Proper identification of samples conserved in genebanks requires careful documentation of data and information about the material. This begins with recording passport data and collecting or donor information if applicable. Such information should also be recorded for older collections in genebanks for which passport data was not previously recorded or is incomplete. Identification of accessions in the field is especially important since inadequate labelling can lead to much genetic erosion. Field labelling needs also be complemented with field layout plans, which should be properly documented in order to ensure proper identification of accessions in field genebanks. Field labels are prone to loss due to various external factors, e.g. bad weather conditions. Modern techniques such as accession labels with printed barcodes and molecular markers can greatly facilitate the management of germplasm by reducing the possibility of error, further ensuring the identity of accessions. An additional factor in the case of species with seeds of unknown post-storage behaviour (i.e. whether recalcitrant, otherwise non-orthodox or orthodox) is the *a priori* requirement of ascertaining seed responses (generally to slow dehydration) before any germplasm storage strategy can be put in place.

Maintenance of quality and viability

8. Maintaining viability, genetic integrity and quality of seed samples in genebanks and making them available for use is the ultimate aim of genebank management. It is therefore critically important that all genebank processes adhere to the standards necessary to ensure that acceptable levels of viability are maintained. For recalcitrant and other non-orthodox seed types, this is assessed by visual inspection for lack of damage, and by rate and totality of germination. However, the frequent occurrence of macroscopically undetectable fungal and bacteria within the seeds may compromise seed quality. Collection of fruits and seeds from the ground should generally be avoided. Thus upon acquisition, seed components need to be tested to ascertain their status with respect to such contaminants. In the context of field genebank the term of propagability (i.e. the quality and state of being propagable) is more relevant than the term viability which specifically relate to the capacity of seeds to germinate and produce a plantlet. Field genebanks are vulnerable to the impacts of environmental factors such as weather conditions, incidences of pests, and such impacts will be different according to the different species type and growth cycles e.g. annual, biannual or perennial.

Maintenance of genetic integrity

9. The need to maintain genetic integrity is closely related to maintenance of the viability and diversity of the original collected sample. All genebank processes, starting from collection and acquisition, through to storage, regeneration and distribution, are important for

the maintenance of genetic integrity. Ensuring that viability is maintained according to the standards contributes to the maintenance of genetic integrity. Various molecular techniques, including surveys of possible epigenetic changes which may or may not be reversible, are needed to assess whether genomic stability has been maintained when samples are retrieved from cryostorage. In plants requiring long intervals from sowing to reproductive maturity, seed regeneration in the field would be highly impractical. Re-sampling the original population should be undertaken when there are signs of declining vigour and viability. Maintenance of genetic integrity is equally as important for germplasm which is conserved *in vitro*, especially in view of the risk of somaclonal variation. This is the main reason for avoiding indirect somatic embryogenesis (i.e. via a callus stage) to generate forms of germplasm to be conserved, except if there is no alternative. Adequately representative samples of good quality and sufficient quantity should be obtained during acquisition as far as possible. However, it is recognized that when the objective is to collect particular traits, then the sample may not be representative of the original population. To minimize genetic erosion it is important to follow recommended protocols for regenerating accessions with as few regeneration cycles as possible, sufficiently large effective population sizes, balanced sampling, as well as pollination control. Special mention is made here of the importance of safety duplication to respond to risks that can occur in genebank facilities.

Maintenance of germplasm health

10. Genebanks should strive to ensure that the accessions and the planting materials being distributing are free from diseases and regulated pests (bacteria, virus, fungi and insects). External surfaces can usually be effectively eliminated using surface decontamination procedures. Field genebanks often do not have the capacity or necessary resources to test whether samples collected or otherwise acquired, and samples harvested from multiplication plots, are free from diseases and pests. The problems are exacerbated for *in vitro* conservation and cryopreservation of recalcitrant-seeded species. Internally-borne contaminants are revealed only when recalcitrant seeds are maintained in short- to medium-term hydrated storage, or when seed-derived explants are placed in tissue culture. The presently unsatisfactory solution is to discard any contaminated seed/explant as it is the only way to ensure uncontaminated germplasm. Thus, it is important that relevant import and phytosanitary certificates accompany seed material when exchange of germplasm takes place to ensure the health status of samples received. Despite certification, screening should be carried out, for imported consignments as well as locally-collected accessions, in a demarcated area of the genebank.

Physical security of collections

11. An underlying principle of germplasm conservation is that the fields and the structures of the genebank facilities in which germplasm are conserved are of adequate standard to secure the materials from any external factors. This may include natural disasters and human-caused damage. Adequate security systems should be in place to ensure that germplasm collections are not threatened. As cryogenic storage requires liquid nitrogen (LN), supplies of this cryogen must always be available. Furthermore, it is vital that levels of LN are maintained, whether filling/top-up of the special storage vats or LN freezers used is done manually or automatically. Another important security issue for genebanks is to ensure materials are safely duplicated in other locations so that if a collection suffers loss, for any reason, material can be restored from duplicated sets.

Availability and use of germplasm

12. The conserved material must be available for current and future use. It is, therefore,

important that all processes in genebank operations and management contribute to this goal. Although there are a few individuals of accessions in field genebanks, and thus a limited capacity for distributing to users, the genebank should have a strategy in place to quickly multiply any germplasm for distribution.

Availability of information

13. In order to ensure communication of information and accountability, essential, detailed, accurate, and up-to-date information at all stages should also be recorded, including historical as well as current information, especially in relation to the management of individual accessions, subsequent to their acquisition. Access, availability and sharing of this information should be treated with high priority, as it leads to better and more rational conservation. Search-query interactive databases containing phenotypic evaluation data can assist germplasm clients in the targeting of germplasm requests, and in turn feedback of further evaluation data adds to the value and utility of the collection. If information on the conserved germplasm is made easily available and accessible it will enhance germplasm use. Further this will help the genebank curators to better plan their multiplication and regeneration activities in order to keep adequate stocks of their accessions. For such genebank-based information systems, a search-query interactive database is recommended. The Seed Information Database (SID[2]) of the Millennium Seed Bank (MSB) Kew provides a good example of the value of this type of database. *BRAHMS* (Botanical Research and Herbarium Management System)[3] is a system developed for the purposes of curation and germplasm data management.

Proactive management of genebanks

14. Sustainable and effective conservation of genetic resources depends on active management of the conserved germplasm material. Proactive management is critical for ensuring that germplasm is efficiently conserved and made timely and in adequate quantity available for further use by plant breeders, farmers, researchers and other users. It emphasizes the importance of securing and sharing material as well as the related information, and sets in place a functional strategy for management of human and financial resources for a rational system. It should be mentioned that the maintenance of field collection is costly and all efforts should be made to develop complementary collections *in vitro* or in cryopreservation. It includes a risk management strategy and encourages a participatory role of genebanks in the efforts to conserve biodiversity. Adherence to the legal and regulatory frameworks at national and international levels, in particular as they relate to access, availability and distribution of materials and plant and seed health is necessary. A Standard Material Transfer Agreement (SMTA) should be used for crops under the Multilateral System of the ITPGRFA. The IPPC regulations provide the framework for quarantine and health regulations to prevent the introduction and spread of plant pests and diseases. Above all, there is a need for long-term and continuous commitment of the institutions holding genebanks with regards to the availability of human and financial resources.

[2] http://data.kew.org/sid/
[3] http://dps.plants.ox.ac.uk/bol

III. Genebank Standards for *In Vitro* Culture and Cryopreservation

3.1 Introduction to *In vitro* culture and Cryopreservation Standards

15. The conservation of plant germplasm *in vitro* and cryopreservation is for plants which produce non-orthodox seeds or are propagated vegetatively. They can either be conserved through slow growth (*in vitro*) for short/medium-term storage, or cryopreservation for long-term conservation. The former method involves cultures (especially shoot tips, meristems, somatic embryos or embryogenic callus) being maintained under growth-limiting conditions on artificial culture media. The growth rate of the cultures can be limited by various methods, including temperature reduction, lowering of light intensity, or manipulation of the culture medium by adding osmotic agents or growth retardants.

16. Cryopreservation is the storage of biological materials (plant embryos, shoot tips/meristems, and/or pollen) at ultra-low temperatures usually that of liquid nitrogen at -196°C (Engelmann and Tagaki 2000; Reed 2010). Under these conditions, biochemical and most physical processes are halted and materials are able to be conserved over the long term. These modes of conservation constitute a complementary approach to other modes and are necessary for a safe, efficient and cost effective conservation (Reed 2010). For example, cryopreserved lines can be maintained as a backup for field collections, as reference collections for available genetic diversity of a population, and as a source for new alleles in the future.

17. Specific standards have been developed for eight key areas and include the following sections:

- acquisition and initial handling
- testing for non-orthodox behaviour and assessment of water content, vigour and viability
- hydrated storage for recalcitrant seeds
- *In vitro* culture and slow growth storage
- cryopreservation
- documentation
- distribution and exchange
- security and safety duplication.

18. It should also be noted that the Standards used in this document are broad and generic in nature. This is because of the marked variation among non-orthodox seeds and vegetatively propagated plants. This variability is a function of the inherent biology and metabolic status of the plants concerned, which influences their differing responses to various manipulations and often requires modifications of basic approaches to be made on a species-specific basis. These various features necessitate an introduction to the phenomenon of non-orthodoxy and storage behaviour of non-orthodox seeds to better understand the scientific basis of the standards.

3.1.1 Phenomenon of non-orthodoxy

19. Understanding the desiccation tolerance and sensitivity in orthodox compared to non–orthodox (intermediate and recalcitrant) seeds is of fundamental importance for cryopreservation. At maturity, orthodox seed water content would generally be in the range

0.05 – 0.16 g g^{-1}[14](5% - 14% [wmb]), although some species are shed at much higher water content, undergoing substantial dehydration after this. Unlike recalcitrant seeds, all orthodox seeds acquire desiccation tolerance, which is genetically-programmed and entrained before, or at the start of maturation drying. Recalcitrant seeds do not dry during the later stages of development and are shed at water contents in the range of 0.3 – 4.0 g g^{-1}. Because they are desiccation-sensitive, the loss of water rapidly results in decreased vigour and viability, and seed death at relatively high water contents. This is due to their metabolic activity (Berjak and Pammenter 2004) with little or no intracellular differentiation occurring, thus exposing membranes to the damaging consequences of dehydration stress (Walters *et al.* 2001; Varghese *et al.* 2011). A spectrum of differences in post-shedding physiology also occurs in intermediate seeds. Seeds showing intermediate behaviour can withstand water loss to ~ 0.11 to ~ 0.14 g g^{-1} (Berjak and Pammenter 2004). They have the capacity to perform some of the important mechanisms and processes governing desiccation tolerance. However, they are not long-lived in the dehydrated condition, particularly at chilling temperatures for some species.

20. The variability in physiology of recalcitrant seeds is frequently also intra-specific. Seed or, embryo/embryonic axis water content can vary significantly in collections from the same locality from year-to-year, and also for material from the same locality, within any one season. This means that the parameters (water content, response to drying) must be assessed for each species. Additionally, seeds harvested late in a season are usually of considerably inferior quality compared with those harvested earlier (Berjak and Pammenter 2004). The provenance of the population from which seeds are collected is also a major factor in the properties and responses of recalcitrant seeds. Thus, even if they are of the same species, seeds developing along a latitudinal gradient can show remarkably different characteristics. (Daws *et al.* 2006; Daws *et al.* 2004).

21. Seed developmental status has emerged as a critical consideration when recalcitrant germplasm is to be cryostored. Early during seed ontogeny, all seeds are highly desiccation-sensitive. Desiccation sensitivity in recalcitrant seeds increases as the processes of germinative metabolism are manifested (Berjak and Pammenter 2004). The early events of germination in recalcitrant seeds are initiated soon after they are shed, without the 'punctuation' between the end of development and the start of germination imposed on orthodox seeds by maturation drying.

22. Depending on the species, recalcitrant seeds will initiate germinative metabolism after being shed. Those species with fully developed embryos on shedding, generally initiate germination virtually immediately, with a concomitant increase in desiccation sensitivity. In some other species, seeds are shed with under-developed embryos, necessitating the completion of development prior to the onset of germinativemetabolism. These developmental differences dictate the duration for which the seeds can be wet-stored (i.e. hydrated storage at the shedding water content). It is now known that recalcitrant seeds cannot be dehydrated to a water content precluding germination (so-called sub-imbibed storage), as this actually shortens the hydrated storage life span. Slight dehydration actually stimulates the onset/progression of germination, thus shortening the time before an extraneous water supply is required to support the process (Drew *et al.* 2000; Eggers *et al.* 2007).

[4]In this document, the term water content is used in preference to moisture content, as recalcitrant seeds are hydrated (wet) rather than moist (barely wet). Also, the figures given are expressed on a dry mass basis (g H_2O g^{-1} dry matter [g g^{-1}]), which is considered to be more explicit than expression as a percentage of the wet mass.

23. In general, recalcitrant seeds from temperate provenances are chilling-tolerant, while those from the tropics and sub-tropics and of the same species, are more likely to be chilling-sensitive. Chilling sensitivity is also an issue for the storage of intermediate seeds, particularly those from the tropics and sub-tropics. When dried to water contents which are not injurious in themselves, the storage life span of such seeds is curtailed at temperatures ≤10°C (Hong *et al.* 1996).

24. Seed-associated microflora (fungus and bacteria), especially those associated with the interior surfaces, e.g. of the cotyledons or embryonic axis, is generally a major problem with recalcitrant seeds, particularly of tropical and sub-tropical origin (Sutherland *et al.* 2002). The conditions of hydrated storage, being moist and often necessarily at benign temperatures, encourage fungal proliferation, with the probability of hyphae penetrating the embryo tissues. This has a major deleterious effect and curtails hydrated storage life span significantly.

25. Under field conditions, unless seedling establishment is rapid, recalcitrant seeds will gradually lose water, the rate depending on the species-specific nature and morphology. Under conditions of slow water loss (days to a week or more), desiccation damage accumulates and the seeds of most species will have lost viability when the embryos/embryonic axes are at a water content of around 0.8 g g^{-1} (Pammenter *et al.* 1993). Thus when handling or storing recalcitrant seeds, great care is normally exercised to maintain water contents at the levels characteristic of shedding.

26. The response of explants[5] to dehydration depends on the rate of drying and size of the explants. Often recalcitrant seeds are too large to dry rapidly, and too large to cool rapidly on exposure to cryogen (as is required to obtain successful cryopreservation). Thus, excised embryos or embryonic axes are explants of choice, since they can be dehydrated to water contents which will minimize ice crystallisation, which are ≤0.4 g g^{-1}. Embryos/axes can be dried in a stream of air (flash-dried) (Pammenter *et al.* 2002), which significantly curtails the time during which metabolism linked desiccation damage can occur. It is *not* that the embryos/axes have become desiccation-tolerant, but simply that they dry before lethal damage has accumulated, providing the time needed to subject them to cryogenic temperatures. In cases where embryo/axes prove impossible to manipulate for successful cryostorage, alternative explants, such as shoot apical meristems excised from seedlings developed from seeds germinated *in vitro,* can be used.

27. In addition to cryopreservation, other means of conservation for species producing recalcitrant or otherwise non-orthodox seeds, include *in vitro* conservation which could involve slow-growth of seedlings/young plants/plantlets. In some instances, slow-growth conditions may be imposed *ex vitro*. In the last instance, plantlets may be derived from embryogenic callus (which itself might be amenable to cryopreservation) and conserved *in vitro*, possibly under slow-growth conditions.

Selected References

Benson E.E. Harding K., Debouck D., Dumet D., Escobar R., Mafla G., Panis B., Panta A., Tay D., Van den houwe I. & Roux N. 2011. Refinement and standardization of storage procedures for clonal crops - Global Public Goods Phase 2: Part I. Project landscape and

[5]In the strict sense, the term, explant, refers to any segment or part of plant tissue which is transferred to a nutrient medium. In the context of this document, explants include embryos, embryonic axes, shoot apical meristems, or any other entity which is excised for the purposes of germplasm conservation of species producing non-orthodox seeds.

general status of clonal crop *in vitro* conservation technologies. System-wide Genetic Resources Programme, Rome, Italy.

Berjak, P. & Pammenter, N.W. 2004. Recalcitrant Seeds. pp. 305-345 *in* Benech-Arnold, RL., Sánchez, R.A. (eds) *Handbook of seed physiology: applications to agriculture*. Haworth Press, New York.

Daws, M.I., Cleland, H., Chmielarz, P. Gorian, F., Leprince, O., Mullins, C.E., Thanos, C.A., Vandvik, V. & Pritchard, H.W. 2006. Variable desiccation tolerance in *Acer pseudoplatanus* seeds in relation to developmental conditions: a case of phenotypic recalcitrance? *Functional Plant Biology* 33: 59-66.

Daws, M.I., Lydall, E., Chmielarz, P., Leprince, O., Matthews, S., Thanos, C.A. & Pritchard, H.W. (2004) Developmental heat sum influences recalcitrant seed traits in *Aesculushippocastanum* across Europe. *New Phytologist* 162: 157-166.

Drew, P.J., Pammenter, N.W. & Berjak, P. 2000. 'Sub-imbibed' storage is not an option for extending longevity of recalcitrant seeds of the tropical species, *Trichiliadregeana* Sond. *Seed Science Research* 10: 355-363.

Eggers, S., Erdey, D., Pammenter, N.W. & Berjak, P. 2007. Storage and germination responses of recalcitrant seeds subjected to mild dehydration. pp. 85-92 *in* Adkins, S., Ashmore, S., Navie, S.C. (eds) *Seeds: Biology, Development and Ecology*. CABI, Wallingford, UK.

Engelmann F. &Takagi H. (eds). 2000. Cryopreservation of tropical plant germplasm. Current research progress and application. Japan International Research Centre for Agricultural Sciences, Tsukuba Japan/ International Plant Genetic Resources Institute, Rome Italy.

Hong, T.D., Linington, S. & Ellis, R.H. 1996. *Seed storage behaviour: A compendium*. Handbooks for genebanks: No. 4. IPGRI, Rome.

Lync P., Souch G., Trigwell. S., Keller J & Harding K. 2011. Plant Cryopreservation: From Laboratory to Genebank. *AsPac J. Mol. Biol. Biotechnol*. 18 (1): 239-242

Pammenter, N.W., Vertucci, C. & Berjak, P. 1993. Responses to dehydration in relation to non-freezable water in desiccation-sensitive and -tolerant seeds. pp. 867-872 *in* Côme, D, Corbineau, F. (eds) *Proceedings of the Fourth International Workshop on Seeds: Basic and Applied Aspects of Seed Biology*, Angers, France. ASFIS, Paris. Vol. 3.

Pammenter, N.W., Berjak, P., Wesley-Smith, & Vander Willigen, C. 2002. Experimental aspects of drying and recovery. pp. 93-110 *in* Black, M, Pritchard, H.W. (eds) *Desiccation and survival in plants: drying without dying*. CABI, Wallingford, UK.

Reed B.M. 2010. *Plant cryopreservation. A practical guide*. Springer.

Reed B., Engelmann F., Dulloo M.E. & Engels J.M.M. 2004. Technical Guidelines on management of field and in vitro germplasm collections. Handbook for genebanks No.7, IPGRI, Rome, Italy

Sutherland, J.R., Diekmann, & Berjak, P. (eds). 2002. *Forest Tree Seed Health*. IPGRI Technical Bulletin No. 6, International Plant Genetic Resources Institute, Rome, Italy.

Varghese, B., Sershen, Berjak, P., Varghese, & Pammenter, N.W. 2011. Differential drying rates of recalcitrant *Trichiliadregeana* embryonic axes: A study of survival and oxidative stress metabolism. *PhysiologiaPlantarum*142, 326-338.

Walters, C., Pammenter, N.W., Berjak, & Crane, J. 2001. Desiccation damage, accelerated ageing and respiration in desiccation-tolerant and sensitive seeds. *Seed Science Research*11, 135-148.

3.2.1. STANDARDS FOR ACQUISITION AND INITIAL HANDLING

A. Standards

1.1 All germplasm accessions added to the genebank should be legally acquired, with relevant technical documentation.

1.2 All material should be accompanied by at least a minimum of associated data as detailed in the FAO/IPGRI multi-crop passport descriptors.

1.3 Only material in good condition and of consistent maturity status should be collected, and the sample size should be large enough to make genebanking a viable proposition.

1.4 The material should be transported to the genebank in the shortest possible time and in the best possible conditions.

1.5 All incoming material should be treated by a surface-decontaminating agent to remove all adherent microorganismsand handled so that its physiological status is not altered, in adesignated area for reception.

B. Context

28. Acquisition is the process of collecting or requesting germplasm (seeds and other propagules[6]) for inclusion in the genebank, together with related information. Adherence to legal requirements is essential, and both national and international requirements must be fulfilled. During the acquisition phase, it is important to ensure that passport data for each accession is as complete as possible and fully documented (Alercia *et al*. 2001).

29. There is a need to ensure maximum seed quality and avoid conservation of immature seeds and seeds that have been exposed for too long to the elements. The way that seeds are handled after collection and before they are transferred to controlled conditions is critical for seed quality. Unfavourable extreme temperatures and humidity during the post-collecting period and during transport to the genebank could cause rapid loss in viability and reduce longevity during storage. The same applies to post-harvest handling within the genebank. The seed quality and longevity is affected by the conditions experienced prior to storage within the genebank. As recalcitrant seeds are metabolically active and have high water contents at maturity, the way they are handled after collection is critical for successful long term conservation of the material. As field-grown material is frequently contaminated with fungi and/or bacteria, it is necessary to have a set of measures in place to reduce the risk of deterioration of the material in the post-harvest state.

30. Material must be as clean as possible. Therefore, transfer of field material into pots and short periods of glasshouse growth is recommended. In these cases, plants should be watered from the bottom and, in extremely infected material, pesticides may support later decontamination of the explants. Visibly infected material should be excluded from the beginning or eliminated when found.

C. Technical aspects

[6] In this context, a propagule refers to vegetative portions of a plant, such as seeds, buds, corms, cuttings and other offshoots, used to propagate a plant.

31. Access to plant genetic resources within the Multilateral System of the ITPGRFA are accompanied by a Standard Materials Transfer Agreement (SMTA). For material acquired or collected outside the country where the genebank is located the acquirers should comply with the relevant national and international legislation. In addition, when required by the providing country, the accession should be subject to the prior informed consent of the country (ENSCONET 2009). Phytosanitary regulations and any other import requirements must be sought from the relevant national authority of the receiving country.

32. Passport data is needed to identify and classify the accessions. Recalcitrant-seeded species of interest will constitute wild populations, making collection of accurate field data absolutely imperative. The multi-crop passport descriptors should therefore include a voucher specimen, as well as GPS coordinates and photographic images of habit, habitat, and the substratum as much as possible. If fallen material is collected, it should be recorded as such and kept separate from that harvested from the parent plant. The sample size should include an adequate number of individuals/accessions, large enough to establish an appropriate protocol for cryopreservation, and/or to place samples in long-term cryo-storage.

33. There is a need to ensure maximum seed quality and avoid conservation of immature or over-ripe material that has been exposed for too long to the elements. Collecting well-matured clean and high quality propagules, will ensure maximum longevity in storage. Fallen material and fruits (seeds) showing abrasions or signs of weathering should be avoided. Late-season seeds appear often to be of inferior quality to those produced earlier (Berjak and Pammenter 2004). It is advised not to collect late-season recalcitrant seeds of any species. Seasonality needs also to be considered when using bulbs and tubers, which develop new shoots only in some seasons, in woody plants which have dormant buds only in winter, and young inflorescence explants or pollens which are available only in the flowering period.

34. Many of the fruits bearing recalcitrant seeds harbour fungal contaminants, even when not visible. This is a serious problem, and surface decontamination prior to transport is important for removal of any superficial contaminant High temperatures and humidity during the post-collecting period and during transport to the genebank exacerbate this problem and could cause rapid loss in viability and reduce longevity during storage. However, seeds and other propagules may be chilling-sensitive and elevated temperatures may either hasten germination or damage the seeds. Thus transport temperature must neither be too low nor too high, generally not below ~16 °C and not above ~25 °C.

35. The problem of fungal contamination also applies to post-harvest handling within the genebank and fruits should be thoroughly surface-decontaminated prior to opening. Similarly, for any imported accessions, contamination can result from containers and wrappings, which need to be incinerated as is generally stipulated by national Plant and Seed Health regulations. Fruit pulp, fibres, etc. must be completely removed from seed outer surfaces, but water must not be used, as seeds could well become (further) hydrated and affect the water content of the seeds. It is also important to gather information about the fruit and seeds weight of the seeds prior to water content determination (see Standard 2).

36. Wherever possible (as in the case of hard-coated fruits), seeds should be transported within the fruits, both for protection and to avoid dehydration. Water loss both stimulates germinative metabolism and shortens storage life span, thus it is important that water content are maintained upon collection and during transport, by maintaining high relative humidity (RH) in the storage containers. Special plastic bags should be preferred which are not vulnerable to breaking as are glass tubes .Insulating packaging will help in keeping the temperature stabile, and can be especially relevant during long transportation.

37. Recalcitrant seeds produced in hard-coated fruits generally remain in better condition for longer periods, than if the seeds are removed from the fruits. Soft fruits, or those which are damaged or have dehisced should immediately be surface-decontaminated, the seeds extracted and the fruits removed and destroyed. If long transport periods are involved, it is advisable to extract, manually clean and surface-decontaminate the seeds prior to transport. Ideally, a decontamination kit comprising water purification tablets or sodium hypochlorite (NaOCl), water (sterile, if possible, or boiled on site) and sterile paper towelling should be carried on field expeditions.

38. Under tropical conditions, other measures such as the storage of plantlets under shade (Marzalina and Krishnapillay 1999) or *in vitro* field collecting (Pence *et al.* 2002; Pence and Engelmann 2011) may be applied. Minimum transportation times are also necessary when *in vitro*-collected material is used.

39. For *in vitro*-cultured explants, surface decontamination starts often by 70% ethanol followed by sodium hypochlorite (NaOCl) diluted from pure stock solution or as constituent of a commercial bleach with a concentration of active chlorine amounting to about 3%. Detergent droplets may support the effect. Other substances may be used as well (e.g. calcium hypochlorite) in appropriate concentrations.The explant needs to be trimmed to the final size after surface decontamination. Note that the decontamination agent will enter cut surfaces resulting in dead zones which need to be removed upon trimming.

D. Contingencies

40. When a consignment is contaminated or deteriorated, all material and its packaging must be incinerated, despite the financial implications.

41. Delays of a consignment in national quarantine facilities are a known hazard. In such cases, steps must be taken to minimize such delays, including the use of couriers.

42. Under conditions of a 'poor' fruiting season, it is preferable to postpone collection to a subsequent fruiting season. If circumstances dictate that fallen fruits have to be collected, only those which are newly-abscised, should be considered.

43. Occasionally, seeds of particular species react badly to NaOCl and/or to the commonly-used commonly used fungicides, in which case safe alternatives must be used (Sutherland *et al.* 2002). Note that 70% (v/v) ethanol in sterile/boiled water could be used.

E. Selected references

Berjak, P. & Pammenter, N.W. 2004. Recalcitrant Seeds. pp. 305-345 in Benech-Arnold, RL., Sánchez, R.A. (eds) *Handbook of seed physiology: applications to agriculture*. Haworth Press, New York.

Engelmann, F. 1997. *In vitro* conservation methods. pp. 119-161 *in* Callow, J.A., Ford-Lloyd, B.V., Newbury, H.J. (eds) *Biotechnology and plant genetic resources*. CABI, Wallingford, Oxon, UK.

ENSCONET. 2009. *Seed collecting manual for wild species*. ISBN 978-84-692-3926-1 (www.ensconet.eu).

Alercia, A., Diulgheroff, S. & Metz, T. 2001. FAO/IPGRI. Multi-crop Passport Descriptors http://www.bioversityinternational.org/.../faoipgri_multi_crop_passport_descriptors

Marzalina, M. & Krishnapillay, B. 1999. Recalcitrant seed biotechnology applications to rainforest conservation. In: Benson, E.E. (ed.) *Plant conservation biotechnology* Taylor & Francis, London, UK. pp. 265-276.

Pence, V.C. 1996. *In vitro* collection (IVC) method. pp. 181-190 *in* Normah, M.N., Narimah, M.K., Clyde, M.M. (eds) *In vitroconservation of plant genetic resources*. PercetakanWatanSdn.Bdh, Kuala Lumpur, Malaysia.

Pence V. C., Sandoval J., Villalobos V. & Engelmann F. (eds.). 2002. *In vitro* collecting techniques for germplasm conservation. IPGRI Technical Bulletin N°7. IPGRI, Rome;

Pence V.C. & F. Engelmann. 2011. Chapter 24: Collecting *in vitro* for genetic resources conservation. In Guarino L., Ramanatha Rao V., Goldberg E. 2011. *Collecting Plant Genetic Diversity: Technical Guidelines*. 2011 update. Bioversity International, Rome. "Available online: http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=661

Sutherland, J.R., Diekmann, M. & Berjak, P. (eds). 2002. Forest Tree Seed Health. IPGRI Technical Bulletin No. 6, International Plant Genetic Resources Institute, Rome, Italy.

3.2.2. STANDARDS FOR TESTING FOR NON-ORTHODOX BEHAVIOUR AND ASSESSMENT OF WATER CONTENT, VIGOUR AND VIABILITY

A. Standards

2.1 The storage category of the seed should be determined immediately by assessing its response to dehydration.

2.2 The water content should be determined individually, on separate components of the propagule, and in a sufficient number of plants.

2.3 The vigour and viability should be assessed by means of germination tests and in a sufficient number of individuals.

2.4 During experimentation, cleaned seed samples should be stored under conditions that do not allow any dehydration or hydration.

B. Context

44. Maintaining seed viability is a critical genebank function that ensures germplasm is available to users and is genetically representative of the population from which it was acquired. As a first step to preservation, it is important to ascertain the seed storage category by assessing the response of the propagule to dehydration. The response to drying will in turn determine the treatment needed for cryo-storage. A number of factors influence drying rate, including RH, seed size, the nature of seed coverings, the flow rate of air over the seeds, and the depth of the layer of seeds (Pammenter *et al.* 2002).

45. The rate and uniformity of germination of a seed sample, or of seed-derived explants, is a reliable indicator of vigour, while the totality of germination (i.e. what proportion/percentage of seeds or explants tested finally germinated) reveals the overall viability of the sample. Viability should not be not less than 80% in a sample.

C. Technical aspects

46. Water content determinations and assessment of vigour and viability should be carried out as a single operation, and are issues to determine before selectingthe type of drying technique. The number of procedures that can be investigated is determined by the number of seeds available. Three methods for screening seeds can be used for seed categorization. These includes a method that can discriminate between intermediate and recalcitrant seeds (Hong and Ellis 1996), one which is designed for cases when seeds are limited (Pritchard *et al.* 2004), and one that assesses axis water content, rather than that of whole seeds. Irrespective of the method chosen, dehydration imposed during the screening procedure must never be carried out at elevated temperatures, which are damaging. The recommended temperatures for tropical/sub-tropical species and those of temperate origin are 25 °C and 15 °C respectively (Pritchard *et al.* 2004). A drying time-course assessing loss of viability with declining water content should be determined for each new accession.

47. The water content present within different components of recalcitrant seeds is critical for their successful cryopreservation. Water content determined on a whole recalcitrant seed basis, gives no indication of the water content of the axis. Therefore, water content determinations must be carried out separately for axes, embryos, fleshy cotyledonary tissue or endosperm (Berjak and Pammenter 2004) and measured individually (not on pooled samples).

In many cases, the dry mass of axes of recalcitrant seeds may be as little as a few milligrams, necessitating a 6-place balance.

48. It is important to determine water content of each newly-arrived accession immediately after the propagules have been cleaned, to avoid further drying. Even if other accessions of the same species have been collected, one cannot make the assumption that water contents will be similar. Because the composition of the axes and storage tissues of recalcitrant or otherwise non-orthodox wild species is generally unknown, drying is recommended to be carried out at 80 °C until constant weight is attained. When tissues are dried at 80 °C, the time taken to attain constant weight is generally between 24 and 48 h. After the drying period, it is imperative that samples reach room temperature, without absorbing water, before being re-weighed.A minimum of 10 seeds is recommended to be tested for water content (determined on an individual seed/embryo/axis basis). Additional seeds will be required for any biochemical analyses undertaken.

49. Seeds and the embryos/axes excised from them should be at their most vigorous when newly-harvested. Intact seeds are best germinated on 0.8-1% water agar in closed plastic containers, which will provide common conditions for all such assessments. It is important that the seeds are surface-decontaminated prior to being set to germinate, or prior to excision of embryos or embryonic axes. Dormancy is not a common feature of recalcitrant seeds, and seeds should normally commence germination in a relatively short interval after being set out. However, the time will vary among species depending on the extent of embryo development. It is essential that all germination/viability testing is done under the same controlled conditions per species. Production of morphologically abnormal seedlings/plantlets (Pammenter *et al.* 2011) should be noted and quantified, as abnormality can occur as a result of imposed stress (e.g. dehydration of recalcitrant seeds, embryos or embryonic axes). A minimum of 20 seeds is recommended for viability testing.

50. When handling recalcitrant seeds, great care is normally exercised to maintain water contents at the levels characteristic of shedding. However, intact recalcitrant seeds are almost invariably too large to be cooled to cryogenic temperatures. Hence the explants, embryos or the embryonic axes, need to be excised from the seeds and dehydrated. Further to this, it is essential that the bulk of the cleaned seed sample be stored under conditions which preclude changes in water status. If exposed to the atmosphere for any length of time, the water content of seeds will change and seeds shedding at relatively high water contents would become somewhat dehydrated.

D. Contingencies

51. If a genebank does not have a temperature- and humidity-controlled drying room then, for whole seeds, bench-top drying in bell jars or drying in the shade in monolayers could be used. Specimens in any Petri dish not closed before extraction from the drying oven, will have to be replaced in the oven, as dry tissues rapidly adsorb water vapour, especially in a humid environment.

52. Excised embryos/embryonic axes will generally not germinate as rapidly as will the intact seeds. When working with excised embryonic axes, often shoot development will not occur. In such cases, root production will be the criterion on which vigour and viability are assessed.

53. In cases where embryos/axes prove impossible to manipulate for successful cryo-storage, alternative explants must be used. These can be of a variety of types, but the most

suitable are shoot apical meristems excised from seedlings developed from seeds germinated *in vitro*.

E. Selected references

Berjak, P. & Pammenter, N.W. 2004. Recalcitrant Seeds. pp. 305-345 in Benech-Arnold, RL., Sánchez, R.A. (eds) *Handbook of seed physiology: applications to agriculture*. Haworth Press, New York.

Hong, T.D. & Ellis, R.H. 1996. *A protocol to determine seed storage behaviour*. IPGRI Technical Bulletin No. 1.International Board for Plant Genetic Resources, Rome, Italy.

Pammenter, N.W., Berjak, P., Wesley-Smith, J. & Vander Willigen, C. 2002. Experimental aspects of drying and recovery. pp. 93-110 in Black, M, Pritchard, H.W. (eds) *Desiccation and survival in plants: drying without dying*. CABI, Wallingford, UK.

Pammenter, N.W., Berjak, P., Goveia, M., Sershen, Kioko, J.I., Whitaker, C., Beckett, R.P. 2011. Topography determines the impact of reactive oxygen species on shoot apical meristems of recalcitrant embryos of tropical species during processing for cryopreservation. *Acta Horticulturae* 908: 83-92.

Pritchard, H.W., Wood, C.B., Hodges, S., Vautier, H.J. 2004. 100-seed test for desiccation tolerance and germination: a case study on eight tropical palm species. *Seed Science and Technology* 32: 393-403.

3.2.3. STANDARDS FOR HYDRATED STORAGE OF RECALCITRANT SEEDS

A. Standards

3.1 Hydrated storage should be carried out under saturated RH conditions, and seeds should be maintained in air-tight containers, at the lowest temperature that they will tolerate without damage.

3.2 All seeds should be decontaminated prior to hydrated storage and infected material should be eliminated.

3.3 Stored seeds must be inspected and sampled periodically to check if any fungal or bacterial contamination has occurred, and whether there has been any decline in water content and/or vigour and viability.

B. Context

54. For provision of planting stock for re-introductions and restoration programmes, or simply for the maintenance of seeds whilst undertaking experimentation, it is sometimes necessary to store recalcitrant seeds in the short- to medium-term (weeks to months). The basic principle for maximising the storage life span of recalcitrant seeds is that water contents should be retained at essentially the same levels as those characterising the newly-harvested state. Thus the seeds must not lose water either before or after being placed in storage. Even very slight degrees of dehydration can stimulate the initiation of germination, and further dehydration can initiate deleterious changes which impact on vigour and viability and shorten the period for which the seeds can be stored. Keeping recalcitrant seeds under conditions which will maintain their water content is termed, hydrated storage, and is achieved by holding the seeds in a closed under saturated relative humidity (RH) conditions.

C. Technical aspects

55. To avoid any water loss from the seeds, hydrated storage must be carried out at saturated RH, achieved by maintaining a saturated atmosphere in the storage containers. Ideally sealing polythene bags with an inner paper bag inside (‘bag within a bag’) or sealing plastic buckets of appropriate size for the seed numbers, are favoured for storage (Pasquini *et al.* 2011). As an essential precaution, storage containers such as buckets with sealing lids, as well as internal grids, must be sterilised prior to the introduction of seeds. Irrespective of the container chosen, a means for absorbing any condensate needs to be included, and changed on becoming damp.

56. Storage temperatures should be the lowest that seeds of individual species will tolerate, without any deleterious effect on vigour and viability. This will slow both progress towards germination and fungal proliferation. The temperature of the store must be kept constant to minimize condensation on the interior surfaces of the storage containers. For recalcitrant seeds of temperate origin, temperatures of 6 ± 2 °C are generally suitable for storage, while for the majority of seeds of tropical/sub-tropical origin, 16 ± 2 °C is normal range. Exceptions occur, particularly for seeds of some equatorial species.

57. Under hydrated storage conditions, fungi (or less frequently bacteria) are likely to proliferate, so vigilance and appropriate action to curtail seed-to-seed infection is required. If infected seeds are not removed they will contaminate the entire batch in a storage container.

This renders the stored seeds useless and eliminates their potential for supplying explants for cryopreservation. Hence, regular inspection right from the outset, and appropriate action such as the application of fungicidal agents should be done to eliminate surface and internal contaminants from seeds at the earliest possible opportunity (Calistru *et al.* 2000).

58. Seeds need to be surface-decontaminated, dried of any residual sterilant, and dusted with a broad-spectrum fungicide. Internally-borne fungi, largely located immediately below the seed coverings, would be most effectively eliminated by the uptake of appropriate systemic fungicides by the seeds. However, these may well affect the seeds adversely. Thermotherapy, as applied to infected acorns (Sutherland *et al.* 2002), is another possibility, but this can be used only when seeds are resilient to transitorily-raised temperatures – which is not always the case. To decontaminate inner surfaces directly, it is necessary to ensure that the seeds survive well in hydrated storage after removal of the coverings, and that presence of systemic fungicides in the seed tissues are not damaging.

59. Depending on the duration of hydrated storage, containers should be briefly and periodically ventilated to avoid development of anoxic conditions at which time the contents of containers must be inspected and any contaminated seeds discarded. Storing seeds in a monolayer is ideal, but if seeds are stored in several layers, the seeds should be mixed about during aeration. After removing any seed showing signs of contamination, the container must be emptied, all apparently uncontaminated seeds disinfected and the seed lot replaced in a sterilised container.

60. Stored seeds must be sampled periodically to check whether there has been any decline in water content and vigour and viability. If water content has remained essentially what it was when the seeds were placed in hydrated storage, and there is no apparent fungal (or bacterial) proliferation, but viability has declined, then the end of the useful storage period will have been reached. Similarly, if visible signs of germination of many of the seeds are apparent, the end of the useful storage period will have been reached. A decline in viability of seeds which have not lost water to any marked extent, or root protrusion by most of the seeds, gives a measure of the time for which hydrated storage is possible under the specific temperature regime used.

D. Contingencies

61. Loss of water from seeds indicate that high RH was not maintained, probably because the storage containers were not properly sealed. This leaves uncertain results for the sample, which should be discarded. Loss of viability of seeds during storage may also be the result of maintenance at inappropriate temperatures. This parameter needs to be resolved by trials testing seed responses to a range of temperatures. Seeds may have lost viability because they were originally of poor quality, or of being too immature at harvest.

62. In cases where there is a high incidence of internally-contaminated seeds in an accession, the contaminants should be isolated and identified, with a view to developing effective means to eliminate them from future collections. Identification of fungi, certainly to genus level, could assist in selection of fungicides which may be more efficacious in combination ('cocktails'), specifically targeting those fungi. Sometimes viruses are present in the seeds, which cannot be eliminated by any treatments. If they can cause serious diseases, the plants must be discarded as soon as the viruses are observed.

63. Contamination may prove to be intractable to any remedial treatment, in which case seeds cannot be stored in this manner, and alternate forms of conservation of the genetic resources must be sought. In such cases, seeds should be set to germinate, with seedlings

developed from any seeds which are uninfected being maintained under slow-growth conditions, and/or used to provide alternative explants for *ex situ* conservation, for instance transferred and planted in field genebanks, or other gardens, as appropriate.

E. Selected references

Calistru, C., McLean, M., Pammenter, N.W. & Berjak, P. 2000. The effects of mycofloral infection on the viability and ultrastructure of wet-stored recalcitrant seeds of *Avicennia marina* (Forssk.) Vierh.*Seed Science Research* 10, 341-353.

Pasquini, S., Braidot, S., Petrussa, E. & Vianello, A. 2011. Effect of different storage conditions in recalcitrant seeds of holm oak (*Quercus ilex* L.) during germination. *Seed Science and Technology* 39, 165-177.

Sutherland, J.R., Diekmann, M. & Berjak, P. (eds). 2002. *Forest Tree Seed Health*. IPGRI Technical Bulletin No. 6, International Plant Genetic Resources Institute, Rome, Italy.

3.2.4. STANDARDS FOR *IN VITRO* CULTUREAND SLOW GROWTH STORAGE

A. Standards

4.1 Identification of optimal storage conditions for *in vitro* cultures must be determined according to the species.

4.2 Material for *in vitro* conservation should be maintained as whole plantlets or shoots, or storage organs for species where these are naturally formed.

4.3 A regular monitoring system for checking the quality of the *in vitro* culture in slow growth storage, and possible contamination, should be in place.

B. Context

65. *In vitro* conservation is used for maintenance of plant organs or plantlets in a medium-term time frame (some months up to some years) under non-injurious, growth-limiting conditions. Generally it is not desirable for long-term conservation (Engelmann 2011). *In vitro* conservation is preferentially applied to clonal crop germplasm as it also supports safe germplasm transfers under regulated phytosanitary control. Technical documents provide detailed information on the possibilities offered by *in vitro* storage, on the main parameters to consider and on the links and complementarity with other storage technologies, such as field genebanks (Reed *et al.* 2004; Engelmann 1999).

66. *In vitro* cultures serve as sources of disease-free materials for distribution, multiplication and a source of explants for cryo-preservation. Safe removal and disposal of infected materials is essential, as it ensures that a pathogen or pest is not released into the environment. Permanent regular monitoring is necessary to avoid accumulation of contamination which might take place during transfers, be transmitted through the air from vessel to vessel or actively transported by vectors like mites and thrips. Breakdown by hyperhydration is another danger, which usually starts in some vessels a little earlier so that a chance exists to rescue the other material when noticed early enough.

C. Technical aspects

67. Optimal conditions for slow growth need to be identified prior to storage. These may be achieved by manipulating variables, including light-regime, temperature, and medium composition, individually, or in combination (Engelmann 1991), but experimentation is generally required to achieve optimal results.

68. The type and physiological condition of explants is basic to success or failure of *in vitro* slow growth. *In vitro* culture is also used as a preparatory phase to cryopreservation as well as for recovery phases after cryopreservation. Thus, suitable media and conditions for *in vitro* growth of explants need to be developed as a first step. This involves appropriate surface decontamination procedures and germination medium (starting with a standard medium (Murashige and Skoog 1962), which may need refining). The basal medium may be determined from the literature concerning culture of similar species. Standard protocols have been published and can be used for guidance (including George 1993; Hartmann *et al.* 2002; Chandel *et al.* 1995) but in many cases detailed trials using explants media and growth conditions are critical and custom-made protocols using explants media and growth conditions are needed even if the species are closely related.

69. Ensuring that the materials are maintained as whole plantlets or shoots, can avoid hyper-hydricity ('vitrification'). For explants of species which naturally grow slowly, no manipulation of media or of culture conditions may be necessary.

70. Experimentation with a range of permutations and combinations of the means to achieve satisfactory slow growth are imperative when first working with explants of a species. For example, very variable responses to manipulations for slow growth have been recorded for different species of single genera. Maintenance of long-term genetic stability of material stored under slow-growth conditions is imperative (Engelmann 2011). Optimal storage temperatures for cold-tolerant species may be from 0 to 5 °C or somewhat higher; for material of tropical provenance the lowest temperatures tolerated may be in the range from 15 to 20 °C, depending on the species (Normah *et al.* 2011; INIBAP 2011; Engelmann 1999a; Engelmann 1991).

71. Various modifications are generally made to culture media, especially reduced levels of minerals, reduction of sucrose content and/or manipulation of the type and concentration of growth regulators, while inclusion of osmotically-active substances (e.g. mannitol) may also be effective (Engelmann 2011; Engelmann 1999a). Activated charcoal in the medium may adsorb exuded polyphenolics (Engelmann 1991).

72. The type, volume, and means of closure of, and atmosphere in, culture vessels constitute an important parameter (Engelmann 2011; Engelmann 1991), which can be established only by experimentation when working with new material.

73. Although traditionally, slow growth storage is used for material cultured *in vitro*, plantlets may also be maintained *ex vitro* under growth-restricting conditions. Seedling slow growth in shaded,light-limitingconditions under natural canopies is an inexpensive alternative (Chin 1996). Furthermore, induction of storage organs *in vitro* can be used for effectively enhancing the conservation period in natural storage organ forming crops (e. g. ginger [Engels *et al.* 2011], taro, yam, potato etc.).

D. Contingencies

74. *In vitro* culture of explants of woody species may pose particular problems, especially regarding exudation of polyphenolics (Engelmann 1999b). Associated problems include poor rooting and the explants becoming hyperhydric. Hyperhydration and leaf necrosis developed during slow growth can lead to deterioration of quality and in some cases death of the whole propagules.

75. In some material, accumulation of covert bacteria may become a gradually increasing obstacle for prolonged slow growth storage. It may be counteracted by temporary removal of vitamins from the medium or addition of antibiotics, but rarely these measures will be of permanent success. Thus, it may be necessary to discard these cultures from the storage (Abreu-Tarazi *et al.* 2010; Leifert and Cassels 2001; Senula and Keller 2011; Van den Houwe 2000; Van den Houwe 1998)

76. Within a genepool, there may be large differences in the response to *in vitro* storage between species/varieties, some responding well while others cannot be conserved using this technology, thus making its application impossible (e.g. for coffee (Dussert 1997))

77. In some species (e.g. yam), storage organs may be formed *in vitro*, but their germination is difficult to attain. This is true also for *in vitro*-derived bulblets in some accessions of a species (e.g. garlic (Keller 2005)).

78. In some species intrinsic genetic instability (e.g. sugar cane) may be enhanced by *in vitro* culture techniques, whereas in others (e.g. cassava) stability over extended storage periods has been demonstrated (IPGRI/CIAT 1994). In the latter cases somaclonal variation may occur in higher frequencies. In most cases somaclonal variation is minimized by consequent use of techniques which avoid induction of adventitious shoots or any formation basal callus after cutting. Where callus has formed this needs to be cut off during transfer to the next culture period. To avoid confusion about the reasons for any genetic deviations occurring, thorough observation of uniformity of source explants is needed and chimerism should also be excluded from the donor material (or carefully maintained if needed in variegated plants). Since regular screening by means of molecular markers seems to be too expensive, regular sampling may be undertaken in cases where somaclonal variation is expected to occur.

79. Dormancy of organs may become a problem, when shoots stop developing (often occurring in species which form *in vitro* storage organs). Additional cutting or application of cytokinins may break dormancy. If this is not successful, then waiting for some time until spontaneous sprouting may be the only (even though uncertain) solution.

E. Selected references

Abreu-Tarazi, M.F., Navarrete, A.A., Andreote, F.D., Almeida, C.V., Tsai, S.M. & Almeida, M. 2010. Endophytic bacteria in long-term *in vitro* cultivated "axenic" pineapple microplants revealed by PCR-DGGE. *World J. Microbiol.Biotechnol.* 26: 555-560.

Benson, E.E., Harding, K. & Johnston, J.W. 2007. Cryopreservation of shoot-tips and meristems. pp. 163-184 in Day, J.G., Stacey, G. (eds) Methods in molecular biology vol. 368. Cryopreservation and freeze drying protocols 2nd edition. Humana Press, Totowa, NJ.

Chandel, K.P.S., Chaudhury, R., Radhamani, J. & Malik, S.K. 1995. Desiccation and freezing sensitivity in recalcitrant seeds of tea, cocoa and jackfruit. *Annals of Botany* 76: 443-450.

Chin, H.F. (1996). Strategies for conservation of recalcitrant species. In *In vitro Conservation of Plant Genetic Resources*, edsNormah, M.N., M.K. Narimah and M.M. Clyde, Kuala Lumpur, Malaysia: PercetakanWatanSdn.Bhd, pp. 203-215.

Dussert S., N. Chabrillange, F. Anthony, F. Engelmann, C. Recalt & S. Hamon. 1997. Variability in storage response within a coffee (Coffea spp.) core collection under slow growth conditions. *Plant Cell Reports* 16: 344-348.

Engelmann, F. 1991. *In vitro* conservation of tropical plant germplasm – a review. *Euphytica* 57: 227-243.

Engelmann F. 1999a. (ed.) *Management of field and in vitro germplasm collections*, Proceedings of a consultation meeting, 15-20 January 1996, CIAT, Cali, Colombia. International Plant Genetic Resources Institute, Rome, Italy, 165 pages.

Engelmann, F. 1999b. Alternative methods for the storage of recalcitrant seeds – an update. pp. 159-170 in Marzalina, M., Khoo, K.C., Jayanthi, N., Tsan, F.Y.M Krishnapillay, B. (eds) *Recalcitrant seeds*, FRIM, Kuala Lumpur, Malaysia.

Engelmann, F. 2011. Biotechnologies for conserving biodiversity. *in vitroCellular and Developmental Biology – Plant* 47, 5-16.

Engels, J. M. M., Dempewolf H. & Henson-Apollonio V. 2011. Ethical Considerations in Agro-biodiversity Research, Collecting, and Use.J Agric Environ Ethics 24:107–126

George, E.F. 1993. Chapter 10 in: *Plant propagation by tissue culture. Part 1: The technology* 2nd edition. Exegenics Limited, Whitchurch, Shropshire, U.K.

Hartmann, H.T., Kesler, D.E., Davies, F.T. & Geneve, R.L. 2002. Chapters 17 & 18 in: *Plant propagation – Principles and practices* 7th edition. Prentice Hall, New Jersey, USA.

INIBAP. 2011. http://www.biw.kuleuven.be/dtp/tro/_data/itc.htm. Leifert, C., Cassels, A.C. (2001) Microbial hazards in plant tissue and cell cultures. *in vitro* Cell. Dev. Biol. – Plant 37: 133-138.

IPGRI/CIAT. 1994. *Establishment and operation of a pilot in vitro active genebank.* Report of a CIAT-IBPGR collaborative project using cassava (*Manihotesculenta*Crants) as a model. A joint publication of IPGRI and CIAT, Cali, Colombia.

Keller, E.R.J. 2005. Improvement of cryopreservation results in garlic using low temperature preculture and high-quality *in vitro* plantlets. *Cryo-Letters* 26: 357-366.

Murashige, T. & Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum* 15: 473-497.

Normah, M.N., Kean, C.W., Vun, Y.L. & Mohamed-Hussein, Z.A. 2011. *In vitro* conservation of Malaysian biodiversity – achievements, challenges and future directions. *In vitro* Cellular and Developmental Biology – Plant 47: 26-36.

Reed B.M., F. Engelmann, E. Dulloo & J.M.M. Engels (eds.). 2004. Technical guidelines for the management of field and *in vitro* germplasm collections. IPGRI/FAO/SGRP, Rome.

Senula, A. & Keller, E.R.J. 2011. Cryopreservation of mint – routine application in a genebank, experience and problems. *Acta Hort.* 908: 467-475.

Van den Houwe, I., Guns, J. & Swennen, R. 1998. Bacterial contamination in Musa shoot tip cultures. *Acta Hort*. 490:485-492.

Van den Houwe, I. & Swennen, R. 2000. Characterization and control of bacterial contaminants in *in vitro* cultures of banana (*Musa* spp.). *Acta Hort.* 530:69-79.

3.2.5. STANDARDS FOR CRYOPRESERVATION

A. Standards

5.1 The explants selected for cryopreservation should be of highest possible quality, and allow onward development after excision and cryopreservation.

5.2 Each step in the cryo-protocol should be tested individually and optimized in terms of vigour and viability in retention of explants.

5.3 Means should be developed to counteract damaging effects of reactive oxygen species, (ROS), at excision and all subsequent manipulations.

5.4 Following retrieval, explants should be decontaminated using standard sterile procedures.

B. Context

80. Cryopreservation permits cells or tissue to be stored for an indefinite period in liquid nitrogen (-196 °C) where metabolic activities are suspended. Four steps are essential in any cryopreservation protocol: (i) selection, (ii) cryopreservation techniques, (iii) retrieval from storage, and, (iv) seedling or plantlet establishment.

81. Cryo-protocols should be developed to prevent cryopreservation damages, and could include possible cryo-protection, partial drying, cooling, storage at cryogenic temperatures, rewarming and rehydration. There are two main types of cryopreservation procedures: conventional slow freezing, based upon freeze-induced dehydration; and, flash-freezing (vitrification), which involves dehydration prior to cooling (Engelmann 2011a).

C. Technical aspects

Selection

82. Dehydration rate, and how evenly cells and tissue dry, depend on size, and since the vast majority of recalcitrant seeds are too large to be dried rapidly and evenly, they cannot be cryopreserved intact. In addition, cells with water contents ≥ 1.0 g g^{-1}, cannot survive after exposure to cryogenic conditions. Excision and cultivation of suitable explants should therefore be developed specifically for the purpose of cryopreservation. The explants should be as small as possible, but big enough to allow onward development after excision, and after cryopreservation. Explants for cryopreservation can be produced from embryonic axes, shoot tips, meristematic and embryogenic tissues. For recalcitrant seeds, excised embryos/axes are the explants of choice for cryopreservation. In the event they are too large, do not withstand the required degree of dehydration, are sensitive to all common modes of surface decontamination, and/or are intractable to culture conditions, explants such as shoot apical meristems are a better option.

83. For vegetatively propagated species, explants of choice are buds, shoot-tips, meristematic, and embryogenic tissue. Not all types of explants are amenable to similar cryoprotection procedures, even when the parent species are relatively closely related taxonomically (Sershen *et al.* 2007), and responses to cryoprotection procedures need to be ascertained per species. Under-developed material is generally more susceptible to excision damage; likewise seeds that have developed/germinated to the stage of visible protrusion of radicals (or other parts of the embryo) should not be selected (Goveia *et al.* 2004).

84. Whole anthers or isolated pollen grains can be used for cryopreservation as well. They represent the inherited genetic diversity like seeds, but bearing the male germ units, they usually have the haploid chromosome set only (see Ganeshan 2008; Rajashekaran 1994; Weatherhead *et al.* 1978, for a review). When pollen is conserved, it needs to be embedded in gelatine capsules or paper pouches or packed on a paper strip, with some species requiring dehydration of the pollen prior to storage.

85. To retrieve the material, anthers or pollens are shed from capsules, pouches or strips at room temperature. Viability may be tested by pollen staining, but results may led to overestimation of survival, since stained pollen may finally not be able to germinate. The assessment of pollen germination is best undertaken either in a germination medium or, better, on the stigma of a test plant. When the behaviour of a species is still not known, test pollinations are needed to confirm successful fertilization by seed set (Ganeshan 2008; Rajashekaran 1994; Weatherhead *et al.* 1978).

86. Probabilistic tools are available which facilitate calculation of the number of propagules to store and retrieve, depending in the objectives, survival after cryo-storage, and other parameters (Dussert *et al.* 2003). Furthermore, protocol development can require more than a single collection and may spread over two or more years due to the seasonal nature of seed availability.

Cryopreservation techniques

87. It is important that a drying time-course of excised embryos/axes be conducted to identify the drying time required to reduce material to an appropriate water content. An additional drying time-course needs to be done after any pre-growth or cryoprotectant treatment.

88. Cooling rate to LN temperatures is important and needs to be considered in relation to the explant water content. The cryo-protocol should be selected to ensure the water content lies within the range that prevents intra-cellular ice-crystal formation on cooling and warming, but also avoids desiccation damage to sub-cellular structure. At the higher end of the water content range to which axes are dried, the faster the cooling rate the better, as very rapid cooling of small specimens tends to be even and minimizes the duration in the temperature range which would permit ice crystallisation. On the other hand, cooling rate is less critical for recalcitrant axes flash-dried (using evaporative dehydration) near to their lower limits of tolerance.

89. Techniques based on dehydration during controlled rate cooling, has an application when the material to be cryopreserved consists of embryogenic cultures and of shoot tips from temperate species (Engelmann 2011a). For vegetative material, many protocols and examples of cryopreservation of a range of explants across species using one or more of the procedures, are documented (Benson *et al.* 2007). In addition, there is a vast number of publications on cryopreservation of apices, other meristematic tissues, embryogenic tissues and dormant buds, and the journal, *CryoLetters*, is a good source for many of these. Once a successful protocol has been developed for a species, periodic testing of samples extracted from cryopreservation should be carried out, initially after a short storage interval. The embryos/axes, generally constitute only an insignificant fraction of seed mass and volume, and are suitable for flash-drying, thus overcoming the problem of metabolism linked damage.

90. Most plant vitrification protocols use cryoprotectants (usually a mixture of penetrating and non-penetrating types). Evaporative dehydration has generally been employed for zygotic

embryos/embryonic axes. Although originally developed for apices and somatic embryos, encapsulation-dehydration and the procedure termed vitrification (employing various plant vitrification solutions (PVS), have also been used in procedures to cryopreserve seed-derived embryos and embryonic axes. A recent overview (Engelmann 2011b) provides the information that all vitrification protocols developed for somatic embryos, utilizing PVS2. Vitrification using PVS2 has also been used for cryopreservation of shoot tips of a wide range of species from both tropical and temperate provenances, the former including several recalcitrant-seeded and vegetatively-propagated species. Another common vitrification solution is PVS3 (Nishizawa 1993) which does not use DMSO and can, therefore, be preferred for species which are damaged by DMSO. A range of alternative loading and vitrification have been developed recently solutions, which can be efficiently used for cryopreserving materials which prove sensitive to PVS2 and PVS3 (Kim *et al.* 2009a; Kim *et al.* 2009b).

91. At the lower limits of dehydration tolerated by recalcitrant embryos and axes, generally a proportion of freezable water is retained. During both slow cooling and rewarming, ice crystallisation can occur in the freezable water fraction between about -40 and -80 °C. Rewarming at ~37 to 40 °C prevents this, noting that transfer from cryogenic temperatures must be very rapid.

Retrieval from cryostorage

92. Rewarming of vitrified germplasm is often undertaken in two steps, the first is slow to allow for glass relaxation, usually at ambient room temperatures. This is followed by more rapid rewarming at ca. 45°C to avoid ice nucleation (Benson *et al.* 2011).

93. Specimens processed by encapsulation-dehydration may be transferred directly onto recovery/germination medium for rapid rewarming, or the cryotubes containing the alginate beads may be placed in a water-bath at 40°C for 2-3 min. Alternatively, the beads may be rehydrated by transferring them for ~10 min in liquid medium. The removal of the capsule has also been shown to be advantageous (ww). Encapsulation-dehydration has proved to be consistent and successful for shoot tips of many species (Gonzalez and Engelmann 2006), somatic embryos of conifers (Engelmann 2011b), a range of citrus species and varieties, and temperate fruit species (Damiano *et al.* 2003; Damiano et al 2007).

94. To restore metabolic activity in the cell upon rewarming, toxic cryoprotectants must be removed from the cell and the normal water balance gradually restored as the cell is returned to a normal functioning temperature. The original composition of the recovery medium may have to be slightly modified after explants have been dehydrated or cryogen-exposed. With the use of plant vitrification solutions (PVS), after rapid rewarming, a dilution or unloading step (removal of toxic PVS) is necessary (Sakai *et al.* 2008; Kim *et al.* 2004).

95. All steps in cryopreservation could compromise survival, and particularly, warming and rehydration can be accompanied by a burst of ROS (Whitaker et al. 2010; Berjak et al. 2011). Rewarming and rehydration media should ideally also counteract the deleterious effects of ROS, but it is imperative that means are established to reduce the bursts of reactive oxygen species (ROS) accompanying excision (Whitaker *et al.* 2010; Berjak et al 2011; Engelmann 2011a; Gioveia *et al.* 2004). Treatments with cathodic water (an electrolysed dilute solution of calcium chloride and magnesium chloride) had potent anti-oxidative properties, which counteracted the effects of ROS bursts at all stages of a cryopreservation protocol for recalcitrant embryonic axes of *Strychnos gerrardii*, and promoted shoot development (Berjak et al 2011). The beneficial effects of the treatment are more marked when development of

embryos/axes progress during an hydrated storage period, indicating the importance of developmental status of the seeds. It appears that treatment of axes with the non-toxic anti-oxidant, cathodic water, offers both an explanation for previous failures of axes to produce shoots, and an ameliorative treatment to counteract stress-related ROS bursts. Furthermore, the instruments used for embryo/axis excision can exacerbate ROS production. In this regard, use of a hypodermic needle is likely to cause less trauma, than will a surgical blade (Benson *et al.* 2007). The use of dimethyl sulphoxide, (DMSO), a hydroxyl radical scavenger, as a pre-culture step (before complete severing of the cotyledonary remnants) and as a treatment after their removal, has shown to facilitate shoot development. Other antioxidant substances are also used to counteract ROS formation, e.g. ascorbic acid and tocopherol (Chua and Normah 2011; Johnston *et al.* 2007; Uchendu *et al.* 2010).

Seedling and plantlet establishment

96. Once excised embryos/embryonic axes have been rewarmed, the next step is to generate and establish a seedling or plantlet to complete the regeneration cycle. Seedling and plantlet establishment requires two steps: (i) its establishment *in vitro* and (ii) establishment *ex vitro* and hardening-off or acclimation. The material recovered from cryo-storage, must be introduced to recovery medium initially in the dark. For introduction into *in vitro* culture, explants require to be surface-decontaminated and handled with sterilised instruments, with all procedures being carried out in a laminar air-flow. In conditions where no laminar flow box (clean bench) is available, it may be possible to perform the work in closed clean rooms with thorough room and air disinfection. Embryos and embryonic axes need to be rehydrated for 30 min at ambient temperature in the dark. Where they are directly exposed to a re-warming medium, rehydration should be in a solution of the same composition. Resultant seedlings each producing both a root and a shoot are a measure of successful axis cryopreservation. For vegetatively propagated material, cryo-storage is considered successful when shoots are obtained, which can be either rooted or further micropropagated.

97. After a precautionary culture period in the dark (Touchell and Walters 2000), explants are usually exposed to conventional growth room lighting conditions and temperature regimes which should be established at the outset as suiting the species and its provenance. Light regimes and temperature for *in vitro* germination and seedling/plantlet development are parameters may need to be fine-tuned, and transferring explants through several culture phases may be necessary. It is critical that the seedlings and plantlets produced *in vitro* are initially maintained under high RH, which is gradually reduced.

98. The establishment *ex vitro* and its hardening-off essentially involves transfer of the seedlings/plantlet from slow growth culture or cryopreservation of vegetative material from the heterotrophic *in vitro* condition to a sterile soil-based medium in which the autotrophic condition will develop. Recovery media must contain macro- and micro-nutrients, essential minerals and a carbon source, but may also require addition of growth regulators. Media must have been autoclaved during preparation, and any heat-labile components (if required) filter-sterilised and added subsequently. Suitable germination media for embryos/axes of a variety of species are based on MS (Murashige and Skoog 1962): however, the MS nutrient medium may be utilized at full-strength, or half- or quarter-strength, as indicated by explant responses when first working with seeds of particular species. Depending on the objective sought, explants recovered from cryopreservation are directly grown into a seedling/plantlet for acclimatization, or a multiplication phase can occur before acclimatization, thus offering the possibility of producing the desired number of copies of the retrieved accession.

D. Contingencies

99. It should be noted that material can either be conserved in LN or above LN in the vapour phase. Storage in the vapour phase is much more expensive and less safe than storage directly in liquid nitrogen. Even if some microbes are suspended in LN there is not essentially the consequence that they would contaminate the samples, because they pass some washing procedures under sterile conditions upon rewarming. Even if spores may adhere to the surface of the explants, microbes cannot enter them in LN because all such processes are stopped at such low temperatures.

100. Excised axes may not germinate because of their maturity status. Hence the collected propagule need to be placed in hydrated storage and sampled periodically for germination and for performance of excised axes. In the event that neither seed/ propagule nor excised embryos/axes geminate, the material may be dead, or dormant. Performing a tetrazolium test will determine whether or not the seeds are viable. If so, then dormancy may be assumed, and investigations to break the dormant condition need to be undertaken.

101. In the case of most recalcitrant-seeded species, regeneration as practised for orthodox-seeded species is not an option. If there is an unacceptable decline in quality of cryostored embryos/axes, the only option would be re-sampling of seeds from the parent population(s) and refining of the procedures. In cases where embryos/embryonic axes continue to be intractable to cryopreservation, then attention must be focused on the development of suitable alternative explants, ideally derived from seedling/plantlets established *in vitro*.

102. Material from prolonged *in vitro* culture or *in vitro* storage may no longer be suitable for extracting shoot tips for cryopreservation, since this material may have accumulated covert bacteria (endophytes) which will break out during recovery from cryopreservation and, thus, hamper cryopreservation entirely. There are instances where explants (e.g. nodal segments) of source material from long-term in-vitro-maintained cultures are excessively hydrated. In such cases, source material should be cultured *de novo*.

103. Cultures which have become infected need to be immediately removed from the growth room and destroyed. The most devastating contingency in any growth room is infestation by mites. After removing any cultures showing 'mite tracks', rapid response by disinfesting the facility is required. This is followed by inspection of each culture vessel and removal and destruction of any left which show evidence of mites (which bite through Parafilm™, and spread fungal spores from any infected culture to others).

104. Depletion of LN in a cryo-storage vat or LN freezer would lead to irretrievable loss of all samples. If not detected, electrical or other failure of the temperature control system in a growth room, could cause overheating with consequent loss of *in vitro* material.

E. Selected references

Benson, E.E. & Bremner, D. 2004. Oxidative stress in the frozen plant: a free radical point of view. pp. 205-241 *in* Fuller, B.J., Lane, N., Benson, E.E. (eds) *Life in the frozen state*. CRC Press, Boca Raton.

Benson, E.E., Harding, K., Johnston, J.W. 2007. Cryopreservation of shoot-tips and meristems. pp. 163-184 *in* Day, J.G., Stacey, G. (eds) *Methods in molecular biology* vol. 368. *Cryopreservation and freeze drying protocols* 2nd edition. Humana Press, Totowa, NJ.

Benson E.E., Harding K., Debouck D., Dumet D., Escobar R., Mafla G., Panis B., Panta A., Tay D., Van den houwe I. & Roux N. 2011. Refinement and standardization of storage procedures for clonal crops - Global Public Goods Phase 2: Part I. Project landscape and general status of clonal crop *in vitro* conservation technologies. System-wide Genetic Resources Programme, Rome, Italy.

Berjak, P., Sershen, Varghese, B. & Pammenter, N.W. 2011. Cathodic amelioration of the adverse effects of oxidative stress accompanying procedures necessary for cryopreservation of embryonic axes of recalcitrant-seeded species. *Seed Science Research* 21:187-203.

Chua, S.P. & Normah, M.N. 2011. Effect of preculture, PVS2, and vitamin C on survival of recalcitrant *Nepheliumramboutan* Ake shoot tips after cryopreservation by vitrification. *Cryo Letters* 32: 596-515.

Damiano C., Frattarelli A., Shatnawi M.A., Wu Y., Forni C. & Engelmann F. 2003. Cryopreservation of temperate fruit species: quality of plant materials and methodologies for gene bank creation. *Acta Horticulturae* 623: 193-200.

Damiano C., Arias Padrò M. D., & Frattarelli A. 2007 Cryopreservation of some mediterranean small fruit plants. *Acta Horticulturae* 760: 187-194

Dussert S., Engelmann F. & Noirot. M. 2003. Development of probabilistic tools to assist in the establishment and management of cryopreserved plant germplasm collections. *CryoLetters* 24: 149-160.

Engelmann, F. 2011a. Germplasm collection, storage and preservation. In Plant Biotechnology and Agriculture – Prospects for the 21st Century. A. Altman & P.M. Hazegawa (eds.), Oxford: Academic Press,pp. 255-268.

Engelmann, F. 2011b. *Cryopreservation of embryos: an overview.* Chapter 13 in Thorpe, T.A., Yeung, E.C. (eds) Plant embryo culture methods and protocols. Methods in Molecular Biology, vol. 710, DOI 10.1007/978-1-61737-988-8_13, Springer Science+Business Media, LLC 2011.

Ganeshan, S., Rajasekharan, P.E., Shashikumar, S. Decruze, W. 2008. Cryopreservation of pollen. In: Reed, B.M. (ed.) *Plant Cryopreservation: A practical Guide*. Springer, pp. 443-464.

Gonzalez Arnao M.T. & Engelmann. F. 2006. Cryopreservation of plant germplasm using the encapsulation-dehydration technique: review and case study on sugarcane. *Cryo Letters* **27**, 155-168.

Goveia, M., Kioko, J.I. & Berjak, P. 2004. Developmental status is a critical factor in the selection of excised recalcitrant axes as explants for cryopreservation: A study of *Trichiliadregeana* Sond. *Seed Science Research* 14: 241-248.

Johnston, J.W. Harding, K. & Benson, E.E. 2007. Antioxidant status and genotypic tolerance of Ribes *in vitro* cultures to cryopreservation. *Plant Sci.* 172: 524–534.

Kim H.H., Cho E.G., Baek H.J., Kim C.Y., Keller E.R.J. & Engelmann F. 2004. Cryopreservation of garlic shoot tips by vitrification: Effects of dehydration, rewarming, unloading and regrowth conditions. *Cryo Letters* 25, 59-70.

Kim H.H., Lee Y.G., Shin D.J., Kim T., Cho E.G. & Engelmann F. 2009a. Development of alternative plant vitrification solutions in droplet-vitrification procedures. *Cryo Letters* 30: 320-334.

Kim H.H., Lee Y.G., Ko H.C., Park S.U., Gwag J.G., Cho E.G. & F. Engelmann. 2009b. Development of alternative loading solutions in droplet-vitrification procedures. *Cryo Letters* 30: 291-299.

Murashige, T. & Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum* 15: 473-497.

Nishizawa S, Sakai A, Amano Y, Matsuzawa T. 1993. Cryopreservation of asparagus (*Asparagus officinalis* L.) embryogenic suspension cells and subsequent plant regeneration by vitrification. *Plant Sci* 91: 67-73.

Rajasekharan, P.E., Rao, T.M. Janakiram, T. & Ganeshan, S. 1994. Freeze preservation of gladiolus pollen. *Euphytica* 80: 105-109.

Sakai, A., Hirai, D. & Niino, T. 2008. Development of PVS-based vitrification and encapsulation-vitrification protocols. pp. 33-57 in Reed, B.M. (ed.) Plant cryopreservation A practical guide. Springer, New York.

Sershen, Pammenter, N.W., Berjak, P. & Wesley-Smith, J. 2007. Cryopreservation of embryonic axes of selected amaryllid species. *Cryo Letters* 28, 387-399.

Sershen, Berjak, P., Pammenter, N.W. & Wesley-Smith, J. 2011. Rate of dehydration, state of subcellular organisation and nature of cryoprotection are critical factors contributing to the variable success of cryopreservation: studies on recalcitrant zygotic embryos of *Haemanthus montanus*. *Protoplasma* 249(1): 171-86.

Shatnawi M.A., Engelmann F., Frattarelli A. & Damiano C. 1999 Cryopreservation of apices of *in vitro* plantlets of almond (*Prunusdulcis* Mill.). *CryoLetters* 20: 13-20.

Touchell, D. & Walters, C. 2000. Recovery of embryos of *Zizaniapalustris* following exposure to liquid nitrogen. *Cryo Letters* 21: 26 –270.

Uchendu, E.E., Leonard, S.W. Traber, M.G. & Reed, B.M. 2010. Vitamins C and E improve regrowth and reduce lipid peroxidation of blackberry shoot tips following cryopreservation. *Plant Cell Rep.* 29: 25–35.

Weatherhead, M.A., Grout, B.W.W. & Henshaw, G.G. 1978. Advantages of storage of potato pollen in liquid nitrogen. *Potato Res.* 21: 331-334.

Whitaker, C., Beckett, R.P., Minibayeva, F. & Kranner, I. 2010. Production of reactive oxygen species in excised, desiccated and cryopreserved explants of *Trichiliadregeana* Sond. *South African Journal of Botany* 76: 112-118.

3.2.6. STANDARDS FOR DOCUMENTATION

A. Standards

6.1 Passport data for all accessions should be documented using the FAO/IPGRI multi-crop passport descriptors. In addition accession information should also include inventory, orders, distribution and data user feedback.

6.2 Management data and information generated in the genebank should be recorded in a suitable data base, and characterization and evaluation data (C/E data) should be included when recorded.

6.3 Data from 6.1. and 6.2 should be stored and changes updated in anappropriate database system and international data standards adopted.

B. Context

105. Comprehensive information about accessions is essential for genebank management. Passport data is a minimum, but additional information including geographical (GPS coordinates) environmental (overlaid climate and soil maps) data of the collection site and historical information as well as data on characterization and evaluation of the material are all very useful.

C. Technical aspects

106. Due to advances in information technology, it is now relatively simple to record, manage and share information about accessions. All genebanks should use compatible data storage and retrieval systems. The FAO/IPGRI multi-crop passport descriptors (Alercia *et al.* 2001) should be used by all genebanks as it facilitates data exchange.

107. Characterization and Evaluation data are produced by users. Such data are useful to the genebank in the management of their collections (BRAHMS 2011) and to facilitate the consecutive use. Genebanks are recommended to request information feedback on these data.

108. Management data should be as complete as possible to enable an effective handling of the collection. Most management data are only of internal use to the curator and of limited or no value to others, users and/or recipient genebanks. Therefore, management data should be restricted for use of the collection holder only; a set of the accession history, life form and availability can be extracted for public use. Beside the key data for the accession (passport and characterization data) they should contain the following:

(i) History (date of acquisition, preliminary numbers, date of changing the numbers, taxonomical determination, name of the specialist who determined the material, cultivation of any donor material in field or greenhouse, way of extracting the in-vitro- and cryo-material from this donor material)
(ii) Type of storage (*in vitro* or cryopreservation, or hydrated storage in the case of recalcitrant seeds)
(iii) Place of the stored material (cultivation rooms, cryo-tank with concrete placement in rack and box)
(iv) Splitting of the accession in several parties (when material is split in sub-clones, several cryopreservation sets, number of stored tubes)

(v) Safety duplication (duplication date, duplicated in which institution/country, responsible person there, reference to duplication agreement documents)
(vi) reference to the protocol used for *in vitro* culture and/or cryopreservation
(vii) Labelling of the culture vessels (colour codes, barcodes). LN-resistant labels are available, which, if necessary, can be wrapped around already frozen cryotubes.

109. Further advances in biotechnology will allow phenotypic data to be complemented by molecular data. Bar coding of germplasm will be helpful in managing the information and the material and reduces the possibility of making mistakes.

110. A majority of genebanks now have access to computers and the internet. Computer-based systems for storing data and information allow for comprehensive storage of all information associated with the management of field collections. Germplasm information management systems such as GRIN-GLOBAL (2011) have specifically been developed for universal genebank documentation and information management. The adoption of data standards which today exist for most aspects of genebank data management helps make the information management easier and improves use and exchange of data. Sharing accession information and making it publicly available for potential germplasm users is important to facilitate and support the use of the collection. Ultimately, conservation and usability of conserved germplasm are promoted through good data and information management.

D. Contingencies

111. Loss or incomplete documentation reduces the value of an accession, to the point of making it unusable. Inappropriate material (e.g. not LN-resistant labels) can cause loss of data.
112. In large collections, skill of the workers becomes a very important factor. Risks of inadequate data entries must be clearly indicated. In complicated collections active access to management data should be limited to the responsible persons only.

E. Selected references

Alercia, A., Diulgheroff, S. & Metz, T. 2001. FAO/IPGRI. Multi-crop Passport Descriptors http://www.bioversityinternational.org/.../faoipgri_multi_crop_passport_descriptors

BRAHMS. 2011. Botanical Research and Herbarium Management System. http://dps.plants.ox.ac.uk/bol

GRIN-GLOBAL. 2011. Germplasm Resource Information Network Database- Version 1. http://www.grin-global.org/index.php/Main_Page

3.2.7. STANDARDS FOR DISTRIBUTION AND EXCHANGE

A. Standards

7.1 All germplasm should be distributed in compliance with national laws and relevant international conventions.

7.2 All samples should be accompanied by a complete set of relevant documents required by the donor and the recipient country.

7.3 *In vitro*/cryopreserved material should be supplied only after the supplier and recipient have established the condition under which the material is transferred and that the prerequisite for adequate re-establishment of plants is ensured.

B. Context

113. Germplasm distribution is the supply of a representative sample from a genebank accession in response to requests from germplasm users. There is a continuous increase in demand for genetic resources to meet the challenges posed by climate change, by changes in virulence spectra of major insect pests and diseases, by invasive alien species and by other end-users needs. This demand has led to wider recognition of the importance of using germplasm from genebanks –- which ultimately determines the germplasm distribution. It is important that distribution of germplasm across borders adheres to international norms and standards relating to phytosanitary regulations and according to provisions of international treaties and conventions on biological diversity and plant genetic resources.

C. Technical aspects

114. The two international instruments governing the movement of genetic resources for food and agriculture are the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) and the Convention on Biological Diversity (CBD). The ITPGRFA has established a multilateral system which is a virtual international pool of PGRFA of 35 crops and 29 forage species (commonly referred to as Annex 1 crops of the Treaty) as well as related information, to be distributed using a Standard Material Transfer Agreement (SMTA). The SMTA can also be used for non-Annex1-crops as is for instance being done within Europe as part of AEGIS. The CBD and its Nagoya Protocol focuses on the conservation and sustainable use of biological diversity, and the fair and equitable sharing of benefits arising from its utilization of biodiversity. The ITPGRFA and the CBD emphasize this continuum between conservation and sustainable utilization, along with facilitated access and equitable sharing of benefits arising from use.

115. All accession should be accompanied with the required documentation such as phytosanitary certificates and import permits, as well as passport information. The final destination and the latest phytosanitary import requirements for the importing country (in many countries, regulations are changed frequently) should be checked before each shipment. Germplasm transfer should be carefully planned in consultation with the national authorized institute, for the appropriate documentation, such as an official phytosanitary certificate, that complies with the requirements of the importing country.The recipient of the germplasm, should provide the supplying genebank with information concerning the documentation required for the importation of plant material, including phytosanitary requirements.

116. Most recalcitrant-seeded species are long-lived perennials that do not reproduce until they are several years old. Thus regeneration is not a rapid way to bulk up sample sizes to meet demand. If the sample is in the form of alternate explants *in vitro*, multiplication before the production of independent plantlets is possible, but a request must be made in advance.

117. Germplasm should reach its destination in good condition and so adverse environmental conditions during transport and clearing customs should be minimized. A reliable courier service having experience in dealing with customs is recommended. The time span between receipt of a request for germplasm and the dispatch of the materials should be kept to a minimum to enhance the efficiency of the genebank function. If the sample is in the cryopreserved state and is being transferred to another genebank where it will continue to be cryopreserved, the sample must be shipped in a LN 'dry shipper'.

118. If the sample will be set out to grow immediately on receipt, it can be rewarmed, rehydrated and encapsulated in a calcium alginate bead prior to despatch. Such synthetic seeds were originally developed for somatic embryos, but can successfully maintain in good condition cryopreserved excised embryos/axes that have been rewarmed and rehydrated, for at least 10 days at 16 °C without germination (radicle protrusion) being initiated. Germination and seedling/plantlet establishment of synthetic seeds is possible both *in vitro* and could succeed in sterile seedling mix. It is an option also for other small explants from cryopreservation, but the technique is applied in few cases only.

119. Plantlets derived from *in vitro* slow growth storage or cryopreservation should be sent in appropriate containers. Recipients of *in vitro*/cryopreserved material need to have the possibility to transfer the material to pots or to the field, or be able to make such arrangements.

120. Sterile plastic bags which may contain special aeration zones are recommended for sending of *in vitro* plantlets. If glass is used, sufficient stuffing of the containers and declaration of fragility need to be ensured. In cases of glass and plastic vessels also the right orientation of the containers needs to be indicated.

D. Contingencies

121. Poor handling, including inappropriate packaging or delay in shipment, can lead to loss of viability and the loss of material. Thus, it is very important that the supplier and recipient have established the condition under which the material is transferred and that the prerequisite for adequate re-establishment of plants is ensured.

E. Selected references

Rao N.K., Hanson J., Dulloo M.E., Ghosh K., Nowell D. & Larinde M. 2006. Germplasm distribution. Chapter 7 in *Manual of seed handling in genebanks*. Handbooks for Genebanks No. 8. Bioversity International, Rome, Italy.

3.2.8. STANDARDS FOR SECURITY AND SAFETY DUPLICATION

A. Standards

8.1 A risk management strategy should be implemented and updated as required that addresses physical and biological risks identified in standards including issues such as fire, floods and power failures.

8.2 A genebank should follow the local Occupational Safety and Health (OSH) requirements and protocols. The cryo-section of a genebank should adhere to all safety precautions associated with using liquid nitrogen (LN).

8.3 A genebank employs the requisite staff to fulfill all routine responsibilities to ensure that the genebank can acquire, conserve and distribute germplasm.

8.5 A safety duplicate sample of every accession should be stored in a geographically distant genebank under conditions the same, or better than those for the original sample.

8.6 The safety duplicate sample should be accompanied by relevant documentation.

B. Context

122. It is of the utmost importance that the physical infrastructure of any genebank as well as the safety of its staff be protected so as to ensure that the conserved germplasm is safe from any threatening external factors. To manage a germplasm collection successfully, a genebank also requires skilled and trained staff. Management involves not only the maintenance of the collection and its data but an assessment of risks from human activity or those naturally caused. There are particular hazards associated with the use of LN.

123. The physical security of the collections also requires a safe duplication of the collections in a geographically distant location, under the same conditions. In case of natural/physical catastrophe (fire, flood), this duplication might be used to re-build the collections. In addition to the sample itself, safety duplication involves the duplication of information which implies database backup.

C. Technical aspects

124. A genebank should implement and promote systematic risk management that addresses the physical and biological risks in the every-day environment. It should have in place a written risk management strategy on actions that need to be taken whenever an emergency occurs in the genebank concerning the germplasm or the related data. This strategy and an accompanying action plan must be regularly reviewed and updated to take account of changing circumstances and new technologies, and well publicized among their genebank staff.

125. The occupational health and safety of the staff should also be considered. The cryo-storage area should be well ventilated with forced air extraction, and oxygen monitors should be in place. Leakage of LN into cryovials is potentially dangerous; therefore, appropriate vessels that are specifically designed for the purpose should be used, and the manufacturers' instructions should be strictly adhered to. To reduce risk of personal injury operators should wear protective clothing, gloves and face masks.

126. Supplies of LN must always be available, and it is vital that levels of LN are maintained. The cryogenic storage tanks are supposed to be placed in an appropriated location: aerated and with temperature less than 50 °C. Maintenance of the level of LN in storage containers is absolutely critical; if all the LN evaporates, the entire contents of the storage container must be discarded.

127. For the maintenance of viability of samples, the temperature of the tissue must be kept below the glass transition temperature. Care must be taken that when removing a vial from a cryo-cane or from a cryo-box that the temperature of the remaining vials does not increase to the glass transition temperature. Vials should not be labelled with conventional adhesive labels, as they will come off at LN temperatures. The use of a dedicated PC-operated label printer allows specific cryovial labels to be printed, recording information and a unique barcode. The manufacturer's recommendations about which vial to use for which particular purpose should be adhered to.

128. Active genebank management requires well-trained staff, and it is crucial to allocate responsibilities to suitably competent employees. A genebank should therefore have a plan in place for personnel, and a corresponding budget allocated regularly so as to guarantee that a minimum of properly trained personnel is available to fulfil the responsibilities of ensuring that the genebank can acquire, conserve and distribute germplasm. Access to disciplinary and technical specialists in a range of subject areas is desirable. Staff should have adequate training acquired through certified training and/or on-the-job training and training needs should be determined as they arise.

129. For the physical security of the collections, safe duplication of the collections in a geographically distant location under the same conditions should be considered. In case of natural/physical catastrophe (fire, flood), this duplication might be used to re-build the collections. The duplicating bank should be located somewhere that is politically and geologically stable, and at an elevation that rising sea levels will not be a problem. The storage conditions for the safety duplicate should be as good as those of the initial collection.

130. Safety duplication requires a signed legal agreement between depositing and storing or repository genebank. The latter has no entitlement to the use and distribution of the germplasm. The access to the collections should be controlled to avoid unauthorized usage.

131. Samples for the safety duplicate should be prepared in the same way as the initial collection. It is the responsibility of the depositor to ensure that the safety duplicate is of good quality. To prevent deterioration in transit to the receiving bank, cryopreserved samples should be despatched in a LN dry shipper, and transit should be as rapid as possible.

D. Contingencies

132. When suitably trained staff is not available, or when there are time or other constraints, it might be a solution to outsource some of the work or call for assistance from other genebanks.

133. Unauthorized entry to the genebank facilities can result in direct loss of material, and jeopardize the collections through introduction of pest and diseases.

134. LN containers are often contaminated with fungi or bacteria. If samples are stored in the liquid phase of the nitrogen, contamination of the sample can occur.

135. Liability issues may arise if material deteriorates in transit. Therefore, all eventualities need to be adherent to the consignment agreement.

E. Selected references

Benson, E.E. 2008. Cryopreservation of phytodiversity: a critical appraisal of theory and practice. *Critical Reviews in Plant Sciences* 27: 141-219.

IV. FIELD GENEBANK STANDARDS

4.1 Introduction to Field Genebank Standards

136. Field genebanking is the most commonly used method for non-orthodox seed producing plants. It is also used for plants that produce very few seeds, are vegetatively propagated and/or plants that require a long life cycle to generate breeding and/or planting materials. While technical guidelines and training manuals exist for the management of germplasm collections held in field genebanks published by Bioversity International (e.g. Bioversity International *et al*., 2011; Reed *et al*., 2004; Said Saad and Rao, 2001; Engelmann, 1999; Engelmann 2001; Engelmann and Takagi, 2000; Geburek and Turok, 2005), there are no published international standards for these genebanks to help guide curators to effectively and efficiently manage collections to high quality standards.

137. These Standards are intended as a guideline for genebanks conserving living collections, but should be used critically because continuous technological advances are occurring in conservation, much of them species-specific. It is therefore recommended that the *Field Genebank Standards* should be used in conjunction with other reference sources, particularly with regards to species-specific information. This is especially true, considering that there exists a wide variety of plant species producing non orthodox seeds of different life forms (herbs, shrubs, trees, lianas/vines) and life cycles (annual, biennial, perennial) for which it is difficult to establish standards that are valid for all species.

138. Due to the diversity of life forms and life cycles of plant species maintained in field genebanks, the standards discussed here are necessarily generic, while taking into account the differences between species in terms of their life forms, life cycles as well as between cultivated plants and their wild relatives. For enhanced utility, it is equally necessary that guidelines be developed that account for the critical aspects to be considered in the setting up of field genebanks.

139. Specific standards have been developed for ten key areas and include the following sections:

- choice of location
- acquisition of germplasm
- establishment of field collection
- field management
- regeneration and propagation
- characterization
- evaluation
- documentation
- distribution
- security and safety duplication.

Selected references

Bioversity International, Food and Fertilizer Technology Center, Taiwan Agricultural Research Institute-Council of Agriculture. 2011. A training module for the international course on the management and utilisation of field genebanks and *in vitro* collections. TARI, Fengshan,Taiwan.

Crop genebank knowledge base.

http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=97&Itemid=203&lang=english

Engelmann, F. ed. 1999. Management of field and in vitro germplasm collections. Proceedings of a Consultation Meeting, 15-20 January 1996

Engelmann, F. & Takagi, H. eds. 2000. Cryopreservation of tropical plant germoplasm . Current research progress and application. Japan International Research Center for AgriculturalSciences, Tsukuba, Japan/ International Plant Genetic Resources Institute, Rome, Italy.

Geburek, T. & Turok, J. eds. 2005.Conservation and Management of Forest Genetic Resources in Europe. Arbora Publishers, Zvolen, 693p.

Reed, B.M., Engelmann, F., Dulloo, M.E. & Engels, J.M.M. 2004. Technical guidelines for the management of field and in vitro germplasm collections. IPGRI Handbooks for Genebanks No. 7, International Plant Genetic Resources Institute, Rome, Italy.

Said Saad, M. & Ramanatha Rao, V. 2001. Establishment and management of field genebank a training manual. IPGR-APO, Serdang.

4.2.1. STANDARDS FOR CHOICE OF LOCATION OF THE FIELD GENEBANK

A. Standards

1.1 The agro-ecological conditions (climate, elevation, soil, drainage) of the field genebank site should be as similar as possible to the environment where the collected plant materials were normally grown or collected.

1.2 The site of the field genebank should be located so as to minimize risks from natural and manmade disasters and hazards such as pests, diseases, animal damage, floods, droughts, fires, snow and freeze damage, volcanoes, hails, thefts or vandals.

1.3 For those species that are used to produce seeds for distribution, the site of the field genebank should be located so as, to minimize risks of geneflow and contamination from crops or wild populations of the same species to maintain genetic integrity.

1.4 The site of the field genebank should have a secured land tenure and should be large enough to allow for future expansion of the collection

1.5 The site of the field genebank should be easily accessible to staff and supplies deliveries and have easy access to water, and adequate facilities for propagation and quarantine.

B. Context

140. Considering the long-term nature of a field genebank, the selection of an appropriate site for its location is critical for the successful conservation of germplasm. There are many factors which need to be taken into account when selecting a site for a field genebank including appropriate agro-ecological condition for the plants being conserved at the site, associated natural and manmade disasters, secure long term land tenure, accessibility of the site for staff and availability of water resources.

C. Technical aspects

141. Plants will grow strong and well if planted under appropriate agro-ecological conditions. Field genebanks are particularly vulnerable to losses caused by poor adaption of material that has originated in environments that are very different from that of the genebank location. The selected site for the field genebank should therefore have an environment and soil type best suited for the species to reduce the risk of poor adaptation. One solution to poor adaptation is to take a decentralized approach to genebank management, i.e. to collocate the collections in different agro-ecologies rather than in a centralized genebank. Accessions of similar adaptation are kept together in a station located in an agro-environment similar to their origin or similar or near to their natural habitat. The natural conditions of the original environment can be simulated by providing higher shade intensity or drainage, for example for crop wild relatives that originated in natural forests versus cultivated plants which are adapted to higher light intensity.

142. Avoidance of pests and diseases and insect vectors are very important for field collections (see standards for germplasm health). If possible, the field genebank should be located in a location that is free from major pathogenic diseases and pests or away from known infected regions for fungi and virus to reduce risk and management costs related to

plant protection and ensure a clean source of material for distribution. Soils should be checked before planting to ensure they are free from fungi, termites or other soil borne parasites and appropriate treatment provided to clean soil before planting. Where this is not possible, the selected site should be located at some distance from fields of the same crop to reduce threats from insect pests and diseases with a vigorous rouging program. If possible, maintain collections in areas with a hot and dry climate which are less favorable for vector movement, pests and diseases.

143. The assessment of risk from natural disasters such as floods, fires, snow/ice, volcanoes, earthquakes and hurricanes is an important criterion for ensuring the physical safety of collections. In addition, physical security and potential of man-made threats such as theft and vandalism should be taken into account. These characteristics should be considered when locating and designing a field genebank to help reduce loss of germplasm (see also standards on safety).

144. Insect netting and cages can be used for protection against insect or bird damage for smaller plants. Out-crossing species such as fruit trees with recalcitrant seeds or grasses that are grown for seed as well as maintained as plants require isolation from potential pollinators. Selecting a site away from crop stands or wild populations of the same species to avoid gene flow or weed contamination is important for ensuring genetic integrity in these species. Recommended isolation distances, isolation caches or pollination control measures should be established and followed for propagation. Crop specific information about isolation distance in regenerating accessions is available on the Crop Genebank Knowledge Base (see references).

145. A field genebank should be located in a secure site with a long-term agreement and guaranteed or gazetted land tenure and funding, taking into consideration the development plan for the area. The land-use history can give information about the pest or weed status of the land and the quantity of fertilizer used. High use of fertilizer in previous years could affect the growths of root and tubers. High residual fertilizer can for example prevent tuber development in sweet potatoes. Drought stress can be avoided when the availability of adequate rainfall or water supply for supplementary irrigation is included as a selection criterion. Apart from land-use history it is recommended to include measures that can be taken to ascertain and correct the physical and nutritional status of soils. This basically entails doing soil physical and chemical analysis with subsequent corrective measures. Areas with high potassium usage need to be balanced with supplemental calcium and magnesium applications, especially for tropical fruit trees.

146. The size of the chosen site should provide sufficient space for the type of species to be conserved as well as for possible future expansion when the collection grows, especially in the case of perennial species. Required space for tree crops can be considerable. Also sufficient space should be available to accommodate annuals that require continuous replanting and rotation between plots to avoid any possible contamination from previous plantings, as well as rotation of annuals and perennials to control disease and manage soil fertility. Sufficient and appropriate storage facilities are required if plant material needs to be stored after harvest before the next planting.

147. Easy physical access to germplasm will aid monitoring and plant management. The site should be suitable for access of labour and machinery for mulching, fertilizer and pesticide applications and have access to adequate year-round irrigation, propagation, and *in*

vitro or cryopreservation facilities as required. A good security system should be in place to avoid theft or damage to germplasm and facilities.

D. Contingencies

148. When accessions from different eco-geographical origins are planted in one location careful attention by the curatorial field staff is required to monitor the reproductive phenology and seed production and identify and transfer poorly adapted accessions to possible alternative sites, greenhouses, or *in vitro* culture to avoid genetic loss. Special management practises may be required for some accessions. Protected areas such as screenhouses or cages may be required to protect the plants from predators.

E. Selected references

Anderson, C.M. 2008. Recursos genéticos y propagación de variedades comerciales de cítricos. XII Simposium Internacional de Citricultura. Tamaulipas, México. En CD.

Anderson, C.M. 2000. Citrus Germplasm Resources and their use in Argentina, Brazil, Chile, Cuba and Uruguay. Proc. IX ISC. Vol I: 123-125, Florida, USA.

Borokini, T.I, Okere, A.U., Giwa, A.O., Daramola, B.O. & Odofin, T.W. 2010. Biodiversity and conservation of plant genetic resources in Field genebank of National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria. *International Journal of Biodiversity and Conservation* 2(3): 037-050. http://www.academicjournals.org/ijbc/pdf/pdf%202010/mar/borokini%20et%20al.pdf

Crop Genebank Knowledge Base – http://cropgenebank.sgrp.cgiar.org/

Davies, F.S. & Albrigo, L.G. 1994. Citrus. CAB International, Wallingford, UK.

Gmitter, F.G. & Hu, X.L. 1990. The possible role of Yunnan, China, in the origin of contemporary citrus species (*Rutaceae*). Economic Botany 44: 267-277.

Said Saad, M. & Ramanatha Rao, V. 2001. Establishment and management of field genebank a training manual. IPGR-APO, Serdang.

4.2.2. STANDARDS FOR ACQUISITION

A. Standards

2.1 All germplasm accessions added to the genebank should be legally acquired, with relevant technical documentation.

2.2 All material should be accompanied by at least a minimum of associated data as detailed in the FAO/IPGRI multi-crop passport descriptors.

2.3 Propagating material should be collected from healthy growing plants whenever possible, and at an adequate maturity stage to be suitable for propagation.

2.4 The period between collecting, shipping and processing and then transferring to the field genebank should be as short as possible to prevent loss and deterioration of the material.

2.5 Samples acquired from other countries or regions within the country should pass through the relevant quarantine process and meet the associated requirements before being incorporated into the field collection.

B. Context

149. Acquisition is the process of collecting or requesting such materials for inclusion in the field genebank, together with related information. The nature of plants with recalcitrant seed and clonally propagated plants requires special attention when acquiring germplasm for conservation in field genebanks. The propagules required for establishing a field genebank may come in different forms such as seeds, cuttings, tubers, corms, scionwood, tissue cultures, graftwood, or cryopreserved material. The plant materials may be obtained from existing genebanks, research and breeders' collections, landraces and cultivated forms grown by farmers and from plant explorations/expeditions. There are many relevant national and international regulations, such as phytosanitary/quarantine laws and national laws for genetic resources access, the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) or the Convention on Biological Diversity (CBD), that govern the movement and acquisition of germplasm, which must be taken in to account.

C. Technical aspects

150. Adherence to Standard 2.1 will allow the safe movement of germplasm both from collection sites within the country and outside the country to the site hosting the genebank. When germplasm material is collected from *in situ* it is important to adhere to the national regulations, which normally require that collecting permits are obtained from relevant national authorities. If the collection is from farmers' fields or community areas prior informed consent (PIC) may be required in accordance with relevant national, regional or international law. If germplasm material has to be exported from a country, an appropriate material transfer agreement should be used. In the case of PGRFA, which are included in Annex 1 and therefore the multilateral system of the International Treaty on Plant Genetic Resources for Food and Agriculture, the export has to be accompanied with the Standard Material Transfer Agreement (SMTA). For other germplasm material not included in Annex 1 of the Treaty, national regulations of access and benefit sharing that may be based on the NAGOYA protocol should be adhered to. Import permit regulations, which specify

phytosanitary and any other import requirements must be sought from the relevant national authority of the receiving country.

151. During the acquisition phase, it is important to ensure that passport data for each accession are as complete as possible, especially georeferenced data are very useful as they give a precise account of the location of the original collecting sites and help to identify accessions with specific adaptive traits in accordance to the agro-climatic conditions of the original collecting sites. Passport data are crucial in identifying and classifying each accession and will function as an entry point in selecting and using the accession. Appropriate collecting forms should be used to capture comprehensive collecting data. These forms should include information such as the initial taxonomic classification of the sample, the latitude and longitude of the collecting site, a description of the habitat of the collected plants, the number of plants sampled and other relevant data that are important for proper conservation, as provided in the FAO/IPGRI multi-crop passport descriptors (Alercia *et al.* 2001). Very useful additional information, such as cultural practices, methods of propagation, history and origin, and uses can be obtained with interviews when material is collected from farmer fields. Whenever possible a herbarium voucher specimen, collected from the same population as the samples, should be kept as a reference collection, and a record should be made of the method and reason for acquisition.

152. In the case of donations (from research programme or genebank), the taxonomic classification, donor name, donor identification number, and names of germplasm in addition to the available passport data should be provided. Adequate information about how the germplasm received was maintained, including pedigree or lineage information, as well as chain of custody information where available should be sought from the donor. Materials should be assigned a unique identification number (either temporary or permanent, according to the practice used in the genebank) that will link the material to the passport data and any other collected information, guaranteeing the authenticity of the sample.

153. Although it is not possible to ensure that plant material collected from *in situ* is in completely healthy condition (no diseases and insect pest infestation) it is important that as far as possible propagules are collected from plants that appear healthy, devoid of disease and insect pest infestations or damage. During collecting, the collector should also be sensitive to the depletion of the natural population targeted for collecting. It may also be useful to repeat sampling from a particular site to maximize capture of genetic variability that may be present at various points in time (Guarino *et al*. 1995). In the collection phase of clonally propagated perennial samples, especially when collecting shoots suitable for taking cuttings or grafting, it would be desirable to stimulate the formation of adequate shoots by scoring the trunk or the branches; these shoots could then be collected during a second visit.

154. It is important to highlight that the time taken to transfer the original seed from the time of collecting to the genebank is critical. This is especially true for species which produce recalcitrant seeds and clonal stock, which do not retain their viability for very long and for vegetative propagules which decay easily. In some cases germplasm material may need to be shipped over long distances, as the case may be when the material is acquired from other countries. Due consideration of the shipping period including transit and processing period, should be taken into account and appropriate measures taken to ensure that the material reaches the destination genebank in good condition. It is also important to properly prepare the propagules (scion woods, seeds or cuttings) to improve viability during postal or parcel transportation. For example, recalcitrant seeds and scions should be packed in sterile cotton or

other suitable material in a perforated plastic bag to ensure sufficient air exchange. Seeds should be protected from crushing by mechanical mail sorter in a rigid cushioned shipping. For scion wood, the two cut ends of the cleaned scion should be wrapped using a para-film strip to reduce moisture loss. Collections sent from tropical areas need to be mindful of high temperatures during transportation.

155. Given that field collections cannot accommodate many samples (see standards for establishment of collection), the sample size for collecting will usually be limited compared to orthodox seeds. Nevertheless, all attempts should be made at maximising the collection for the target population's genetic diversity. However, in collecting for a field genebank, the collector will need to take decisions on how many plants within a population can practically be collected. The actual figure will largely depend on the breeding system of the plant, the plant type and the part of the plant being collected.

D. Contingencies

156. Collecting should not take place without meeting the legal requirements especially if the germplasm is taken out of the country of collecting afterwards. In the event that materials cannot be taken out of the country due to phytosanitary requirements, efforts should be made to establish field collections in the country of origin and/or to establish *in vitro* cultures that are more amenable for export. Allowances in terms of the sample size should be made for wild and rare species where propagation material might not be available in optimal conditions or quantity.

E. Selected References

Alercia, A., Diulgheroff, S. & Metz, T. 2001. FAO/IPGRI. Multi-crop Passport Descriptors http://www.bioversityinternational.org/.../faoipgri_multi_crop_passport_descriptors

Bioversity International, Food and Fertilizer Technology Center, Taiwan Agricultural Research Institute-Council of Agriculture. 2011. A training module for the international course on the management and utilisation of field genebanks and *in vitro* collections. TARI, Fengshan,Taiwan.

Brown, A.H.D. & Hardner, C.M. 2000. Sampling the genepools of forest trees for ex situ conservation. Pp.185-196: IN A. Young, D. Boshier and T. Boyle Forest conservation genetics. Principles and practice. CSIRO publishing and CABI.

Brown, A.H.D. & Marshall. 1975. Optimum sampling strategies in genetic resources conservation. Pp 3-80. IN: O.H. Frankel and J.H. Hawkes (eds.) Crop genetic resources for today and tomorrow. Cambridge University Press.

Bustamante, P.G. & Ferreira, F.R. 2011. Accessibility and exchange of plant germplasm by EMBRAPA. Crop Breeding and Applied Biotechnology S1: 95-98, 2011.

Crop genebank knowledge base. Field genebanks. http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=97&Itemid=203&lang=english

Engelmann, F. ed. 1999. Management of field and in vitro germplasm collections. Proceedings of a Consultation Meeting, 15-20 January 1996. CIAT, Cali, Colombia. International Plant Genetic Resources Institute, Rome, Italy.

FAO Forest Resources Division. 1995. Collecting woody perennials. Pp 485-511. IN: L. Guarino, V.R. Rao and R. Reid (eds.) Collecting plant genetic diversity. Technical guidelines.

CABI.

Ferreira, F. R. & Nehra, N. 2011. Forestry Germplasm Exchange and Quarantine in Bazil. In: National Convention da Society Americam Foresters, realizada no Havai no período de 02 a 06 de Novembro de 2011. http://www.eforester.org/natcon11/program/2011conventiononsitebook.pdf

Frison, E.A. & Taher, M.M. eds. 1991. FAO/IBPGR Technical Guidelines for the Safe Movement of Citrus Germplasm. Food and Agriculture Organization of the United Nations, Rome, Italy; International Board for Plant Genetic Resources, Rome, Italy.

Guarino, L., Ramanatha Rao, V. & Reid, R. eds. 1995 Collecting Plant Genetic Diversity: Technical Guidelines, Wallingford: CAB International on behalf of IPGRI. in association with FAO, IUCN and UNEP, 748 pp.

Reed, B.M., Engelmann, F., Dulloo, M.E. & Engels, J.M.M. 2004. Technical guidelines for the management of field and in vitro germplasm collections. IPGRI Handbooks for Genebanks No. 7, International Plant Genetic Resources Institute, Rome, Italy.

Said Saad, M. & Ramanatha Rao, V. 2001. Establishment and management of field genebank a training manual. IPGR-APO, Serdang.

Veiga, R., Ares, I., Condon, F. & Ferreira, F.R. 2010. Intercambio seguro de recursos fitogenéticos. In: Estrategia em los recursos fitogenéticos para los países del Cono Sur/IICA. Montevideo: PROCISUR, IICA. p. 75-83 (ISBN 13:978-92-9248-327-2).

Walter, B.M. & Cavalcanti, T.B. 2005. Fundamentos para a coleta de germoplasma vegetal, Embrapa Recursos Genéticos, D.F. Brasil, 778p.

4.2.3. STANDARDS FOR ESTABLISHMENT OF FIELD COLLECTIONS

A. Standards

3.1 A sufficient number of plants should be maintained to capture the genetic diversity within the accession and to ensure the safety of the accession.

3.2 A field genebank should have a clear map showing the exact location of each accession in the plot.

3.3 The appropriate cultivation practices should be followed taking into account micro-environment, planting time, rootstock, watering regime, pest, disease and weed control.

B. Context

157. It is difficult to provide specific standards for the establishment of a field genebank collection. It will depend very much on the nature of the species which are intended to be conserved. Species specific standards will have to be developed depending on the biological characteristics of the species, its phenology, reproductive mechanism and population structure. There are three main considerations which should be taken in to account in establishing a field genebank collection: (a) how many plants per accessions should be maintained; (b) how the plants are laid out within the genebank; and (c) what cultivation practices need to be applied to ensure optimal growing conditions of the accessions in the collections.

C. Technical aspects

158. The decision for determining how many plants per accession should be planted in a field genebank hinges on the balance of the need to maintain the genetic diversity of accessions, space considerations and economic conditions of the field genebank. It will be different for annual and perennial plants and whether species are seed or clonally propagated. In the case of seed propagated species, the sample size needs to be sufficiently large to capture the genetic diversity contained in the accession that has been collected. It is worth noting here that during collecting non-orthodox seed material, a proper sampling design that prioritises plants for collection has to be made as it will be difficult to harbour a lot of 'within accession genetic diversity' in a field genebank collection. For clonally propagated species, only a small number of plants is necessary to represent the genetic diversity within the accession and to ensure the security of the accession. Sample size can also depend on the purpose for establishing the collection, i.e. evaluation and/or distribution, which may determine different number of individuals per accession as compared to conservation purposes.

159. In establishing a field genebank collection, it is very important to know what accessions are being planted where. A proper planned layout and well prepared field plan will enhance efficiency of space use and management of the collection. The location of individual accessions should be clearly defined. In this respect, plot layout, design, electronic and print maps, as well as barcodes and field labels should be incorporated at the field genebank establishment phase. Considerations should be given to placing accessions in the most appropriate micro-environment in the genebank. Some plants require special environmental conditions and may need to be housed in greenhouses to have a greater environmental control (e.g. to avoid heat or cold) or require shading by other plants.

160. The growth habits and the adult size of the plants as well as irrigation structures and the ease of maintenance need to be considered when calculating the size of the plots. For perennial species, appropriate spacing of plants within the plot allows for proper growth of the individual plant, e.g. a tree, and avoids admixture of those crops that develop tubers on long underground stolons. In addition physical barriers should be implemented between plots to avoid admixture (gene flow), for example by separating the plots with different species that do not cross pollinate. It helps avoid competition that will result in weak plants or could favor rapid spread of disease or insect pests. Invasive clones may require planting in cans, pots or boxes to reduce mixing or competition with less vigorous accessions. Accessions with easily distinguishable morphologies may be planted in adjacent plots when creeping, spreading or shedding of bulbils or seeds to the adjacent plot is a problem. For out-crossing species sufficient isolation distance between plots of different accessions or measures such as isolation caches are required to maintain the genetic integrity of any seeds collected for distribution.

161. It should be emphasised that the layout and field plan are not fixed in time, and will change according to planting schedules. In the case of annuals, rotation is essential and this requires proper scheduling and additional space. It is also important to design layout so as to ensure that there are no pesticide drifts to the immediate environment.

162. Correctly and clearly written labels with two water resistant indelible tags are extremely important in field collections. The tags should contain information on: date, common name and field collection number. If possible, computer-produced labels should be used because they reduce transcription errors in names and numbers. Field maps (as hard copy and in digital form) are essential documents for field genebanks and provide a backup to field labels that are easily lost or destroyed. They should be developed before planting and kept updated regularly.

163. The establishment of field genebank collection requires that the appropriate cultivation practices, specific to the species, be adopted to ensure successful establishment of plants in the field genebank. Planting material needs to be selected carefully. Selecting only strong plants to retain in the field genebank could reduce genetic variation. The quality of initial planting material from a phytosanitary perspective is extremely important when planting new fields or replanting empty plots or when rejuvenating entire collections as long as no genetic selection would be undertaken. Only healthy material and vigorous parts of the plant should be used. Simple sanitary care, like using clean disinfected tools in the preparation of planting materials should be observed. The possibility of indexing for non-apparent diseases such as viruses prior to establishment should be considered where possible.

164. Plants should be planted at the right time. Where recommendations on planting time for different species from different areas have been developed these should be followed. These should take into account optimal conditions for plant establishment, which could include temperature, moisture levels, soil type and rootstock etc. For plants propagated by grafting, one must be careful to get the rootstocks in a standardized way to do the grafting of all samples at the right time. Specific types of species are grafted on a rootstock of the same species, or a closely related one with proven good compatibility. In those cases, the same rootstock should be used for all the accessions of that species. The rootstocks must be selected for their adaptation to soil characteristics and minimum influence on the behaviour of the grafted material. Trees should be planted on their own roots, not grafted, except if the use of

rootstocks is needed to prevent disease or if the graft is the normal form of cultivation of a species.

165. Crops that require cross-pollination should be planted in groups by bloom date. In dioecious species, a suitable amount of male/female plants should be planted. For self incompatible species asexually propagated the curator has to know which SI system is presented by the species and the allelic combination in order to have a good field collection and to guarantee fruit or seeds formation. It is also important to observe the land treatment (agro-technical measures) during establishment of field collections.

166. Some species require additional support by planting shade trees in an appropriate design (e.g. coffee), which need to be chosen according to the local conditions and the requirements of the species. Some species grow as lianas (e.g. vanilla, many beans, cucurbits and others) and need trees, wooden sticks, wires or other installations for proper growth. It may be necessary to install very special beds for special species (mainly those from arid climates), e.g. “table beds” and shelters to keep away precipitation in certain periods of the year. The same may be true for special shading periods, irrigation or flooding times (rice), or covers to protect against frost etc. Some fruit tree species need regular pruning to express their typical appearance and remain healthy. For tree crops, another practice that should be strongly encouraged is the use of dwarfing rootstocks.

D. Contingencies

167. Some genotypes may not respond well to general propagation methods established for particular species types and research should be carried out to develop new methodologies. In the case of plants propagated with rootstock in a planting site requiring the use of a closely related species as rootstock, an interstock should be used.

168. It is important to consider maintaining the collection duplicated at another location (see safety duplication standard). Some genotypes, for example those living in the shadow under the trees of the forest or may be disease susceptible, may not adapt well to conditions of full sun in the field and thus needs to be provided with adequate shelter. This is exacerbated by resource constraints, causing a dual role for field genebanks (conservation plus crop improvement) which can lead to conflicts, e.g., in genebank layout, management, and duplication of accession, etc.

E. Selected references

Crop genebank knowledge base.
http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=97&Itemid=203&lang=english

Reed, B.M., Engelmann, F., Dulloo, M.E. & Engels, J.M.M. 2004. Technical guidelines for the management of field and in vitro germplasm collections. IPGRI Handbooks for Genebanks No. 7, International Plant Genetic Resources Institute, Rome, Italy.

Sebbenn, A.M. 2002. Número de árvores matrizes e conceito genéticos na coleta de sementes para reflorestamentos com espécies nativas. Revista do Instituto Florestal de São Paulo, V.14, n.2, 115-132.

4.2.4. STANDARDS FOR FIELD MANAGEMENT

A. Standards

4.1 Plants and soil should be regularly monitored for pests and diseases.

4.2 Appropriate cultivation practices such as fertilization, irrigation, pruning, trellising, rootstock and weeding should be performed to ensure satisfactory plant growth.

4.3 The genetic identity of each accession should be monitored by ensuring proper isolation of accessions wherever appropriate, avoiding inter-growth of accessions, proper labeling and field maps and periodic assessment of identity using morphological or molecular techniques.

B. Context

169. Field management refers to the day to day curating of the field collections to ensure that plant accessions are in good health, are easily accessible and available for use. This involves many different activities including pest and disease control, proper nutrition of the plants, watering, weeding, pruning and monitoring of accessions to ensure genetic integrity of the collections.

C. Technical aspects

170. Germplasm losses due to poor health can be a major cause of genetic erosion in field genebanks. Maintaining healthy plant accessions in germplasm collections is a major challenge, especially when accessions are collected from a wide area of distribution where different pest and diseases exist. Accessions within collections can also be a source/focus of pest and disease spread if not properly managed. Therefore it is important that strict control of plant introductions into the field genebank be exercised. In addition, current and historical levels of both insect and diseases populations must be considered. Careful inspections and recording are very important in all pest management operations. The timing of disease control is also of paramount importance since after the plant material is infected the damage is often irreversible. Modelling of climatic scenarios and diseases could also assist in the on control of new emerging pests and diseases.

171. Insect pests and diseases may include a very wide range of organisms depending on the target collections. Some of the most commonly associated plant germplasm pests include insects, mites, fungi, bacteria, nematodes, viruses, viroids, spiroplasma, phytoplasma, slug, snails as well as weeds. Clonally propagated plants may be virus-infected, leading to impairment of vigour, hardiness, and graft incompatibility, among others. During quarantine or maintenance, insect pests and diseases maybe detected through a number of techniques including visual examination, isolation by agar plate method/streak plate method, moist chamber incubation, grafting, bioassays, electron microscope examination and plant diagnostic kits. The latter may include enzyme-linked immunosorbent assay (ELISA), which is easy to use, and already available for diseases of root crops (cassava, potato, beet), fruits (banana, pome, stone, and soft fruits) and vegetables. Major plant fungal and bacterial diseases must be controlled by prophylaxis, or prevention. DNA-based diagnostic kits are also extremely efficient in detecting diseases through PCR analysis of specific genes of pathogens. It is advisable to have staff trained in agronomy, horticulture, micropropagation, and pathology performing disease assessments.

172. Correct identification, at the time of delivery, of accessions susceptible to insect pests and diseases is desirable. It is important that field genebanks have a system in place for the identification of all associated pests and diseases for the range of crops they hold in their collection. This is especially true for those crops for which quarantined high risk pathogens have been described. Genebanks should also have procedures in place for the application of relevant diagnostic methodologies that give rigorous assurance on pest and disease status, as directed by local, regional and country requirements. In cases where a genebank does not have this capacity, these tasks should be outsourced to specialised institutions for quarantining incoming plants.

173. Genebank staff need to apply management practices which would reduce the risks of spreading diseases among the collection. It is necessary to ensure that tools and implements, soil and footwear are properly sanitized. Integrated pest management (IPM) is a recommended approach for pest control, where possible. This program uses biological control where possible, supplementing it with pesticides and mechanical control.

174. The field genebank management staff must be proactive to meet the individual needs of diverse germplasm. After planting the plot, staff need to aid the growth of plants only by supplying favourable conditions for their development. Watering plants regularly during the dry season is far more important than fertilizing them. The irrigation system should be appropriate for the type of plant and the ecological conditions where the field is established. Fertilization of the field collection is complicated by the fact that many different types of plants are grown together. Each type of plant has special nutrition requirements due to genetic differences, size, or age. Compound mixtures can be used with low amount per plant and proper care to assure distribution. Small amounts applied at intervals may be more effective than the same total amount applied at intervals of several months. Pruning is necessary in most plants to keep their size within acceptable parameters within the plantation and in the case of trees to shape their canopy. Sometimes only a light thinning should be made in order to have space to develop properly without excessive competition for light. This shaping and thinning operation should be entrusted to an experienced person. Due to the importance of a germplasm collection, labour must be high quality and field maintenance should be done by collection personnel.

175. Competition for weeds is a much more serious problem for young plants than for old ones because of their shallower rooting system. Weed control is important for a rapid and vigorous plant growth. Weeds can be controlled by mechanic ways or using chemicals (herbicides). Herbicides can be used to reduce to a minimum the necessity of hand labour and mechanical cultivation. The type of weed control should be the recommended for each species.

176. In some accessions other protection practices are needed such as frost and/or hail protection or against insect disease vectors using screenhouses. Fruit removal is also an important management practice for disease control, to avoid competition with the next year crop and to reduce stress on the plant.

177. In order to ensure the genetic identity of each accession, any contamination among accessions, geneflow from neighbouring plants and inter-growth of accessions should be avoided. Monitoring and periodic checks should be made to ensure that each accession is properly identified and mapped in the field. Labelling is extremely important and needs to be constantly verified on site and compared to plot plans of the field genebank. Labels should be

clear, concise and be as weather-proof as possible. The use of barcodes or other computer-generated labels are encouraged to reduce transcription errors. Identity of each accession should be periodically checked using morphological and molecular markers when possible (see standard on characterization).

178. The maintenance practices are usually crop-specific and may vary according to the intended use of the collection (conservation, evaluation, distribution). All germplasm accessions should be monitored, however frequency depends on whether the plant is herbaceous (with higher frequency of monitoring) vs. woody (less frequently monitored). All germplasm should be monitored for new animal, insect and disease pests that may be introduced into the germplasm collections. All germplasm must be monitored for vandalism as well (see security standard).

D. Contingencies

179. The lack of expertise in genebanks in dealing with pest and diseases can be a major limiting factor for maintaining healthy plants in the collection, for which skilled plant pathologists may be required. Genebanks should have contingency plans in place to deal with outbreaks of diseases. They should be in contact with specialised plant pathology services such as national plant pathology authorities, university laboratories or commercial laboratories, all of which may provide the services they require.

180. Another good practice is to rotate planting sites (where possible, especially for annually propagated species and perennials highly susceptible to soil sickness) so as to reduce the perpetuation of any soil-borne pests and diseases. Another option is to disinfect the soil. In some cases, plants can be grown in a nursery where phytosanitary conditions can be easier managed, and then be planted out in the field when plants have been acclimated.

181. Some accessions may be very valuable and vulnerable to pathogens. For such cases, it is important to keep them in screen houses and to keep duplicates *in vitro* or in cryopreservation as a complementary conservation back-up.

182. Hand weeding might be required where plants might be injured by herbicide applications. Utilizing sites that do not favour pests and disease development for regeneration purposes is advisable.

F. Selected References

Crop genebank knowledge base.
http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=97&Itemid=203&lang=english

Mathur, S.B. & Kongsdal, O. 2003. Common Laboratory Seed Health Testing Methods for Detecting Fungi. International Seed Testing Association, Bassersdorf, Switzerland.

Navarro, L., Civerolo, E.L., Juarez J. & Garnsey, S.M. 1989. Improving Therapy Methods for Citrus Germplasm Exchange. XI IOCV Conf: 400-408.

Navarro, L. 1988. Application of shoot-tip grafting in vitro to woody species. Acta Horticulturae 227: 43-55.

Reed, B.M., Engelmann, F., Dulloo, M.E. & Engels, J.M.M. 2004. Technical guidelines for

the management of field and in vitro germplasm collections. IPGRI Handbooks for Genebanks No. 7, International Plant Genetic Resources Institute, Rome, Italy.

Roistacher, C.N., Navarro, L. & Murashige, T. 1976. Recovery of citrus selections free of several viruses, exocortis viroid, and Spiroplasma citri by shoot-tip grafting in vitro, p. 186-193. In Proc. 7th Conf. IOCV. IOCV, Riverside.

Sheppard, J.W. & Cockerell, V. 1996. ISTA PDC Handbook of Method validation for the detection of Seed-borne Pathogens. ISTA, Basserdorf, Switzerland.

Sutherland, J.R., Diekmann, M. & Berjak, P. 2002. Forest Tree Seed Health. IPGRI Technical Bulletin N° 6. International Plant Genetic Resources Institute, Rome.

4.2.5. STANDARDS FOR REGENERATION AND PROPAGATION

A. Standards

5.1 Each accession in the field collection should be regenerated when the vigour and/or plant numbers have declined to critical levels in order to bring them to original levels and ensure the diversity and genetic integrity is maintained.

5.2 True-to-type healthy plant material should be used for propagation.

5.3 Information regarding plant regeneration cycles and procedures including the date, authenticity of accessions, labels and location maps should be properly documented and included in the genebank information system.

B. Context

183. In the context of field collections the terms regeneration and propagation refers to the re-establishment of germplasm samples which are genetically similar to the original collection when vigour or plant numbers are low (Dulloo *et al*., 2008). Standards for regeneration and propagation procedures would need to be species specific. When available, protocols or guidelines for particular species should be used. Regeneration and propagation should aim at ensuring that there is no loss of any plants within the collection. However, it is inevitable that the loss of any single individual would entail genetic erosion within the accession because there are normally only a few plants for each accession (see standard of establishment of collection –sample size). Regeneration and propagation are costly and should therefore be carefully planned. They may require changing sites for security or to avoid diseases, pests and soil sickness processes.

C. Technical aspects

184. Regeneration and propagation may be necessary for a variety of reasons depending on the plant type, threats and distribution needs. A plant may decline in vegetative vigour or even die from many different causes, due to climatic, edaphic and/or biotic factors. For maximum efficiency in a field collection plot, it is essential that every dead plant be replaced. This is especially important since the number of individuals per accession is generally low in field collections (see standard 3- establishment of field collection).

185. The method of propagation of the target species is an important consideration. Some species can be propagated by seeds while other species are propagated clonally. In principle, seeds should not be used for propagation in a field collection even if the species can reproduce by seeds unless the population size is represented by a sufficiently large number of individuals. As the objective of regeneration is to maintain the genetic integrity of the accession and given that there is only a limited number of plants per accession, propagation through seeds can lead to significant genetic drift in the accession. In addition, in cross pollinated species, hybridization between accessions may effectively reduce the genetic variance between accessions and change the integrity of individual accessions. Whenever possible, plants should be propagated clonally in which case each offspring is an exact replica of the parent and hence genetic integrity of the accession is maintained.

186. The time at which regeneration should be carried out is another important factor, which often depends on climate and planting season of the crop. FAO has published a series

of crop calendars for Latin America and Africa (FAO, 2004, 2012), which can be a useful guide in determining the appropriate time for planting, and thus for regeneration. The FAO crop calendars provide information for more than 130 crops, located in 283 agro-ecological zones of 44 countries Again the timing will be species- and possibly site specific. A good indication of when to best initiate propagation is provided when propagules start to sprout or mother plants start to die continuously. Another consideration will be whether or not the collection is to be ratooned, i.e. suckers are allowed to develop to produce the next crop, as is the case for aroids (Jackson, 2008).

187. Propagation should be done using true to type and healthy plant material. If available the new plant has to be regenerated using propagation material stored in special facilities (greenhouses, in vitro, or freezer) to ensure its health. Available protocols or guidelines for particular species should be used. Regeneration of accessions of out-crossing species should be made in isolation using special facilities and protection for weeds, pests and diseases.

188. It is important that all information relating to the regeneration of the accession be properly documented and included in the genebank documentation system. This should include *inter alia* information about the accession number and the plant sequence number within each accession, the site where regeneration is carried out, the type of propagation and materials used (cuttings, tuber, corms, bulbs), planting date, survival rate of the propagated materials , the protocol for seed dormancy breaking, management practices employed, method of planting, field conditions, number of plants established, and harvest dates (if any).

D. Contingencies

189. Climatic factors may be more harmful to young plants than to older ones. Because a few plants are likely to be lost during the first year due to various causes, it is a wise precaution at planting to keep some plants for use as replacement if needed. This assures to have plants of the same type and age as the original for replacing lost individuals.

190. Field collections are extremely vulnerable to climatic and other environmental disturbances and it is very important for field genebanks to have a contingency plan for urgent regeneration of the collection. A safety backup may be maintained *in vitro* or cryopreserved as a complementary measure. Contingencies may also occur with wild relatives of crops and native species for which there are no regeneration protocols. These may often require different treatments compared to cultivated relatives.

E. Selected references

Costa, N., Plata, M.I. & Anderson, C. 2004. Plantas cítricas libres de enfermedades. En: Biotecnología y Mejoramiento vegetal. V. Echenique, C. Rubistein; L. Mroginski (eds). Cap 7: 317-318. Ediciones INTA. Argentina.

Crop genebank knowledge base. http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=97&Itemid=203&lang=english

Dulloo, M.E., Thormann, I., Jorge, A.M. & Hanson J. eds. 2008. Crop specific regeneration guidelines [CD-ROM]. CGIAR System-wide Genetic Resource Programme (SGRP), Rome, Italy. (CD-ROM)

ICRISAT. Online. Germplasm regeneration. http://www.icrisat.org/what-we-

do/genebank/genebank-manual/germplasm-regeneration-9.pdf

FAO. 2004. Calendario de cultivos. América Latina y el Caribe. Estudio FAO producción y protección vegetal, No 186.

FAO. 2012. Crop calenders. http://www.fao.org/agriculture/seed/cropcalendar/welcome.do

Jackson G.V.H. 2008. Regeneration guidelines: major aroids. In: Dulloo M.E., Thormann I., Jorge M.A. and Hanson J., editors. Crop specific regeneration guidelines [CD-ROM]. CGIAR System-wide Genetic Resource Programme, Rome, Italy. 16 pp.

Plata, M.I. & Anderson, C.M. 2008. *In vitro* Blueberry (*Vaccinium* spp.) Germplasm management in Argentina. 9th International Vaccinium Symposium, ISHS, Corvallis, OR, USA.

Sackville Hamilton, H.R. & Chorlton, K.H. 1997. Regeneration of accessions in seed collection: a decision guide. Handbook for genebanks No 5. International Plant Genetic Resources Institute, Rome, Italy.

4.2.6. STANDARDS FOR CHARACTERIZATION

A. Standards

6.1 All accessions should be characterized.

6.2 For each accession, a representative number of plants should be used for characterization.

6.3 Accessions should be characterized morphologically using internationally used descriptor lists where available. Molecular tools are also important to confirm accession identity and trueness to type.

6.4 Characterization is based on recording formats as provided in internationally used descriptors.

B. Context

191. Characterization is the description of plant germplasm, and a tool for the description and fingerprinting of the accessions, confirmation of their trueness to type, and identification of duplicates in a collection. It determines the expression of highly heritable characters ranging from morphological, physiological or agronomical features, including agrobotanic traits such as plant height, leaf morphology, flower colour, seed traits, phenology, and overwintering ability for perennials. These are essential information for curators to distinguish among samples in the collection.

192. For field collections, characterization can be carried out at any stage of the conservation process. However, it is essential that the accessions being conserved are known and described to the maximum extent possible to assure their maximum use for customers and stakeholders. Therefore, characterization should be carried out as soon as possible to add value to the collection. The time will vary from species to species depending on their life cycle. The use of a minimum set of phenotypic, physiological and morphological descriptors and information on the breeding system, selected from internationally used descriptor lists (eg. those published by Bioversity International, the International Union for the Protection of new Varieties, or the USDA-ARS NPGS Crop Germplasm Committees) increases the usefulness and cross-referencing of the characterization data.

193. With the advances in biotechnology, molecular marker technologies and genomics are increasingly used for characterization (de Vicente, *et al*. 2004). Characterization will allow true-to-type identification, detecting gene flow and setting reference profiles, identifying mislabelling and duplications, detecting diversity within and among accessions and coefficient of parentage. Measures, such as splitting samples, may be necessary for ensuring the preservation of rare alleles or for improving access to defined alleles. Documentation of observations and measures taken is extremely important.

C. Technical aspects

194. In contrast to seed collections, phenotypic characterization of field collections is easier to perform, given that the plants are in the field and the scoring of the relevant traits for characterisation can be done at the appropriate time and repeated over the years.

195. Some relevant characterization data can be obtained when collecting in the field, so

the time for collecting expeditions should be carefully planned whenever possible. Accessions could then be characterized side by side in the field when collected. Historical and cultural information obtained from farmers, botanists, horticulturalists, or native people during collecting expeditions usually provide valuable information. Local knowledge about the origin of an accession and disease and insect resistance can decrease characterization costs and limit duplication.

196. Descriptors for crops are defined by crop experts and/or curators in consultation with crop experts and genebank managers for relevancy to increase utilization of collections. A wide range of crop descriptor lists has been developed for example by Bioversity International, UPOV, OIV and the USDA-ARS NPGS Crop Germplasm Committees; also minimum sets of key descriptors for utilization have been established for several crops. Data recording needs to be conducted by trained staff using calibrated and standardized measuring formats as indicated in the descriptor lists. The data need to be validated by curators and documentation officers before being uploaded into the genebank database and made publicly available to encourage the use of the collection. It is also recognized that reference accessions planted in the same field are needed to score the traits. Reference collections (herbarium specimens, high quality voucher images) play an essential role for true-to-type identification.

197. The number of plants characterized within an accession should be a representative sample and depends on its diversity. In general, there should be a minimum of 3 plants for diverse accessions, whereas for clonal plants 1-2 are sufficient. A lot of characterization data are heavily influenced by the location where they are recorded, and may also vary over the years depending on the concrete weather conditions. Therefore, the characterization should be repeated over enough years to confirm the traits are correctly scored; three representative years are usually considered as the minimum for reliable characterization of a trait. In species prone to mutate (such as Citrus), annual characterizations for key characters should be done for true-to-type verification.

198. Molecular marker technologies and genomics are increasingly used for characterization in combination with phenotypic traits, because they have advantages on ensuring the identity of clonal plants, identifying mislabelling and duplications, detecting genetic diversity and parentages within and among accessions. The technologies develop fast and costs are also decreasing quickly, allowing a more extensive use in the field collections, and should be used when resources do allow it. There are many markers and techniques available (e.g. SSR, EST-SSR, AFLP, RAPD) but, for characterization purposes, only well-established, repeatable markers such as SSR should be used. For many crops, a wide range of marker primers suitable for their use in characterization has been developed; also minimum sets of key markers have been established. In order to ensure that the results of different analysis batches are comparable, some genebank accessions should be included as reference on each batch. The inclusion of reference accessions in molecular characterizations also plays an essential role for comparison among different genebanks.

199. One of the most advanced techniques employed in the improvement of tree species is genome-wide selection (GWS) (Grattapaglia and Resende, 2011; Fonseca *et al*., 2010). GWS requires the use of molecular markers that allow for wide coverage of the genome and high density genotyping such as SNP and DArT. Although this technique is applied for improvement the information generated can be used to characterize and conserve new accessions or superior genotypes.

D. Contingencies

200. Reliability of data might vary among data collectors if they are not well trained and experienced. Therefore trained and experienced technical staff in the field of plant genetic resources should be available during the entire growth cycle to record and document characterization data. Access to expertise in taxonomy, seed biology, plant pathology and molecular characterization (in-house or from collaborating institutes), during the process of characterization is desirable. For those crops for which there are no internationally used descriptor lists, it should be necessary to develop them. Existing descriptor lists for related crops or species could be used as references.

E. Selected references

Bioversity International. 2007 List of crop descriptors published. http://www.bioversityinternational.org/fileadmin/bioversityDocs/Research/Conservation/Sharing%20Plant%20Information/Descriptor_lists/LIST_OF_CROP_DESCRIPTORS_PUBLISHED.pdf

Bioversity International. 2007. Developing crop descriptor lists, guidelines for developers. Bioversity Technical Bulletin no. 13. http://www.bioversityinternational.org/index.php?id=19&user_bioversitypublications_pi1[showUid]=3070.

Bioversity International. Descriptor lists and derived standards. http://www.bioversityinternational.org/?id=3737

Crop genebank knowledge base. http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=97&Itemid=203&lang=english

De Vicente, C., Metz, T. & Alercia, A. 2004. Descriptors for genetic markers technologies. International Plant Genetic Resources Institute, Rome Italy.

Engels, J.M.M. & Visser, L. eds. 2003. A guide to effective management of germplasm collections. IPGRI Handbooks for Genebanks No. 6. IPGRI, Rome, Italy.

Fang, D.Q., Roose, M.L., Krueger, R.R. & Federici, C.T. 1997. Fingerprinting trifoliate orange germ plasm accessions with isozymes, RFLPs, and inter-simple sequence repeat markers. Theor. Appl. Genet. 95: 211-219.

FAO/IPGRI. 2001. Multi-crop passport descriptors. Food and Agriculture Organization of the United Nations, Rome, Italy; International Plant Genetic Resources Institute, Rome, Italy.

Fonseca, S.M., Resende, M.D.V., Alfenas, A.C., Guimarães, L.M.S., Assis, T.F. & Grattapaglia, D. 2010, Manual prático de melhoramento genético do eucalipto, UFV, Viçosa, MG.

Grattapaglia, D. & Resende, M.D.V. 2011, Genomic selection in forest tree breeding, Tree Genetics & Genomes (Print), 7, 241.

Lateur, M., Maggioni, L. & Lipman, E. 2010. Report of a Working Group on Malus/Pyrus. Third Meeting, 25-27 October 2006, Tbilisi, Georgia. Bioversity International, Rome, Italy.

Maggioni, L., Lateur, M., Balsemin, E. & Lipman, E. 2011. Report of a Working Group on Prunus. Eighth Meeting, 7-9 September 2010, Forlì, Italy. Bioversity International, Rome,

Italy.

OIV. 2009: OIV descriptor list for grape varieties and Vitis species (2nd edition).Organisation International de la Vigne et du Vin, 18 rue d'Aguesseau, 75008 Paris, France.

UPOV Descriptor lists. http://www.upov.int/test_guidelines/en/list.jsp

USDA/ARS/GRIN. Evaluation/characterization Data Queries http://www.ars-grin.gov/cgi-bin/npgs/html/croplist.pl

4.2.7. STANDARDS FOR EVALUATION

A. Standards

7.1 Evaluation data on field genebank accessions should be obtained for traits of interest and in accordance with internationally used descriptor lists where available.

7.2 The methods/protocols, formats and measurements for evaluation should be properly documented with citations. Data storage standards should be used to guide data collection.

7.3 Evaluation trials should be replicated (in time and location) as appropriate and based on a sound statistical design.

B. Context

201. Evaluation is the recording of those characteristics whose expression is often influenced by environmental factors. It involves the methodical collection of data on agronomic and quality traits through appropriately designed experimental trials. Evaluation data frequently includes insect pest and disease resistance and quality evaluations (e.g. oil, protein or sugar content density), production (wood, grain, fruits, seeds, leaf, other) and abiotic traits (drought / cold tolerance and others). These data sets are all highly desired by users to incorporate useful traits into breeding programs and help to improve utilization of the collections. These traits for which the germplasm accessions are assayed are defined in advance by crop experts in collaboration with gene bank curators. Reliable evaluation data that are easily retrievable by plant breeders and researchers facilitate greatly the use of plant germplasm accessions. Germplasm may be systematically evaluated using a network approach, at either an international level or national level.

202. Obtaining evaluation data by genebanks is time consuming and frequently more expensive than obtaining characterization data. Collaboration with breeders and other specialists (virologists, entomologists, mycologists) is recommended in this endeavour. . Curators should make all possible efforts to obtain at least some minimum records of evaluation data. Possible sources of evaluation data may be obtained from users to whom germplasm materials have been distributed previously. The genebank should solicit the user to share the evaluation data and practical arrangements in this regard should be worked out between the gene bank and the recipients/users of the material. Such information could address resistances to biotic and abiotic stresses, growth and development features of the germplasm, quality characteristics of yield, etc. Adding this type of information to the genebank database allows more focused identification of germplasm to meet prospective client needs. Such data should be included in the genebank's documentation system after appropriate verification and validation.

C. Technical aspects

203. A wide range of crop descriptor lists have been developed for example by Bioversity International and the International Union for the Protection of New Varieties of Plants (UPOV). Furthermore, there are evaluation descriptor lists developed by regional and national organizations such as USDA National Plant Germplasm System (NPGS).

204. Data collection should be conducted by trained staff using as much as possible calibrated and standardized measuring formats with sufficiently identified check accessions and published crop descriptor lists. The results of greenhouse, laboratory or field evaluations,

following standardized protocols and experimental procedures are usually presented as either discrete values (e.g. scores for severity of disease symptoms; counting) or continuous values (based on measuring). The data need to be validated by curators and documentation officers before being uploaded into the genebank database and made publicly available.

205. Many agronomic traits required by breeders are too genetically complex to be screened for in preliminary evaluations of germplasm accessions. Data on agronomic traits are usually obtained during the evaluation of germplasm in a breeding program, and many of these traits result from strong genotype by environment (G x E) interactions and hence are site-specific. It is essential to use replications for the evaluation of desired traits in different environments and to clearly define and identify check accessions to be used over time. Check accessions facilitate comparisons across years of data collected.

206. The use of molecular markers in combination with phenotypic observations facilitates the estimation of uniqueness of a source of variation within or among accessions. Genotypic data obtained from characterizing germplasm using molecular techniques has the advantage over phenotypic data in that variations detected through the former are largely devoid of environmental influences (Bretting and Widrlechner 1995). However, molecular evaluations require advanced laboratory facilities and technical capability, and could be expensive, particularly considering the large number of entries to be evaluated (Karp et al., 1997). Thus, the adoption this technology is difficult by some institutions, especially when it is necessary to develop the genetic markers for specific species (SSRs).

207. A number of molecular markers are available and some of the most commonly used in germplasm characterization include, Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs), and Single Nucleotide Polymorphisms (SNP), sequencing of coding and non-coding regions and genotyping-by-sequencing (GBS). In addition next generation sequencing platforms and whole genome sequencing should be mentioned as these are becoming more of a reality as expenses are dropping. These markers vary in the way they detect genetic differences, in the type of data they generate, in the taxonomic levels at which they can be most appropriately applied, and in their technical and financial requirements (Ayad *et al*., 1997). With the increasing use of Marker Assisted Selection techniques the determination of traits at the molecular level such as disease and insect pest resistance, quality and environmental traits has become cheaper and more accurate than field evaluations and can be readily generated. However, molecular data referring to disease or insect pest resistance always need to be confirmed through phenotypic observations in the field. There is a need to ensure the molecular data are loaded into documentation systems appropriately. An important element related to the use of molecular data is the need to match DNA sequence data to phenotypic traits and its appropriate recording in information systems..

D. Contingencies

208. Reliability of data might vary among data collectors if they are not well trained and experienced and when data collection procedures are not harmonized. Therefore trained technical staff in the field of plant genetic resources should be available to collect and document evaluation data. The participation of multi-disciplinary teams with expertise in plant pathology, entomology, and environmental (abiotic) stress tolerance, both in-house and from collaborating institutes, during the process of evaluation is highly desirable.

209. The evaluation of plant germplasm is very labour-intensive and requires adequate and continuous levels of sustainable funding to allow for the assemblage of reliable high quality data. In situations where carrying out the full evaluation of all accessions, which though desirable may not be economically feasible, the selection of genetically diverse accessions (based for instance on previously delineated sub-sets of germplasm collections) is recommended as a starting point.

210. Variations in the incidences of pests and diseases, the severity of abiotic stresses and the fluctuations in environmental and climatic factors in the field impact on the accuracy of data and should be mitigated through reasonably replicated, multi-locational, multi-season and multi-year evaluations. Also, the laboratory assays for the measurements of some traits like oil or protein contents, starch quality, nutritional factors, require specialized equipment and skilled staff which are not always available or could be costly. This again underscores the need for the participation of multi-disciplinary teams from several organizational units or institutions as the case may be. The sanitary status (viruses) of the accession may have incidence in the evaluation as well as in morphological descriptions

211. Using the evaluation data generated by others could pose significant practical challenges. For instance, the data may be in different formats, and if published already may involve copy right and intellectual property rights issues. In order to facilitate the use of externally sourced data, it is important to standardize data collection, analysis, reporting and inputting formats.

212. It should be noted that many characters may appropriately be evaluated within a field-planted genebank itself. However, stresses that impose risks to the collection, and may result in accession losses if uncontrolled, should be evaluated in separate, specially designed trials. Serious insect pests and diseases or major soil problems are examples. The field collection often is not an appropriate place to evaluate yield or quality because of inappropriate plot design or the need to leave plants in the ground well beyond the normal harvest period

E. Selected references

Ayad, W.G., Hodgkin, T., Jaradat, A. & Rao, V.R. 1997. Molecular genetic techniques for plant genetic resources. Report on an IPGRI workshop, 9-11 October 1995. Rome, Italy. International Plant Genetic Resources Institute, Rome, Italy. 137pp.

Bretting, P.K. & Widrlechner, M.P. 1995. Genetic markers and plant genetic resource management. Plant Breeding Reviews 13:11-86.

De Vicente, M.C. & Fulton, T. 2004. Using molecular marker technology in studies on plant genetic diversity. International Plant Genetic Resources Institute (IPGRI), Rome, Italy and Institute for Genetic Diversity, Ithaca, New York, USA.

Karp, A., Kresovich, S., Bhat, K.V., Ayad, W.G. & Hodgkin, T. 1997. Molecular tools in plant genetic resources conservation: a guide to the technologies. IPGRI Technical Bulletin No. 2. International Plant Genetic Resources Institute, Rome, Italy. 47pp.

4.2.8. STANDARDS FOR DOCUMENTATION

A. Standards

8.1 Passport data for all accessions should be documented using the FAO/IPGRI multi-crop passport descriptors. In addition accession information should also include inventory, map and plot location, regeneration, characterization, evaluation, orders, distribution data and user feedback.

8.2 Field management processes and cultural practices should be recorded and documented.

8.3 Data from 8.1. and 8.2 should be stored and changes updated in an appropriate database system and international data standards adopted.

B. Context

213. Comprehensive information about accessions, including regularly updated and detailed field maps as well as information about field management processes is essential for a field genebank to manage and maintain its field collections. Documentation of characterization and evaluation data is particularly important to enhance the use of the respective collection and to help in the identification of distinct accessions.

C. Technical aspects

214. All data and information generated throughout the process of acquisition, establishment of the collection, field management, regeneration, characterization, evaluation, and distribution should be recorded. Such data and information ranges from details of the genetic characteristics of individual accessions and populations to distribution networks, clients and user feedback. Types of data to be recorded in a field genebank other than passport data and standard crop descriptors are for example plant catalogues, voucher images (photos, drawings), planting and harvest dates, and notes on the verification (identity) history.

215. The FAO/IPGRI List of Multi-crop Passport Descriptors should be used for documenting passport data as they are instrumental for data exchange among different genebanks and countries. Standards for documenting characterization data such as the Bioversity crop descriptors as well as genetic marker descriptors exist (de Vicente et al., 2004)) and should be used. With advances in biotechnology, there is a need to complement phenotypic trait data with molecular data. Efforts should be made to record the molecular data being generated through genomics, proteomics, metabolomics and bioinformatics.

216. Record keeping about the field management processes including daily interventions, is extremely important for good management of the field collection. Good records of field maps (as hard copy and in digital form) (see standard on field establishment) are essential to properly document. Old maps should be retained and dated for reference.

217. Different cultural practices are required for the proper management of accessions of different types of species and should be carefully documented to guarantee their consistent employment over time and the appropriate treatment of accessions.

218. A majority of genebanks now have access to computers and the internet. Computer-based systems for storing data and information allow for comprehensive storage of all

information associated with the management of field collections. Germplasm information management systems such as GRIN-GLOBAL (http://www.grin-global.org/index.php/Main_Page)have specifically been developed for universal genebank documentation and information management. The adoption of data standards which today exist for most aspects of genebank data management helps make the information management easier and improves use and exchange of data. Sharing accession information and making it publicly available for potential germplasm users is important to facilitate and support the use of the collection. Ultimately, conservation and usability of conserved germplasm are promoted through good data and information management.

219. All data should be kept up to date. They should also be duplicated at regular intervals and stored at a remote site to guard against loss from fire, computer failure etc. (see standards for security and safety). It can be useful to have written records of the main passport data and hard copies of the field maps.

D. Contingencies

220. Lack, or loss, of documentation, field plans or labels compromises the optimal use of the germplasm or can even lead to its loss, if it impedes proper management and regeneration.

221. Lack of adequate identification of species does not allow to record all necessary information for proper management of the accession and to identify appropriate cultural practices.

222. Many wild species are notoriously difficult to maintain either as field collections or as *in vitro* plantlets. These species can be recovered from cryopreservation.

E. Selected references

de Vicente, M.C., Metz, T. & Alercia, A. 2004. Descriptors for genetic markers technologies. International Plant Genetic Resources Institute., Rome, Italy.30p.

Lipman, E., Jongen, M.W.M, van Hintum, Th.J.L., Gass, T. & Maggioni L. compilers. 1997. Central Crop databases: Tool for plant genetic resources management. International Plant Genetic Resources Institute, Rome, Italy. CGN, Wageningen, The Netherlands.

Fabiani, A., Anderson, C. & Tilleria J. 1996. Desarrollo de una Base de datos para la evaluación de Germoplasma cítrico. (Abstr.). VIII Congreso latinoamericano y VI Nacional de Horticultura. Soc.Urug. Hortic. Montevideo, Uruguay.

Painting, K.A, Perry, M.C, Denning, R.A. & Ayad, W.G. 1993 Guidebook for Genetic Resources Documentation. International Plant Genetic Resources Institute, Rome, Italy.

Tillería, J., Andrade, R. & Zamuz, J. 2011. Documentación de la colección de chirimoya (Annona cherimola Mill) del INIAP con la herramienta curatorial DBGERMOWeb, VIII SIRGEALC, Quito, Ecuador.

Tillería, J., Paniego, N., Zamuz, J. & Luján, M. 2009. El Sistema DBGERMO Web para la Documentación de Colecciones Vegetales, VII SIRGEALC, Pucon, Chile.

Tillería, J. & Zamuz, J. 2011. La Herramienta Curatorial DBGERMOWeb para la Documentación de Colecciones Vegetales. Demostración de la aplicación web en tiempo real con colecciones documentadas, VIII SIRGEALC, Quito, Ecuador.

Tilleria, J. 2001. Sistema DBGERMO para la Documentación de Bancos Activos de Germoplasma, Memoria, Reunión Técnica para Latinoamérica y el Caribe del Sistema Mundial de la FAO de Información y Alerta para los Recursos Filogenéticos:85-115. Turrialba, Costa Rica.

Tilleria, J. & Anderson, C.M. 2004. The DBGERMO II desktop system for an easy documentation of germplasm collections. Proc. ISC. (Abstr.), Morocco.

4.2.9. STANDARDS FOR DISTRIBUTION

A. Standards

9.1 All germplasm should be distributed in compliance with national laws and relevant international treaties and conventions

9.2 All samples should be accompanied by all relevant documents required by the donor and the recipient country.

9.3 Associated information should accompany any germplasm being distributed. The minimum information should include an itemized list, with accession identification, number and/or weights of samples, and key passport data.

B. Context

223. Germplasm distribution is the supply of a representative sample from a genebank accession in response to requests from germplasm users. There is a continuous increase in demand for genetic resources to meet the challenges posed by climate change, by changes in virulence spectra of major insect pests and diseases, by invasive alien species and by other end-user needs. This demand has led to wider recognition of the importance of using germplasm from genebanks – which ultimately determines the germplasm distribution. It is important that distribution of germplasm across borders adheres to international norms and standards relating to phytosanitary regulations and according to provisions of international treaties and conventions on biological diversity and plant genetic resources.

C. Technical aspects

224. There are two major international instruments governing the movement of genetic resources, namely the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) and the Convention on Biological Diversity (CBD). The ITPGRFA aims to facilitate access to plant genetic resources for food and agriculture , and to share, in a fair and equitable way, the benefits arising from the utilization of these resources. It has established a multilateral system which is a virtual international pool of PGRFA from 35 crops and 29 forage species (commonly referred to as Annex 1 crops to the Treaty) as well as related information, which can be distributed using a Standard Material Transfer Agreement (SMTA). While other distribution models also exist, the SMTA can also be used for non-Annex1 crops. The CBD is one of the major conventions on biodiversity conservation with the principal objectives of conservation and sustainable use of biological diversity. The ITPGRFA and CBD emphasize this continuum between conservation and sustainable utilization, along with facilitated access and equitable sharing of benefits arising from use.

225. All accessions should be accompanied with the required documentation such as phytosanitary certificates and import permits. The final destination and the latest phytosanitary import requirements for the importing country (in many countries, regulations are changed frequently) should be checked before each shipment and germplasm transfer be carefully planned in consultation with the national plant protection organization or the officially authorized institute, which needs to supply the appropriate documentation, such as an official phytosanitary certificate, that complies with the requirements of the importing country. The recipient of the germplasm, should provide the supplying genebank with information concerning the documentation required for the importation of plant material, including phytosanitary requirements.

226. Vegetative materials from a field genebank accession should be subjected to therapy and indexing procedures before being distributed to germplasm users. Indexing for difficult-to- detect pathogens, such as viruses, is important for limiting their spread. When virus indexing capabilities are unavailable, in particular for material known to have come from virus-infected areas, the sanitary status should be included in the passport data and the material distributed if the recipient has a quarantine facilities or if it meets the criterion of the import permit of the requesting country or region.

227. The type of shipping container, packing materials and the choice of shipping company will depend greatly on the plant part to be distributed. Phytosanitary certificates and quarantine and import permits often document how the specific germplasm has to be packaged and shipped. Dormant or storage organs require fewer precautions and may spend a longer time in transit without damage than actively growing propagules. Accessions should be kept separate during shipment, they must not mix. Standard operating procedures (SOPS) available in many genebanks cover technical issues such as packaging, treatment, shipping method, sample size, etc. and should be referred to.

228. Timing shipments to avoid severe weather (either hot or cold) and notifying the recipient or customs official prior to the plant's arrival will improve the likelihood that the plants will arrive in good condition. Fragile propagules may require express delivery services. International shipments are facilitated if necessary papers are attached to the outside of the container for easy access by officials without disturbing the plants, with copies inside for the recipient. The requestor may need to purchase services of a courier to carry the germplasm through customs into the country.

229. All accessions should be accompanied with the minimum information necessary to the requester to make appropriate use of the material. This information should include at least an itemized list, with accession identification, number and/or weights of samples, and key passport data. Also the pathogen testing history is usefully included. Distribution records (records with date of request, plants requested, plant form, requester's name and address, shipment date and shipping cost) should be maintained and included in the genebank's documentation system (see standards for documentation). Distributed plant material may become a source of propagative material in case of a catastrophic loss of original material at the originator genebank.

D. Contingencies

230. Simultaneous conservation of accessions *in vitro* provides protection from pests, pathogens and climatic hazards and increases their availability for distribution if the materials are maintained virus free. In some cases, such as cassava (*Manihot esculenta* L.) and cacao (*Theobroma cacao* L.) cuttings from field banks can generally only be disseminated within a country, and sometimes only within certain regions of a country, due to pest and disease quarantine regulations. Other forms of propagation, e.g. *in vitro* cultures or seeds should be used to exchange germplasm between countries or quarantine regions. Distribution of materials from greenhouses or screenhouses may be necessary for crops with insect- or mite-borne viruses and *in vitro* cultures may be required.

231. Political decisions, crisis situations or bureaucratic delays might extend the time span between receipt of a sample request and the distribution of the material. Limitations related to regeneration and/ or multiplication of the accessions may also affect and delay the

distribution process. A delay in checking quarantine regulations until the shipment is ready to send will result in a waste of resources. Consignments of germplasm infested with pests or without proper documentation will be refused entry into the importing country or be destroyed.

E. Selected references

Crop Genebank Knowledge Base
http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=59&Itemid=208&lang=english

4.2.10. STANDARDS FOR SECURITY AND SAFETY DUPLICATION

A. Standards

10.1 A risk management strategy should be implemented and updated as required that addresses physical and biological risks identified in standards.

10.2 A genebank should follow the local Occupational Safety and Health (OSH) requirements and protocols.

10.3 A genebank should employ the requisite staff to fulfill all routine responsibilities to ensure that the genebank can acquire, conserve and distribute germplasm according to the standards.

10.4 A field genebank accession should be safety duplicated at least in one more site and/or backed up by an alternative conservation method/ strategy such as *in vitro* or cryopreservation where possible.

B. Context

232. Given that a field genebank is a live assemblage of plants collected from different areas that will stay in one location for many years, it is extremely vulnerable to a number of threats, including environmental conditions, pests and diseases, land tenure and development. A field genebank is also expensive to maintain and requires constant care compared to other means of conservation. This standard provides the elements which a genebank need to fulfil in order to secure the collection for these threats and ensure that no loss in genetic diversity occurs.

C. Technical aspects

233. A field genebank should implement and promote systematic risk management that addresses the physical and biological risks in the every-day environment. It should have in place a written risk management strategy on actions that need to be taken whenever an emergency occurs in the genebank concerning the germplasm or the related data. This strategy and an accompanying action plan should be regularly reviewed and updated to take advantage of changing circumstances and new technologies and be well publicized among the genebank staff.

234. Field genebanks are exposed to many threats. These include extreme weather conditions like drought, freezing, hail, cyclones, typhoons, hurricanes, which are partially predictable and precautions can be undertaken to give plants additional protection during unfavourable periods. If plants are held in pots, they can be taken into a sheltered place. For smaller plants in the open field, depending on the plant type, little can be done except for reinforcing stakes or covering with a protective cover where feasible. For fruit trees pruning branches can be done to reduce the impact of strong winds which may lead to the uprooting of trees.

235. Other extreme events such as fire outbreaks or earthquakes are hardly predictable, and in such cases precautionary measures to prevent damage to plants in the field genebank need to be taken. Fire breaks across the field genebank need to be established and maintained at all times. In addition fire fighting equipment has to be in place and regularly checked. Fire fighting equipment will include extinguishers and fire blankets. Field genebank buildings

including greenhouses and nurseries need to be earthquake-proof if situated in a seismic-prone area.

236. Other threats to field collections relate to biotic factors including pests and diseases, predators, alien species, rodent pests and native material of the same species growing wild in the area that can enter the field as weeds. Precautionary measures need to be taken against these threats. Pesticides should be used with caution as this not only has a negative impact on the environment, but also on the health and safety of personnel applying these. Where appropriate, the use of traps to catch predators or ditches to prevent access to the plots can be more ecologically friendly and the invasion of animals into field genebanks should be avoided by using humane protocols approved by relevant societies.

237. Vandalism or theft of planting material can also be major problem to the security of collections. Field genebanks should be appropriately fenced and access to the premises of the field genebank should be well controlled. In some places, additional security guards or security fencing may be required. Considering the long-term nature of field genebanks, especially for fruit and other tree species, securing the land tenure and development plan for the site is important to reduce the need for moving to a new site and to allow expansion.

238. The occupational health and safety of the staff should also be considered. Properly functioning protective equipment and clothing should be provided and used in the field, especially when using chemical pesticides and fertilizers. Choice of agrochemicals is important to reduce risk. A list of chemicals that are generally safe for various crops and a "black list" of chemicals that are dangerous and are forbidden should be establish. Staff should be instructed in the correct and safe use of equipment with regular training provided in health and safety in field environments.

239. Active genebank management requires well-trained staff, and it is crucial to allocate responsibilities to suitably competent employees. A genebank should therefore have a plan or strategy in place for personnel, and a corresponding budget allocated regularly so as to guarantee that a minimum of properly trained personnel is available to fulfil the responsibilities of ensuring that the genebank can acquire, conserve and distribute germplasm. Access to disciplinary and technical specialists in a range of subject areas is desirable, depending on the mandate and objectives of each individual genebank. However, staff complements and training will depend on specific circumstances. Staff should have adequate training acquired through certified training and/or on-the-job training and training needs should be determined as they arise.

240. The use of complementary conservation methods for safety duplication of accessions maintained in field genebanks is an important strategy to reduce risk mentioned above and may be more economical. Accessions may be backed-up as slow growth *in vitro* cultures or cryo-preserved in liquid nitrogen, whenever protocols for the target accessions are available. For those species that produce short-lived or recalcitrant seeds, short-term seed storage, where seeds are renewed before viability is lost, is a feasible and cost effective back-up method. A duplicate field genebank in another area with a suitable climate and agroecology where the plants will thrive but that is not subject to the risks of the main genebank, can also be used for safety backup. It also provides an additional site from which material can be distributed and can be located in an area with different pest and disease risks for safety of the collection and to ease quarantine restrictions for distribution within regions. Pollen and DNA storage also complements field genebanks by providing a cost effective way to maintain a larger amount

of diversity within an accession than can be maintained as plants in the field genebank. It can later be transferred back to the population through use of the mother plants in the field genebank.

241. Any safety duplication arrangement requires a clear signed legal agreement between the depositor and the recipient of the safety duplicate that sets out the responsibilities of the parties and terms and conditions under which the material is maintained. This is particularly important for field genebanks where the plants have to be managed on a daily basis.

D. Contingencies

242. When suitably trained staff is not available, or when there are time or other constraints, the solutions would be to include outsourcing some of the genebank work or approaching other genebanks for assistance. It is important to develop networks and collaborations with other genebanks.The international community of genebanks should be instantly informed, if the functions of the genebank are endangered.

243. Unauthorized entry to genebank facilities by humans, animals, wildlife or birds can result in direct loss of material, but can also jeopardize the collections through inadvertent introduction of insect pests and diseases and interference in management systems. Working closely with local communities to raise awareness of the purpose and value of the collection can give a sense of ownership and increased protection to the field area.

E. Selected references

Crop genebank knowledge base. Safety duplication. http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=58&Itemid=207&lang=english

Engels, J.M.M. & Visser, L. eds**.** 2003. A guide to effective management of germplasm collections. IPGRI Handbooks for Genebanks No. 6. IPGRI, Rome, Italy. Available in English and Spanish.

Nordgen. 2008. Agreement between (depositor) and the Royal Norwegian Ministry of Agriculture and Food concerning the deposit of seeds in the Svalbard Global Seed Vault. The Svalbard Gloal Seed Vault. [online] The Nordic Genetic Resource Centre, ALNARP. Available from: http://www.nordgen.org/sgsv/scope/sgsv/files/SGSV_Deposit_Agreement.pdf.

Reed, B.M., Engelmann, F., Dulloo, M.E.,& Engels, J.M.M. 2004. Technical guidelines for the management of field and *in vitro* germplasm collections. IPGRI Handbooks for genebanks No. 7. International Plant Genetic Resources Institute, Rome, Italy. Available from http://cropgenebank.sgrp.cgiar.org/images/file/learning_space/genebankmanual7.pdf

V. **Annexes and List of Acronyms** *to be added*